Clinical evaluation of the HINS-light EDS for the continuous light based decontamination of the burns unit inpatient and outpatient settings

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Declaration of author's rights

This work is dedicated to the staff on the burns unit at GRI, whose support, dedication, and sense of humour are inspiring.

And Michelle, I would not even have known where to start without your help.Thank you.

Abstract

The consequences of sustaining a burn are potentially devastating to a person. Even a relatively smooth recovery from a burn injury can be traumatic to both the body and mind of the patient. Complications, such as infection, only serve to augment this traumatic period through prolonging recovery and worsening outcome. Notwithstanding the great advances in burn treatment made during the last half century, the presence of infection has remained a major influence in dictating the path of recovery for an individual. In fact, advances in resuscitation, surgery, and intensive care support have only served to emphasise the role played by infection. Patients with even severe burns are now surviving their initial injury and remaining in hospital for prolonged periods of rehabilitation. Coupled with a worldwide increase in multi-drug resistant bacteria, and an endemic overuse of increasingly complex regimens of antibacterials, the threat from nosocomial pathogens is greater than ever.

As bacteria become increasingly resistant to antibiotics, novel bactericidal technologies must be explored. Furthermore, emphasis has shifted from treatment to prevention, specifically prevention of cross-contamination between patients. The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is one such weapon in the armamentarium against cross-infection. It works using a safe blue light to kill bacteria in the air and on surfaces around patients and staff. When considering the setting for the first clinical trials of the effectiveness of this light, no area was considered to be more appropriate than the burns unit, due to the high density and great significance of bacteria in this unique environment.

This thesis has not just examined the HINS-light EDS. It has taken a holistic view through considering every step of the route by which one nosocomial strain of bacteria is passed from burns patient to burns patient: the cycle of cross-contamination. Every step in this cycle has been examined in order to determine when the HINS-light EDS could have its maximum efficiency. This has been coupled with extensive clinical studies of the HINS-light EDS in a variety of inpatient and outpatient scenarios in the burns unit, to determine the optimal utilisation of this technology and achieve maximum impact on bacterial populations in the environment.

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Chapter 8: Conclusions and future work

Chapter 1

Introduction

Burns patients are exceptional in their propensity to dissipate large numbers of bacteria into the environment and their susceptibility to infection. This combination renders the burns unit an area liable to facilitate cross-contamination of multi-drug resistant hospital acquired infections (HAI) – also termed 'nosocomial' infection – between patients. The propagation of such infections between burns patients confers multiple consequences to the individual patient, the unit and the hospital at large. These include prolonged admissions; cost and staffing implications; poor wound healing and scar formation; organ failure or death; outbreaks and ward closures.[1]The value of reducing the rate of cross-contamination of nosocomial infections between burns patients cannot be underestimated.

Modalities of transmission of bacteria can be broadly categorised into airborne spread, and direct or indirect contact via a healthcare workers (HCW) or the environment. The initial aim of this study was to examine the effect of a novel light-based method for continuous patient-safe decontamination of the hospital environment. This system is termed the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS). In addition to this, during the course of the work, further possible applications of the HINS-light EDS, and its potential to impact on both airborne and HCW-transmitted bacterial transfer were identified and explored.

The study begins in the laboratory, where the effect of the HINS-light EDS on a range of important pathogenic bacteria collected from a burns unit was examined. This laboratory work aimed to verify previous experiments identifying the HINS-light EDS as a viable method of disinfecting relevant bacteria that may be present in the clinical environment.[2] Furthermore, it was the intention to replicate the clinical environment as closely as possible, by exposing bacteria on solid surfaces, as they would be in a hospital room, rather than in a liquid suspension, as had been performed in the majority of the previous work.

Following the generation of inactivation curves for these bacteria, the clinical part of the work began. These studies were carried out in a burns unit and are divided into two main parts: studies carried out in inpatient facilities and studies carried out in the outpatient clinic. The inpatient studies were performed in individual isolation rooms containing a single patient. They evolved over the course of the work, with initial studies being of a similar model to those previously reported.[3] Later inpatient studies each

identified novel aspects of the use of the EDS, with a view to recognising the most efficient and economical methods of achieving effective environmental decontamination. This section produced several significant findings, with considerable implications as to how use of the EDS may best be optimised.

Studies in the outpatient clinic represented a new direction for the use of the EDS, and one that had not previously been considered achievable given the relatively short time period during which a clinic takes place. Successful results demonstrated that the EDS had a significant impact on the environmental bacterial contamination levels generated during clinics. Again, this noteworthy finding indicated an entirely new application of the HINS-light EDS that had previously been thought to be unobtainable.

Throughout the course of the work, the cycle of cross-contamination through which bacteria are transmitted between patients was examined. This was initially with the aim of identifying specific high-risk 'events' that may provide further opportunities for the use of the EDS. However, it also became apparent that some points during the cycle were less well understood than others, leading to inconsistencies in infection control practices and guidelines between burns units. Therefore, the final part of this thesis is the consideration of specific aspects of the cycle of cross-contamination. Where it was felt that further investigation was warranted, experiments were performed. If it was felt that the HINS-light EDS might play a role in impacting on this specific part of the cycle, its potential contribution has been explored.

Although the initial goal of this work was the examination of the impact of the HINS-light EDS on the environment of the burns unit, the remit evolved to become much broader than this, all the while considering this initial aim primarily. Consequently, a comprehensive overview of the cycle of cross-contamination of infections between burns patients is presented, and what was previously poorly understood has been addressed as far as possible. Alongside this, experimental clinical data has been created, supporting the efficacy and practicality of the HINS-light EDS as a valuable and feasible adjunct to infection control practices in the modern clinical environment.

The following is an overview of each of the chapters, and a brief description of the content of each.

Chapter 2: Background and literature review. This provides background to the current management of burn injuries and the potentially devastating consequences of burn wound infections. It outlines the particular relevance of infection control on the burns unit, and the increasing recognition of an environmental reservoir of bacteria. New and developing technologies to overcome this are discussed, before the basic principles behind the HINS-light EDS are introduced. These include the use of

photodynamic inactivation (PDI); light emitting diodes (LED); the potential hazards of light-based technologies; and the results of previous HINS-light work.

Chapter 3: Materials and methods. This chapter provides information on the media, equipment and microbiological methods used throughout the course of this work. It includes a description of the HINS-light EDS used during laboratory and clinical studies, and reports safety analyses performed on the system.

Chapter 4: Laboratory inactivation of bacterial isolates from the burns unit. The next chapter investigates the laboratory inactivation of bacteria isolated from the burns unit environment by exposure to the HINS-light EDS.

Chapter 5: Inpatient studies. This marks the start of clinical studies. It examines the decontamination efficacy of the HINS-light EDS in various clinical scenarios, all taking place within individual patient isolation rooms. It has the overall aim of identifying the most efficient use of the EDS, and determining the mechanism by which it takes effect.

Chapter 6: Outpatient studies. An entirely new application of the HINS-light EDS. This chapter presents the results of using the HINS-light EDS continuously in a communal area, in the presence of several different patients and staff.

Chapter 7: The contribution of nursing 'events' to the cycle of cross-contamination. As mentioned, the cycle of cross contamination was closely examined during the course of this work, and any areas of knowledge that were previously limited were addressed where possible. Potential applications of the HINS-light EDS are considered at each point of the cycle, with areas for future work being of particular relevance to this chapter.

Chapter 8: Conclusions and future work. The final chapter provides a brief summary of results, discussions and potential areas of future work that have been provided in Chapters 4 to 7.

Chapter 2

Background and literature review

2.0 Outline

This chapter begins with a general overview of the classification and current treatment modalities of burn injuries. The common but potentially devastating consequences of burn wound infections are then described. Attention turns to the spread of nosocomial infection on burns units and the concept of a cycle of cross-contamination between burns patients is introduced. Routes of transfer of bacteria between burns patients are outlined, including the important contribution made by the environmental reservoir to cross-contamination. Current guidelines and the evidence basis of infection control practices in burns units are then explored, with emphasis placed on environmental decontamination.

The focus of the chapter then shifts towards new and developing technologies for improving cleaning practices within hospitals. These include gaseous and airborne decontamination, persistent antimicrobial coatings and ultraviolet light. The advantages and disadvantages of each technology are discussed in turn. Photodynamic inactivation (PDI) is highlighted as an emerging medical adjunct and the bactericidal properties of visible blue light are discussed. Examination of the potential risks to humans of blue light exposure follows. Finally the background to the development of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is introduced, alongside the general aims of this work.

2.1 An introduction to burn injuries

This section provides a synopsis of burn injuries, clarifies terminology and classification of burns wounds; and briefly discusses common treatment modalities.

Introduction

Burns are a common and potentially devastating cause of trauma. Each year about 250 000 people in the United Kingdom (UK) will sustain a burn injury. Over 90% of these injuries are preventable. About 175 000 people visit emergency departments following a burn each year and 13 000 require admission to hospital. Around 1000 patients per year are admitted with a severe burn requiring resuscitation.[4] Data from the National Centre for Injury Prevention and Control in the United States (US) estimates that approximately 450 000 incidences of medical treatment; 45 000 hospital admissions and 3 500 deaths a year are as a result of burn injuries.[5]

Survival rates for burns patients have improved dramatically in the last six decades. In the 1950s, shock, sepsis and multi-organ failure created a 50% mortality rate in children with burns over 50% total body surface area (% TBSA).[6, 7] More recently, a child sustaining a burn over 95% TBSA will survive more than 50% of the time.[7] This improvement has been due to better management of resuscitation, intensive care and pulmonary support, nutritional care, and the practice of early excision and wound cover.[8, 9] However, infection in the burns patient remains the leading cause of morbidity and mortality and a constant challenge for the burns team.[10] If burn wound infection migrates beneath the dermis, it can lead to bacteraemia, sepsis, multi-organ failure and death.[11] In patients with burns over 40% TBSA, 75% of all deaths are related to burn wound infections or other infectious complications and/or inhalation injury.[1, 12, 13] This indicates a significant shift in the cause of mortality away from shock and hypovolaemia, and towards sepsis.

Classification of burns wounds

Burns are the result of disruption to the skin via injuries including thermal, chemical and electrical. The severity of a burn is assessed by consideration of the factors in Table 2.1.

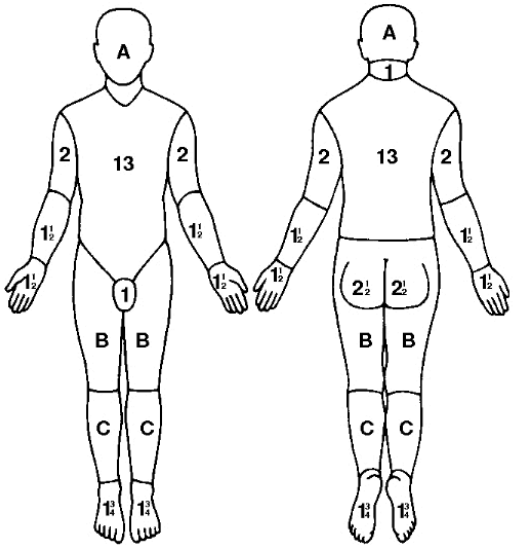
Table 2.1: *Five main factors to consider when assessing a burn injury.*

Size of the burn (measured as %TBSA)
Site of the burn on the body
Depth of the burn
Presence of inhalation injury
Other injuries or pre-existing medical conditions including age

Regression analysis of 1665 patients demonstrated that three of these aid prediction of mortality: age greater than 60 years; burns of more than 40%TBSA; and the presence of inhalation injury. The presence of none of these confers a mortality of 0.3%; one of 3%; two of 33%; and all three of approximately 90%.[14] However, all five factors guide subsequent decisions about treatment and hospital admission and will be considered briefly in turn.

Burn size

Burn size determines fluid resuscitation, nutritional support and surgical requirements. A common way to assess burn size, which takes differing body proportions for different age groups into account, is by using the Lund and Browder chart, which allocates proportional areas to different parts of the body (Figure 2.1).[15] Burned areas are drawn onto the chart and the % TBSA burned is calculated according to the age of the patient. Alternatively the rule-of-nines is used for adults, dividing the body surface into areas, with 9% TBSA assigned to each upper limb and the head, 18% TBSA assigned to the front of the trunk, back of the trunk, and each lower limb, and the remaining 1% TBSA representing the perineum.[16] Finally smaller burns may be estimated by comparing to the patient's palm, which represents 0.78%TBSA, although this is often approximated to 1%TBSA.[17]



Area /Age	A ½ head	B ½ one thigh	C ½ one leg
0 yr	9 ½	2 ¾	2 ½
1 yr	8 ½	3 ¼	2 ½
5 yr	6 ½	4	2 ¾
10 yr	5 ½	4 ¼	3
15 yr	4 ½	4 ½	3 ¼
Adult	3 ½	4 ¾	3 ½

Figure 2.1: *Lund and Browder chart to aid estimation of the percentage of a burned area relative to the total body surface area (TBSA), adapted from[15].*

Site of burn

The site of a burn on the body often determines whether a patient requires hospital admission and surgical intervention. Even small burns to the hands, face and perineum will usually require admission. Deep circumferential burns around a limb or the trunk have the potential to act as a tourniquet, as swelling occurs, and necessitate special consideration, as they may require emergency surgery in the form of escharotomies.

Burn depth

The skin is the largest organ of the body and is divided into two main layers: the epidermis and the dermis. Burn depth is classified according to the level of penetration through the layers of the skin or subcutaneous structures (including fat, tendon, muscle and bone).[18] **Erythema** is temporary and not included in the estimation of burn size and heal within days. **Superficial dermal** burns extend into the superficial (papillary) dermis, forming blisters, with underlying skin having a wet, pink appearance and blanching on pressure. With appropriate care they heal within three weeks without scarring. **Deep dermal** burns breech the deep (reticular) dermis and may blister. The surface is mottled pink and white, with fixed capillary staining and no blanching apparent. Due to prolonged healing times, deep dermal burns often require surgical intervention. **Full thickness** burns involve the entire dermis, and may extend into the deep tissues. The skin may be charred black, if due to flames, or white and leathery and is dry and insensate. Surgical intervention is almost always required. [18]

Burns form three distinct zones. The central focus of the thermal injury is the **zone of coagulation,** where no viable cells remain. Surrounding this is the **zone of stasis**, characterised by a mixture of viable and non-viable cells. [19] The outer rim is the **zone of**

hyperaemia: viable tissue affected by vasodilatation. Tissues in the outer two zones usually recover completely unless complicated by hypoperfusion, oedema or infection.[11] Progressive necrosis into the outer zones may change a burn from one that can be managed conservatively to one that requires operative intervention: hence the significance of a burn wound infection begins to be apparent.[18]

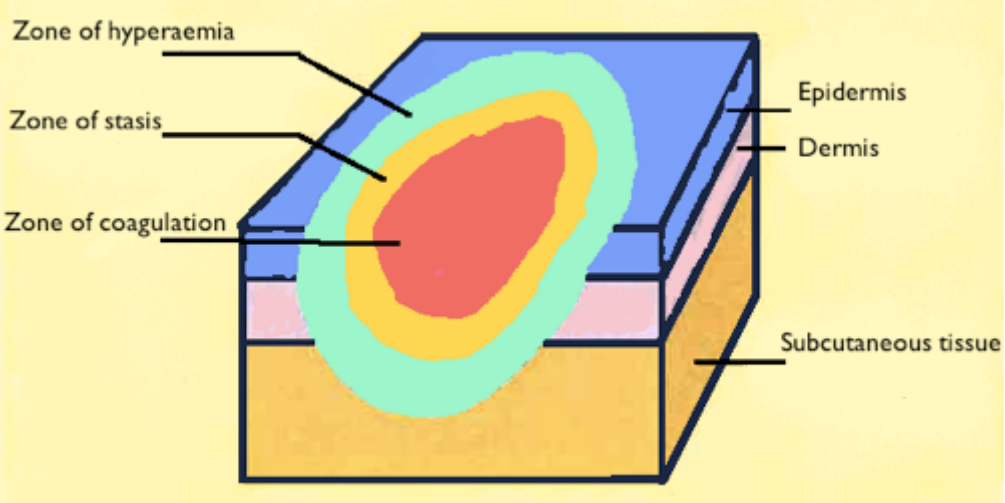


Figure 2.2: *Schematic diagram of three zones of burn injury as described by Jackson[19].*

Inhalation injury

Inhalation injuries can be divided into direct airway injury, usually above the glottis and smoke inhalation, which mainly has its effect below the glottis. Direct thermal airway injuries may follow facial burns, neck burns, chemical ingestion, or hot steam and gases, and are characterised by swelling obscuring the airways. Smoke inhalation is essentially a corrosive burn of the lungs from the products of combustion. Both injuries require admission to a specialist burns unit or an intensive care unit for intubation and oxygen therapy.[11] Burns associated with inhalational trauma have a significantly higher mortality rate than those without any inhalation injury.[20]

Pre-existing conditions

The presence of pre-existing medical conditions or simultaneous traumatic injury is taken into account when deciding on the treatment and admission of a patient to a burns unit. Similarly the age of the patient, their mobility and social circumstances must also be considered.[21]

2.2 Current treatment of burns

Burn injuries result in a wound of protein-rich non-viable burn eschar, which has the potential to act as a rich nutrient medium for the growth of microorganisms. This section will consider the available treatments to prevent the bacterial colonisation of this eschar and promote wound healing.

Topical treatments

Topical agents with antimicrobial properties reduce the bio burden on the wound surface and can reduce infection.[11] They include ingredients such as silver, mupirocin or iodine.[11] Wound washing should be carried out carefully, without causing undue pain to the patient.[22] Commonly used topical agents at Glasgow Royal Infirmary (GRI), where this work was carried out, include povidone iodine preparations and silver sulfadiazine cream (Flamazine™; Smith and Nephew, London, UK), which has been shown to reduce inflammatory cell migration, vascular migration and bacterial density.[22] Alternatively, for patients with extensive burns, a mixture of silver sulfadiazine and cerium-nitrate (Flammacerium™; Solvay Pharmaceuticals, Brussels, Belgium) is often used. The cerium-nitrate binds and denatures the lipid protein complex, rendering the burn eschar firm and impermeable. This decreases oedema from the wound and may reduce the amount of bacteria colonising the eschar.[23-25]

Debridement

Debridement, or the removal of contaminated or necrotic tissue may be achieved using dressings, chemicals, high-pressure water systems, or surgery. Surgical debridement is classified into tangential excision (preserving deep layers of the dermis) or fascial excision (removing all dermis and fat). The timing of surgical debridement is classified as early (within 72 h or burn injury); intermediate (at around one week); or late (after three weeks). The practice of early debridement and coverage of deep or full thickness burns with skin grafts or substitutes is believed to reduce infection rates and systemic inflammation, and is the preferred management in the UK and US.[9]

Wound coverage

Although superficial burns will usually heal, deep burns often require wound coverage with skin grafts. Skin grafts are categorised into full thickness (all layers of dermis) or split thickness (upper layers of dermis only). Autografts are harvested from unburned areas on the patient, creating further wounds that usually heal over two weeks. For reasons including shortage of donor site and the condition of the patient, temporary skin replacements may be used. Human cadaveric skin (allograft) and porcine skin (xenograft) are both used as interim biological dressings.[26] Alternatives include skin

substitutes, which become incorporated into the patient's own dermis including Matriderm and Integra.[27, 28] These require coverage with a split thickness skin graft, although it may be delayed.[29] Biobrane is an alternative biological dressing: a nylon-collagen mesh that can decrease the pain of dressing changes while promoting re-epithelialisation.

Graft 'take'

The process of graft healing or 'take' occurs in four phases. **Adherence** is the immediate formation of fibrin bonds when the graft is in contact with the recipient bed. **Imbibition** is the process through which the graft maintains viability by absorption of nutrient and oxygen containing fluids from the bed, occurring two to four days after application. **Revascularisation** is the process of blood vessel growth from the recipient bed into the skin graft. Finally, **remodelling** is the process by which the graft adopts similar histological architecture to normal skin.[30] Dermal replacements including Integra undergo similar processes. Graft failure is due to the interruption of these stages. This may be due to factors including haematoma or seroma formation between the recipient bed and the graft; shear forces; an inappropriate or inadequately excised recipient bed (such as exposed tendon or bone); technical error (such as placing a graft upside down); or infection.

The importance of burn wound infection has already been noted due to its effects on the progression of a burn wound and the ability of a wound to heal. It is considered in more detail in the next section.

2.3 Infections in the burns patient

This section outlines the aetiology and classification of infections pertinent to burns patients.

Aetiology of infections

The development of an infection requires the breech of defensive barriers and the introduction of microorganisms in sufficient quantities.[31] Humans have three lines of defence against infection: physical; non-specific immune; and specific immune responses. All three are affected following a significant burn. The skin acts as a physical barrier and its destruction permits permeation of microorganisms. The size of the burn is proportional to the rate of wound infection and sepsis.[11] Although the focus of this work is on burn wound infections, other physical barriers are commonly breached following severe burn injury leading to penetration of infective agents into several body systems. Smoke inhalation damages the mucociliary lining of the respiratory tract, which decreases the clearance of invading microorganisms. Disruption of pulmonary endothelial lining also leads to leakage of protein rich plasma, promoting bacterial growth.[11] The gastrointestinal tract may undergo bacterial translocation or adynamic ileus following severe insult, with commensal flora altered through the administration of antimicrobials.[11] The use of urinary, faecal, intravenous and gastric catheters all bypass the normal physical defences to bacterial invasion, as do invasive diagnostic and therapeutic procedures.[31, 32]

Significant burns (i.e greater than 20% TBSA) induce a state of immunosuppression, rendering the burns patient susceptible to infectious complications. The insult sustained through a burn injury has effects on both the innate and adaptive immune responses.[11] The initial immunological response to severe burn injury is pro-inflammatory, with the release of cytokines, mainly by leukocytes. These produce an initial catabolic state, with fever and raised inflammatory markers. However, this is followed by an anti-inflammatory phase with subsequent immunosuppression due to a decreased production and release of monocytes and macrophages. Neutrophil chemotaxis and intracellular killing is reduced and macrophages develop a decreased phagocytic ability. The alternate pathway of the complement cascade is depressed.[11]

This chain of events has the overall effect of increasing the patient's vulnerability to infection, rendering them susceptible to sepsis. Furthermore, areas of deep burn are rendered avascular, impairing migration of host immune cells and restricting the delivery of antimicrobials to the area. This may be further affected by dehydration, hypotension and temperature regulation.[10] In addition, toxins released by burn eschar impair local host immune response.[33] The most significant factors influencing the

development and severity of burn wound infections and sepsis are: increased size of a burn; the amount of full thickness burn; and prolonged open wounds or delayed wound coverage. Factors thought to limit the risk of burn wound infections include early wound closure and stringent infection control.[1]

Classification of burn wound infections

In practice burn wound infection is usually determined by the clinical state of the patient, inflammatory markers and surrounding cellulitis.[34] Most patients with burns over 20%TBSA develop a low-grade fever due to a hypermetabolic state, so pyrexia is a poor indicator of infection. The progression of wound depth, easy separation of eschar and the presence of an offensive discharge are more specific signs of burn wound infection.[1] However, histological analysis of tissue biopsy samples may be required to definitively diagnose burn wound infection. [11]

Burn wound colonisation is the presence of pathogens in a burns wound. It progresses to Burn wound infection when microbes access the underlying tissue and achieve a critical number, usually defined as 1×10^5 colony-forming units (cfu) per gram of tissue. When bacteria progress to involve unburned tissue, (see on histological specimen), it is classified as invasive burn wound infection. [34] Other indicators of invasive infection are the rapid change in the appearance of the unexcised burn wound, such as a dark black colour, or separation of the eschar. The surrounding skin becomes oedematous and warm[10, 11, 34] Sepsis is diagnosed by the presence of positive blood cultures, and is suspected by cardinal signs including high or low temperature; circulatory failure (decreased blood pressure and low urine output); confusion; and failure to absorb feed. [32,34] Frank sepsis is most likely to occur during day six to ten post burn injury.[32]

Sources of infective organisms

Sources of organisms may be endogenous (from the patient's own flora) or exogenous (from an external source). Many studies of infections in burns wound hail from the 1990s, before the practice of early excision and skin cover was common. Although a thermal injury will kill surface bacteria, the native Gram-positive flora such as coagulase-negative staphylococci (CNS) from the skin appendages colonise the wound in the first 48 h.[34, 36, 37] During the next five to seven days there is a gradual decrease in the number of CNS and an increase in methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Pseudomonas aeruginosa*, derived from the gastrointestinal and respiratory tract. There is a gradual pattern from Gram-positive to Gram-negative infection and by day 21 post-burn, 57% of wounds still open will be colonised with resistant Gram-negative bacteria.[8, 34, 36, 38, 39] Later, colonisation with yeasts and fungi follows antibiotic treatment, and resistant nosocomial bacteria become highly virulent infective agents. [40,41]

Common organisms worldwide causing burn wound infections

The spectrum of microbes causing infection in burns patients varies over time and between geographical areas.[35] However, common pathogens have emerged worldwide. Prior to the use of antibiotics, Group A beta-haemolytic *Streptococcus pyogenes* was the major cause of death in patients with severe burns. With the introduction of penicillin, MSSA became the principle infective agent. Although this remains a common cause, Gram-negative infections by organisms such as *P. aeruginosa* have become increasingly common.[33]

A recent twenty-year study in Switzerland examined over five thousand burns patients' samples and found that while MSSA remained the most frequently isolated (20.8%), other common colonising bacteria included *Escherichia coli*, *P. aeruginosa*, CNS and *Enterococcus sp*.[42] However, a study of US burns units reported that *P. aeruginosa* was the most common organism.[43] This was mirrored in studies from India and Egypt, which found high incidences of *P. aeruginosa*, followed by *S. aureus*, *Klebsiella* sp. and *Proteus* sp.[38, 44]

Common organisms on the burns unit at GRI

A database of all burn wound swabs processed between 1st September 2007 and 31st August 2009 in GRI was examined. The total number of organisms isolated was 3336, with 2964 (89%) from non-specific wound swabs, taken for screening or due to clinical indication of infection, and 372 (11%) from methicillin-resistant *S. aureus* (MRSA) specific screening. The commonest organism isolated was MSSA, found on 882 (26%) samples: 17 of these isolated a second strain of MSSA. Following this, MRSA was isolated on 814 (24%) samples, although of note, 295 (36%) of these were from MRSA specific swabs, which may have been carried out more often than usual once a patient was found to have MRSA. Fourteen of these had a second strain of MRSA isolated on the same swab. Other commonly isolated bacteria included coliforms, *P. aeruginosa*, *Proteus* sp., *Streptococcus* sp. (Groups A, B, C and G) and *Enterococcus* sp., with a rare incidence of *Acinetobacter baumannii.* Results are summarised in Figure 2.3.

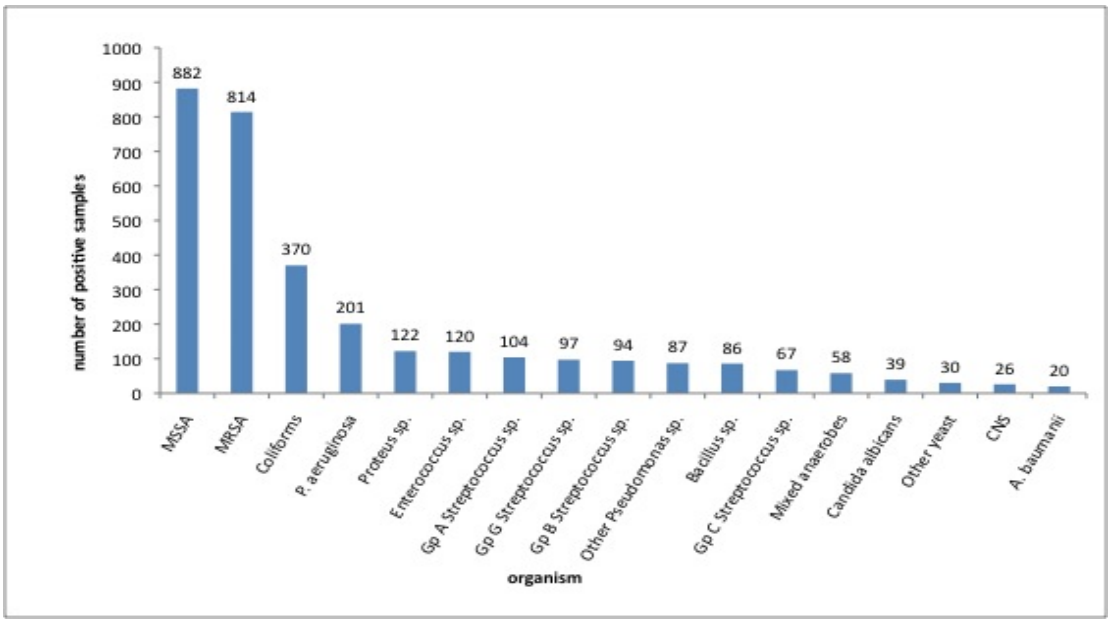


Figure 2.3: *Incidence of organisms isolated from wound swabs taken on the burns unit at GRI over two years from 1st September 2007 to 31st August 2009*

Treatment of burn wound infections

Burn wound infection and sepsis are treated by addressing three aspects: patient optimisation; topical treatment; and systemic antimicrobial treatment. Nutritional support is vital to burns patients, particularly during times of increased metabolic demand, such as infection.[22, 45] Management of pre-existing conditions such as diabetes mellitus or other endocrine abnormalities, and the replacement of deficient vitamins, minerals and ions are also important. Topical treatment and wound cleaning has been previously discussed, and is an important adjunct to the treatment, as well as prevention of wound infections.[46]

Systemic antimicrobials are regularly used in the management of burn infections, although there is little evidence supporting their use as prophylaxes.[47] Afore mentioned difficulties with diagnosing burn wound infection makes the decision to initiate antibiotics complex, and over enthusiastic prescribing of systemic antibiotics carries the additional risk of the development of multi-drug resistant species or fungal infection.[22] The use of broad-spectrum and toxic antimicrobials should be limited as much as possible, and patients treated with a narrow spectrum agent where possible, guided by regular wound cultures.[22]

Consequences of a burn wound infection

The implications of burn wound infections for the patient are manifold. The presence of bacteria in the wound is a burden to the host, as microorganisms compete for nutrients and oxygen. The effect is related to both the number of bacteria present and their virulence.[22] Wound infection therefore leads to delayed healing, graft loss and additional

scar tissue formation, with increased risk of hypertrophic scarring.[22] The incidence of bacterial wound contamination in patients who subsequently developed hypertrophic scarring was 88%, significantly greater than that found in the 27% of patients who did not develop hypertrophic scarring.[48] Spread to other body systems from the burn wound can lead to respiratory, urinary or gastrointestinal infections, with the potential to develop sepsis and multi-organ failure, necessitating in organ support and admission to the intensive care unit.[1] Antimicrobial treatment may contribute to renal or hepatic complications, and prolonged immobility leads to muscle wasting and increased risk of venous thromboembolism. The psychological effects of prolonged hospital admissions are significant.

The burns unit and hospital incur additional costs when a patients' recovery is complicated by infection. Failed grafts necessitate more theatre visits, and increased length of admission has a financial implication due to increased use of beds, staff and resources.[1] Expensive antimicrobial treatment may be required to manage multi-drug resistant nosocomial infections, with the cost of antimicrobials alone estimated at 24% of all medication costs in the burns patient.[49] Burns patients are unique in their susceptibility to infection, and their propensity to disperse pathogens into the environment. This combination has the potential to cause outbreaks of resistant bacteria in a large numbers of burns patients and a place a burgeoning strain on resources. [11]

2.4 The cycle of cross-contamination

This section introduces the concept of a cycle of cross-contamination of infection between burns patients and examines the stages of bacterial transfer.

Events causing bacterial dispersal from burn patients

The main modes of cross-contamination between burns patients are believed to be direct and indirect contact either from the hospital environment and equipment, or via healthcare workers (HCW).[1, 50] The role of the airborne route is less well defined. When a burns patient is at rest in bed, the dispersal of bacteria from their wounds is likely to be negligible. On the instigation of activity however, a proliferation of bacteria are released into the air, and onto surrounding surfaces. Certain events or conditions have been identified as high-risk periods of bacterial liberation. **Bed sheet changes** have been highlighted as an event creating enhanced bacterial dispersion. Mean counts of airborne MRSA from infected patients have been shown to be 4.7 cfu/m^3 during rest periods, rising to 116 cfu/m^3 during sheet changes. Levels remained elevated for at least 15 min after activities cease.[51] **Dressing changes** are a further event shown to liberate bacteria from wounds, with links made between the size of the burn wound and the amount of bacterial release.[52-54] Both of these events will be discussed in detail in Chapter 7.

Transfer of bacteria to environmental surfaces

Bacteria are transferred to environmental surfaces by indirect contact via HCW, direct patient contact with contaminated surfaces, or following the precipitation of airborne bacteria, travelling a distance of up to two metres from whence they came.[55, 56] The contribution of the **airborne route** can be difficult to quantify, as "it is a characteristic of the airborne route…that whenever there is the possibility of aerial transfer there is almost always the possibility of transfer by other routes".[57] A true airborne route is one in which particles remain suspended in the air almost indefinitely as they are so small, and are transmitted over long distances. Examples of these include *Mycobacterium tuberculosis* (TB), *Morbillivirus* (measles) and *Varicella zoster* virus (chickenpox). Other pathogens may behave in similar way to varying degrees. Bacteria may be dispersed as clusters without associated cells or liquid, or carried on skin cells, mucus or saliva, which evaporate leaving smaller, more truly airborne droplet nuclei.[58]

Of particular relevance to burns patients are studies of the airborne spread of staphylococci. The significance of friction on the skin, the desquamation rate, wound infection and agitation during bed making has been emphasised, rather than airborne spread from nasal carriers.[57, 59-61] Air samples conducted in burns units have demonstrated that burns patients generate high levels of infectious MSSA aerosols.[54]

Epidemics of MSSA on burns units have been linked to individual heavy dispersers and a consequential increase in positive air samples.[62-64]

Evidence for airborne bacteria contaminating the inanimate environment includes a study of sterile operating trays, open but untouched in an operating theatre. Within 4 h, 30% of trays were contaminated, with 44% of isolates being CNS.[65] Further work has demonstrated positive air samples and settle plates near carriers of MRSA, indicating an airborne route of dispersal.[66] Air and environmental surface contamination has also been exhibited from a HCW carrying MSSA. The same strain of MSSA was isolated in the surrounding environment during 82/250 surgical procedures in which the HCW was present in theatre, with positive air samples during 19 operations.[68] The airborne route has previously been attributed to 98% of bacteria found in wounds during clean operations: approximately 30% of these being directly precipitated from the air, with the majority being transferred indirectly via the environment or HCW. [68, 69]

Direct contact between an infected patient and the surrounding environment, and **indirect contact** via a HCW, are also central to the transmission of bacteria between patients.[50, 70] There is considerable evidence incriminating **HCW as vectors of transmission** of infection.[71, 72] Strains recovered from the hands of HCW have been shown to correlate strongly with those recovered from patients, and several outbreaks of nosocomial infection on burns units have been attributed to transfer via HCW. These have included MRSA, *P. aeruginosa,* vancomycin-resistant *Enterococcus* (VRE) and multidrug-resistant *A. baumannii* (MDR-*A. baumannii*).[73,-81] Activities associated with high levels of HCW contamination include direct patient contact, spending longer than five minutes in a patient's room, respiratory care, handling of bodily secretions or any indwelling devices, and interruption in the sequence of patient care.[75, 79, 81] Hand hygiene is emphasised as the single most important measure in the prevention of hospital-acquired infections.[50, 75]

Despite widespread hand hygiene practices, studies of HCW carrying out routine nursing tasks have demonstrated up to a 70% transfer rate of VRE or MRSA from the patient and their environment to the HCW.[81, 82] Between 23% and 53% of samples from HCW hands and clothes become contaminated with pathogenic bacteria following contact with the hospital environment alone, despite an absence of contact with the patient themselves.[82-85] One extensive study showed that 65% of HCW caring for patients with MRSA in a wound contaminated their uniforms: but MRSA was also transmitted to 42% of HCW who just touched the environment around the patient.[86] Even following a terminal clean of a room after patient discharge, 24% of volunteers who touched surfaces around the room contaminated their hands.[84] These studies provide evidence that HCW are nearly as likely to become contaminated following contact with the environment as they are following contact with an infected patient, yet even around VRE positive patients, HCW wear gloves only 57% of the time when

touching the surrounding environment, compared with 90% of the time when coming into direct contact with the patient.[82]

Burns units have been identified as one of the few areas in a hospital where the contamination of uniforms poses a serious risk to patients.[50, 87] Bacteria from a HCW's uniform, previously contaminated during the care of a burns patient, were transferred to a second 'patient' during a laboratory reconstruction where the same uniform was worn during the mimicking of routing care activities.[88] Similarly, transmission of *S. aureus* from HCW uniforms to patient bedclothes has been demonstrated during bed making.[89] The transfer of organisms from HCW to the environment was elegantly confirmed in a further study. HCW touched VRE-positive sites such as the skin of colonised patients and contaminated environmental surfaces. They then touched 151 VRE-negative sites. VRE were transferred to 11% of the negative sites. Contamination from a surface led to transfer of VRE to another surface about as often as contamination from a patient.[90]

The environmental reservoir

The inanimate environment surrounding the burn patient has been repeatedly identified as a reservoir for bacteria, and its contribution to nosocomial infection is becoming increasingly topical.[72, 91-95] One systemic review reported that between 31% and 100% (mean 86%) of all sampled hospital equipment was contaminated and that this was frequently linked to hospital-acquired infection.[96] As a marker of nosocomial infection, MRSA is often isolated in clinical studies. Environmental contamination has been demonstrated in the rooms of 73% of MRSA-infected patients and of 69% of MRSA-colonised patients.[86] An increasing proportion of samples taken from surfaces and the air of an MRSA-positive patient's room become MRSA positive over a course of weeks: 54% of surface and 28% of air samples in the first week, compared with 81% surface and 33% air samples by the forth week.[66] Its presence in wounds or urine confers a six-fold increase in environmental load than if it is isolated from other body sites.[86] MSSA or MRSA have been isolated from 63% of HCW tourniquets;[97] 19% of all clinical environmental samples from surfaces including computer keyboards, pulse oximeters, and chairs;[98] television sets;[99] and pens.[100] MRSA has been reportedly present on between 30% and 40% of surfaces in burns units.[62, 101, 102]

One study revealed a 24% total contamination rate of computer keyboards. Species typing revealed that two patients had isolated the same strain of MRSA, which was found on keyboards in their isolation rooms as well as keyboards in unoccupied rooms, and rooms occupied by other patients. The environment was playing a direct role in the propagation of cross-contamination.[103] Similar reports describe patients who acquired MRSA indistinguishable from that in environmental isolates, despite no patients with those strains being present on the ward for the preceding seven days, and control of

outbreaks of type-specific MRSA following identification and decontamination of the environmental culprit.[104-107] There is no doubt that environmental contamination with MRSA occurs in both endemic and epidemic situations and that surfaces within rooms of patients colonised with MRSA should be considered to be contaminated.[95, 108] Epidemic MRSA outbreaks on burn units have led to the closure of the unit, with both staff carriers and environmental surfaces being cited as possible sources of contamination.[109] Conversely, reports of increased patient space and improved air quality significantly decreasing the incidence of MRSA cases on a renovated burns unit have highlighted the role of the environment in the propagation of MRSA amongst burns patients.[53, 74]

An outbreak of *S. pyogenes* infection, followed by environmental sampling of a nursing home, indicated widespread distribution of the bacteria on carpets and soft furnishings of the home. Cleaning measures decreased the levels of environmental contamination and no further cases of skin infections were found.[110] Similar outbreaks of VRE in hospital wards have been ascribed to the environmental reservoir. One study reported 63% of rooms had VRE-positive environmental cultures, including the rooms of 13 patients who were not colonised with VRE: 23% of whom later became infected.[111] A contaminated ECG lead was the source of continuing transmission of VRE in a burn unit outbreak, which again was controlled by environmental cleaning.[112] Discussion of the role of environmental contamination in the transmission of VRE led to the conclusion that change to routine disinfection alone was unlikely to reduce disease transmission as recontamination of the patient environment is so rapid. This heralded a call for innovative, continuous ways to control environmental contamination.[113]

Gram-negative bacteria tend to proliferate in damp areas of the burns unit, such as communal bathrooms and hydrotherapy rooms. *P. aeruginosa* has a predilection for wet environments and is a persistent contaminant of sink taps and drains where it can remain for months, due to an ability to adhere to inert surfaces by fimbriae or by forming a protective glycocalyx.[31, 114-116] An *A. baumannii* outbreak on a burns unit was similarly followed by intensive scrutiny of the environment, leading to its isolation from multiple locations including intravascular drip stands, bed controls, and door handles.[77] Multi-drug resistant *A. baumannii* persists in damp environments for extended periods, which has resulted in outbreaks amongst burns patients.[77, 117] As with MRSA, outbreaks were only controlled following identification of a source and thorough environmental cleaning.[76, 118]

Survival of bacteria in the environment

One critical consideration is the survivability of organisms on inanimate environmental surfaces.[86] There is compelling evidence for the continued existence over several months of species such as MRSA and VRE in a desiccated state.[119, 120] VRE has been shown to be recoverable for 24 hours without significant reduction in colony counts

when inoculated onto bed rails, and for up to 7 days post-inoculation onto counter tops.[121] MRSA demonstrate an extraordinary survivability of up to 38 weeks on inanimate surfaces such as paper and foil at populations of approximately 1 x 10^8 cfu/sample.[122] Laboratory experiments whereby Gram-positive bacteria including MRSA, *S. aureus, Staphylococcus epidermidis, E. coli*, and the Gram-negative *Acinetobacter calcoaecticus,* were spread onto cotton and glass, showed survival of between 2 h and 60 days, with only 2 x $\log_{10}$ reductions in viable colonies after 25 days.[93, 123] In fact, *Acinetobacter* sp. has a significant capacity for long-term survival on dry surfaces or dust particles compared to other Gram-negative bacteria.[124] [125] In one clinical example, the same strain of *A. baumannii* caused two outbreaks on a burns unit, six months apart, with contaminated hydrotherapy equipment found to be the source.[126]

Standards of cleanliness in the hospital environment

There are currently no standards for an acceptable microbial load in the hospital environment.[127] Benchmark values for the number of microorganisms on hand touch sites, or a similar approach to that taken by the food industry have been suggested in the assessment of cleanliness in the hospital.[128, 129] US Department of Agriculture specifications for microbial surface counts on food processing equipment propose acceptable total counts of <5 bacterial cfu/cm^2. In addition, the presence of 'indicator organisms' including MRSA*, Clostridium difficile,* VRE and various Gram-negatives would indicate increased cleaning was needed.[128]

Does a clean environment prevent nosocomial infection?

Several examples have been given where identification and decontamination of environmental reservoirs of bacteria have halted outbreaks. However, hospital-acquired infections have a multi-factorial pathogenesis and effective prevention depends on a multi-faceted approach as demonstrated in Figure 2.5.

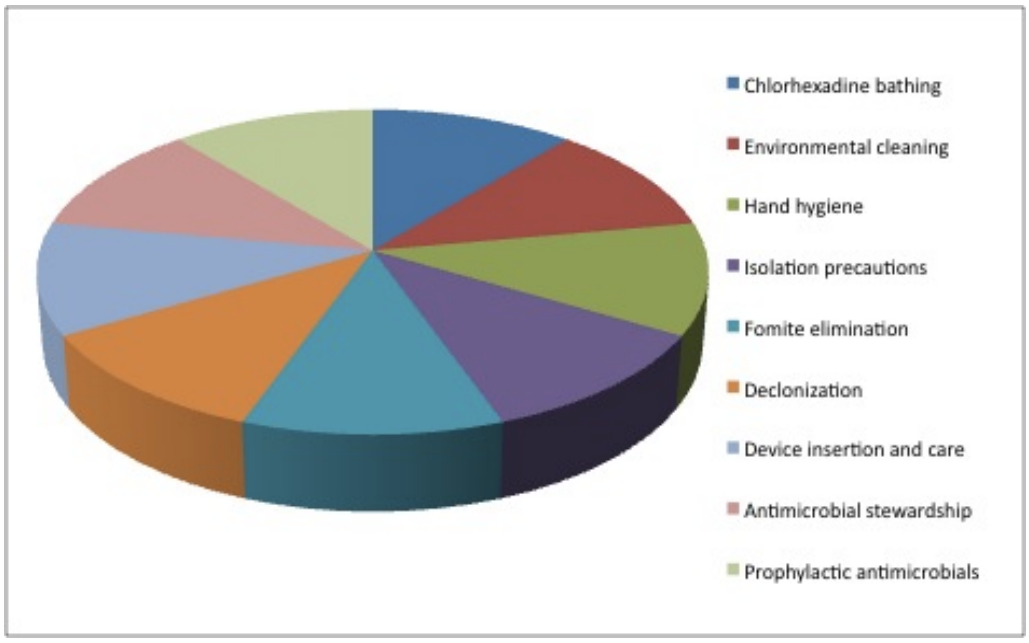


Figure 2.5: *The balanced approach to optimal infection control practices in hospitals. Adapted from*[72]*.*

Studies proving that improved environmental cleanliness results in reduced infection rates are limited due to difficulties with the diagnosis of infection, and the multi-factorial pathogenesis highlighted. Previous attempts to demonstrate reduced infection rates following a period of enhanced environmental cleaning failed to show a reduction in MRSA acquisition by patients, despite a reduction in MRSA isolated from the hands of HCW and the environment.[130] In fact, a paucity of evidence exists that decreasing environmental contamination decreases rates of infection.[131]

2.5 Infection control practices in the burns unit

This section outlines current infection control guidelines, both on the burns unit at GRI and nationally. It details the use of barrier precautions and isolation rooms, cleaning and disinfection, personal protective equipment (PPE), hand hygiene, air filtration, and routine wound culture surveillance.

Barrier precautions

In most hospital wards, strict barrier precautions are reserved for patients with known infections or immunosuppression. However, due to the almost ubiquitous colonisation of large wounds and the predisposition of burns patients to contract infections, pre-emptive barrier precautions are often adopted for all burns patients. The advantages of this were demonstrated following an MRSA outbreak in a burns unit, which was terminated only on the introduction of pre-emptive barrier precautions: a fresh gown and gloves were donned for any physical contact with a patient or their environment. The implementation of this precaution enabled the unit to reduce the rate of MRSA from 7 cases per 1000 patient days, to 1 case per 1000 patient days, which was maintained during a 27 month follow up.[74] Good barrier precautions should always be aspired to, although cost considerations and non-compliance may limit their use in practice.

Isolation rooms

The recognition of the significance of physical separation between burns patients in the prevention of cross-infection has been recognised for over half a century.[132] In 1966, it was stated that "as it is impossible to maintain a deep burn sterile at every stage in its treatment, complete isolation of each patient seemed the safest way of avoiding airborne cross infection".[133] This was an essential concept prior to the advent of early excision, when burns patients were left for weeks with exposed wounds. Within isolation rooms, monitoring equipment including blood pressure monitors and oxygen saturation monitors should be kept at the bedside for the use of that particular patient, and not moved from room to room before without appropriate decontamination.[31]

Cleaning and disinfection

Cleaning and disinfection reduce pathogen load in the environment. Surface disinfection with phenols and quaternary ammonium compounds remains an important weapon in the control of hospital-acquired infections.[134, 135] Any inability of these methods to control infection has been blamed on a failure of the cleaner, rather than the cleaning products and equipment. Conversely, cleanliness of environmental sites has been shown to improve following education programmes amongst cleaning staff.[136]

Personal protective equipment (PPE)

The use of PPE including plastic aprons, gowns and gloves is probably more strictly adhered to on the burns unit than anywhere else in the hospital.[33, 87] The Centre for Disease Control and Prevention (CDC), Royal College of Nursing (RCN) and National Institute for Clinical Excellence (NICE) provide general hospital infection control guidelines, although these are not specific to the unique requirements of burns patients.[137, 138] Guidelines are summarised in Table 2.2.

Table 2.2: *National recommendations for the use of PPE in hospitals, Adapted from NICE infection control guidelines[138].*

Disposable plastic aprons should be worn when there is a risk that clothing may be exposed to blood, body fluids, secretions or excretions, with the exception of sweat, onto the skin or clothing of HCW
Full-body fluid-repellent gowns must be worn where there is a risk of extensive splashing of blood, body fluids, secretions or excretions, with the exception of sweat, onto the skin or clothing of HCW

These recommendations are somewhat ambiguous when considering activities that take place on the burns unit, such as dressing changes on large open wounds. Therefore, the GRI burns unit in conjunction with the GRI department of microbiology created burns-specific guidelines for infection control measures. These are found on the door to each inpatient isolation room and are summarised in Table 2.3.

Table 2.3: *Local guidelines on the use of PPE. Adapted from GRI burns unit infection control ward policy.*

Disposable gloves and plastic aprons must be worn by all staff on entering the room if the patient's dressings are cut down, or if staff are to have any contact with the patient or their immediate environment (including carrying out dressing or bed changes)
Disposable aprons for small burns (< 15%TSBA) or disposable full-body gowns for large burns (> 15%TSBA) should be worn by staff for dressing changes
Hands should be decontaminated before and after entering the room with alcohol gel or soap and water
Relatives and friends visiting should observe hand hygiene rules only

The use of PPE and the shortfalls of current guidelines will be considered in Chapter 7.

Hand hygiene

Meticulous hand hygiene is widely acknowledged to be the single most important factor in the prevention of cross-contamination between spread, and its role is not disputed.[81, 84,, 139] Unfortunately there is evidence that hand hygiene is not always adhered to by HCW.[140] Issues with compliance have always been a problem and hospitals often conduct audits and education programmes for staff to encourage the practice.[43] Guidelines are visible throughout NHS hospitals reminding staff and visitors of the importance of hand hygiene in clinical areas. These are summarised in Table 2.4.

Table 2.4: *National guidelines on hand hygiene. Adapted from RCN and NICE infection control guidelines.*[138, 140]

Keep nails short, clean and polish free, avoid wearing wristwatches and jewellery, artificial nails must not be worn, cuts should be covered with a waterproof dressing
Adequate hand washing facilities should be provided in all patient areas, treatment rooms and sluices, and alcohol hand gel must also be provided at 'point of care'
Hands must be decontaminated, preferably with an alcohol based hand rub unless visibly soiled, between caring for different patients
An effective hand washing technique involves wetting the hands with tepid water; hand wash solution coming into contact with all surfaces of the hand; hands being rubbed together for a minimum of 10-15 seconds; and through rinsing

Air conditioning and laminar airflow

Laminar airflow is the flow of air in a room with uniform velocity along parallel lines, thus reducing turbulence and directing airborne bacteria from within the room to outside. It has been used on burn units for several decades.[141] To limit airborne transmission of infective organisms, heating, ventilation and air conditioning (HVAC) systems containing filters, are used to establish airflow in isolation rooms. A constant negative pressure must be maintained on the inside of the room relative to the surrounding areas, and there must be a sufficient number of air changes per hour (ACH). Guidelines from the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) state that high-efficiency particulate air (HEPA) filters must capture at least 99.97% of 0.3 μm diameter particles.[142,143] Theoretically, these are excellent at reducing levels of airborne particles,[144] however in the hospital setting their maintenance often proves difficult.[55]

The return ducts of isolation rooms should remove air at a faster rate than supply ducts add it to maintain a negative pressure of at least 2.5 Pascals (Pa), as recommended by CDC. There should be at least six, but preferably in a new build, 12 ACH.[145] Scottish guidelines state that there should be at least ten ACH.[146] However, negative pressure

isolation rooms are notoriously difficult to maintain. One study demonstrated that 45% of hospital rooms designed to be under negative pressure were actually under positive pressure.[147] Dramatic pressure changes due to poor room sealing have also been demonstrated and air particles can easily escape into the surrounding environment.[148,149]

Overall, relying on the HVAC systems of isolation rooms to help limit airborne particles is not recommended. Evaluation of the performance of the HVAC systems of 678 hospital isolation rooms in the US, based on six essential criteria demonstrated the limitations of these systems. These criteria included: a negative pressure difference greater than 2.5 Pa; at least 12 ACH; a permanently installed pressure monitor; not having the ability to switch the same room from negative to positive pressure isolation; self-closing doors; and final filters at least 90% efficient. Only 32% rooms met these criteria.[142]

Culture surveillance

Routine surveillance of burn wound microbial colonisations is part of everyday practice in most burns units.[92] This helps to not only treat individual patients but to administer appropriate empirical antibiotics based on the cultures and sensitivities of that particular unit.[36] The routine swabbing of other body regions, such as the nares, axilla, throat, groin and umbilicus can also help to determine which organisms are prevalent in a unit, although not necessarily causing wound infections at that time.[37] Recommendations are that patients should undergo routine swabs on admission and then at least weekly until the wound is closed, although it is often undertaken more frequently than this. The general rule is to obtain a swab culture for each 10% of open burn.[31]

On admission to the GRI burns unit, all patients undergo wound cultures and sensitivities, as well as MRSA-specific swabs of the nares, throat and groin. Following this, burns are swabbed usually twice weekly or more frequently if clinically indicated.

Environmental sampling methods

Environmental sampling is not routine practice in most burns units, but is usually carried out upon the onset of an outbreak within hospital wards in order to identify the source. There are several possible methods used for environmental sampling, the main ones being swabbing and contact agar plating. For **swabbing** a moist swab is rolled or dragged over a surface. The tip of the swab is either used to directly inoculate an agar plate, or is removed and placed into phosphate buffered saline (PBS) and agitated to aid dispersal of bacteria into the solution. Serial dilutions of the bacterial suspension are then carried out, and known dilutions are spread onto agar plates. Both methods are commonly used due to the cheapness and availability of swabs. However, due to

swabbing involving this two-stage procedure, there is potential for inaccurate results due to inconsistent sampling and sample processing methods. The amount of bacteria picked up by the swab depends on the area over which the swab is moved, how much the tip is rolled, how long the sampling takes place for and pressure applied to the surface. When directly transferring to an agar plate, the amount of bacteria transferred varies, depending on how much the swab is moved on the plate, and the amount of rotation of the swab on the agar surface. If the tip is broken off into PBS, the amount of agitation will affect the density of the solution. A further disadvantage in this method is the processing necessary following sampling, by either inoculating agar or preparing a solution with which agar can be inoculated. In a clinically based study with a high number of samples frequently taken swabbing can be impractical.

Contact plate sampling, using either contact plates or dip slides is an alternative method. It involves the direct placement of agar (a bacterial growth medium) onto an environmental surface in order to lift organisms directly from that surface. Contact plates or dip slides are then incubated and the resultant bacterial counts are enumerated directly from the agar surface. This method has numerous advantages:

- The same contact area is always made with the surface being sampled, provided care is taken to cover the whole surface of the agar on curved surfaces such as bed rails.
- There is no second stage where further error could be introduced in the transfer of bacteria to a culture medium.
- The timing of contact with the environmental surface, and the pressure applied can be controlled to some extent by all samples being carried out in the same manner by the same sampler.

Contact plating for environmental sampling is described in detail in Chapter 3.

2.6 New technologies to reduce environmental contamination

The importance of reducing environmental contamination to prevent propagation of nosocomial infections throughout the burns unit has been discussed. This section outlines new technologies that have been developed with this aim. Current cleaning methods are often suboptimal due to the rapid regeneration of surface contamination, and novel disinfection methods for the decontamination of the environment are increasingly necessary.[150, 151]

1. Reducing airborne transmission

Four methods can be used to reduce the risk of airborne transmission: [152]

- **Differential pressurisation**, i.e. measurable differences in air pressure creating a directional airflow between adjacent spaces (e.g. negative pressure airborne infection isolation rooms, and positive pressure operating theatres);
- **Dilution** through frequent ACH, with current guidelines recommending 12 ACH for isolation rooms;
- **Filtration** of air handling units (e.g. pre-filters of 30% particle removal efficiency, followed by HEPA filtration of 99% of 0.3 μm particles;
- **Purification** of air.[152]

Guidelines for the first three are well established and have already been discussed. Technologies for 'whole room' environmental decontamination involving air purification and surface decontamination will now be considered.

Gaseous hydrogen peroxide

Hydrogen peroxide can be generated using either a hydrogen peroxide dry-mist system ('dry fog'; Gloster Santé Europe, Labege Cédex, France), or hydrogen peroxide vapour (HPV; BioQuell Ltd, Andover, UK).[153] HPV has been shown in laboratory conditions to inactivate 7 $\log_{10}$ populations of nosocomial bacterial strains including MRSA, *Acinetobacter* sp. and VRE within 90 min.[154] A systematic review into the decontamination effects of HPV in the clinical setting and its use as an infection control adjunct was encouraging.[151] Five studies evaluated the effectiveness of HPV against MRSA and the remainder considered other pathogens.[155-163] Samples were taken from multiple sites in the patient's immediate environment, such as bed frames, bedside tables, and taps, using a variety of sampling methods such as moistened swabs, contact plates and dip-slides. Before any cleaning took place, 39% (range 19%-81%) of sites were contaminated; after cleaning, 28% (range 12%-66%) of the sites were contaminated; but after disinfection with HPV only 2% (range 0%-4%) of the sampled

sites were contaminated. HPV use has aided termination of MRSA and *C. difficile* environmental contamination and outbreaks in hospitals.[151,156, 160,164]

HPV was used to simultaneously decontaminate air-conditioning ducts and ward surfaces and furniture. Patients were removed into corridors not served by the same HVAC system while the rooms were sealed and fogging took place for 3 h followed by 3 h aeration with fresh air. Environmental samples taken using damp swabs showed a greater than 99% reduction in counts of bacteria and fungi in all areas tested.[165] This study highlights one of the main problems with the use of HPV. Namely the time required for decontamination: between 2 h and 12 h.[158,160,166] Advocates defend the feasibility of routinely using HPV to decontaminate isolation rooms in a busy hospital, with claims that 83% of staff reported no delay in discharges or admissions, although this is just one parameter of patient and staff disruption.[164] Furthermore, as it is toxic, HPV cannot be used continuously in the presence of patients, and bacteria will quickly re-accumulate.[155] One study into HPV decontamination demonstrated that while MRSA-positive samples decreased from 74% to 7% following cleaning and HPV treatment, pre-treatment levels were reached six days later.[163] Even reports of total environmental eradication of MRSA using HPV demonstrated re-colonisation of the environment within 24 h of admission of new patients.[155] Furthermore, the process costs around £10,000 per ward.[155] However, in contrast with other reagents such as formaldehyde, no post fogging treatment is required and the end product, water, is simply wiped off the surfaces.[155]

Chlorine dioxide

Passing a 2% chlorine nitrogen gas mixture over granules of sodium chlorite produces chloride dioxide. This may be applied at room temperature, but needs a relative humidity of > 65% for effective sterilisation. To date there are no studies of its use in a clinical environment, but it has been utilised for the decontamination of large buildings, such as following an outbreak of anthrax.[167] Although its efficacy has been reported in laboratory studies, there are several factors that limit its use in the clinical environment: [168]

- The gas can penetrate plastics including polyvinyl medical device containers;
- It can cause discolouration of porous fabrics;
- A by-product of incomplete chlorine dioxide production is chlorine gas;
- Chlorine dioxide can be explosive when present at high concentrations that preclude it being compressed or stored commercially.[167]

Ozone

Ozone is a powerful oxidising agent used for decontamination applications in the pharmaceutical and food industries, and for water disinfection. It has well documented bactericidal properties and although toxic, is quickly dissociated to oxygen.[169] Its efficacy has been demonstrated against a range of bacteria including MRSA, *S. pyogenes*, *A. baumannii*, and *P. aeruginosa,* that had been dried onto plastic surfaces. However, the room required evacuation and sealing prior to treatment with ozone at a concentration of 25 ppm and relative humidity of 90% for 20 min.[169] One study has detailed the use of ozone in the home of a contaminated HCW to manage MRSA contamination.[170] The main problems with ozone are its toxicity and ability to corrode metal. Its main use in the clinical environment has therefore been limited to the decontamination of laundry.[167]

Hydroxyl radical disinfection units

The Inov8 air disinfection (AD) unit produces hydroxyl free radicals from the reaction between ozone and water vapour, catalysed by an olefin (D-limonene). On encountering an oxidisable substrate, the hydroxyl radical will precipitate a free radical cascade, cause cell injury at sites distant from where initial free radical reaction occurred.[171] The Inovat8 AD can be used in the presence of a patient to improve microbial air quality. The effect of the Inovat8 AD unit on reducing airborne contamination has been assessed in a clinical setting, following a minimum 18 h exposure.[171] Seventeen air samples (volume 1 m^3) were collected from a single patient isolation room on an intensive care unit, at 15 min intervals over 4 h onto TSA plates. Simultaneously, 40 settle plates were exposed for 4 h. The authors reported a reduction of 55% (range -24% to 86%) in the mean air total viable count per plate when the Inov8 was used, compared to when it was not used, over eight paired repetitions.[171] The counts from settle plates were similarly reduced, with a mean cfu/plate of 54% (range 123% to 73%), and a high correlation evident between the air samples and settle plate samples.

A further study in a UK burns unit demonstrated a reduction in mean colony count on agar settle plates from 99.8 cfu/plate without the Innov8 to 28.3 cfu/plate with the Innov8 in use for an unstated period of time.[172] This study shows the potential for such a system, but there is a current lack of experimental data, with samples repeated just five times. The authors acknowledged that reduction in airborne bacteria does not necessarily confer a decrease in environmental surface contamination, although it is likely. Furthermore, a 50% reduction is less than the reduction of 75-93% demonstrated with a portable HEPA filter.[173] Currently, the long-term effects of hydroxyl radicals on human health have not been established.[171]

Super oxidised water (SOW)

SOW is an electrochemically-activated solution, prepared by passing saline through an electrolyser. Water at the positive electrode has a low pH and is rich in dissolved chloride, oxygen, and hydroxyl radical. Water at the negative electrode becomes an electrolyzed base solution, with a high pH, rich in alkaline minerals, and in which organic compounds can be reduced.[174] The exact chemical properties of the resulting anolyte are dependent on characteristics of the electrochemical cell and its operating parameters. Usually conditions conductive to a pH 2-3 and high oxidation-reduction (redox) potential are sought. The efficacy of SOW has been demonstrated on a range of Gram-positive and Gram-negative bacteria as well as viruses and fungi.[174-176] The solutions have the added advantage of low-cost raw materials and ease of production, either remotely or on site.[174]

There are marked differences between the various SOW tested to date. Properties including pH, concentration of chlorine and redox potential, determine germicidal effects and shelf life. Acidic SOW is very effective but highly corrosive, with a short shelf life.[177] The stability of the SOW can be improved by increasing its pH.[174] This has led to the development of near-neutral SOW, such as Microcyn™, which can be bought ready-made, rather than be made fresh each time, and has been shown to have a bactericidal effect against five common hospital organisms.[177] Sterilox® is an established disinfectant for flexible endoscopes. Its main components are hypochlorous acid and chlorine. The disinfectant is generated at the point of use by passing a saline solution over coated titanium electrodes. It is mildly acidic (pH 5.0-6.5) with a high redox potential. Despite this, it contains levels of chlorine below analytical detection levels and is non-toxic orally, and non-irritant to skin and mucous membranes.[174, 175] Near-neutral SOW have decreased the time, toxicity and costs of endoscope disinfection.[177] Their direct use on humans has also been described for the treatment of burn wounds, ulcers, and mediastinal irrigation after open-heart surgery.[178, 179]

Fogging machines may be used to disperse SOW. Laboratory tests demonstrated reductions in levels of 10^4 fold for MRSA and $10^{5.8}$ fold for *A. baumannii,* following fogging of previously inoculated ceramic tiles.[180] However, as with HPV, patients must be removed from the area to be sterilised before fogging can be carried out. Its use confers cost, time, training implications, and disruption to patients. Recontamination of the room is also likely to occur following cessation of its use. The effects of repeated long-term exposure to equipment have not been shown, but at least one manufacturer has voided its warranty on endoscopes cleaned with acidic SOW.[174, 181]

Essential oil (EO) vapours

Essential oils (EO) are naturally occurring organic compounds distilled from plants, and certain EO possess bactericidal properties.[182] For example, *S. aureus* is susceptible to tea tree, patchouli and geranium oil, but not lavender oil.[183,184] Interestingly, MRSA is less affected by these oils, with only a minor susceptibility to tea tree oil. However, combinations of several oils increase the effect.[184,185] EO have an advantage over the aforementioned decontamination technologies as they have a low toxicity, enabling a potential for continuous use in a healthcare environment.[186] Laboratory studies were performed using a blend of geranium and lemongrass EO, termed BioScent™. Minimum inhibitory concentration (MIC) studies (defined as the lowest concentration that completely suppressed visible growth) demonstrated activity of BioScent against a range of Gram-positive and Gram–negative organisms including MRSA, *A. baumannii*, and *Pseudomonas* sp with a mean MIC of 0.54% required for Gram-positives and 0.79% required for Gram-negatives. Of note, exposure was via direct contact, which does not equate to that achieved by vapourisation.[187]

Environmental studies were performed using BioScent dispersed by a ST Pro™ machine (Scent Technologies Ltd, Wigan, UK). This releases vapours into the air by means of a negative and Venturi airflow, and is commercially available as a fragrance generator.[185] Airborne contamination following collection of 500 l air onto Columbia blood agar was shown to be reduced by 89% after 15 h saturation at 100% output. However, these studies were carried out in an unoccupied office, rather than a hospital ward. Also, there was no control period to demonstrate that this reduction was not due to the office being unoccupied rather than the effects of the BioScent™.[185] In short, although EO represent an alternative continuous decontamination of the clinical environment, their efficacy in a hospital setting, and clinical trials have not yet been established.

While physical cleaning remains at the forefront of reducing environmental contamination, alternative technologies, such as gaseous and air purification technologies are being developed for use in hospitals for 'whole room' decontamination. [167] The advantages and disadvantages of the main methods of air decontamination are summarised in Table 2.5

Table 2.5: *Summary of technologies for the 'whole room' decontamination of the hospital environment (air and surfaces). Adapted from* [167]

Technology	Supporting evidence in clinical environment	Advantages	Disadvantages
Gaseous Hydrogen Peroxide	Review of 10 studies showing significant reduction in environmental contamination[151]	No toxic by-products. No residue. Compatible with wide range of materials. Good distribution across all areas	Room must be completely sealed and patients removed before decontamination. Periodic effect. Pre-clean required to remove any organic matter
Chlorine dioxide	No studies in clinical environment	Good distribution across all areas	Room must be completely sealed and patients removed before decontamination. Periodic effect. UV light will break it down. Can cause discolouration of materials. Can penetrate some plastics. Humidity >65% required. Chlorine gas canisters required.
Ozone	No studies in clinical environment	Rapidly dissociates to oxygen. Good distribution across hard to reach areas	Room must be completely sealed and patients removed before decontamination. Periodic effect. Only small volumes can be effectively decontaminated. Requires humidity >45% to be effective.
Hydroxyl radical disinfection units (Inov8)	Effectiveness in reducing airborne bacteria demonstrated in clinical environment[171, 172]	Can be used in presence of patients. Silent unit. Continuous effect.	Less effective than HEPA filters. Long-term effects of hydroxyl radicals on humans not known. Limited experimental data to date
Super oxidised water	No studies in clinical environment. Shown to reduce contamination of endoscopes and kill a range of organisms[180]	Non-toxic by-products. Can be stored for use. Non-corrosive to equipment.	Ineffective in presence of organic matter. Patients must be removed before decontamination. Time consuming. Periodic effect. May corrode equipment with long-term use.
Vapourised essential oils	No studies in clinical environment to date. One study in empty office.	Non-toxic so has the potential to be used continuously, and be acceptable to patients and staff. Inexpensive.	Limited data to date, particularly of the bactericidal properties of essential oils in the vapour phase. Potential for allergic reaction or non-acceptence by patients. Potential discolouration and/or limited penetration of materials.

2. Persistent antimicrobial coatings

Antimicrobial coatings applied to hospital equipment and surfaces have the potential to counteract re-contamination and maintain constant levels of cleanliness. Technologies and materials available are now discussed.[91]

Silver ion containing coatings

Persistent silver-containing coatings such as Surfacine and BioCote® have been increasingly incorporated into hospital equipment in recent years. Silver lends itself as an antimicrobial agent due to its efficacy against a range of microorganisms by interaction with cell membranes. Furthermore it is not toxic to mammalian cells and its ions can be incorporated onto fabrics, plastics and paints.[188, 189] A study was carried out in two comparable NHS clinics: one containing mainly BioCote® treated equipment, and the other containing standard equipment. Environmental contamination levels measured by swabbing surfaces demonstrated a mean reduction in total bacterial counts of 96% on BioCote® treated surfaces and of 44% was on untreated equipment placed in the same room. This suggested decontamination was not limited to the products themselves but produced a wider reduction in environmental contamination.[188] Disadvantages of silver containing coatings are the high cost of installation and possible development of resistance.[188]

Copper

Copper has long been known to have antimicrobial activity. One cross over study demonstrated a reduction in median numbers of microorganisms harboured on a toilet seat, door push handle, and taps of up to 100% when these items contained copper. Samples were taken for 10 weeks, with the copper-containing and non-copper containing items exchanged after five weeks.[127] Again, the cost of refitting a hospital ward with copper-containing items is the main precluding factor of its widespread use.

Polymers

Apeartex® (Appeartex AB, Göteberg, Sweden) is composed of the active polymer A-200, polyhexamethylene biguanide (PHMB) and a surfactant solution to achieve adequate moistening of a treated surface, which is then air dried. A-200 acts by trapping negatively charged particles (including microorganisms) on a surface, where PHMB can exert its effect.[190] It is claimed that A-200 adsorbs to textiles, paper or hard and smooth surfaces. Laboratory studies of MDF bedside tables that had been partially treated with Apeartex® prior to inoculation with bacteria, demonstrated 1 x 10^2 bacterial reductions in the areas treated with Apeartex®.[190] However, much lower reductions were seen when the table was inoculated with wound swabs and urine, indicating the presence of

organic debris and body fluid seemed to compromise the effect of Apeartex®. This limits its usefulness within a hospital environment. The cost and time taken to apply the product as well as its effect on the aesthetic and functional aspects of the surfaces, and the potential for microbial resistance were not discussed.

2.7 Ultraviolet (UV) light technology

This section summarises current knowledge and application of UV light: an established light-based germicidal technology.

History of photobiology

As early as 1845 it was known that microorganisms respond to sunlight.[191, 192] In 1877 the observation was made that test tubes containing Pasteur's solution exposed to sunlight remained bacteria free for several months: a discovery later referred to as "one of the most influential… in all of photobiology."[191-193] The ability of sunlight to neutralize bacteria was shown to be dependent on the dose and wavelength received: shorter wavelengths were most effective. Dose (J/m^2) is the product of light intensity (W/m^2) and exposure duration (sec). In 1885 it was also noted that different bacteria exhibit varying sensitivities to sunlight.[191, 192]

UV radiation

UV light falls between x-rays and visible light in the electromagnetic spectrum, at wavelengths of between 100 and 400 nm. The UV wavelengths are divided into long-wave (UVA), medium wave (UVB), short wave (UVC), and vacuum-UV radiation, based on their effects on tissues. UVA wavelengths, or 'black light', are the most penetrating but least damaging form of UV radiation. Exposure may lead to skin tanning or induce optical damage. UVB can cause skin erythema and cancers: it is responsible for 80% of the carcinogenic effects of sunlight.[194] UVC wavelengths are shorter and are absorbed by deoxyribonucleic acid (DNA) and proteins. UVC instigates cell death, and can produce mutations or carcinogenic effects. This renders UVC wavelengths the most biologically active, but less dangerous to humans as the outer dead layer of skin cells absorbs UVC radiation, while UVA and UVB penetrate deeper.[191] Bacterial inactivation occurs when the absorption of a photon forms pyrimidine dimers between adjacent thiamine bases, rendering the microbes unable to replicate by blocking transcription. Peak bactericidal effectiveness in the UVC range corresponds to the nucleotide bases of DNA having peak absorbencies of 240-280 nm.[191]

Ultraviolet germicidal irradiation (UVGI)

The bactericidal effects of UV light were discovered over 100 years ago, but its use was largely neglected due to anxieties about safety and an inability to reproduce early studies. However, concerns about multidrug resistance and biological warfare led to resurgence of interest in the technology.[191] Several methods are used to deliver UVGI, the commonest being UVC emitting low-pressure mercury discharge lamps. These arc lamps consist of a quartz tube with electrodes at either end, containing mercury and an

inert gas, usually argon. A voltage is established across the electrodes to generate an electrical arc. The optical emission from the arc depends on the gas pressure. **Low-pressure mercury arcs** have a principle output wavelength emission of 254 nm: the wavelength generally referred to as UVGI.[152] About 40% of the input power is converted to light. This wavelength inactivates a wide range of Gram-positive and Gram-negative bacteria, and fungi.[195] **Medium-pressure mercury arcs** are characterised by a polychromatic output. Although less energy efficient than low-pressure arcs, they produce a higher intensity of UVC light, but at increased cost and heat emission. **Flash lamps** are also quartz tubes with electrodes at either end, in this case filled with xenon. They release energy in intense pulses rather than continuously. This pulsed-power technology produces pulses of high intensity polychromatic light, 20 000 times the intensity of sunlight and rich in germicidal UVC waves.[196]

Applications of UVGI

UVGI can be used to disinfect air, water and surfaces, although surface disinfection is limited due to shadowing and absorptive protective layers. Water disinfection is the most advanced and accepted UVGI application.[197-199] Air disinfection is achieved by one of three main methods: upper-room air irradiation; irradiation of the full room; and irradiation of air passing through enclosed HVAC systems.[191] Approximately 60% of all UVGI air disinfection systems are installed in health facilities.[152]

Upper room UVGI is designed for use in occupied rooms. It achieves microbial air decontamination by the movement of air through a UV beam, installed at ceiling height and thus confining radiation to above people's heads. Effective disinfection depends on air movement between the upper and lower parts of the room.[152] Respiratory infection rates reportedly halved through the use of upper room UVGI when natural ventilation was impeded, but no effect was seen when doors and windows were left open.[200] The system is silent, inexpensive and ideal for disinfection of large areas.[152, 191] **In-duct UVGI** is the irradiation of the entire cross section of an HVAC system air duct at high intensities, often with the aid of highly reflective materials to increase irradiance. The effectiveness of the UVGI in achieving room disinfection is dependent on circulating maximal room air through the duct and the velocity with which it is circulated.[191] Its effects were demonstrated in laboratory studies of two sealed chambers containing guinea pigs. The air supply to both chambers was from a tuberculosis ward, but one chamber received in-duct UVGI and the other did not. UVGI was shown to successfully decontaminate the infected air.[201] **Whole-room UVGI** has been mainly used in the absence of patients, for example to clean a room following the discharge of one patient and admission of another.[196,202, 203] Alternatively, protective clothing any eyewear may be worn during exposure.[204-206]

Clinical studies into UVGI

UVGI has been used to disinfect contaminated hospital water supplies during outbreaks of *Legionella* sp. in hospitals. This pneumonia-causing organism is spread mainly by inhalation of aerosols generated from water surfaces. UVGI was more efficient than heat or chlorine at disinfecting high levels of contamination.[198, 199] In 1941 upper-room UVGI was successfully used to prevent an epidemic of measles amongst schoolchildren. The study showed that 54% of susceptible children in non-irradiated schools were infected, compared with 13% of susceptible children in irradiated schools.[207]

Whole-room UVGI has been used to decontaminate hospital isolation rooms between patients: an important adjunct due to the high frequency failure of manual disinfection. [196, 202,, 203] Cleaning of soiled surfaces is required (due to the low penetration of UVC) followed by irradiation. Doses ranging from 160 J/m^2 in shadowed areas to 19 230 J/m^2 in highly exposed sites (irradiance 0.08 W/m^2 to 6.82 W/m^2) significantly reduce bacterial counts on surfaces.[202, 203] The success of surface disinfection using UVC is believed to depend greatly on the consistency of the material to be disinfected. Whole-room UVGI has long been used as an air-cleaning method for intra-operative infection control during clean surgery (e.g. joint replacements)[204-206] Infection rates following total joint replacements were reduced from 1.8% using laminar air flow to 0.6% using UVGI over a course of 5980 joint replacements.[204] However the CDC currently recommends against using UVGI to prevent surgical site infections.[152]

Portable devices including the Tru-D™ Rapid Room Disinfection device (Lumalier, Memphis, TN) have been developed for the disinfection of rooms between patients. Sensors are placed to automatically stop the device should someone enter the room. Levels of UVC received in all areas of the room are monitored so the machine stops when an adequate dose has been administered.[208] The device was effective against MRSA and VRE inoculated onto surfaces in hospital rooms.[208] Pulsed-xenon UV disinfection using a portable flash lamp and hand held UV sources to disinfect computer keyboards have also been described.[196, 209]

Limitations of UVGI

Relative humidity greater than 60% produces a sharp decline in the fraction of organisms killed. However, this is usually not an issue as buildings are kept below this humidity. UVGI is believed to be most effective at preventing spread of infections by droplet nuclei, not by direct contact, although some surface disinfection may occur. The use of upper-room UCGI depends heavily on the air flow in that room, and outside ventilation to the room being minimised. Direct UVGI requires the removal of people from the room or use of protective clothing due to the harmful effects of UV to the skin and eyes.[191] The National Toxicology Programme (NTP) classifies UVC as a probable

human carcinogen.[152] Excessive exposure to the eyes can cause photokeratitis and conjunctivitis, and chronic exposure has been implicated in cataracts.[152] UVC may have some destructive effect over time on materials such as plastic and vinyl, and cause fading of paints or fabric colours.[203] Its action on surfaces is dependent on radiation directly hitting a surface so care must be taken to prevent shadowing.[203] Furthermore, the decrease in efficacy due to the presence of organic matter mean that visibly soiled surfaces must be cleaned before its application.[203]

The future of UVGI

The recent development of UV light-emitting diodes (LED) has delivered an energy efficient alternative to mercury vapour lamps that also removes the problem of disposing of toxic products after use.[197] UVC is quicker and more effective at killing bacteria in hospitals than alternatives such as HPV.[202, 203] It is cheap to run and does not require additional staffing.[208] The current opinion is that UVGI should be considered as a disinfection application in hospitals only in conjunction with other established elements such as appropriate HVAC systems and thorough manual cleaning of the environment. It is not a primary intervention to kill or inactivate microorganisms, but has a role as an adjunct.[152]

2.8 Photodynamic inactivation (PDI)

This section introduces photodynamic inactivation (PDI), with particular emphasis on medical applications of PDI requiring an exogenous photosensitiser.

Background

PDI, also known as photodynamic therapy (PDT), involves the combination of a molecule called a photosensitiser, with light and molecular oxygen to achieve destruction of a target cell or organism.[210] In summary, a photosensitiser molecule is administered to the target cell, following which inactivation is initiated by light of an appropriate wavelength being absorbed by the photosensitiser molecule. This results in its excitation to a higher energy level. The excited photosensitiser molecule then reacts with molecular oxygen resulting in the production of predominantly highly reactive singlet oxygen. Singlet oxygen produces oxidative damage to the target cell, resulting in cell destruction.[211] PDI was first introduced as a cancer treatment, with applications in ophthalmology, dermatology and more recently infectious diseases.[212] Applications in the dental field, and in the management of contaminated wounds and infectious conditions are being explored.[213-215] Sterilisation of blood products and organ transplant tissue is another area that lends itself to PDI.[211]

The PDI process is shown in Figure 2.5. Exposure of the photosensitiser molecule to light of a wavelength corresponding to its absorption maximum, results in the photosensitiser becoming excited from ground state to singlet state. The photosensitiser molecule then undergoes intersystem crossing to a triplet state that is lower in energy but longer lived than the singlet state. The triplet state can then react via a Type I or Type II pathway, both of which are oxygen-dependent and involve the formation of reactive oxygen species (ROS).[216] The Type I reaction involves electron or hydrogen atom transfer from the activated photosensitiser, producing free radicals. These radicals then interact with oxygen to produce toxic products such as hydrogen peroxide or superoxide ions. The Type II reaction involves the interaction of the triplet state photosensitiser molecule with oxygen, forming singlet oxygen as a reactive intermediate. Singlet oxygen has a high reactivity with many biological molecules, including nucleic acids, proteins and lipids, ultimately causing cell death via these substrates.[212] The current data of literature indicate that PDI action is mediated via Type II mechanism for most photosensitisers, particularly porphyrin derivatives. [216]

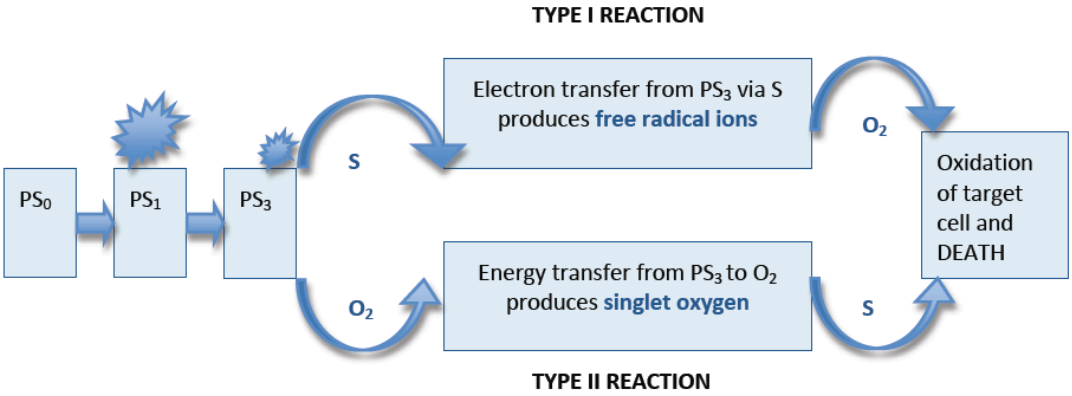


Figure 2.5: *Pathways of Type I and Type II photosensitisation reactions. Light activates ground-state photosensitiser (PS) to become highly excited singlet PS (PS_1), which crosses to the lower energy triplet state (PS_3). This can follow two pathways: Type I involves organic substrates (S) to produce free radicals and Type II transfers energy to oxygen (O_2), producing singlet oxygen. Both result in oxidation and destruction of the target cell.* [216]

Exogenous photosensitisers

Typically, PDI involves the application of an exogenous photosensitiser such as methylene blue, toluidine blue, cationic porphyrins, phthalocyanines and chlorins. This is usually a non-toxic dye, and frequently either a porphyrin or its derivative. Porphyrins are a group of naturally occurring organic compounds, consisting of four modified pyrole subunits interconnected via methane bridges. They exhibit intense fluorescence when illuminated by light of an appropriate wavelength, and may be deeply coloured.[217]

The ideal exogenous photosensitiser should exhibit:

- Low toxicity;
- High selectivity;
- More rapid clearance in healthy than diseased tissue;
- Action at a wavelength that can penetrate the target tissue;
- Production of enough cytotoxic molecules to eliminate the target cells.

In 1975 it was demonstrated that haematoporphyrin derivative (HPD) and red light could selectively destroy cancer cells without affecting normal tissue.[219] PDI was later applied to the treatment of skin conditions including psoriasis, keratoacanthoma, atopic dermatitis, pre-cancerous (including actinic keratosis (AK), Bowen's disease and xeroderma pigmentosa) and cancerous lesions (including cutaneous lymphomas, basal cell carcinoma (BCC) and skin metastases), depilation and acne.[219-224] It is well tolerated, non-invasive, specific to the target tissue, and not associated with cumulative toxicity.

Other topical photosensitisers commonly used on the skin include aminolevulinic acid (ALA, 5-ALA, δ ALA) hydrochloride and methyl aminolevulinate (MAL).[220, 222] ALA is technically not a photosensitiser, but is a naturally occurring amino acid that is a readily converted to protoporphyrin IX (PpIX). HPD was established as a photosensitiser due to its preferential accumulation in malignant tissue.[218, 225] However, ALA induced PpIX has an advantage over HPD as the short half-life of PpIX mean that its photosensitising effects do not last longer than 48 h.[226] The downsides of ALA include its low bioavailability, requiring administration of high concentrations, and its poor penetration into tissues. This has led to the development of ALA pro-drugs to overcome these issues.[226] Other photosensitisers include hypericin, to treat cutaneous lymphomas and psoriasis, and methylene blue.[227, 228] The light source used for PDI requires a wavelength that coincides with the peak absorbance of the photosensitiser, and that is capable of penetrating sufficiently into the target tissue. Red light penetrates the skin to 6 mm and is thus useful in deeper lesions. Blue light is used for more superficial damage as it penetrates up to 2 mm.

Applications of PDI with exogenous photosensitisers

Photodynamic photorejuvination of sun-damaged skin can be carried out using different photosensitisers and different light wavelengths according to the depth of actinic damage. Prolonged sun exposure leads to chronic actinic damage and the development of AK. AK is the commonest epithelial pre-cancerous lesion amongst fair individuals and may progress to invasive squamous cell carcinoma (SCC). ALA mediated PDI has been shown to be an effective and safe treatment of multiple AK of the face and scalp.[222, 224, 229-231] Photo-damaged or rapidly proliferating epidermal cells convert more topical ALA to PpIX than unaffected cells.[232] Fluorescent blue light treatment is then delivered at a dose of 10 J/cm^2, and differentially kills pre-cancerous cells.[222, 228] Similar results have been demonstrated using MAL PDI with blue light to treat photo-damaged skin.[223] ALA followed by intense pulsed light also gives good cosmetic results for the treatment of sun-damaged skin with AK, including an increase in collagen.[233]

PDI has similarly been approved for the treatment of Bowen's disease and superficial BCC. ALA PDI using a xenon short arc lamp filtered to 630 nm cleared over 90% of small lesions of Bowen's disease and BCC.[220] PDI using hypericin or methylene blue and visible white light from fluorescent tubes at 590 nm to 670 nm is an effective treatment of cutaneous lymphoma and psoriasis.[227, 228] It has the advantages over traditional psoralen plus UVA (PUVA) light treatment in the management of these conditions as PUVA is associated with the development of skin cancers and requires specialist UVA lamps.[227]

ALA mediated PDI has also been used to treat acne. It acts via three pathways: killing *Propionibacterium acnes* bacteria by blue 415 nm light; damaging sebaceous glands; and

reducing follicular obstruction through increasing keratinocyte turnover by red 660 nm light.[221, 234 235,] ALA PDI has also been used in the treatment of verruca vulgaris (common warts), again via its anti-inflammatory properties and the destruction of infected keratinocytes. PDI and a photosensitiser have also been used in the management of fungal skin infections.[236]

2.9 The bactericidal properties of visible light

The rapid emergence in antimicrobial resistance has led to increased research interest in the development of PDI as an alternative antibacterial treatment.[211, 225,]This section describes the bactericidal properties of light, in the presence and absence of an exogenous photosensitiser.

Bacterial inactivation with exogenous photosensitisers

The use of visible light and exogenous photosensitizing molecules is well established as being an effective antimicrobial treatment. Results have demonstrated the successful inactivation of a range of bacteria, yeast, fungi, parasites and viruses.[211, 212, 225, 237-244] One specific example is the inactivation of common plaque-causing oral bacteria using visible red light in conjunction with a number of topical photosensitisers including toludinine blue and rose Bengal.[245-247] A clinical study of dentures treated with a topical photosensitiser and irradiated with blue light reported elimination of over 90% of microorganisms.[248] PDI may have further applications in the management of burn wound infections, due to the ease of access of the skin. Most studies to date have been limited to those requiring addition of an exogenous photosensitiser. Experiments on murine burn wound models demonstrated effective killing of *A baumannii* without interfering with wound healing.[249-251]

Gram-positive bacteria are particularly susceptible to PDI using exogenous photosensitisers as their porous cytoplasmic membranes allow passage of substances. However, the cell membrane of a Gram-negative bacterium has an additional outer membrane, which prevents migration of the photosensitiser.[211] Work has been performed on increasing the permeability of the Gram-negative membrane to allow the permeation of exogenous photosensitisers, for example by administering the photosensitiser with a cationic agent.[211, 225] Other theories for inter-species variation in PDI susceptibility include the capacity of the organism to produce extra-cellular slime, enabling an impermeable biofilm formation. As slime production in staphylococci is greater in the stationary phase than the log phase, the growth phase of bacteria may also affect susceptibility.[252]

Bactericidal properties of blue light without exogenous photosensitisers

In 1896 it was observed by Finsen that a combination of blue, violet and UV light killed bacteria.[253] He proceeded to win a Nobel Prize for treatment of lupus vulgaris (*M. tuberculosis* or tuberculosis of the skin). Although Finsen believed the main effect was due to UV light, analysis of his optical lenses over 100 years later revealed their maximum transmission to be at about 400-450 nm: the blue part of the spectrum. *M. tuberculosis* was shown to fluoresce when excited by 395-405 nm light, suggesting that

porphyrins were present in the bacteria, as the absorption spectrum of porphyrins displays a maximum around 405 nm, a peak that is known as the Soret band.[217] This indicated the potential use of blue light PDI.[253]

The bactericidal properties of blue light have been extensively studied.[254-256] Inactivation of *S. aureus* was demonstrated following exposure of broadband visible light source (400 nm-800 nm) without the prior application of an exogenous photosensitiser.[256] Another study demonstrated that the causative visible wavelengths were between 400 nm and 420 nm, with peak inactivation at 405 nm.[255] Testing of multiple Gram-positive and Gram-negative species, demonstrated a species-dependent and dose-dependent response.[257]

Inactivation of *S. aureus* and MRSA was studied using optical filters over a broad-spectrum xenon white-light source to identify the sensitivity of *S. aureus* to wavelengths of visible light. Maximum inactivation was shown to occur at 405 ±5 nm, producing a 5 $\log_{10}$ reduction in populations.[255] Following these findings, a range of medically important pathogens were exposed to a 405 nm wavelength LED array, termed High-Intensity Narrow-Spectrum light (HINS-light). Gram-positive bacteria tested (*S. aureus* and MRSA, *S. epidermis*, *Clostridium perfringens*, *S. pyogenes* and *Enterococcus faecalis*) all behaved in a similar manner, with the exception of *E. faecalis.* Following exposure to 405 nm light at an irradiance of 10 mW/cm^2, an approximately 5 $\log_{10}$ reduction in cfu/ml counts were observed after 60 to 90 min of exposure. *E. faecalis* was less susceptible, with negligible inactivation after 120 min. Gram-negative species (*A. baumannii, Proteus vulgaris, P. aeruginosa, Klebsiella pneumoniae*, and *E. coli)* in general required longer exposure times than Gram-positive species. Inactivation curves were produced demonstrating the reduction in starting populations of bacterial cfu was a function of dose. Similar inactivation responses were seen irrespective of initial starting populations.[2]

P. acnes is also killed by blue light in the absence of exogenous photosensitisers or dyes.[258] The action spectrum for *P. acnes* shows the greatest inactivation was found with exposure to UVA wavelengths, and this decreases with increasing wavelength. However, a secondary sensitivity peak is apparent in the blue region, centered about 415 nm wavelength.[259] This is also true of *S. aureus*, but with the secondary peak found to be in the region of 405 nm.[254] In addition, laboratory studies demonstrated inactivation of *Helicobacter pylori* by exposure to visible light.[260] Using filters, it was shown that violet-blue light (375 nm-425 nm wavelength) was the most effective wavelength at killing the organism. A 405+/-5 nm emitting laser was used for subsequent experiments and all tested strains were killed. The degree of photosensitivity was found to increase with the age of the culture suspension.[260] There is also evidence that blue light is effective at killing yeasts such as *C. albicans*.[261] Studies demonstrating inactivation of a range of

organisms, using violet/blue light in the region of 405 nm is summarised in Table 2.6.[2, 257, 258, 260, 262-264] These include studies on food-borne pathogens such as *Campylobacter jejuni*, *Salmonella enteritidis*, *Shigella sonnei*, *Listeria monocytogenes* and *Mycobacterium terrae.*

Table 2.6: *Summary of experimental data to date, showing inactivation of bacteria by blue light.*

Organisms	Authors	Arrangement	Max irradiance used (mW/ cm^2)	Max. dose used (J/cm^2)	Conclusions
S. aureus	Maclean PhD thesis 2002-2006[254] (Published in Maclean 2008 [255])	Xenon lamp with 405nm bandpass filter	3.27	23.5	405nm light is the most effective bandwidth of visible light for bacterial inactivation
MSSA, MRSA, S. epidermidis, S. pyogenes, E. faecalis, C. perfringens, A. baumannii, P. aeruginosa, E. coli, P. vulgaris, K. pneumoniae	Maclean PhD thesis 2002-2006[254](Published in Maclean 2009[2])	405 nm LED array & liquid suspension	10	53 G+ 216 G-	Gram-positives killed more quickly than Gram-negatives
P. acnes	Ashkenazi 2003[258]	407-420 nm metal halide lamp & liquid suspension	20	100	Cultures grown with ALA exhibited faster decrease in viability than those without
H. pylori	Hamblin 2005[260]	400 nm long pass filter white light & liquid suspension	100	30	375-425 nm was the most effective wavelength
S. aureus, P. aeruginosa	Guffey 2006a[265]	405 nm (blue) and 880 nm (infrared) SLD cluster	40	20	Dose-dependent effect of combined blue and infrared light
S. aureus, P. aeruginosa, P. acnes	Guffey 2006b[257]	405 nm and 470 nm SLD clusters	40	15	470 nm killed *P. aeruginosa* at all doses but *S. aureus* only at high doses. 405 nm killed both. Neither killed *P. acnes*
MRSA (two strains)	Enwemeka 2008 and 2009[262,266]	405 nm and 470 nm SLD & surface plated bacteria	100	60	Dose-dependent reduction seen, although non-linear as increases in irradiance up to 15J/ cm^2 produced more bacteria death than similar increase >15J/ cm^2
C. jejuni, S. enteridis, E. coli	Murdoch 2010[263]	405 nm LED array on liquid suspension	10	288	*C. jejuni* particularly susceptible to light inactivation
S. enteridis, S. sonnei, E. coli, L. mono-cytogenes, M. terrae	Murdoch 2012[264]	405 nm LED array on liquid suspension and on agar, PVC and acrylic	30	288	Reductions of approximately 5 $\log_{10}$ achieved regardless of irradiance in dose-dependent fashion.

Porphyrin-mediated PDI of bacteria

Endogenous porphyrins within bacteria are believed to cause PDI without the need for exogenous photosensitisers. The intensity of the fluorescence exhibited by a porphyrin varies with pH and is affected by the presence of impurities.[217] As mentioned, the absorption spectrum of porphyrins is maximum around 405 nm with a full-width half-wavelength (FWHW) +/-10 nm.[217] Consequently, blue light is more effective than red light at activating porphyrins via the Type II pathway.[267, 268] The first indication of porphyrin-mediated PDI of bacteria was the observation that ALA induces increased endogenous porphyrin production, which in turn increased the susceptibility of bacteria to light inactivation. The addition of ALA caused accumulation of uroporphyrin in *S. aureus* cells and excretion of coproporphyrin (CP) into the growth medium, demonstrated by a fluorescence activated cell sorter. Following ALA induction, the most effective wavelengths for killing *S. aureus* and *E. coli* were found to be between 400 nm and 450 nm.[269] Further studies involved bacteria pre-treated with ALA and exposed to 407-420 nm blue light.[270] Four Gram-positive and five Gram-negative bacteria were tested in order to determine if the rate of inactivation was the result of different types of porphyrins produced by the different species. The predominant porphyrin in the staphylococcal strains (*S. aureus* and *S. epidermidis*) was found to be CP whereas there was no predominant porphyrin produced by the Gram-negative strains tested (*E. coli, Acinetobacter* and *Aeromonas* strains). The amount of CP produced by the Gram-positive strains was three times higher than that produced by the Gram-negative strains.[270] The use of exogenous photosensitisers complicates this study, due to the impermeable membrane of Gram-negative bacteria.[225] However, further work in the absence of an exogenous photosensitiser also supported the theory that efficient photo-inactivation of staphylococci is likely to be due to high levels of CP.[268]

Experiments on *H. pylori* have also indicated that inactivation by visible light centred around 400 nm was due to the accumulation of endogenous porphyrins producing ROS.[260] Fluorescence emission spectroscopy was performed on the cultured supernatants of *H. pylori* following illumination with 405 nm light. The supernatant contained an emission peak centred at 622 nm, almost midway between the emission peaks of CP (610 nm) and PpIX (632 nm). The measures of fluorescent intensity in the culture supernatants correlated with the amount of inactivation demonstrated by different strains and different ages of *H. pylori.* There was an excellent correlation between the porphyrin fluorescence in the medium and the cytotoxicity observed.

Further confirmation was made that *H. pylori* produces both CP and PpIX using analysis by solvent extraction. These studies showed that *H. pylori* naturally accumulate sufficient photoactive porphyrins to be effectively killed after illumination with visible blue light.[260] Fluorescence spectroscopy has also demonstrated that *P. acnes* naturally

synthesise porphyrins including CP and PpIX.[235, 271-273] Extracts from growing cultures of *P. acnes* have demonstrated emission peaks around 612 nm when excited at 405 nm. High-performance liquid chromatography indicated the endogenous porphyrins produced by *P. acnes* to be mostly CP.[258]

In summary, inactivation of bacteria by blue light is thought to involve photodynamic action whereby the endogenous porphyrins absorb light and produce, via an excited state, an interaction with molecular oxygen to produce cytotoxic singlet oxygen.[260]

The role of oxygen in PDI of bacteria

Consideration of the essential role played by oxygen provides further proof that visible-light inactivation of bacteria occurs via photo-excitation of intracellular porphyrins.[210, 268] Both Type I and Type II PDI reaction pathways require oxygen.[274] Studies were performed examining the effect of oxygen depletion and oxygen enhancement on the inactivation rate of *S. aureus* in liquid suspension, exposed to visible light. Ascorbic acid, catalase and dimethylthiourea were used as oxygen scavengers. Following a 30 min exposure time, a reduction in *S. aureus* population of a single $\log_{10}$ order was seen with all three scavengers. By contrast, the control, which had no scavengers, demonstrated near-destruction of the 2.2 x 10^5 cfu/ml starting population. The same study also demonstrated that the addition of oxygen accelerated the rate of inactivation.[268] A number of studies have demonstrated reduced inactivation of bacteria exposed to visible light in anaerobic conditions.[275-277] Clinical studies have also demonstrated that the effectiveness of dermatology PDI can be manipulated by modulating skin oxygen tension.[274]

Evidence for the formation of ROS

Clinical suspensions of isolates of *E. coli* and *S. aureus* were exposed to visible light using filtered halogen lamps or LED arrays at either 415 nm or 455 nm wavelengths.[278] An electron paramagnetic resonance (EPR) spin trapping technique was used to detect the formation of ROS upon exposure of the bacteria to blue light. ROS production following blue light illumination was found to be higher than that of red light. Within the blue light range, 415 nm induced more ROS than 455 nm, which correlated with reductions in the colony count of *S. aureus* and *E. coli* following illumination using the two wavelengths.[278]

Mechanisms of PDI-mediated damage of bacteria

There are two basic mechanisms that have been proposed to account for the toxicity caused to bacteria by PDI: DNA damage, and damage to the cytoplasmic membrane, allowing leakage of cellular contents and inactivation of membrane transport systems.[225] There is reason to believe that some damage does take place to DNA. Breaks in DNA and the disappearance of the plasmid super-coiled fraction have been detected in

bacteria following PDI. Additionally, there is evidence that the ease with which photosensitisers are incorporated into DNA is related to the amount of damage they cause, and DNA repairing systems may repair some damage caused by PDI.[225]

However, protection against PDI does not seem to correlate with the cells' capacity to repair DNA strand breaks.[245] This is supported by the observation that PDI easily kills *Deinococcus radiodurans*, despite it having a very efficient DNA repair mechanism.[245] Furthermore, irradiation with UV-light works via energy absorption by the pyrimidine bases in DNA, altering the structure of the bases, but the peaks of absorption of pyrimidine bases lies outside the blue spectrum.[279]

So, although DNA injury occurs, it likely to be secondary to porphyrin-mediated cytoplasmic membrane damages.[280] Electrophoretic analysis of cytoplasmic membrane proteins and DNA of *S. aureus* cells suggest that the membrane represents the primary target of PDI, while DNA damage, which occurs only at a relatively long irradiation time may be a secondary effect. Furthermore, a correlation was shown between the amount of kill and modification of the electrophoretic pattern of the cytoplasmic membrane proteins.[280] The effect of membrane damage on intracellular pH in *P. acnes* following the application of blue and UV light was studied.[281] The pH gradient across the cell membrane increased after blue light irradiation, with a relationship demonstrated between intracellular pH changes and cell survival. Sub-lethal doses of irradiation resulted in a reversible increase in the intracellular pH. However, lethal doses of irradiation decreased intracellular pH: a pattern obtained in both UV and blue light regions, despite different inactivation mechanisms.

The irradiation-induced pH changes were thought to be a result of membrane damage, with two different mechanisms possible for sub-lethal and lethal dose changes observed. The pH homeostasis of bacteria is due to the action of a number of membrane transport systems, and the decrease in intracellular pH may be the result of proton influx due to increased membrane permeability.[281] The alteration of cytoplasmic membrane proteins and disturbance of cell-wall synthesis with loss of potassium ions from cells has also been demonstrated.[282,283]

Clinical applications of blue light PDI

Clinical uses of blue light PDI as a bactericidal agent are burgeoning.[284] The FDA recently approved of the use of high-intensity narrow-band blue and red light therapy without exogenous photosensitisers, for the treatment of acne vulgaris.[234,285,286] Following laboratory studies of the effect of visible light on *H. pylori,* a controlled, blinded, prospective trial was carried out of endoscopically delivered blue 405 nm wavelength light to the gastric antrum.[260] This achieved a mean reduction in *H. pylori* colonies per

gram of tissue biopsy of 91%.[287] Later work delivered violet phototherapy to the entire stomach, achieving an 86% - 97% reduction in bacterial load.[288]

2.10 Potenital hazards of blue light exposure to humans

Blue light, as with any radiation technology, can pose potential hazards to humans and these are discussed in this section.

The electromagnetic spectrum

Visible light is the region of electromagnetic radiation visible to the human eye. It has a range of between 380 nm and 780 nm, with shorter wavelengths carrying a higher energy. The energy of a photon is proportional to its radiation frequency: E = nf (where n = Plank's constant; f = radiation frequency). The electromagnetic spectrum is illustrated in Figure 2.6.

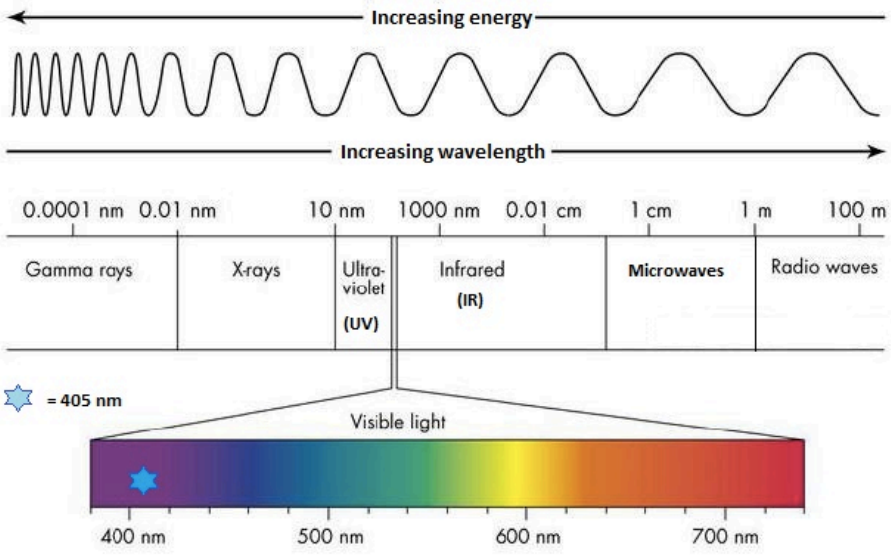


Figure 2.6: *Diagram of electromagnetic spectrum, with visible light and 405 nm light highlighted. Adapted from* [289].

The effects of blue light on skin

With expanding clinical use of visible blue light PDI, a study was carried out into the effects of blue light on the skin. Irradiation doses of 20 J/cm^2 were given on five consecutive days using a lamp with an emission spectrum of 380 nm to 480 nm, and a peak output of 420 nm. These doses were based on those used in the treatment of AK and acne. The effects on melanogenisis (number of melan-A positive cells); skin aging (density and quality of collagen and elastin); and photo-damage (number of p53 positive cells and 'sunburn' cells) on skin biopsies were studied.[194] Reversible minimal hyper-pigmentation and a significant increase in melan-A positive cells were seen in one volunteer. There were no signs of additional skin aging following irradiation. A temporary decrease in p53 cells was observed for 24 h, but no sunburn cells were seen

throughout the treatment. The authors concluded any biological effects observed were transient and that blue light at these doses was safe to the skin.[194]

When using blue light sources, some radiation either side of the desired wavelengths will be emitted, including small amounts of UV light. Effects on the skin from UV radiation include sunburn and skin cancers (depending on the wavelengths emitted and the dose) and exposure to UV light should be minimised. Patients may be predisposed to photosensitivity, either due to pre-existing conditions such as lupus erythematosus or rosacea; genetic disorders including xeroderma pigmentosum; metabolic defects such as porphyria; or certain medicines including tetracycline antibiotics, amiodarone, non-steroidal anti-inflammatory drugs (NSAIDs) and furosemide.[290,291]

Eye damage mechanisms

In recent years the biological effects of different frequencies of radiation to the eye have been analysed and defined in detail.[292] **Radiometric quantities** include: **radiant flux**, describing the energy emission by a light source in all directions (W or mW); **radiance**, that describes the 'brightness' or intensity of a light source (in $W/cm^2 \times sr$ or $mW/cm^2 \times sr$); and **irradiance** that describes the power density on a receiving surface (in W/cm^2 or mW/cm^2). **Photometric quantities** indicate light levels spectrally weighted by the standard photometric visibility curve, which peaks at 555 nm (yellow and green), for the human eye and is relatively insensitive at perceiving red and blue.[293] Photometric quantities include the **luminous flux** (lumen, lm); **luminance**, that describes the 'light' perceived by a standard human observer (in cd/m^2); and the **illuminance** describing the 'light' falling onto a surface (in lm/m^2 or lux).

Interaction of radiation with biological systems occurs through absorption, whereby the radiant energy is transferred to the biological material. There are two main mechanisms for this transfer: photothermal and photochemical.[294] During heat production, radiant energy is converted to kinetic energy of molecules. The radiant energy absorbed per unit time (measured as J/sec, or Watt), per unit volume (in m^3) is the determining factor. **Photothermal** damage occurs when the rate of energy release exceeds the rate of dissipation, inducing a rise in tissue temperature. Radiant energy can also excite atoms by moving outermost (valence) electrons to higher orbit energy levels. This energy can produce **photochemical** damage. When the light absorbed by part of a molecule (the chromophore) leads to an excited state of the chromophore, it can either undergo chemical transformation itself or interact with other molecules that subsequently become chemically reactive.[293] Radicals and ROS may be formed, leading to damage of the retina. Chromophores in the retina and retina pigment epithelium (RPE) include the photoreceptors, haem proteins, melanosomes and lipofuscin (a toxic by-product from the visual cycle that accumulates in RPE cells over years, and a hallmark of the aging eye). Tissues with a large concentration of cell membranes are

particularly vulnerable to free radicals. Retinal photoreceptors, particularly the outer segment, possess large amounts of membrane and are therefore thought to be particularly susceptible to photochemical damage.[294]

Visible light radiation is either absorbed or transmitted by different eye tissues before reaching the retina, depending on its wavelength. These are summarised in Table 2.7.[293]

Table 2.7: *Summary of potential mechanisms of damage to the different eye tissues as a result of different wavelengths of light, adapted from*[293]*. (UV = ultra violet, IR = infrared)*

Tissue/molecule	Wavelength (nm)	Mechanism of injury
Cornea	UVA (315-400), UVB (280-315), UVC (100-280), IR (780-1400)	Absorption/ heat dissipation
Iris (melanin)	UV and visible (300-700)	Absorption/ heat dissipation
Lens	Peak absorption changes with age (365 at 8 years, 450 at 65 years)	Absorption/ heat dissipation UV light induces cataract
Retina	Visible (400-700)	Photochemical damage Class I max at 507 nm; Class II max at 400-480 nm
RPE (melanin)	Visible (400-700)	Absorption/ heat dissipation, photochemical damage Class II max at 400-480 nm
Lipofuscin	Peak absorption changes with age 355-450, with more blue light damage to aging eyes	Photochemical damage

Depending on the wavelength, the absorbed energy and the exposure duration, two classes of photochemical damage have been described: Class I damage is characterised by relatively low level of irradiance (less than 1 mW/cm^2), over hours to weeks. Damage is thought to occur at the outer segment of the neurosensory retina.[295] Class II injury is due to exposure to high irradiances (over 10 mW/cm^2), with relatively short exposure times. Damage occurs mainly at the level of the RPE and is increased in response to shorter wavelengths: a response termed 'blue light hazard'.[292] Related to this is age-related macular degeneration (AMD): the macular being the central area of the retina that provides detailed vision.

Blue light hazard

The interaction of blue light with molecules constituting the retina, or molecules such as lipofusin that accumulate in the retina with age, induces photochemical damage to RPE

cells and photoreceptor cells.[292] The blue light hazard was first described over 40 years ago, and studies have shown that the shortest wavelengths of the visible spectrum (i.e. blue and violet) are the most dangerous to the retina, with an action spectrum peaking at approximately 440 nm.[296-298] Exposure to blue light at 403 ± 10 nm wavelength at intensities of 3.2 mW/cm^2 (Ganzfeld) or 33 mW/cm^2 (spot) for two hours showed severe retinal damage in rats.[299] Damage occurs only in the presence of the visual pigment rhodopsin in the photoreceptor cells, indicating the reaction is rhodopsin mediated.[298.299] When light hits photoreceptor cells, the rhodopsin becomes 'bleached' and effectively useless until it recovers through a metabolic process termed the 'visual cycle'. However, short wavelength light can cause reversal of this bleaching and a premature regeneration of rhodopsin. This increases the potential for oxidative damage of photoreceptors, and lipofuscin build up in the RPE.[299] The degree of retinal injury caused by blue light is dependent on a number of factors, including intensity, duration, intermittence of exposure to light, and spectral distribution. Short wavelength radiation (rhodopsin spectrum) and the blue light hazard have been shown to have a major impact on photoreceptor and RPE cell function. The high-energy photons in the spectrum of blue and UV light create ROS that are particularly deleterious to mitochondrial DNA, resulting in photochemical-mediated apoptosis of retinal cells. [298]

The risk of AMD increases with age due to several factors. [298] There is an age-related loss of RPE cells in the retina, coupled with a decreased density of melanosomes with age, and consequently a reduction in melanin. Melanin – a free radical scavenger, effective anti-oxidant, and pigment responsible for absorption of light - is a powerful defence against light-mediated retinal damage.[298] Population-based studies have shown a strong positive correlation with smoking and AMD development.[300] Also, a continuous increase in lipofuscin in the RPE cells deteriorates cellular function and increases the susceptibility of the retina to radiation damage, particularly by blue light.[301,302] Furthermore, near-UV light radiation has been demonstrated to accelerate the formation of lipofuscin and ageing of RPE cells in tissue culture.[298,303] The combination of these effects makes the aging retina particularly sensitive to light damage. A natural protection against the risk of blue light is the yellowish tinge the aging lens develops: by the age of 75 years scotopic (low light) vision has decreased by 75%, but the protection afforded by the native lens against UV and blue light has increased by 90%.[298] After cataract surgery however, patients lose that protection, so yellow synthetic intraocular lenses have been advocated.[298,304]

Other light hazards

UV radiation poses a risk of cataract or photokeratitis (sunburn of the cornea); IR radiation can induce IR cataracts (known as glass blower's eye); and radiation of all wavelengths at extreme intensities may lead to retinal thermal injuries.[305]

LED lighting and blue light safety

The imminent out-phasing of incandescent light bulbs within the European Union and other countries, followed by their replacement with white LED has led to a heightened interest in the potential safety hazards posed by LED lighting. The European Lamp Companies Federation (ELC) carried out a detailed evaluation of the biological safety of common LED light sources for domestic use, with particular focus on white light sources.[305] White LED sources emit a higher proportion of blue light than traditional sources, as they consist of a blue light LED, modified by a phosphor or green and red LED. Similar risk levels were observed between LED and the traditional light sources they are intended to replace, with levels well within the uncritical range. Nevertheless, looking straight into any bright source should be prevented. However, on accidentally looking into a bright light source, natural defence reflexes occur, including pupillary constriction and aversion of the eyes. Of note, blue light is important for the regulation of the circadian rhythm, and exposure to blue light during daylight hours may help people to keep in tune with natural day-night rhythm.

Blue light retinal injury (photoretinitis) can result from a bright light for a short time, or a low intensity light for longer exposure periods. The American Conference of Governmental Hygienists (ACGIH) has developed 'Threshold Limit Values' (TLV) for visible light. The ACGIH TLV to protect the human retina against blue light photoretinitis is an effective blue light radiance of 100 J/cm^2xsr for less than 10000 seconds (2.8 hours).[306]

2.11 HINS-light technology

This section introduces HINS-light technology and outlines the aims of this work.

Background

As previously discussed in Section 2.9, and summarised in Table 2.6, much work has been done in the past demonstrating the bactericidal properties of visible blue light.[2,257-260,262-266] Several studies identified the most effective wavelength for achieving PDI as being 405 nm wavelength.[2,262-265] In particular, work at ROLEST involved the exposure of multiple pathogenic bacteria in a liquid suspension, generating inactivation curves that showed the PDI effects of a 405 nm emitting LED array to be dose-dependent and species-dependent.[2] The work at the Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST) termed the use of these bactericidal wavelengths High-Intensity Narrow-Spectrum light (HINS-light). Further progression led to the development of the novel High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS).

The HINS-light EDS

The HINS-light EDS is a ceiling-mounted light system for the continuous disinfection of the air and surfaces in the illuminated environment. The system combines blue bactericidal light with white light for aesthetic purposes. The HINS-light EDS mounted in the ceiling is shown in Figure 2.7.

Figure 2.7: *The ceiling-mounted HINS-light EDS.*

It has previously been trialled in Glasgow Royal Infirmary (GRI) in various locations including isolation rooms in a vascular surgery ward, the intensive care unit and the burns unit with successful results. Results demonstrated that use of the HINS-light EDS in a clinical setting reduced environmental surface contamination by between 56% and 86%.[3] The findings suggested the potential for the HINS-light EDS to make a significant

contribution to bacterial decontamination in clinical environments. These studies are discussed in detail in Section 5.2.

The system has clear advantages over other methods of environmental decontamination. It can be used continuously in the presence of patients and staff due to its safety profile, analysis (detailed in Section 3.5). This is in contrast to technologies including UV light exposure and other 'whole room' technologies discussed in Section 2.6. It requires limited operator training, as it simply switches on and off at the mains power supply, and is connected to a timer, so it is off overnight, enabling the patient to sleep undisturbed. As it requires no input from patients or staff there are no compliance issues and no extra staff time is required, and the patient does not need to be removed from the room prior to its decontamination. No chemicals or pre-treatments including photosensitisers are required for decontamination to take effect, and there are no known adverse effects to the materials and equipment within the hospital.

Overview of this work

The studies outlined in this thesis will significantly expand on previous work and generate new knowledge of the efficacy of the HINS-light EDS. It will investigate the application of HIN-light technology in the burns unit, examining its role in various scenarios, including both inpatient and outpatient settings. Particular emphasis will be placed on optimising the use of the EDS in the clinical environment, to obtain the best possible decontamination effect with the most efficient use of the light. In addition, this work will further understanding of the cycle of cross-contamination of infection between burns patients and examine and how the EDS may potentially be used to interrupt this cycle and prevent the propagation of hospital-acquired infections within the burns unit.

Chapter 3

Materials and methods

3.0 Outline

This chapter details the materials and methods used throughout all laboratory and clinical studies. Firstly, the various bacterial culture media, diluents and reagents used are described. The sampling methods used during clinical work are then outlined. These include methods to sample the air; inanimate environmental surfaces in the burns unit; and the gowns worn by healthcare workers (HCW) caring for patients. The third part of the chapter details methods and tests used in the laboratory to enable identification of bacterial species obtained from clinical samples. The preparation of bacteria for use during laboratory inactivation experiments is then outlined. The final section of the chapter details the 405 nm High-Intensity Narrow-Spectrum light (HINS-light) systems used throughout the course of this work for both small-scale laboratory inactivation tests and environmental decontamination.

3.1 Microbiological media and reagents

This section describes culture media and reagents used throughout this research.

Introduction to culture media

Microorganisms were cultured in a culture medium, either as on solid agar plate, or in a liquid broth. Several types of culture media exist, each with particular nutritional properties.[307] The culture media used during this work are detailed in Table 3.1.

Table 3.1: *Culture media used during clinical and laboratory studies.*

Type of culture medium	Preparation	Weight required	Supplier	Product number
Baird Parker agar egg yolk tellurite (BPA) 55 mm contact plates	Ready made	n/a	Cherwell Labs.*	101170
Tryptone soya 1.6% agar + neutraliser (TSA) 55 mm contact plates	Ready made	n/a	Cherwell Labs.*	101560
Tryptone soya agar (TSA) 90 mm petri plates	Laboratory prepared	40 g/L	Oxoid**	CM0131
Tryptone soya broth (TSB)	Laboratory prepared	30 g/L	Oxoid**	CM0129
Nutrient agar (NA) 90mm petri plates	Laboratory prepared	28g/L	Oxoid**	CM0003
Nutrient broth (NB)	Laboratory prepared	13g/L	Oxoid**	CM0001

* Cherwell Laboratories Ltd., Bicester UK ** Oxoid Ltd., Basingstoke, UK

The properties of the different media and rationale for using them are described below. Firstly the contact agar plates used for environmental sampling are outlined, followed by media for the laboratory cultivation of bacteria.

1. Contact agar plates for clinical environmental sampling

Baird Parker agar with egg yolk tellurite (BPA) contact plates

Baird Parker agar with egg yolk tellurite enrichment (BPA) contact plates were used during hospital environmental sampling for the selective growth and enumeration of Gram-positive staphylococcal-type species. BPA contains sodium pyruvate and glycine to promote the growth of staphylococci, and tellurite to inhibit other microbial flora. On this medium, bacteria appear as grey or black colony forming units (cfu) following incubation (Figure 3.1). BPA plates were bought as ready-made 55 mm diameter contact

plates. This agar was chosen for the majority of environmental sampling on the burns unit due to its ability to isolate staphylococcal-type organisms – a good indicator of contamination from human sources. This enabled the study of organisms pertinent to burns patients.

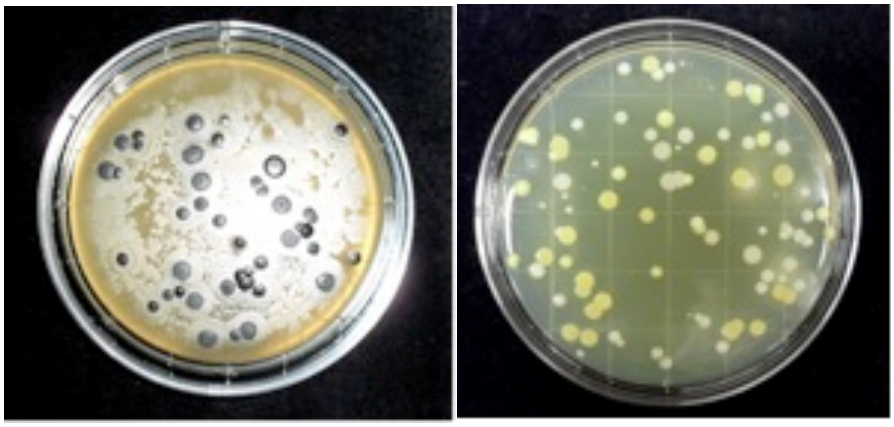


Figure 3.1: *Appearance of contact agar plates following environmental sampling and incubation: BPA plates demonstrating the grey-black appearance of colonies (left); TSA plates demonstrating the variety of different bacterial colonies (right).*

Tryptone soya agar (TSA) contact plates

Tryptone soya culture medium was used as either an agar or a broth. It is a non-selective medium that supports the growth of a wide variety of organisms.[308] TSA contact plates were used for some environmental sampling in the clinical environment, permitting quantification of total environmental bacterial load, including pathogenic and non-pathogenic bacteria. Colonies cultured on TSA demonstrate various morphologies, aiding identification of species (Figure 3.1).

2. **Media for the laboratory cultivation of bacteria**

Two main types of media were used for the laboratory cultivation of bacteria. Tryptone soya broth and agar, and nutrient broth and agar.[308] Both Tryptone soya media and nutrient media are non-selective general-purpose media that support the growth of most bacteria. Bacteria were cultured in either Tryptone soya or nutrient liquid broth overnight in order to prepare a suspension of bacteria with a known population density for experimental purposes. TSA and nutrient agar plates (prepared in 90 mm Petri dishes) were used for the plating and enumeration of bacteria. 90 mm TSA plates were also used for air sampling in the clinical environment.

Preparation of agars and broths

Laboratory-prepared culture media were prepared as required by dissolving the appropriate amount of agar or broth in powder form into distilled water (Table 3.1). This was sterilised by autoclaving at 121 °C for 15 min. After cooling, broths were ready to use. To prepare agar plates, the molten agar was held in a water bath at 48 °C until manually poured into 90 mm single vent Petri dishes and allowed to solidify. For longer-term storage of bacterial isolates agar slopes were prepared. These were made by dispensing ~10 ml molten agar into a sterile 30 ml Universal bottle, resting at an angle, and allowing to solidify.

Diluents and reagents

Diluents and reagents were used during laboratory experiments and identification of environmental bacterial cultures are listed in Table 3.2.

Table 3.2: *Diluents and reagents used for experiments and species identification.*

Type of diluent or reagent	Preparation	Weight required	Supplier	Product number
Phosphate buffered saline (PBS)	Laboratory prepared	1 tablet / 100 ml	Oxoid*	BR0014G
Gram-stain reagents (crystal violet, Lugol's iodine, ethanol and safronin)	Ready-made	n/a		
Oxidase identification sticks	Ready-made	n/a	Oxoid*	BR0064A
PBP2' test kit	Ready-made	n/a	Oxoid *	DR0900A
API 20 E test kit	Ready-made	n/a	bioMérieux**	20 100
API 20 NE test kit	Ready-made	n/a	bioMérieux**	20 050
Staphaurex Plus test kit	Ready-made	n/a	Remel***	30950201ZL34

* Oxoid Ltd., Basingstoke, UK ** bioMérieux® SA, Lyon, France

*** Remel Products, Thermo Fisher Scientific, Lenexa, USA

3.2 Hospital environmental sampling methods

This section details the general bacteriological techniques used during the collection, incubation and enumeration of environmental isolates during clinical studies. Methods used in clinical studies included sampling from environmental surfaces, sampling from healthcare worker (HCW) gowns, and air sampling.

Environmental sampling with contact plates

As discussed in Chapter 2, there are several possible methods for the collection of environmental samples from surfaces. For reasons already discussed, contact plate sampling was the preferred method used during this study.

Contact plate preparation

Contact plates were not prepared in the laboratory due to the accuracy required in pouring a proud meniscus and the quality control afforded by pre-prepared plates. Contact plates, sometimes known as RODAC (replicate organism detection and counting) plates have a proud convex surface that evenly contacts the environmental surface being sampled. Environmental samples were collected using 55 mm diameter contact plates with a sampling surface of 25 cm^2, and containing a volume of 17 ml ±1 ml (Cherwell Laboratories Ltd., Bicester, UK). BPA and TSA contact plates were both used for environmental sampling, with the choice of agar dependent on the study being performed.

Contact plate sampling and enumeration

Samples were taken by removing the lid of the plate and gently but firmly pressing the whole surface of the agar onto the environmental surface being sampled. The agar surface was either pressed flat or rolled evenly over a round object, such as a bed rail, taking care to ensure the whole agar surface had been in contact with the sampling surface. The agar must have made contact with the environmental surface only once, with pressure being applied for approximately 2 sec. Surface bacteria are consequently picked up onto the agar, and the lid was immediately replaced for transport to the laboratory.

Contact plates were then incubated at 37°C for 24 h or 48 h for TSA and BPA contact plates, respectively. Incubation was always carried out at 37 °C in order to allow for the growth of human pathogens. Post-incubation, bacterial colony-forming units (cfu) are visible on the agar surface, and the total number of cfu per plate was enumerated manually using a colony counter (Stuart Scientific, UK). Figure 3.2 shows a TSA contact plate following incubation, when the colonies are visible as pigmented raised growths. It also illustrates how the agar stands proud of the plastic container.

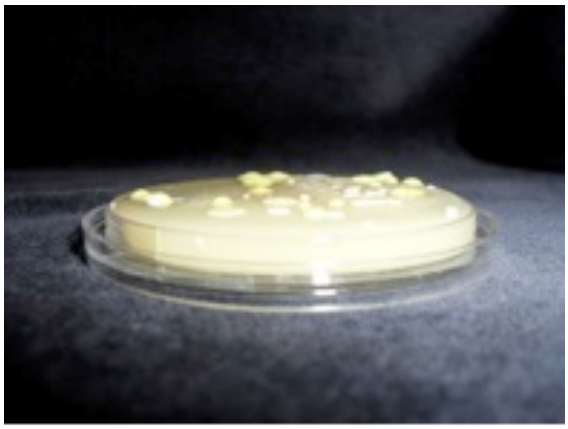


Figure 3.2: *Photograph of TSA 55 mm diameter contact plate following clinical environmental sampling and incubation. This illustrates the agar standing proud of plastic casing to enable sampling and the different morphologies of bacteria.*

HCW sampling with contact plates

To collect bacteria that had been dispersed onto HCW during the course of caring for burns patients, contact plate sampling from the surface of a sterile, impermeable, disposable gown was the preferred method. This method is described fully in Chapter 7.

Air sampling

For sampling airborne microorganisms, the sieve impaction method was chosen for the collection of air samples in the burns unit environment. TSA was selected as the non-selective nutrient-rich medium for the recovery of airborne microorganisms.

SAS Super 180 air sampler

A Surface Air System (SAS) Super 180 air sampler (Cherwell Laboratories Ltd., Bicester UK) was used for all air samples in this work. Air is aspirated at a fixed speed for a variable time through a perforated cover. Particles in the air land onto the agar surface of a 90mm laboratory prepared TSA plate (Figure 3.3).

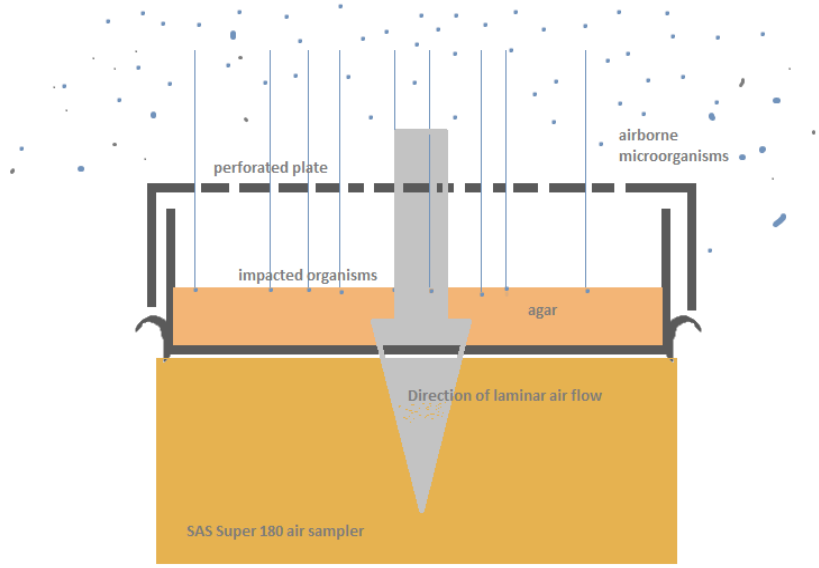


Figure 3.3: *Schematic diagram illustrating the sieve impaction method utilised by the SAS Super 180 air sampler.*

The SAS Super 180 air sampler was the chosen method for air sampling as it has several advantages:

- It uses simple and readily available laboratory prepared agar plates
- It can sample air for a variable amount of time and volume, and thus can be used in various situations on the burns unit when air contamination levels are expected to be different
- It aspirates air with sufficient velocity to impact organisms onto the agar plate
- It is portable and small enough to be used in an inpatient isolation room
- It is easy to clean between uses using detergent wipes
- It has a re-chargeable battery, and does not need mains power during use
- It has a short sampling time, enabling multiple samples to be taken at different points during an 'event', such as a dressing or bed sheet change
- It is an established method for measuring air contamination

In contrast, the Anderson air sampler, while a preferred sampling method for monitoring hospital airborne contamination levels is large and cumbersome. It needs a separate pump and is difficult to clean. Therefore, it was deemed too inconvenient for discrete sampling during potentially sensitive patient activities.

Operation of the SAS Super 180 air sampler

For operation, the sampler was first powered on and the programme set to sample for the required sampling time at a fixed rate, thus sampling a known volume of air. A pre-prepared 90 mm TSA plate was inserted (lid removed and the agar facing outwards), and the head replaced. The sampler was switched on and air samples collected for the programmed amount of time (Figure 3.4).

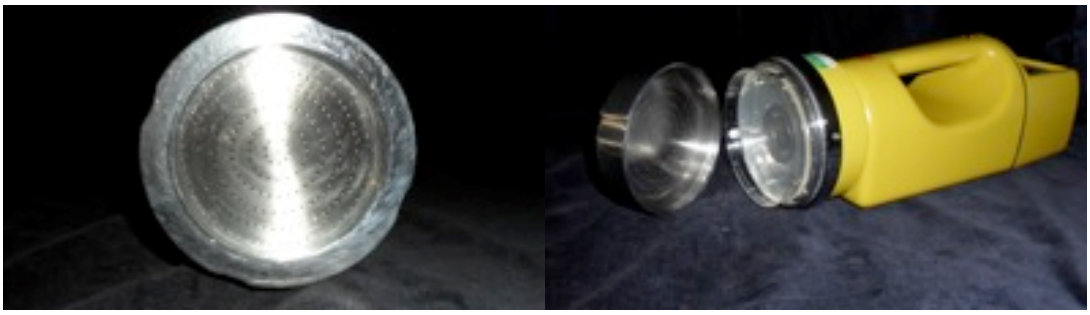


Figure 3.4: SAS Super 180 air sampler head (left) with 219 machine-perforated holes, and placement of agar plate within air sampler (right).

The SAS Super 180 air sampler is programmed to aspirate 180 litres of air per minute. The sampling times required for the aspiration of different volumes of air are detailed in Table 3.3.

Table 3.3: *Sampling times required to sample fixed volumes of air using the SAS Super 180 air sampler.*

Litres of air	Time required
10	3 sec
30	10 sec
50	17 sec
90	30 sec
100	33 sec
180	1 min
500	2 min 47 sec
900	5 min
1000	5 min 30 sec

Incubation and enumeration of air samples

Following sampling, the perforated head was removed and the TSA plate removed and transported to the laboratory for incubation at 37 °C for 24 h. The visible colonies were then enumerated (Figure 3.5). The raw total number of cfu counted on the surface of the agar plate was then corrected for the statistical probability of multiple particles passing through the same hole, by referring to correction tables supplied with the equipment (Appendix A).

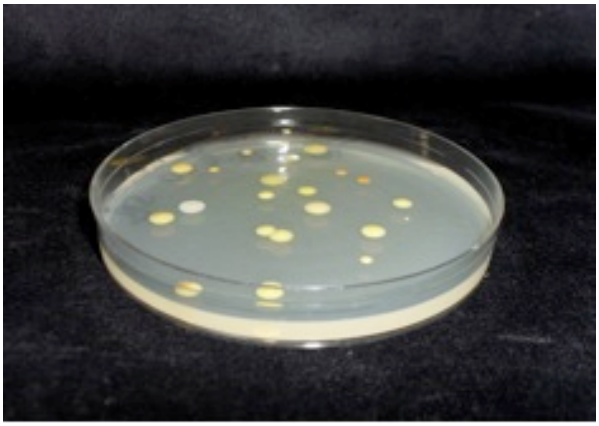


Figure 3.5: *Photograph of an incubated 90 mm TSA plate used in the SAS Super 180 air sampler for collection of airborne bacteria.*

The probable count (Pr) was then used to calculate the cfu per cubic metre of air sampled using the Equation 3.1:

$$X = \frac{Pr \times 1000}{V}$$

Where: V = volume of air sampled
Pr = probable count
X = cfu per 1000 litres of air (1000 litres = 1 m^3)

Equation 3.1: *Calculation of probable bacterial cfu count per* 1 m^3

3.3 Identification of bacterial isolates from clinical samples

Following clinical sampling using the previously described methods, further work was carried out to identify some of the bacteria isolated from the hospital environment. This section outlines the methods employed to identify bacterial species from samples taken from the hospital environment during the course of clinical studies.

Subculturing isolates from environmental sample plates

Subculturing enables the further study and identification of bacteria collected during environmental sampling. For the isolation and identification process to begin, a colony (from either a contact agar plate or an air sample plate) is selected. This colony must then be streak-plated to ensure that it is a pure culture. To do this, the individual colony was lifted from the hospital sample plate using a sterile loop and streaked onto a 90 mm TSA plate to obtain individual colonies, as demonstrated in Figure 3.6. The agar plate is then incubated at 37 °C for 24 h to enable growth of the bacteria. If the culture is pure, a single colony was then used to inoculate an agar slope, which was then incubated at 37°C. After incubation, this agar slope was then stored at 4°C for experimental use.

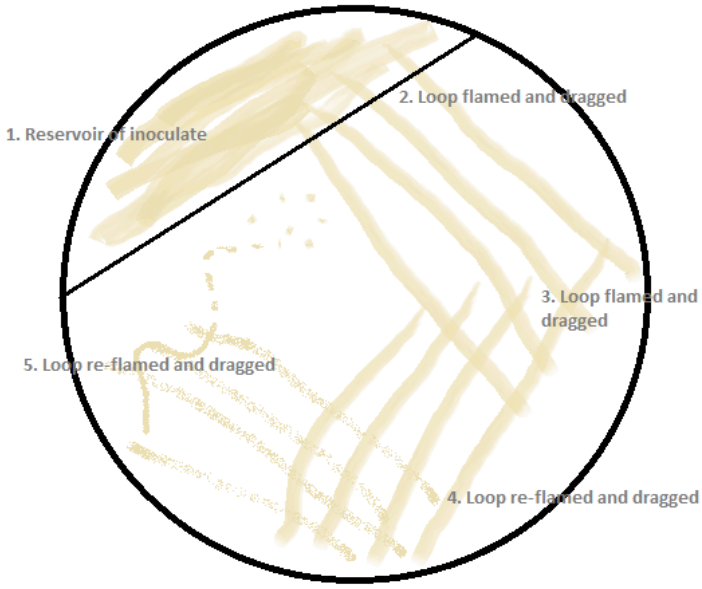


Figure 3.6: *Diagram to illustrate how individual colonies of bacteria are obtained using the streak-plating method. Adapted from* [309].

Microbiological and biochemical identification tests

A selection of microbiological and biochemical tests were used for the identification of bacterial isolates. The general principle was to obtain a pure culture, then work down from general tests which could broadly identify bacterial Genus to more specific tests which could identify bacterial species, using as few tests as possible.[310]

1. Appearance on culture media

As previously mentioned, hospital environmental sampling was carried out using either BPA or TSA plates. The appearance of organisms on these specific culture media was the first step towards bacterial identification.

BPA (with egg yolk tellurite) enables selective growth of staphylococcal-type species. Colonies appear as grey/black following incubation. Typically *Staphylococcus aureus* grow as grey-black shiny rounded convex colonies, surrounded by a halo of clear agar from the proteolytic reaction between the organism and the egg yolk.[308] This appearance was sought when attempting to isolate *S. aureus* or methicillin resistant *S. aureus* (MRSA) from hospital samples.

TSA is a less selective medium, supporting Gram-positive and Gram-negative bacterial growth. The appearance of colonies following incubation is much more varied in terms of colour, size and morphology. Again, the initial appearance was noted as the primary step in identifying environmental bacteria.

2. The Gram stain

The Gram stain is used for the microscopic identification of bacteria, allowing differentiation between Gram-positive and Gram-negative bacteria. It is a basic step of bacterial identification. To prepare a smear for staining, a flamed wire loop was used to lift a colony of bacteria from an agar plate, which was emulsified with a few drops of water on a glass slide. After air-drying, the slide was passed through a Bunsen flame in order to heat-fix it the sample. Following this, the slide was flooded with crystal violet for approximately 30 sec, before being drained and rinsed with iodine. It was covered with iodine for one minute, and then rinsed with ethanol. Finally, it was covered with safranin for a further 30 sec, before being rinsed with water and blot dried.

The slide was viewed under an oil immersion lens at 100 × magnification. Gram-positive cells stain purple due to retention of crystal violet within the thick peptidoglycan cell wall layer. In contrast the Gram-negative cells have a thin wall and a lipid-rich outer membrane that allows the easy removal of the crystal violet stain. The safranin red counter-stain is taken up instead, so cells appear pink. Bacterial morphology can also be classified (e.g. cocci, rods, etc).

3. Oxidase test

This test determines the whether cytochrome oxidase enzyme is present in the bacteria. Oxidase sticks (Oxoid Ltd., Basingstoke, UK) with one end impregnated in N-N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and α-napthol, are touched onto a colony of bacteria. After 30 sec the stick is examined. If there is no colour change from the initial grey/brown colour, the stick is left for a further three minutes. A test is deemed positive if the tip changes colour to a deep purple-blue. Pseudomonas sp. are usually oxidase positive.[311]

4. Catalase test

This is used to determine whether catalase enzyme is present in the bacteria. One or two drops of 40% concentration hydrogen peroxide are dropped onto a colony of bacteria. The release of hydrogen gas and oxygen, demonstrated by the formation of small bubbles on the agar surface, signifies a catalase positive test. Staphylococci are catalase positive, whereas streptococci are catalase negative.

5. Staphaurex Plus latex agglutination test

Staphaurex Plus (Remel Products, Thermo Fisher Scientific, Lenexa, USA) is a rapid latex agglutination test for the identification of Staphylococcus aureus. The test uses latex beads coated with fibrinogen and rabbit immunoglobulin G (IgG) for the detection of clumping factor (bound coagulase), protein A and/or surface antigens characteristic of S. aureus.[312] For this test the unknown isolate was first grown on TSA. A colony of the organism was then mixed with a drop of reagent. If the organism is *S. aureus*, rapid agglutination occurs through the interaction of fibrinogen and clumping factor, and the IgG protein interacting with cell surface antigens. Control latex is provided to ensure a non-specific aggregation of latex particles has not been observed (Figure 3.7).

Figure 3.7: *Example of Staphaurex Plus test. Three tests are along the bottom row and three controls are along the top row. Results can be seen from left to right: positive test, negative control (positive for S. aureus); negative test, negative control (negative for S. aureus); positive test, positive control (indeterminate).*

6. Penicillin-binding protein (PBP 2') latex agglutination test

PBP2' (Oxoid Ltd., Basingstoke, UK) is a rapid latex agglutination assay, detecting PBP2' in isolates of *S. aureus* in order to identify MRSA.[313] The *S. aureus* isolate (confirmed by Staphaurex Plus test) was first grown on TSA. A sterile 5 mm loop was then used to lift cells that were added to a microcentrifuge tube containing four drops of Extraction Reagent 1 and suspended. The tube was placed into a water bath at 95 °C for 3 min. The microcentrifuge tube was removed and cooled to room temperature. One drop of Extraction Reagent 2 was then added and mixed well. The tube was centrifuged at 1500 × g for 5 min. 50 µl of the cell supernatant was then mixed with one drop of test latex and one drop of control latex and mixed. The card was rocked for 3 min to look for evidence of agglutination. Again, the test is positive if the test latex agglutinates and the control does not (Figure 3.8).

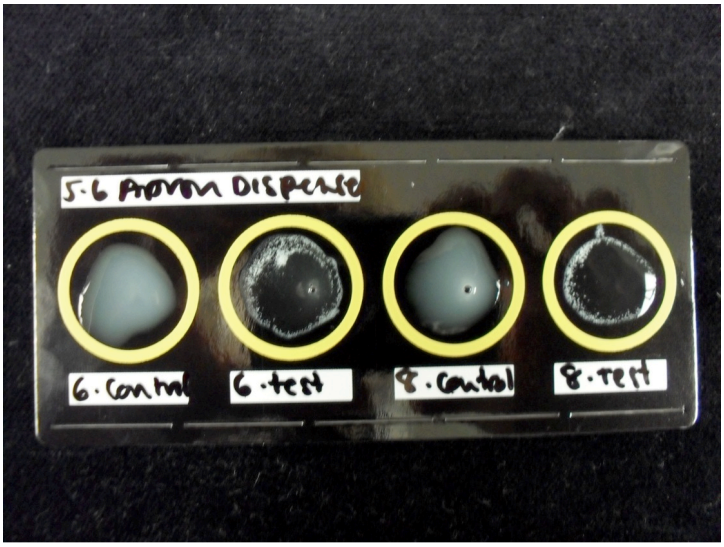


Figure 3.8: *Example of PBP2' latex agglutination test. A positive reaction, and confirmation of MRSA, is demonstrated by agglutination of both test but no agglutination in either control sample.*

7. API identification tests

API strips are bacterial identification strips that use miniaturised biochemical tests and a computer database (bioMérieux® SA, Lyon, France).[314] Each strip has 20 microtubules containing dehydrated substrates. Three kits were used:

- **API 20 NE**: identification of non-fastidious, non-enteric Gram-negative rods (e.g. *Pseudomonas, Acinetobacter, Moraxella* etc.)
- **API 20 E**: identification of Enterobacteriaceae and other non-fastidious Gram-negative (e.g. *Escherichia coli, Salmonella, Proteus* etc.)
- **API Staph**: identification of the genera *Staphyloccus, Micrococcus* etc.

A homogenised bacterial suspension is prepared and used to inoculate the microtubules on the strip. Mineral oil is added to any tubules testing for anaerobic growth. The test strip is incubated at 29 °C for 24 h. Positive metabolism of each test substrate (either spontaneous or following the addition of reagents) is indicated by a colour change. The results are recorded (positive or negative for each tubule), and the information is entered into a computerised database, which confirms the identity of the bacterial species being tested, and the percentage certainty for this result being correct.

3.4 Bacterial preparation for laboratory experiments

This chapter describes methods used in laboratory experiments studying the effect of HINS-light on bacterial isolates from the burns unit. These isolates were obtained either from environmental samples, or burns patients wound swabs (supplied by GRI microbiology laboratory), and their identity confirmed using methods detailed in Section 3.3.

Preparing a bacterial suspension

Bacterial cultures were stored at 4 °C on NA slopes as described in Section 3.1. For experimental use, a loopful of bacteria was aseptically transferred to 100 ml broth (NB or TSB), and this was incubated at 37 °C for 18 h -24 h in a rotary incubator (120 rpm). The broth was then centrifuged at 3400 rpm for 10 min and the supernatant discarded. The cell was resuspended in 100 ml PBS and thoroughly mixed using a vortex mixer (Fisherbrand FB15024, Fisher Scientific, Loughborough, UK). This produced a bacterial suspension with a population of approximately 10^9 cfu/ml depending on the bacterial species.

Serial dilutions

For experimental use, the 10^9 cfu/ml bacterial population needed to be diluted to the appropriate starting population before exposure to light-treatment. To do this, serial dilutions of the original bacterial suspension were prepared by adding 1 ml of suspension to 9 ml PBS and mixing well: this provided a 10^{-1} dilution. This was repeated serially, adding 1 ml of that suspension to 9 ml of sterile PBS, until the desired dilution of bacteria was obtained. For example, a 10^{-6} dilution was needed to obtain starting populations of 10^3 cfu/ml for experimental use.

Plating techniques

In this study bacteria were exposed to HINS-light treatment using two methods:

- Bacterial suspensions were plated onto the surface of agar plates and exposed to the HINS-light EDS.
- Bacterial populations in liquid suspension were exposed to HINS-light.

Plating techniques enable bacterial samples to be deposited on, or held within, agar, where they can then be incubated and the resultant colony growth enumerated – thus establishing the effect of the HINS-light on the exposed bacterial population. Various plating techniques were required throughout this work to (i) seed bacterial suspensions onto agar surfaces for light-exposure, and also (ii) to plate out bacterial suspensions after they have been light-exposed. The volume of suspension and method of plating to

be used was selected depending on the expected sample population. The different methods for plating bacterial samples will now be described.

1. Spread plate method

Spread plates were prepared by one of two ways. A Whitley automated spiral plater (Figure 3.12; Don Whitley Scientific, Shipley, UK) was used to prepare spread plates by automatically dispensing 100 µl of bacterial sample onto the surface of a rotating agar plate in the shape of a linear Archimedes spiral.

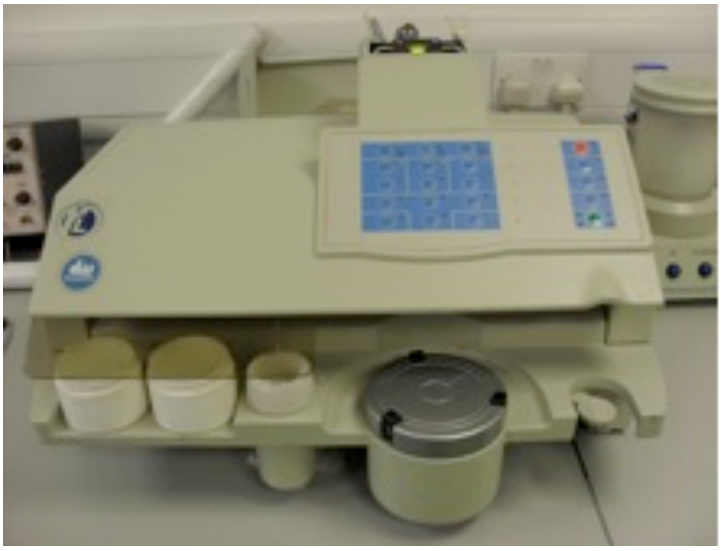


Figure 3.9: *The automated spiral plater (Don Whitley Scientific, Shipley, UK). The agar plate is placed on the central rotating disk, and liquid samples drawn under negative pressure and distributed over the agar surface: linear distribution for spread plates and logarithmic distribution for spiral plates.*

Alternatively a 100 µl volume of bacterial suspension is manually pipetted, using an automatic pipette, onto an agar plate and distributed evenly over the surface using a sterile L-shaped spreader. The lid is replaced and following drying, the plate is incubated. After incubation, a cfu count of the entire plate was obtained and this was then used to quantify the number of cfu in the bacterial sample:

- If a liquid bacterial suspension exposed to HINS-light was being enumerated then the bacterial count was multiplied by 10 to get the number of cfu per millilitre of sample, with results recorded as 'cfu/ml';
- If bacteria were exposed to HINS-light whilst seeded on an agar surface the entire plate count was used, and results were recorded as 'cfu/plate'.

2. Spiral plate method

To allow enumeration of high-density bacterial populations, bacterial samples were plated using the spiral plater. In this instance, the plater was used to dispense a 50 µl volume of bacterial suspension as an Archimedes spiral, with logarithmic deposition. Following incubation, colonies are counted by centering a grid over the plate, with each marked grid segment corresponding to a known constant volume of deposited sample. The number of cfu/ml was then calculated with reference to the manufacturer's supplied charts.

3. Pour plate method

If a sample is expected to contain < 250 cfu/ml, the pour plate method may be used. For this method, 1 ml of undiluted sample was pipetted into an empty sterile petri dish. Approximately 20 ml of molten agar was poured onto the sample and the plate gently rotated clockwise, then anti-clockwise to ensure mixing of suspension. After solidifying at room temperature, the plate is incubated. For enumeration, the total number of cfu on the plate was counted, thus providing the number of cfu/ml of sample.

3.5 High-Intensity Narrow-Spectrum light (HINS-light) sources

During this research, two different sources were used to deliver 405 nm HINS-light to bacteria in the environment and laboratory:

- A small 405 nm LED array for laboratory exposures of bacteria in liquid suspension;
- A ceiling-mounted HINS-light Environmental Decontamination System (HINS-light EDS) for decontamination of the clinical environment, and laboratory exposures of bacteria on an agar surface.

This section will describe these two light sources, and a safety analysis of the HINS-light EDS, carried out by the University of Strathclyde is also reported.

Light-Emitting Diodes (LED)

A LED consists of two regions of different semi-conducting materials, that is, materials with electrical conductivity intermediate between that of a conductor and an insulator. A LED emits light in a narrow spectrum, determined by the value of the energy gap between the materials forming the p-n junction.[293] The n-type material acts as a source of electrons. It is a crystal of a compound semi-conductor that has been 'doped' with an element such as silicone. This impurity has an extra electron in its outer shell, which is added to the crystal lattice and moves through it, carrying a negative charge. Opposite this is the p-type material, with an excess positive charge due to doping with an element such as zinc, which has a deficit of electrons in its outer shell. The mobile vacancy left due to this electron shortage acts as a positive charge that can move throughout the crystal, and is termed the 'hole'.[315] When a current is applied to the active region between the n-type and p-type regions, charge-carriers (electrons and holes) flow into the junction from electrodes with different voltages. Recombination of an electron and a hole cause the electron to become excited into a lower energy level, releasing energy in the form of a photon.[293] This phenomenon is termed electroluminescence, whereby light is emitted by a material in response to the passage of an electric current.

The first report of light emission from a semiconductor was in 1907, when silicon carbide (SiC) was observed to emit a yellow glow.[315] This was followed by further observations in the 1920s of electroluminescence in zinc oxide and silicon carbide, alongside documentation of the spectrum of light emitted and constituting the discovery of the LED.[316] In the 1960s several groups of researchers simultaneously reported a LED semiconductor laser emitting orange light.[316-320] The mid 1990s saw the first commercial bright-blue LED, which led to the production of white light by coating the

blue LED with a yellow phosphor.[321] Alternatively, blue, green and red LED can be combined to produce white light.[322]

The 405 nm HINS-light LED array

Laboratory studies exposing bacteria in a liquid suspension to 405 nm light were carried out using a small 405 nm LED array, as used in previous studies.[2] The array is composed of a rectangular block of 99 individual 405 nm-emitting LEDs arranged in an 11×9 matrix (OD-405-99-070, OptoDiode Corp, CA, USA). The manufacturer's stated output emission has a bandwidth of 405 nm (10 nm full-width at half-maximum, FWHM). The array is bonded to a heat sink and fan to minimise heat production, with the temperature maintained at around 30°C. The LED array is powered by a direct current (DC) supply with output controllable in the range 0-15 Volts and 0-3 Amps. The LED array is pictured in Figure 3.10.

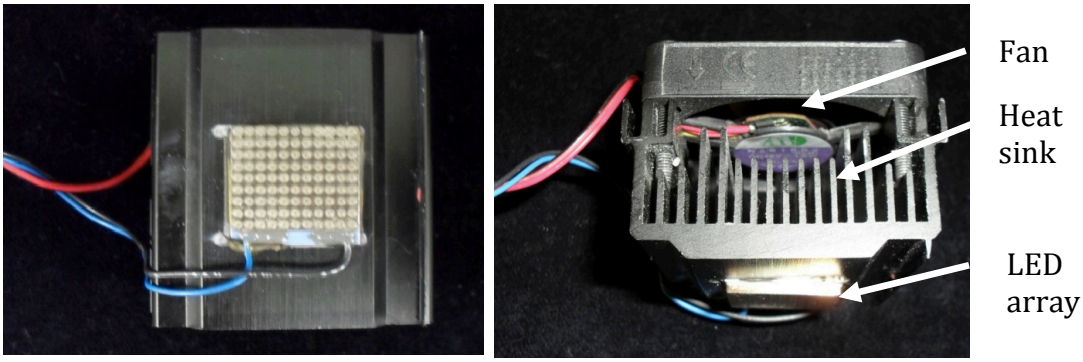


Figure 3.10: *405nm-light LED array. The 99-DIE LED array is visible in the picture on the left. The picture on the right shows the fan and heat sink arrangement connected to the LED array.*

The HINS-light Environmental Decontamination System (HINS-light EDS)

The HINS-light EDS units were developed for use in the hospital environment, and a full description of the technology was given in Section 2.11. During the course of this work the HINS-light EDS was used for:

- Clinical investigations into the efficacy of the system for environmental decontamination in the burns unit;
- Laboratory-based studies to investigate the bactericidal efficacy of the HINS-light EDS against known populations of bacterial isolates on agar surfaces.

The HINS-light EDS used throughout this work was a Mark I prototype unit developed by the University of Strathclyde. It is a ceiling mounted light source consisting of a matrix of 16 405 nm 99-LED arrays (OD-405-99-070, OptoDiode Corp, CA, USA) and five white LED (GE-VHD-1A3B8, General Electric, USA), mounted onto a heat sink, with the configuration illustrated in Figure 3.11.[323] The white illuminating element is of a higher

illuminance to that of the 405 nm light. A Fresnel lens directs light onto the target region and a diffuser blends the two different light sources (blue and white), to produce an aesthetically pleasing violet/white hue (Figure 3.11). The HINS-light EDS light source has a diameter of 30cm, designed to irradiate an area of approximately 10 m^2 from a distance of 2 m, with sufficient intensity to cause inactivation of exposed bacteria. The light source is held in a 58×58 cm housing designed to allow retro-fit into a false ceiling by replacement of a ceiling tile. The HINS-light EDS in a clinical environment is shown in Figure 2.7.

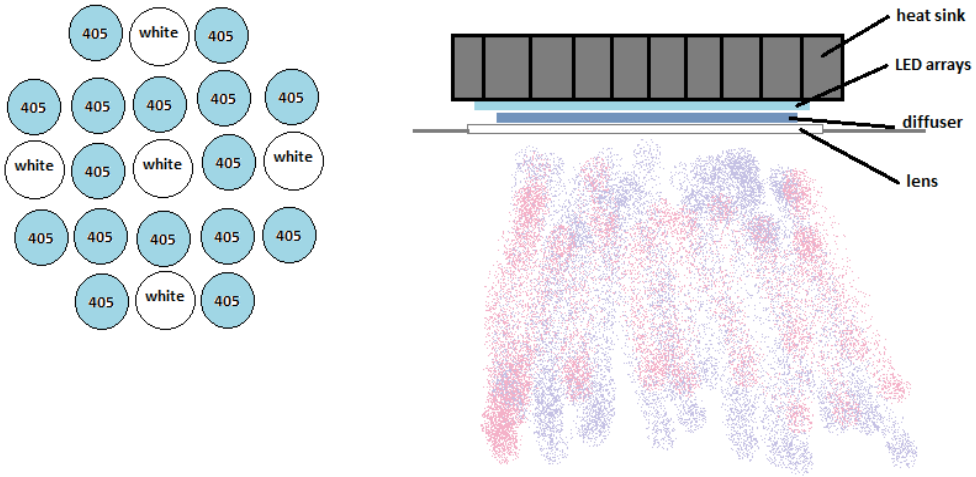


Figure 3.11: *Diagram of the HINS-light EDS design. On the left is a diagram of the matrix arrangement of blue (405 nm) and white LED arrays. On the right is a cross sectional diagram of the ceiling mounted EDS. Adapted from*[323].

The optical emission spectrum of the HINS-light EDS was measured using a HR2000 spectrometer and SpectraSuite spectroscopy software platform (Ocean Optics, Dunedin, FL, USA). This is illustrated in Figure 3.15. A peak can be seen centred around 405 nm wavelength, with secondary peak output in the range of around 500 nm to 700 nm due to the white LED element of the HINS-light EDS.

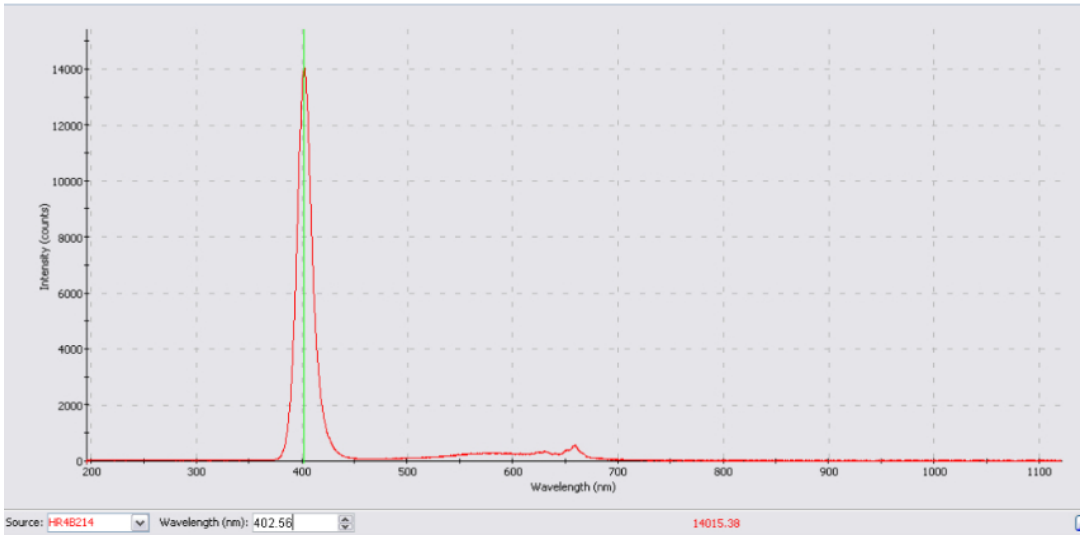


Figure 3.12. *Emission spectrum of the HINS-light EDS measured using HR2000 spectrometer and analysed using SpectraSuite spectroscopy software (Ocean Optics).*

The irradiance of light emitted by the HINS-light EDS varies with distance. This was measured for each of the two HINS-light EDS installed into one isolation room in the burns unit, with results shown in Figure 3.13. Irradiance measurements of the bactericidal 405 nm blue light component only were taken using a radiant power meter and photodiode detector head (Oriel Instruments, Stratford CT, USA), with all measurements being taken at a vertical distance of 1.66 m below the ceiling (approximate bed-table height).

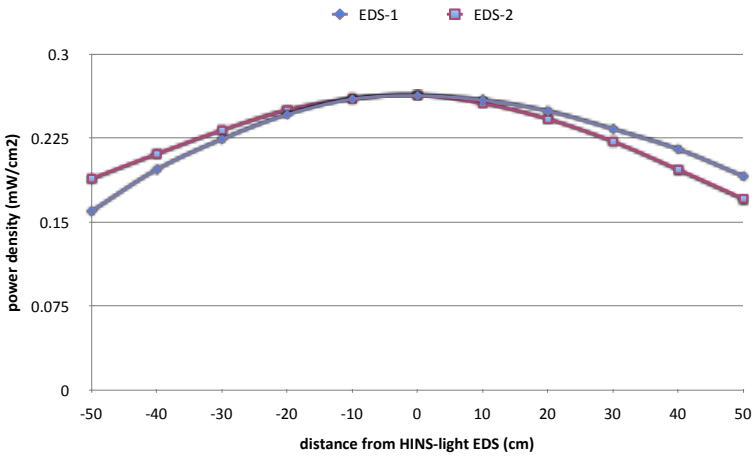


Figure 3.13: *Irradiance measurements of the two HINS-light EDS units in an isolation room in the burns unit. Measurements are taken at a distance of 1.66m below the ceiling, with the distance between the point below the EDS and point at which reading is being taken along x-axis.*

The system uses two power supplies to drive the LED, one for the blue LED (~18 A and 15 V), and one for the white LED (~1.5 A and 10.5 V), so they can be controlled independently. The power supplies are contained in a lockable mobile cabinet and are run off standard mains electricity, with the units being switched on and off at the wall via a standard electrical plug on/off switch.

For the majority of the clinical studies, two HINS-light EDS units were installed in each isolation room. These were placed so they were never directly above a patient head as they lay in bed, but were at a distance of at least 1 m from the patient's head. When the HINS-light EDS units were in use in an inpatient isolation room, a timing device was fitted to switch the unit on at 0800 h and off at 2200 h each day, so as not to disturb patient sleep. Minimal staff training was required and there was no disruption of the normal hospital routine.

Safety analysis

Researchers at the ROLEST facility at the University of Strathclyde have carried out safety analysis of the HINS-light EDS.[323] The safety analysis of the Mark I prototype used throughout this study is unpublished, however the safety analysis of a Mark II prototype has recently been published, and although based on a newer prototype, this had been developed to provide the same light emission and light distribution as the Mark I prototype: the analysis is therefore comparable.[323]

The safety analysis carried out to standards set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and the American Conference of Governmental Industrial Hygienists (ACGIH), both of whom collaborate with the World Health Organisation (WHO) in the establishment of guidelines for human exposure limits to electromagnetic radiation.[324-327] In addition, reference is made to guidelines by The Health Protection Agency (HPA), who also provides information on light exposure limits.[328]

Three regions of the electromagnetic spectrum were considered with reference to their potentially harmful biological effects, as discussed in Chapter 2:

- Ultraviolet (UV) light (180 nm to 400 nm);
- Visible light (400 nm to 750 nm); and
- Infrared (IR) (750 nm to 1mm).

The UV region is further divided into UVA (320 nm to 400 nm), UVB (290 nm to 320 nm) and UVC (less than 290 nm).[323,329]

Measurements carried out to establish the luminous flux (measured in lumens, lm) and illuminance at 200 cm distance from the source (measured in lux) were performed on

the blue 405 nm wavelength component, the white component and the total output of an EDS unit. These are summarised below (Table 3.5). For comparison, the illuminance from home lighting is usually between 30-300 lux; an office desk light is 100 lux; and an operating theatre light 10,000 lux.[323,330,331]

Table 3.5: *Summary of luminous flux and illuminance at 2m for the HINS-light EDS Mark II, adapted from.*[323]

Light source	Luminous flux (lm)	Irradiance (mW/ cm^2)	Illuminance at 2 m (lux)
Blue (405 nm) LED (x4)	64		107
White LED (x12)	1080		102
Total output from Mark II EDS	1144	0.32	209

Thermal hazard, IR radiation and UV light

Figure 3.12 shows that the HINS-light EDS emits a spectrum with no IR component. ICNIRP regulations state that retinal thermal hazard calculations must be performed in the wavelength 380 nm to 1400 nm if the luminance of the source is greater than 1 candela (cd) per cm^2 (1 x 10^4 cd/m^2).[325] The luminance of the Mark II HINS-light EDS was calculated to be 0.239 x10^4 cd/m^2: below the exposure limit.[323]

Although no region of the HINS-light falls within the UVB or UVC wavelength region, around 20% of HINS-light power falls within the UVA spectrum. ICNIRP state that the total UV (315 nm to 400 nm) radiant exposure should not exceed 1 J/cm^2, and that maximum irradiance should be 1 mW/cm^2.[324,326] Published data show the Mark II HINS-light EDS to have irradiance values in the range of 2.5x10^{-4} mW/cm^2 to 4.9x10^{-4} mW/ cm^2, which is 0.025% to 0.049% of the exposure limits established by ICNIRP.[323]

When the whole UV spectral range was assessed, the maximum permissible exposure (MPE) time (as defined by HPA) was found to be > 8 h at a distance range of 30-200 cm. This is in line with HPA recommendations which state that the UV element of a light source is safe for any length of exposure time at the distance tested if the MPE time is > 8 h. However, the spectral range for UVA demonstrated an MPE > 8 h only for distances greater than 115 cm. For a distance less than 115 cm between the HINS-light EDS and the patient's eyes the MPE was less than 8 h, with shorter distances producing shorted MPE times.[323] The ceiling height of the rooms on the burns unit was 250 cm, and the maximum height of the bed 82 cm. A tall patient sat in bed directly underneath the EDS would have a height of approximately 100 cm from the bed, producing a distance between the ceiling and their eyes of 68 cm. Using the Pythagorean theorem, the distance from the point below which the EDS is placed, and the patient's head must be greater than 93 cm (Figure 3.14). Of note, the calculations represent an extreme

situation that would never occur: that of a tall person, sat in a bed at maximum height, staring continually at the HINS-light EDS. Although staring may occur with a patient lying in bed for long stretches, they would be much more than 115 cm from the light source.

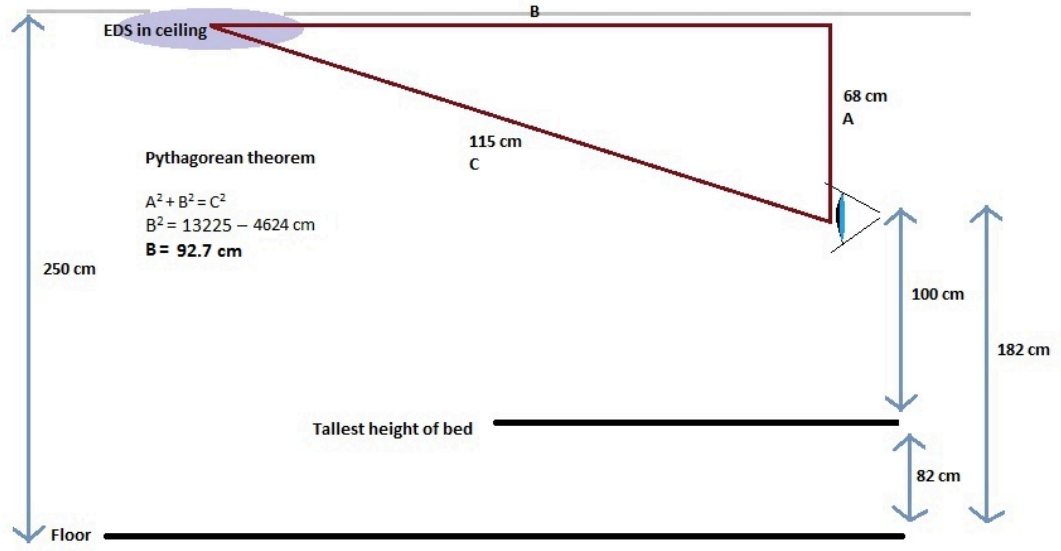


Figure 3.14: *Diagram to illustrate safe distance between patient and HINS-light EDS so not exceed safe UVA exposure, and calculation according to the Pythagorean theorem.*

Blue light hazard

According to ACGIH guidelines, long-term exposure to a particular light source is acceptable when the **effective** blue light radiance is < 10 mW/cm^2 x sr. These are calculated with different adjustments for the phakic (containing a crystalline lens) and aphakic (with the lens removed and replaced by a clear lens) eye.

The irradiance of the Mark II HINS-light EDS source, at a distance of 200 cm was reported to be 0.32 mW/cm^2.[323] From this measurement, effective radiance of the EDS was found to be 2.97 W/m^2 x sr (0.297 mW/cm^2 x sr) for normal eyes, and 13.24 W/m^2 x sr (1.3 mW/cm^2 x sr) for aphakic eyes (following removal of the crystalline lens and replacement with a clear lens) .[323] Both values are well below the HPA and ICNIRP guidelines which state that long term exposure to a particular source is permissible when the blue light radiance is < 10 mW/cm^2 x sr. Of note, HPA guidelines state that if the effective blue light radiance is less than the exposure limit, there is no risk that the exposure limit will be exceeded at any distance from the light source.[325,328]

Summary of safety data

According to calculations performed at the University of Strathclyde, the Mark II HINS-light EDS is safe for use within the hospital environment, provided it is installed with a direct distance of at least 115 cm from the patient's head; a distance of 93 cm between the point on the floor below the patient's head and the point on the floor below the HINS-light EDS. This is in order to satisfy HPA guidelines on the UVA emission from the light. From the point of view of the blue light hazard, blue light radiance was found to be less than the recommended limits. According to the HPA, this renders the light safe at any distance. All calculations are summarised in Table 3.6. Of note, the calculations were performed in-house, and before use of the EDS unit can become widespread, an independent review of all safety aspects of the unit would be required. Furthermore, all calculations have been undertaken at a distance of 2 m from the light source. As the patient may well be closer to the source, these independent assessments should be carried out from shorter distance to ensure total safety.

Table 3.6: *Safety analysis of Mark II HINS-light EDS at a distance of 200 cm and irradiance of* 0.32 mW/cm^2. *Adapted from* [323].

Interaction	% Threshold Limit Value (TLV)
Thermal energy to skin and eyes	Not calculated (<20)
UV to unprotected eyes	11
Blue light to unprotected phakic eyes	3
Blue light to unprotected aphakic eyes	13

Chapter 4

Laboratory inactivation of bacterial isolates from the burns unit

4.0 Outline

Chapter 2 (Sections 2.9 and 2.11) reviewed previous laboratory investigations using 405 nm wavelength high-intensity light-emitting diode (LED) arrays to inactivate bacteria in liquid suspension and on agar surfaces.[2,264] The bulk of this chapter outlines clinically focused laboratory studies investigating the inactivation of clinical bacterial isolates using a ceiling-mounted High-Intensity Narrow Spectrum light Environmental Decontamination System (HINS-light EDS) identical to those used in hospital studies. The aim of the experiments was to establish the inactivation kinetics of clinically derived isolates, exposed to the relatively low levels of irradiance emitted by the HINS-light EDS in conditions mimicking the clinical environment. To achieve this, low-density bacterial contamination (similar to the levels found on hospital surfaces) was seeded onto agar surfaces, and exposed to the laboratory ceiling-mounted HINS-light EDS. These results, although generated under laboratory conditions, provide important information about the decontamination efficacy of the HINS-light EDS system in the hospital environment. All work was carried out on bacteria isolated from the burns unit or burns patients, rather than a national depository.

In addition to investigating the efficacy of the HINS-light EDS unit, a study was completed to investigate the use of a single high-intensity 405 nm emitting LED array for bacterial inactivation, similar to the studies by Maclean et al.[2] The use of the single array permitted liquid suspensions of high density bacterial populations to be exposed to 405 nm light, thus generating information about bacterial susceptibility. A clinical isolate of *Pseudomonas stutzeri* was chosen for this work: this organism was isolated from the burns unit environment and has not been previously studied.

4.1 Background

As discussed in Section 2.8, there is a growing body of evidence to support the use of photodynamic inactivation (PDI) of bacteria using visible light, by way of a variety of laboratory experiments using LED, lamps, lasers and Super Luminous Diodes (SLD). [2,257,258,260,262-266] The majority of these studies used either:

- High-density liquid bacterial suspensions and high irradiance light exposure, or;
- Low-density surface seeded bacteria and high irradiance light exposure

The HINS-light EDS was developed to deliver much a lower irradiance than that delivered by the high irradiance LED arrays used in experimental conditions, enabling its continuous use in the presence of people. Therefore the aim of the majority of experiments outlined in this chapter was, for the first time, to examine the rate of inactivation of clinical isolates using the EDS, rather than a high-intensity 405 nm LED array, before moving into the dynamic environment of the hospital. This allowed the generation of inactivation kinetics, which provide information about the time taken to inactivate known low-density populations of bacteria with significantly lower irradiances to those used previously. Central to these studies is the requirement to demonstrate that the system can inactivate pathogenic clinical isolates with particular significance to the burns population. One study completed in this chapter was carried out in a similar manner to that performed by Maclean et al.[2] That was the inactivation of a liquid suspension of a *P. stutzeri* clinical isolate with a high-intensity 405 nm LED array. This permitted the verification of the susceptibility of a previously untested organism in a high population liquid suspension rather than low-density surface inoculated bacteria.

With the exception of the *P. stutzeri* experiment, the focus throughout was on the performance of laboratory studies with particular clinical relevance to the burns unit. In order to replicate the inactivation happening in the hospital environment using a laboratory model, the following changes were made to Maclean *et al*'s experimental methods:[2]

- Bacterial isolates from environmental samples or patient wound swabs, all taken from the burns unit at GRI were used throughout, rather than isolated from a national depository. Five pathogens pertinent to burns patients were tested. These were methicillin sensitive and methicillin resistant *Staphylococcus aureus* (MSSA and MRSA), *Streptococcus pyogenes*, multi-drug resistant *Acinetobacter baumannii* (MDR-*A. baumannii*), and *Pseudomonas aeruginosa*.

- Rather than being suspended in an inert solution (phosphate buffered saline, PBS), isolates were spread onto an agar surface in similar densities to those shown to occur in the burns unit by environmental contact plate samples.
- The light source was not a 405 nm LED array (irradiance around 10 mW/cm^2), but a ceiling mounted HINS-light EDS unit, identical to those installed on the burns unit (irradiance around 0.5 mW/cm^2).
- The distance between the light source and agar plates was similar to distances between the EDS and surfaces sampled in the hospital (approximately 1.5m).
- Finally, as the irradiance was much less than that used during Maclean *et al*'s study, longer exposure times were used, in the order of hours, to achieve sufficient dose to inactivate bacteria.[2]

4.2 Experimental arrangements and general methods for inactivation of clinical isolates on agar surfaces

This section describes the experimental arrangements used for exposure of bacterial clinical isolates on an agar surface to the HINS-light EDS. Low-density bacterial starting populations (approximately 200 cfu/plate) were used, replicating the typical bacterial density that can be found in the burns unit environment. Use of set population densities of known bacteria allowed the generation of more accurate inactivation data than would be possible in the dynamic clinical environment.

Preparation of bacterial seeded surfaces

All bacteria used were isolated either from contact agar plates collected from the burns unit environment, or from routine patient burn wound swab surveillance samples, supplied by the GRI microbiology laboratory. All isolates were identified using the tests described in Section 3.3. The species used for this work were:

Methicillin-sensitive *Staphylococcus aureus* (MSSA)

MSSA was isolated from a sample taken using contact agar plates from a healthcare worker's (HCW) gown, worn during a dressing change on a burns patient.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA was isolated from an environmental contact plate sample from the room of an MRSA positive patient on the burns unit.

Streptococcus pyogenes

S. pyogenes was isolated from a routine wound surveillance swab from a patient on the burns unit and identified by the GRI microbiology laboratory.

Multi-drug resistant *Acinetobacter baumannii* (MDR-*A. baumannii*)

MDR-*A. baumannii* was isolated from a routine wound culture swab from a patient on the burns unit and identified by the GRI microbiology laboratory.

Pseudomonas aeruginosa

P. aeruginosa was isolated from a routine wound culture swab from a patient on the burns unit and identified by the GRI microbiology laboratory.

Isolates were stored and prepared for exposure as described in Section 3.4. In brief, the day prior to an exposure experiment, a bacterial broth was prepared by inoculating 100 ml Nutrient Broth (NB) with a loop of the chosen organism. The broth was

incubated at 37°C for 18 h in a rotary incubator (120 rpm). This provided a bacterial population of approximately 109 cfu/ml, depending on the organism. After incubation, broths were centrifuged at 3,939 g for 10 min, and the resultant pellet re-suspended in 100 ml PBS. The suspension was diluted in PBS to achieve a 103 cfu/ml population density. An appropriate volume of this suspension, usually 100 µl or 50 µl, was spread onto a 90 mm Nutrient Agar (NA) plate, giving starting populations of approximately 100-200 (1-2 x 10^2) bacterial cfu/plate. NB and NA were used throughout as standard non-selective media that supported the growth of all five organisms.

Experimental set-up

Laboratory work was carried out using a single HINS-light EDS, identical to those installed in the burns unit at GRI for clinical studies. For experiments, the HINS-light EDS was installed into the ceiling in the laboratory annex room, as shown in Figure 4.1. Experiments were carried out here rather than the main laboratory, as the room had less activity and therefore there was a lower risk of contamination of the agar plates with environmental organisms. The electrical input settings used throughout all experiments were approximately 15 V and 18 A. Full details of the HINS-light EDS are found in Section 3.5.

For exposure of bacterial seeded agar plates, a table was placed directly beneath the ceiling-mounted HINS-light EDS at a distance of 1.56 m from the light source. A grid was marked out on the table surface and the irradiance received at each point on the grid was measured at the start of the experiment using a radiant power meter and photodiode detector (Oriel Instruments, Stratford CT, USA). The irradiance at this distance was approximately 0.5 mW/cm^2 across the grid surface.

The seeded agar plates were then placed on the table surface for exposure. The inoculated agar plates were positioned so that the agar plate exposed for the longest length of time would be placed on the square receiving the highest irradiance, and thus the greatest dose. This also enabled more accurate repetition of the experimental arrangement. An example of a layout used to expose seven bacterial-seeded agar plates for between 1 h and 7 h, including the irradiance levels and doses used for exposure, is shown in Figure 4.2.

During experiments, bacterial seeded agar plates were exposed to a set irradiance for a set exposure time. From these values it was possible to calculate the total energy (dose) to which the bacteria were exposed. Dose is calculated using the following equation:

$$\text{Dose (J/cm}^2\text{)} = \text{irradiance (W/cm}^2\text{)} \times \text{exposure time (s)}$$

Equation 4.1: *Calculating dose*

Figure 4.1: *Ceiling-mounted HINS-light EDS in laboratory annex room. Table positioned underneath with marked grid for the placement of seeded agar plates*

Approximately 40 x 40 cm

Not used	0.52 mW/ cm^2 2 h 3.7 J/cm^2	0.52 mW/ cm^2 2 h 3.7 J/cm^2	Not used
Not used	0.55 mW/ cm^2 6 h 11.8 J/cm^2	0.55 mW/ cm^2 7 h 13.8 J/cm^2	0.50 mW/ cm^2 1 h 1.8 J/cm^2
Not used	0.54 mW/ cm^2 4 h 7.7 J/cm^2	0.55 mW/ cm^2 5 h 9.8 J/cm^2	Not used

Figure 4.2: *Schematic diagram of the grid layout of bacterial-seeded agar plates, exposed to the HINS-light EDS for between 1 h and 7 h. Each grid box details the measured irradiance at that position (mW/cm^2); the exposure time (h); and the dose applied to each seeded agar plate (J/cm^2).*

Generation of bacterial inactivation curves

For each experiment, two inoculated agar plates were prepared which were not exposed to the HINS-light EDS: these were used to determine the starting populations (0 h exposure). Two inoculated agar plates, one 'test' and one 'control' plate were prepared for each hourly exposure time. Test plates were placed on the table directly beneath the HINS-light EDS. Control plates were placed away from the EDS but in the same room and exposed to the same environmental conditions. At the start of the experiment all lids were removed from the agar plates. At hourly intervals, the lids would be replaced on one test plate and one control plate, and they would be removed and stored in the dark at room temperature until the experiment was completed. At the end of the experiment, all test and control plates were placed into an incubator at 37°C for 24 h before enumeration of bacterial cfu was performed. Each experiment was repeated in triplicate for each isolate tested. Graphs of the raw data were produced for each experiment, plotting the bacterial cfu count per plate every hour for each test and control plate.

Statistical analysis

Data is initially reported as raw bacterial cfu/plate counts versus exposure time. To account for variation between starting populations, bacterial inactivation at each hourly interval was also expressed as a percentage (%) of the control bacterial cfu/plate count, calculated thus (Equation 4.2):

$$\frac{\text{Total bacterial cfu on test plate at that hourly interval}}{\text{Total bacterial cfu on control plate at that hourly interval}} \times 100$$

Equation 4.2: *Percentage bacterial inactivation*

Further statistical analysis was carried out using Minitab v16. One-way analysis of variance (ANOVA) was performed for each hourly interval, comparing differences between the test and control samples. This was first carried out on raw data for each of the three experiments per isolate. Further analysis was carried out on % reductions achieved for each of the three experiments per isolate, again comparing the test plate and control plate bacterial count at each hourly interval. A significance level of <0.05 was accepted throughout.

4.3 Methicillin sensitive *Staphylococcus aureus* (MSSA)

This section describes the clinical relevance of MSSA, and the results obtained following exposure of MSSA on agar surfaces to the HINS-light EDS.

Background of MSSA

MSSA are Gram-positive cocci, about 1μm diameter and arranged in clusters. They grow well on most culture media aerobically, and less well anaerobically. Their optimum temperature for growth is 37˚C. They typically occur as round golden colonies on tryptone soya agar (TSA) or NA, and black with an opaque halo on Baird Parker agar (BPA). Identification of MSSA is by detection of Protein A as detailed in Section 3.3.[312] MSSA was isolated on 25% of routine wound surveillance swabs in the burns unit at GRI in the two years preceding this work.

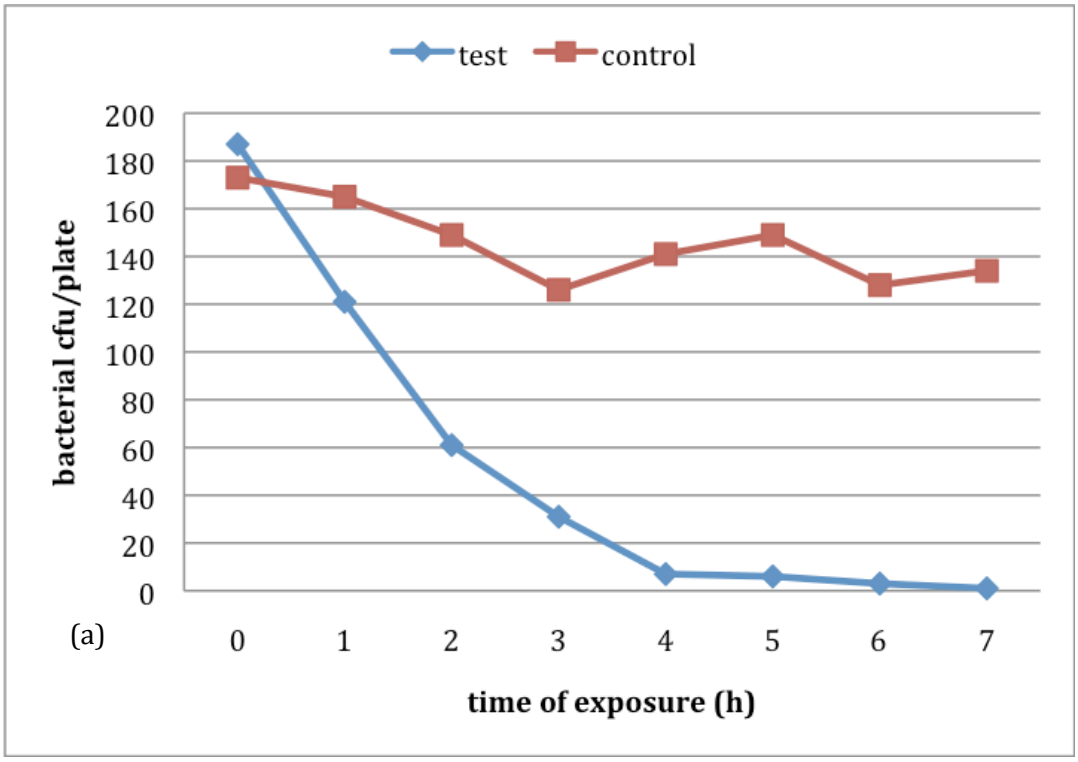
S. aureus is the main staphylococcal pathogen responsible for pyogenic infections. However it is carried in the nose in around 50-75% of healthy people, and less often in the gut, skin or throat. It is often isolated on burn wounds during routine wound surveillance swabs, where it may cause few symptoms. However it also has the potential to infect the burn wound, cause skin graft loss or lead to invasive infections, such as sepsis, endocarditis or osteomyelitis. *S. aureus* is also capable of producing virulent exotoxins, known as 'super antigens', leading to rapidly progressing toxic shock syndrome (TSS).[333] Children with small burns are particularly at risk of this devastating disease.

In the context of a clean, healthy-looking wound and a well patient, MSSA may be considered to be contaminating, rather than infecting the wound, and the patient merely observed for any deterioration. However in the presence of surrounding cellulitis, raised inflammatory markers or pyrexia, or any co-existing positive blood cultures, it will be classed as wound infection and treated, usually with flucloxacillin. Of note, over 80% of hospital strains of MSSA are now resistant to penicillin.[332]

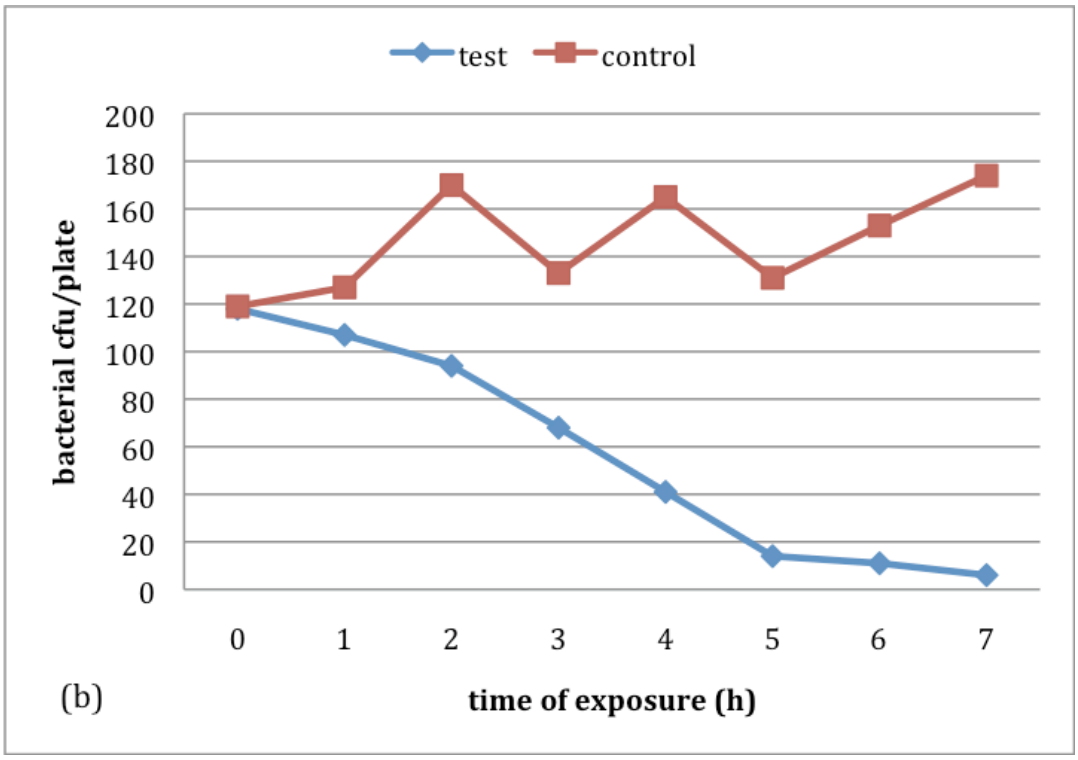
Results of HINS-light EDS exposure of MSSA on agar surfaces

Figure 4.3 displays the inactivation data for the exposures of MSSA on agar plates to the HINS-light EDS, conducted in triplicate (Figures 4.3 a, b and c). It can be seen that during all three experimental runs, the inactivation kinetics were similar, whereby the control population remained fairly static, but the test population (exposed to the HINS-light EDS) decreased. Initially, this inactivation is fairly rapid on the first 2 h, but the remainder of the population appeared to be slightly harder to inactivate, with the graphs displaying slight 'tailing' effect. Following a 7 h exposure, between 1 and 16 cfu/ plate survived. Based on raw data, one-way ANOVA demonstrated that a significant

difference between the test and control bacterial cfu count was achieved by 2 h (p=0.006).



(a)



(b)

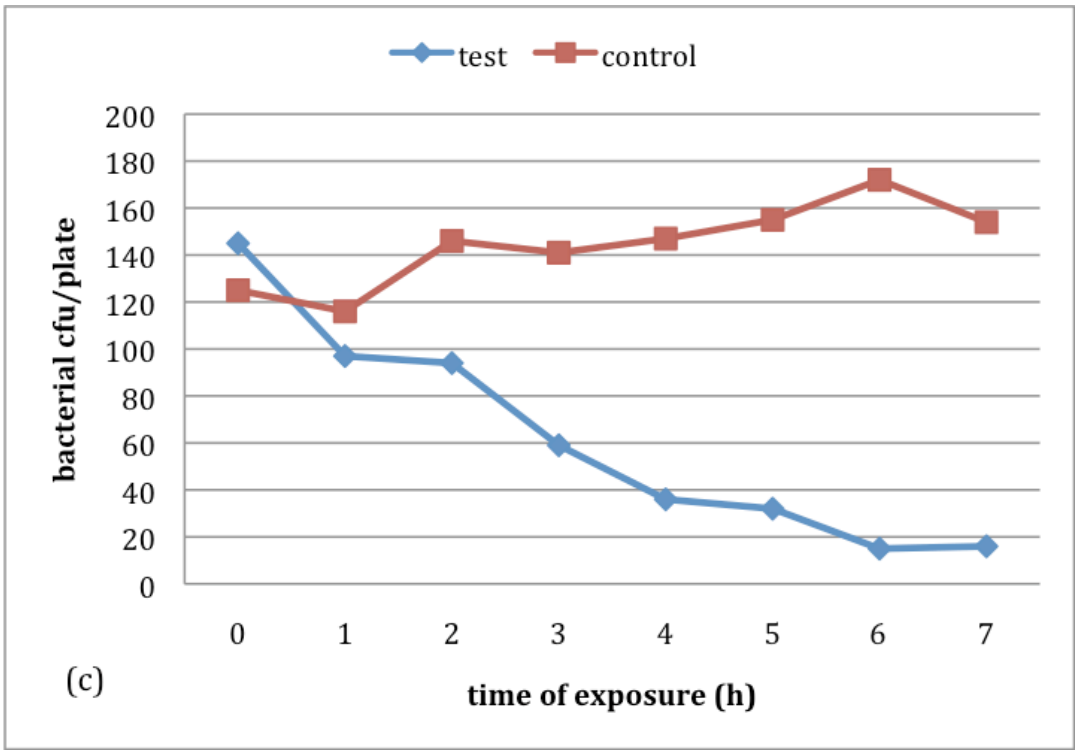


Figure 4.3: Inactivation of *MSSA seeded onto* an agar surface *by exposure to the HINS-light EDS. Results for the three experiments are shown in Figures a, b and c.*

The % surviving cfu/plate, as compared to the control counts, at each hourly interval is plotted in Figure 4.4. It can be seen that overall, an approximate 50% reduction in bacterial contamination was achieved by 2-4 hours, and 90% reduction by 6-7 hours. Based on % reductions, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu counts was also achieved at 2 h ($p=0.002$).

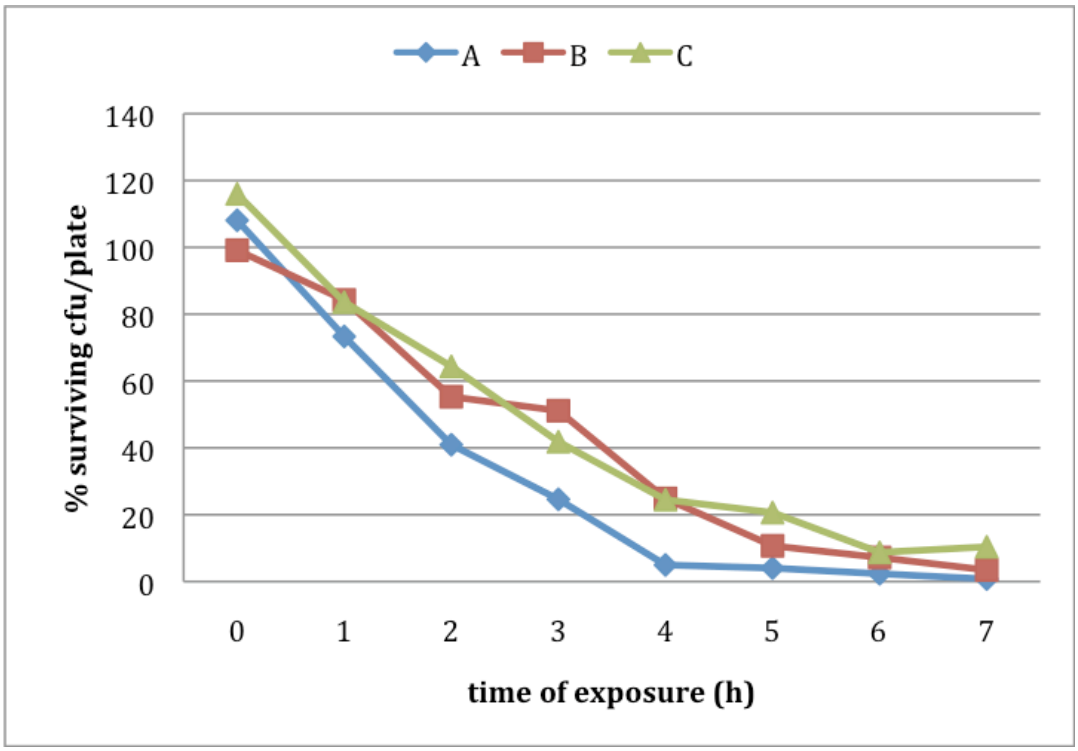


Figure 4.4: *Combined results of MSSA experiments, demonstrating the effect of HINS-light EDS exposure on MSSA seeded on agar surfaces, with test bacterial cfu counts expressed as a % of the control count at each hour.*

4.4 Methicillin resistant *Staphylococcus aureus* (MRSA)

This section describes the clinical relevance of MRSA, and the results obtained following exposure of MRSA on agar surfaces to the HINS-light EDS.

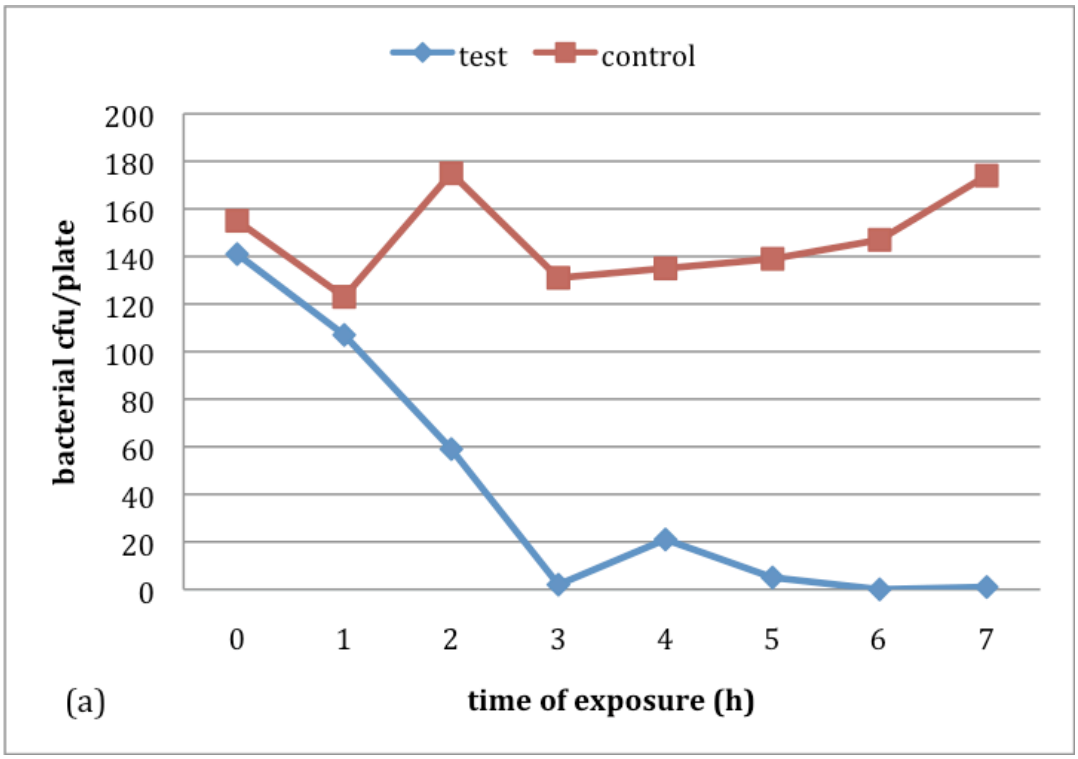
Background of MRSA

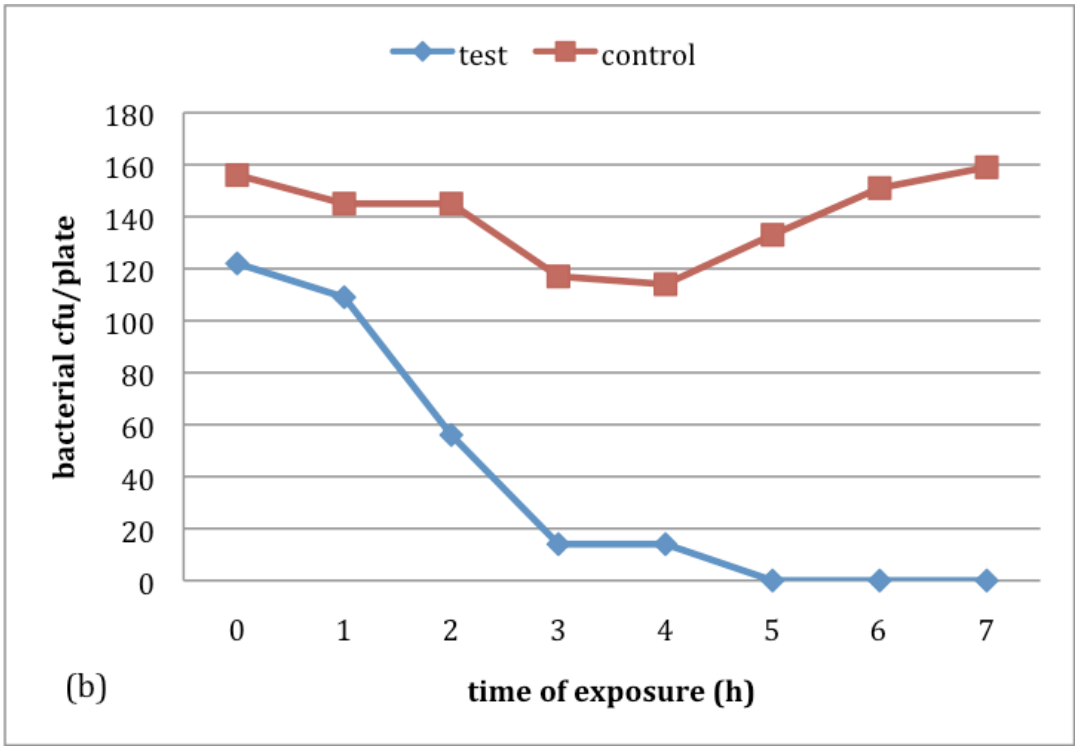
Few nosocomial bacteria have achieved the notoriety of MRSA in the past decade. MRSA includes any strain of *S. aureus* that has developed resistance to beta-lactam antibiotics, which include the penicillins (for example methicillin and oxacillin) and cephalosporins. While the development of such resistance does not increase virulence, it does make MRSA infections more difficult to treat with standard antibiotics and thus more of a threat to individual patients and hospitals. The characteristics and laboratory culture of MRSA are the same as those described for MSSA in the previous section, with an additional gene for antibiotic resistance.[333] Identification of methicillin resistance is confirmed by confirmation of the presence of PBP'2, as described in Section 3.3. MRSA was isolated on 24% of routine wound surveillance swabs in the burns unit at GRI in the two years preceding this work.

MRSA presence in the hospital environment is becoming almost endemic. Previous studies have demonstrated environmental contamination with MRSA in the rooms of 73% of patients infected with MRSA, and 69% of patients colonised with MRSA.[86] In a further study, MRSA was isolated from environmental samples at every one of 24 screenings, with no correlation between the number of infected patients and the number of positive environmental samples. The authors concluded that reservoirs of MRSA were resulting in widespread contamination of the environment even when small numbers of patients were colonised.[104] Outbreaks of MRSA on the burns unit have been repeatedly shown to be most likely when environmental contamination levels are high.[101,109,334] Furthermore, the presence of an MRSA outbreak on the burns unit leads to a significant increase in the acquisition of MRSA by patients on other wards, an effect only halted by closing the burns unit.[109,334]

Results of HINS-light EDS exposure of MRSA on agar surfaces

Figure 4.5 (a, b and c) displays the inactivation data for the exposure of MRSA on agar plates to the HINS-light EDS. As before the inactivation kinetics for all three runs were similar, with fairly static control populations, and test populations demonstrating a rapid decrease in colonies, followed by a tailing effect. Based on raw data, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu count was also achieved at 2 h exposure ($p<0.001$).


test
control
200
180
160
140
120
100
80
60
40
20
0
bacterial cfu/plate
0
1
2
3
4
5
6
7
time of exposure (h)
(a)


test
control
180
160
140
120
100
80
60
40
20
0
bacterial cfu/plate
0
1
2
3
4
5
6
7
time of exposure (h)
(b)

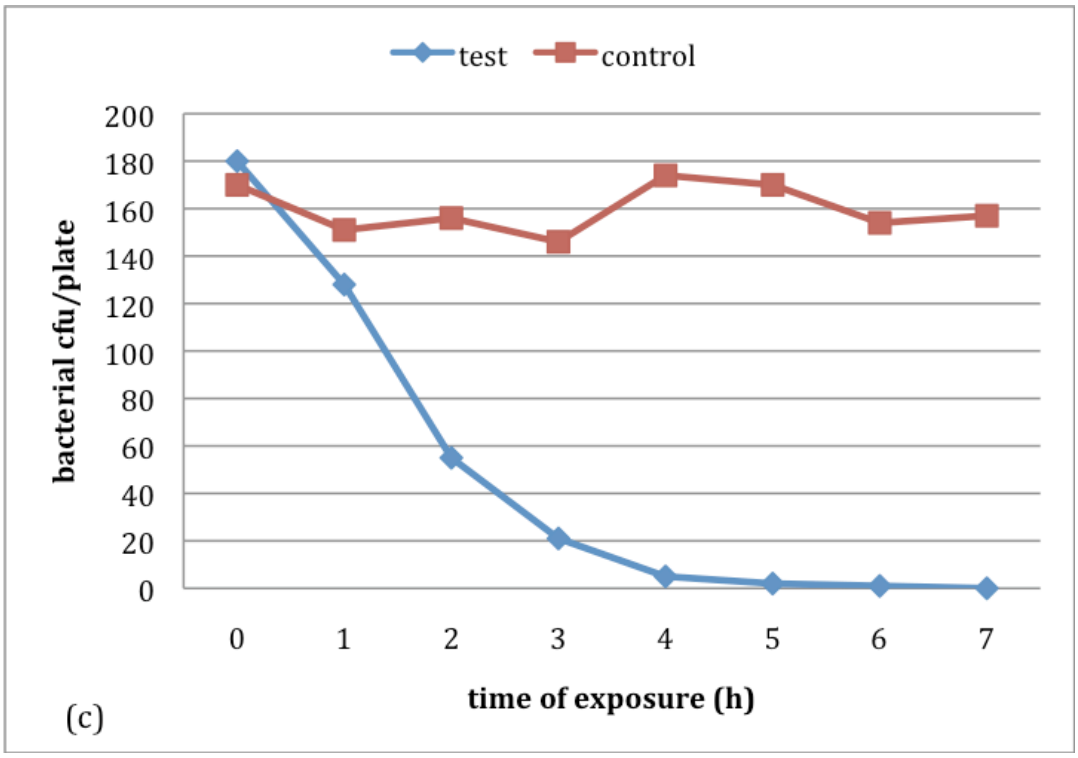


Figure 4.5: *Inactivation of MRSA seeded onto an agar surface by exposure to the HINS-light EDS. Results for the three experiments are shown in Figures a, b and c.*

The % surviving cfu/plate, as compared to the control counts, at each hourly interval is plotted in Figure 4.6. It can be seen that overall, an approximate 50% reduction in bacterial contamination was achieved by approximately 2 h, and 90% reduction by 3-5 h. Based on % reductions, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu counts was achieved at 1 h ($p=0.008$).

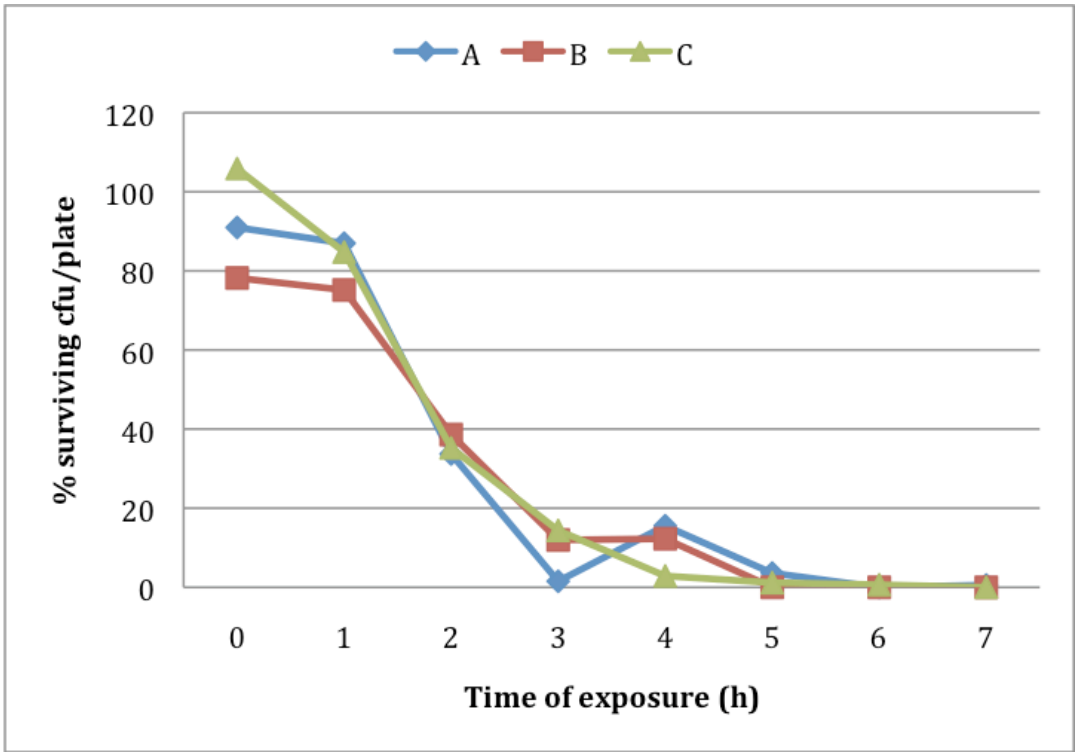


Figure 4.6: *Combined results of MRSA experiments, demonstrating the effect of HINS-light EDS exposure* on MRSA seeded on agar surfaces*, with bacterial cfu counts expressed as a % of the control count at each hour.*

4.5 *Streptococcus pyogenes*

This section describes the clinical relevance of *S. pyogenes*, and the results obtained following exposure of *S. pyogenes* on agar surfaces to the HINS-light EDS.

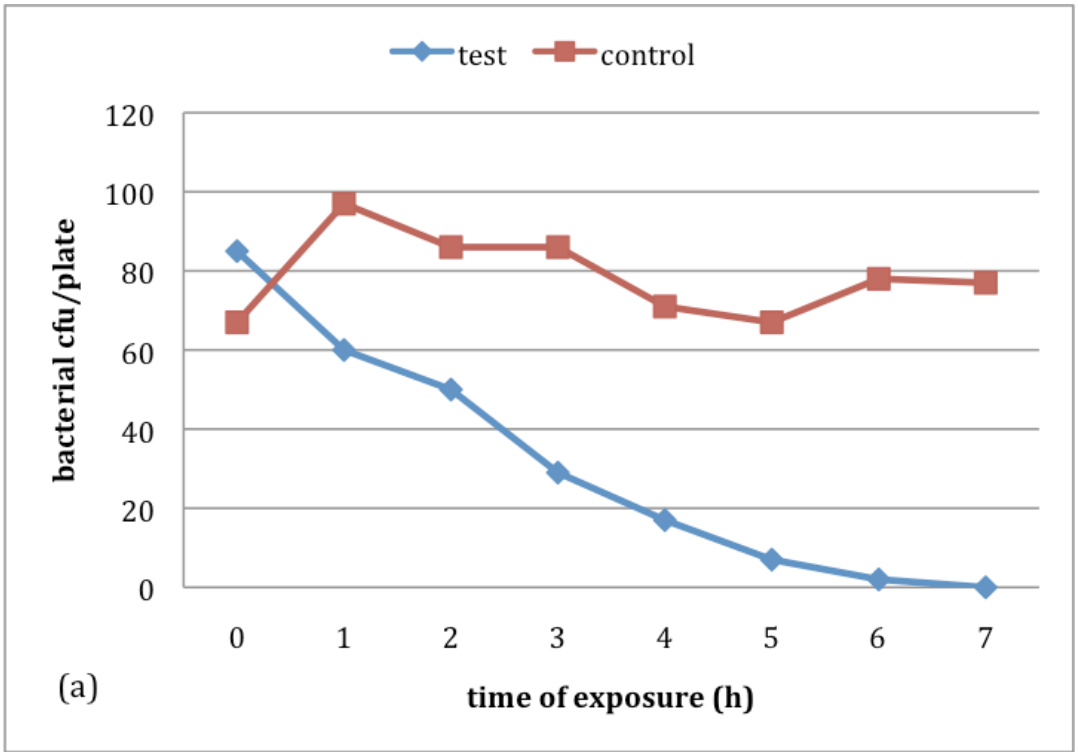
Background of *S. pyogenes*

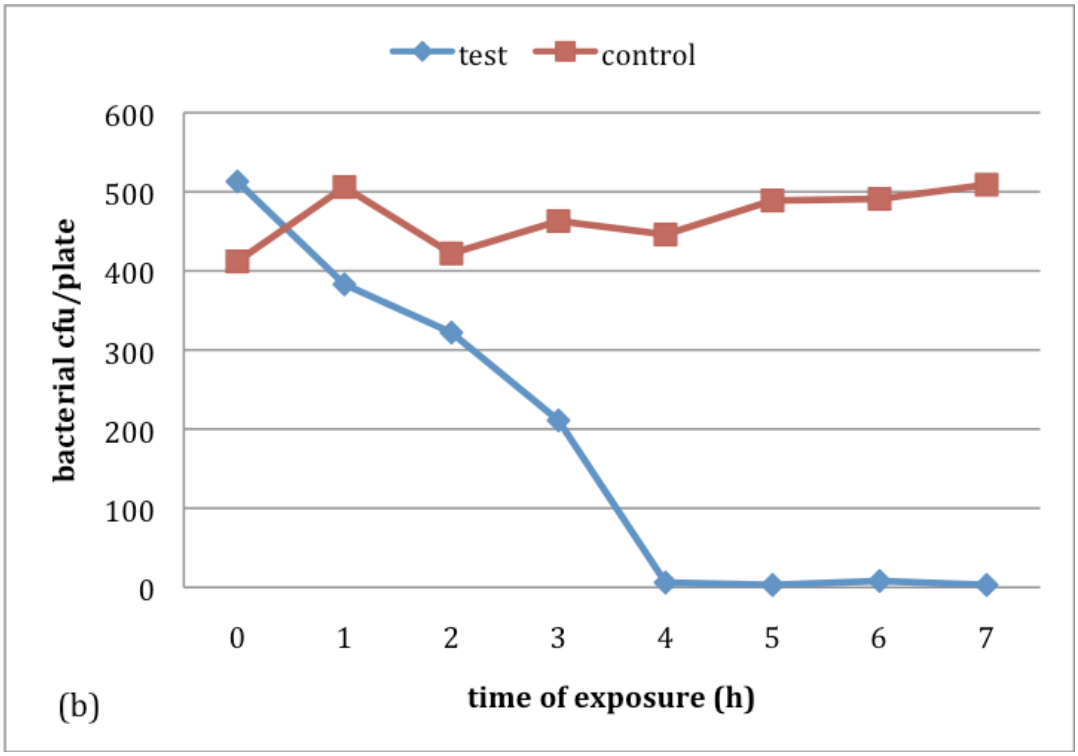
S. pyogenes are streptococci from Lancefield group A, so named due to the presence of streptococcal group A antigens on their cell walls. These Gram-positive cocci are approximately 1μm diameter and occur in pairs or chains. They grow particularly well on blood agar, at an optimum temperature of 37˚C, as small matt colonies, surrounded by zones of β-haemolysis, due to erythrocyte disruption and haemoglobin release. *S. pyogenes* is also referred to as group A β-haemolytic *Streptococcus*, or GABHS. It is the most pathogenic member of the genus, although other β-haemolytic streptococci may cause similar symptoms in burns patients.[335] *S. pyogenes* exhibits several virulence factors enabling it to evade host immune systems: a capsule composed of hyaluronic acid protects it from phagocytosis by neutrophils; M proteins embedded in the cell wall inhibit opsonisation by blocking the binding of complement and binding to host fibrinogen; exotoxins produced including proteases and hydaluronidase, which alongside streptokinase allow the organism to spread rapidly through tissues and prevent the adhesion of skin grafts.[332] *S. pyogenes* is often present as a nasal commensal, particularly in children, who probably have a baseline rate of carriage of approximately 5%.[336] The commonest *S. pyogenes* infection is pharyngitis, followed by cellulitis (a superficial spreading infection of the dermis) and erysipelas (a severe cellulitis of the most superficial dermal layer). It can also cause impetigo, necrotising fasciitis (a rapidly progressive subcutaneous tissue infection), surgical site infections, and sepsis.[337]

In the pre-antibiotic era, S*. pyogenes* was one of the most serious infections to burn patients, thought to be responsible for approximately 50% of burn-related deaths before World War II.[337] Although the discovery of penicillin reduced the mortality from streptococcal infection, it remains a significant threat. *S. pyogenes* is destructive to healing epithelium and new skin grafts, thus its presence in wounds is often a contra-indication to skin grafting.[335] Infection with the organism increases the depth of the burn wound and destroys skin grafts, prolonging healing and producing poor scarring.[338,339] Streptococci are also capable of causing exotoxin mediated TSS, the commonest cause of unexpected mortality in children with small burns.[335] The threat of *S. pyogenes* has been pre-empted in the past by the prophylactic administration of penicillin in the first five days, but this has been discontinued as it was shown to not be effective in further reducing the low baseline incidence of infection in the current era of early excision and grafting.[337,338]

Results of HINS-light EDS exposure of *S. pyogenes* on agar surfaces

Figure 4.7 (a, b and c) displays the inactivation data for the exposure of *S. pyogenes* on agar plates to the HINS-light EDS. *S. pyogenes* inactivation followed a similar pattern during each of the three exposure experiments. Reasonably rapid inactivation occurred in the first few hours, but the remainder of the population took up several hours more, yet again producing a 'tailing' effect. The non-exposed control bacteria again remained fairly constant throughout the experiment. *S. pyogenes* proved difficult to culture consistently, therefore mean starting populations for these experiments varied considerably: Experiment A had a starting population of 75 cfu/plate, and Experiments B and C (which were carried out on the same day) were 463 and 551 cfu/plate, respectively. Due to the huge difference in starting population, Experiment A was omitted and ANOVA was performed on Experiments B and C only. This showed significant kill was achieved at 1 h (p=0.038)





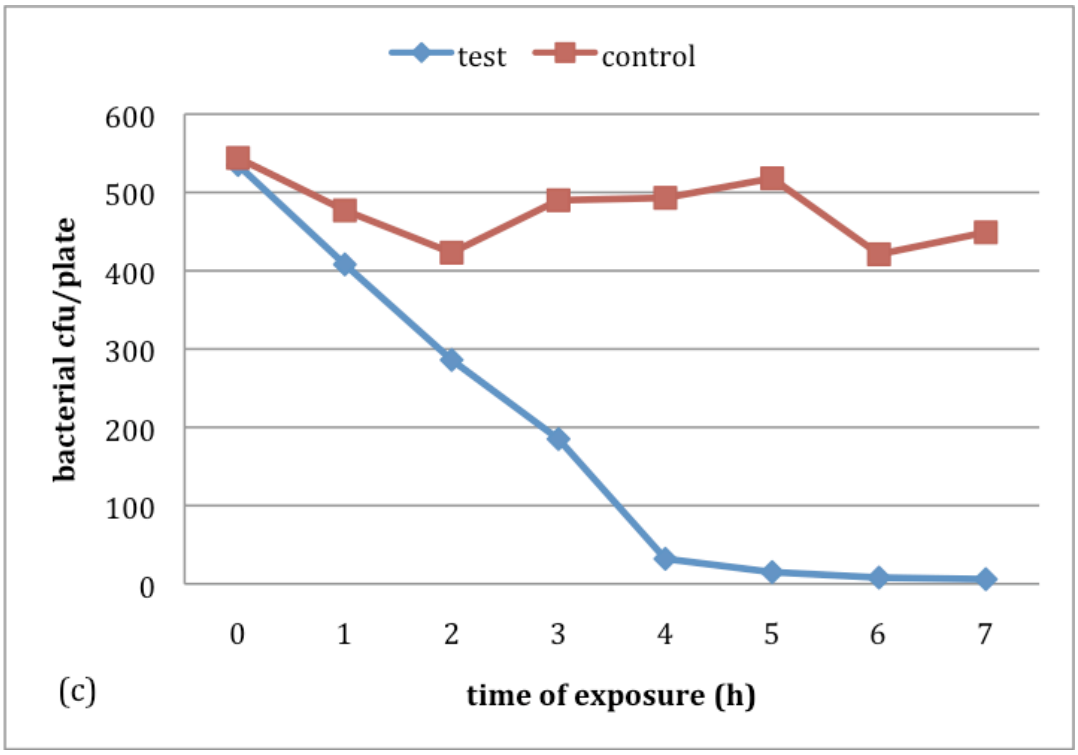


Figure 4.7: *Inactivation of S. pyogenes seeded onto an agar surface by exposure to the HINS-light EDS. The results for the three experiments are shown in Figures a, b and c.*

The % surviving cfu/plate, as compared to the control counts, at each hourly interval is plotted in Figure 4.8. This demonstrates an approximate 50% reduction in bacterial contamination was achieved by 3 h, and 90% reduction by 4-6 h. Based on % reductions of all three experiments, one-way ANOVA demonstrated a significant difference between the test and control bacterial cfu counts was achieved at 1 h (p=0.020).

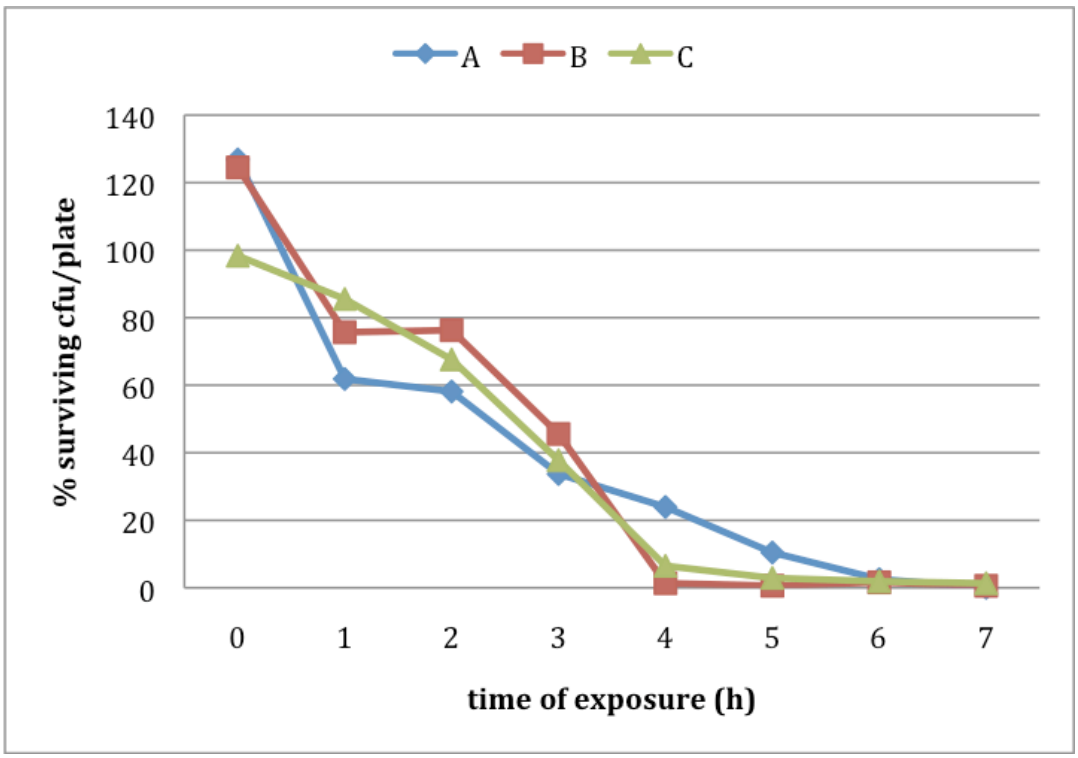


Figure 4.8: *Combined results of S. pyogenes experiments, demonstrating the effect of HINS-light EDS exposure* on *S. pyogenes seeded on agar surfaces, with bacterial cfu counts expressed as a % of the control count at each hour.*

4.6 Multi-drug resistant *Acinetobacter baumannii*

This section describes the clinical relevance of and the results obtained following exposure of MDR-*A. baumannii* on agar surfaces to the HINS-light EDS.

Background of MDR-*A. baumannii*

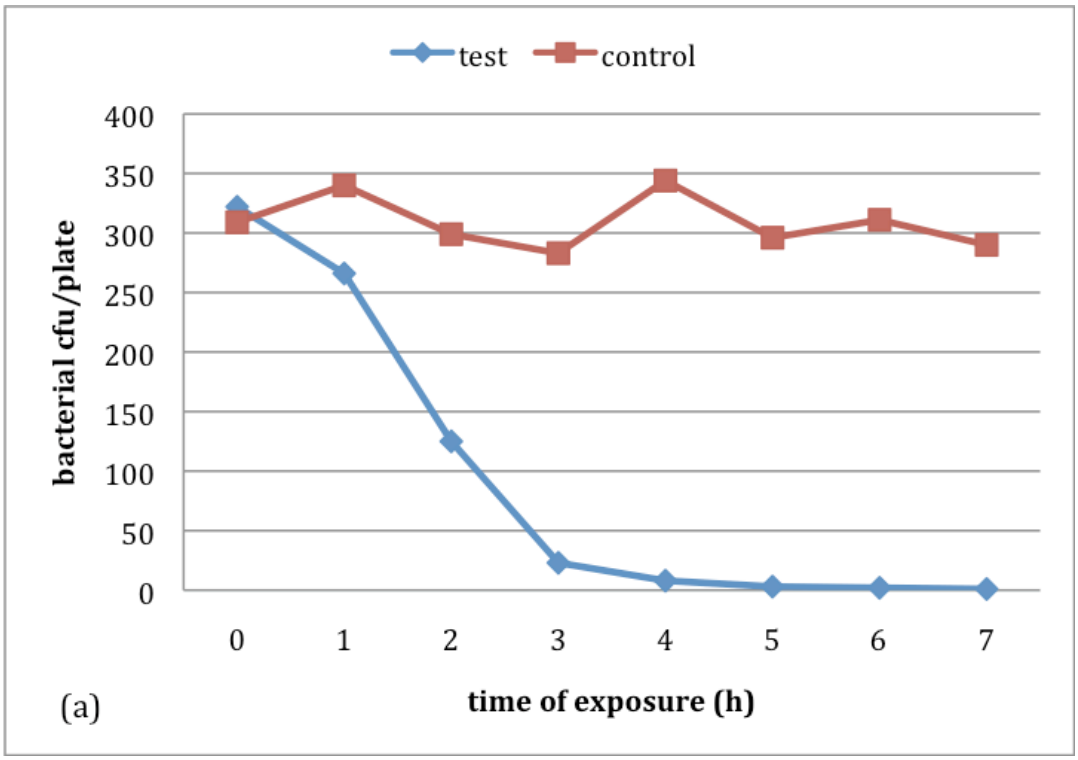
A. baumannii (formally known as *Acinetobacter calcoaceticus* var. *anitratus*) is a Gram-negative bacteria that is emerging as an increasing problem on burn units worldwide.[340] It is part of the normal flora of human skin, gastrointestinal and upper respiratory tracts and is found in soil and water, but can form opportunistic infections.[341] During growth, *A. baumannii* is rod shaped and up to 2.5µm long, and found in pairs or groups. Although it has no flagellum, it exhibits 'gliding' motility.[341] Its ability to form biofilms and survive on fomites and epithelial surfaces makes it difficult to eradicate. Species of the genus *Acinetobacter* are strictly aerobic, non-fermentive and oxidase-negative. *A. baumannii* develops antimicrobial resistance very quickly, leading to nosocomial outbreaks especially in intensive care units and burns units. The multi-drug resistant strains show resistance to many antibiotics including beta-lactams, cephalosporins, aminoglycosides and carbapenems. For this reason MDR-*A. baumannii* is becoming of increasing concern on burn units worldwide.[117,341,343] Although only three patients in the burns unit at GRI have isolated *A. baumannii* in the last five years, it is becoming increasingly prevalent in burns units worldwide. In one unit in Singapore, 76% of major burns patients were colonised or infected with *A. baumannii*, with 43% being infected by multi-drug resistant strains.[341] A further study from the USA, reported a 16% incidence in all patients admitted to the burn unit.[343] Other reported prevalence rates vary from 1 to 62%.[344-346] A recent increase in the incidence of infections caused by the organism has been noted by the US military health-care system, particularly in soldiers returning from Iraq or Afghanistan.[347] Risk factors for the acquisition of *A. baumannii* have been suggested to include high % TBSA; the presence of full thickness burns; a high number of intravascular lines; a high number of operations; the use of artificial ventilation; a high APACHE II score (Acute Physiology and Chronic Health Evaluation II; ICU severity of disease classification score); and prior use of antibiotics.[347]

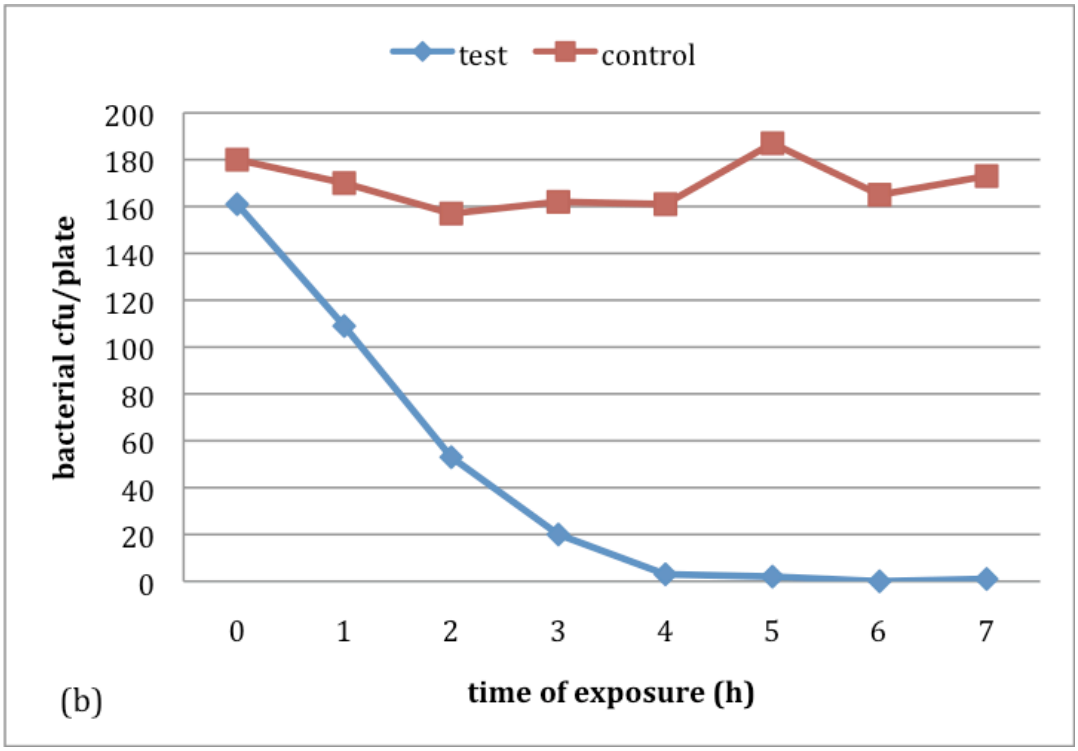
A. baumannii infections are associated with a high mortality in burn patients, although the pathogen was shown in a retrospective cohort study to not independently affect mortality. Rather, patients with *A. baumannii* infection had more severe burns and co-morbidities, and longer length of stays than those without infection or with colonisation alone.[347] However, it has been estimated following a case-control study of burns patients that infection with MDR-*A. baumannii* is associated with a significant increase in cost. Mean total hospital cost for 34 patients was $ 201 558 for those patients with MDR-*A. baumannii* compared to a total of $ 102 983 for controls matched for age, sex,

extent of burn injury and Zawacki trauma score ($p<0.01$). The mean length of stay was 37 days for the cases and 26 days for the controls ($p=0.06$) .[343]

Results of HINS-light EDS exposure of MDR-*A. baumannii* on agar surfaces

Figure 4.9 (a, b and c) displays the inactivation data for the exposure of MDR-*A. baumannii* on agar plates to the HINS-light EDS. Moderate variation was seen in the starting populations of MDR-*A. baumannii* of between 161 and 322 cfu/plate. However, MDR-*A. baumannii* inactivation kinetics were similar for each of the three exposure experiments. Initial inactivation was fairly rapid, within the first 2 h exposure. The remainder of the population displayed a 'tailing' effect over the next 5 h, although only one or two cfu remained at 6 or 7 h. The non-exposed control bacteria again remained fairly constant throughout. Based on raw data, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu count was achieved at 3 h ($p=0.007$).





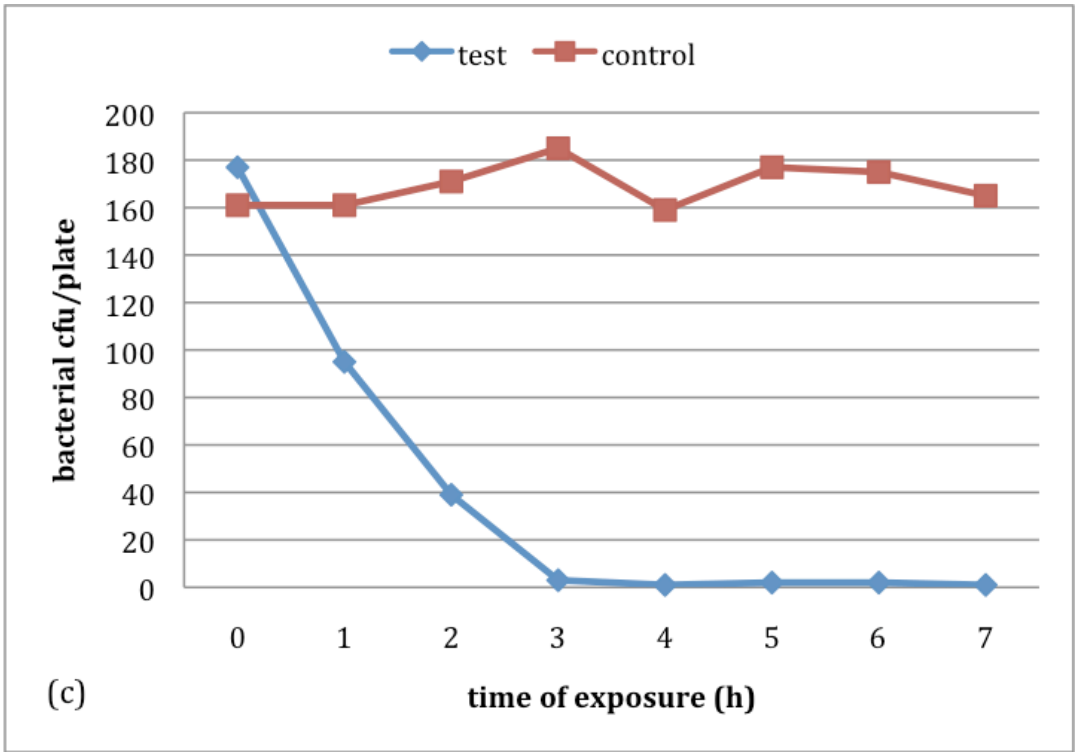


Figure 4.9: *Inactivation of MDR-A. baumannii seeded onto an agar surface by exposure to the HINS-light EDS. The results for the three experiments are shown in Figures a, b and c.*

The % surviving cfu/plate, as compared to the control counts, at each hourly interval is plotted in Figure 4.10. This demonstrates an approximate 50% reduction in bacterial contamination was achieved by 2 h, and 90% reduction by 3-4 h. Based on % reductions, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu counts was achieved at 1 h (p=0.005).

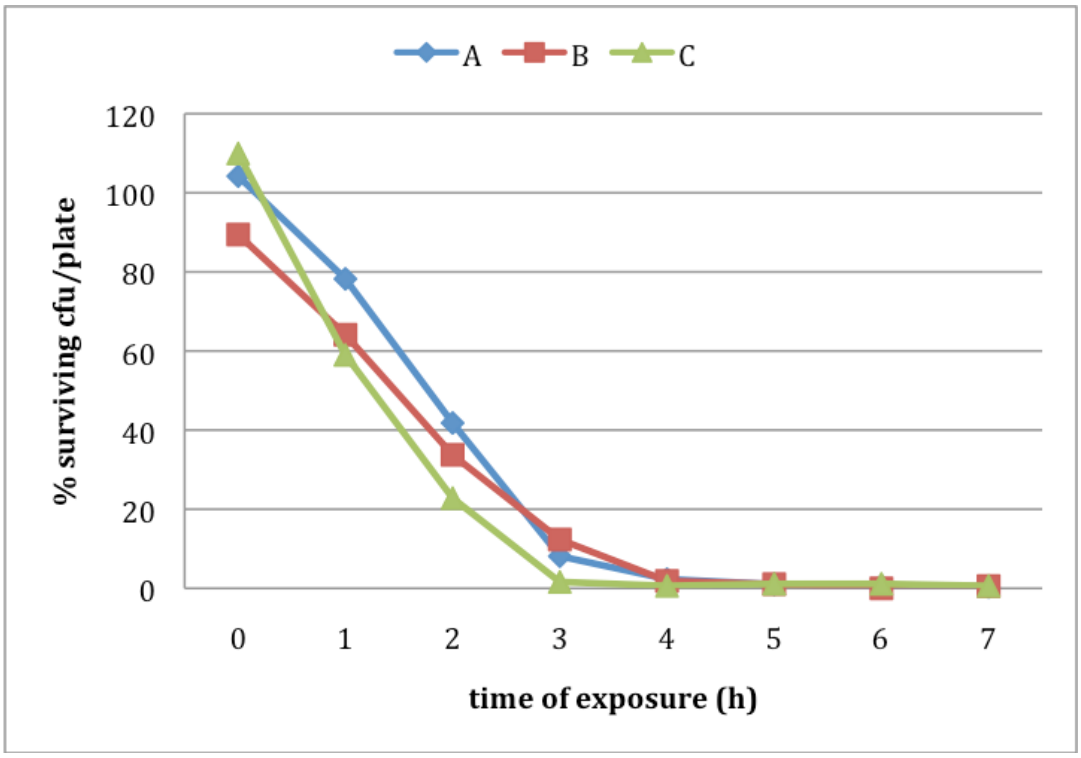


Figure 4.10: *Combined results of MDR-A. baumannii experiments, demonstrating the effect of HINS-light EDS exposure on MDR-A. baumanni seeded on agar surfaces, with bacterial cfu counts expressed as a % of the control count at each hour.*

4.7 *Pseudomonas aeruginosa*

This section describes the clinical relevance of *P. aeruginosa*, and the results obtained following exposure of *P. aeruginosa* on agar surfaces to the HINS-light EDS.

Background to *P. aeruginosa*

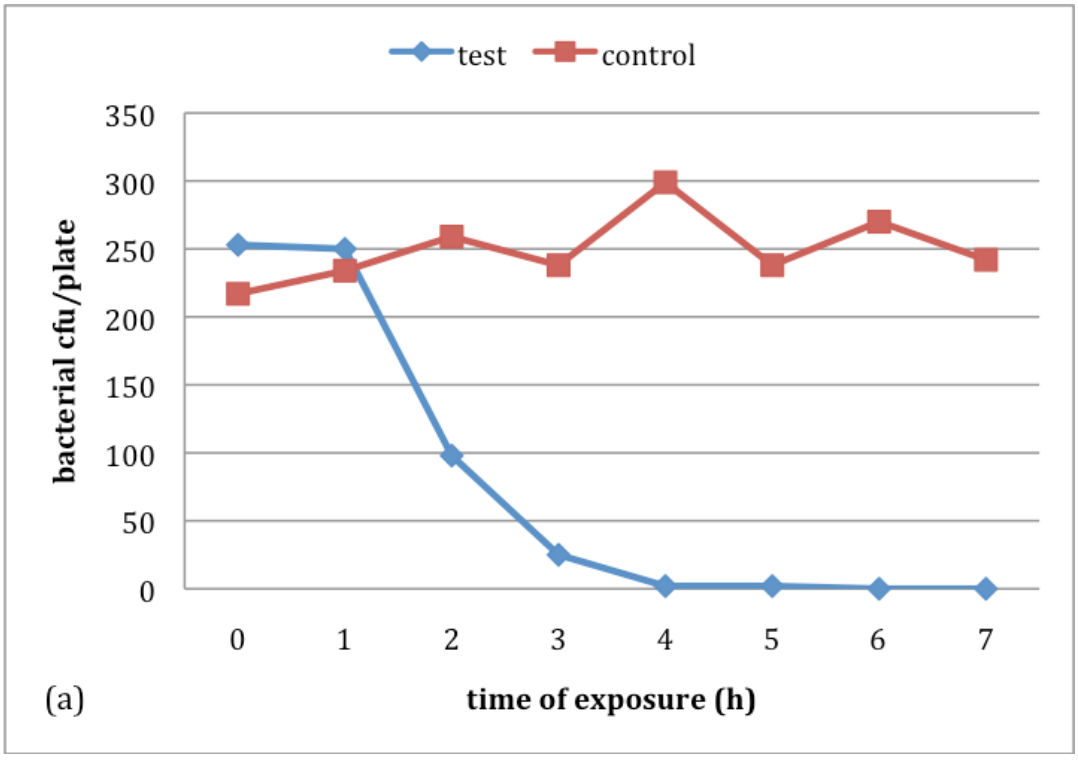
P. aeruginosa is a common Gram-negative rod-shaped bacterium, which readily grows on routine media over a wide range of temperatures, and produces positive catalase and oxidase tests. It can be between 1-5μm long and forms large irregular colonies with a greenish appearance due to its ability to secrete a variety of pigments including pyocyanin and fluirescein.[348] It is often identified by a pearlescent appearance and 'flower-water' smell. The *Pseudomonas* organism possesses flagellum rendering it motile. Although classified as aerobic, it may adapt to propagate in anaerobic conditions. It is able to utilise a variety of organic matter for food. It remains an important nosocomial pathogen and a significant cause of infection and mortality in burns patients, as well as cycstic fibrosis sufferers, with a majority of isolates displaying intrinsic plasma-mediated resistance against antimicrobial agents.[349,350] Imipenem is one of the most effective drugs against *P. aeruginosa*, but resistance to imipenum is being increasingly reported.[349]In a case-control investigation examining burns patients with aminoglycoside-resistant *Pseudomonas* sp., a significant increase in mortality and morbidity in terms of length of stay, ventilator days, number of surgical procedures and the amount of blood products required were all demonstrated in the case group. Costs associated with antibiotic use were also significantly higher.[350] In one survey of 176 burn centres in North America, *Pseudomonas* sp. was considered the most serious cause of life-threatening infections in burn patients.[351] Furthermore, a 25 year review undertaken in 1985 documented an overall mortality of 77% in burn patients with *Pseudomonas* sp. bacteraemia, 28% above predicted.[352] Further studies in 2007 demonstrated a significantly higher mortality rate in burns patients with gentamicin-resistant *P. aeruginosa* (33% vs. 8%) when matched with controls for age, TBSA burn, admission year and the presence of inhalation injury.[114]

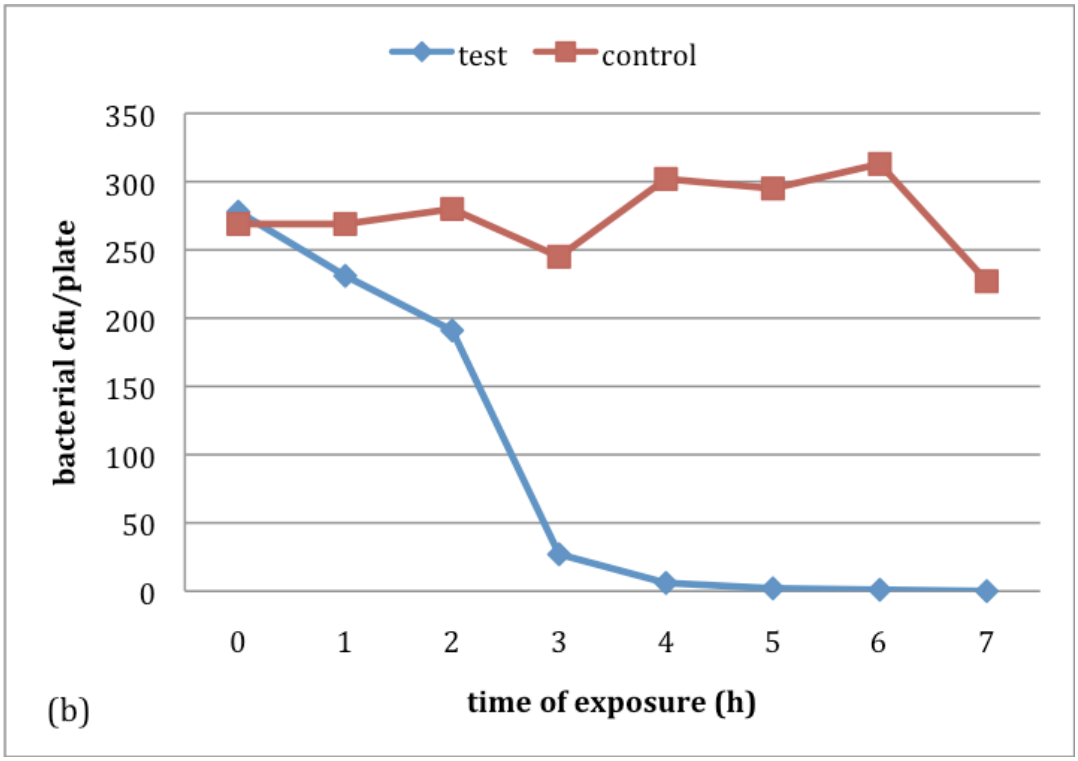
The ability of *P. aeruginosa* to form biofilms is critical for its survival in humans and the environment, and enhances its resistance to antimicrobials and host defence mechanisms.[114] *P. aeruginosa* biofilms are common on medical devices such as catheters and communal hydrotherapy equipment. The risks of cross-contamination of biofilm-forming Gram-negative organisms such as *P. aeruginosa* have led to new designs for sinks and a reduction in the use of common treatment rooms for burns patients.[1,350,354] At least three exopolysaccharides (alginate, Psl and Pel) have been shown to contribute to the formation of biofilms by this organism. Furthermore, alginate production has been shown to decrease the susceptibility of biofilms to antibiotic treatment and human

immune defence systems.[353] In burns patients, the clinical features of infection with *P. aeruginosa* range from low-grade skin infection; through to graft loss; widespread infection of skin donor site wounds; and severe sepsis. Clinically, a green slime on the wound and dressings, and musty-sweet smelling, wound, with the skin graft "floating" and non-adherent, is typical of *Pseudomonas* infection. Treatment is usually topical, with judicious wound cleaning, and the use of silver sulfadiazine or mafenide acetate.[350] *P. aeruginosa* was isolated on 6% of routine wound swabs in the burn unit at GRI in the two years preceding these studies.

Results of HINS-light EDS exposure of *P. aeruginosa* on agar surfaces

Figure 4.11 (a, b and c) displays the inactivation data for the exposure of *P. aeruginosa* on agar plates to the HINS-light EDS. It can be seen that the inactivation kinetics for *P. aeruginosa* were similar for each of the three experiments. Initial inactivation was fairly rapid, with the majority of the bacterial population inactivated by 2-3 h exposure. The remainder of the population took between 5 and 7 h to kill, producing a 'tailing' effect. The non-exposed control bacteria were fairly constant throughout. Based on raw data, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu count was achieved at 3 h (p=0.005).





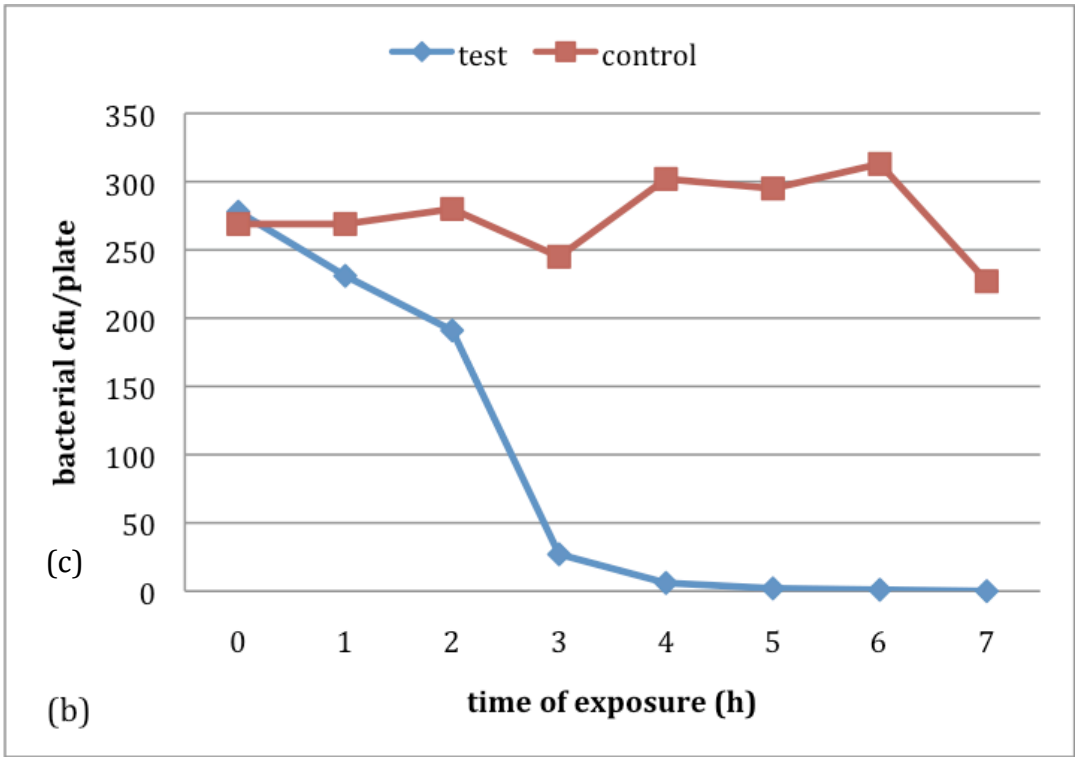


Figure 4.11: *Inactivation of P. aeruginosa seeded onto an agar surface by exposure to the HINS-light EDS. The results for the three experiments are shown in Figures a, b and c.*

The % surviving cfu/plate, as compared to the control counts, at each hourly interval is plotted in Figure 4.12. An approximate 50% reduction in bacterial contamination was achieved by between 2 - 3 h, and 90% reduction by 4 h. Based on % reductions, one-way ANOVA demonstrated that a significant difference between the test and control bacterial cfu counts was achieved at 2 h (p=0.010).

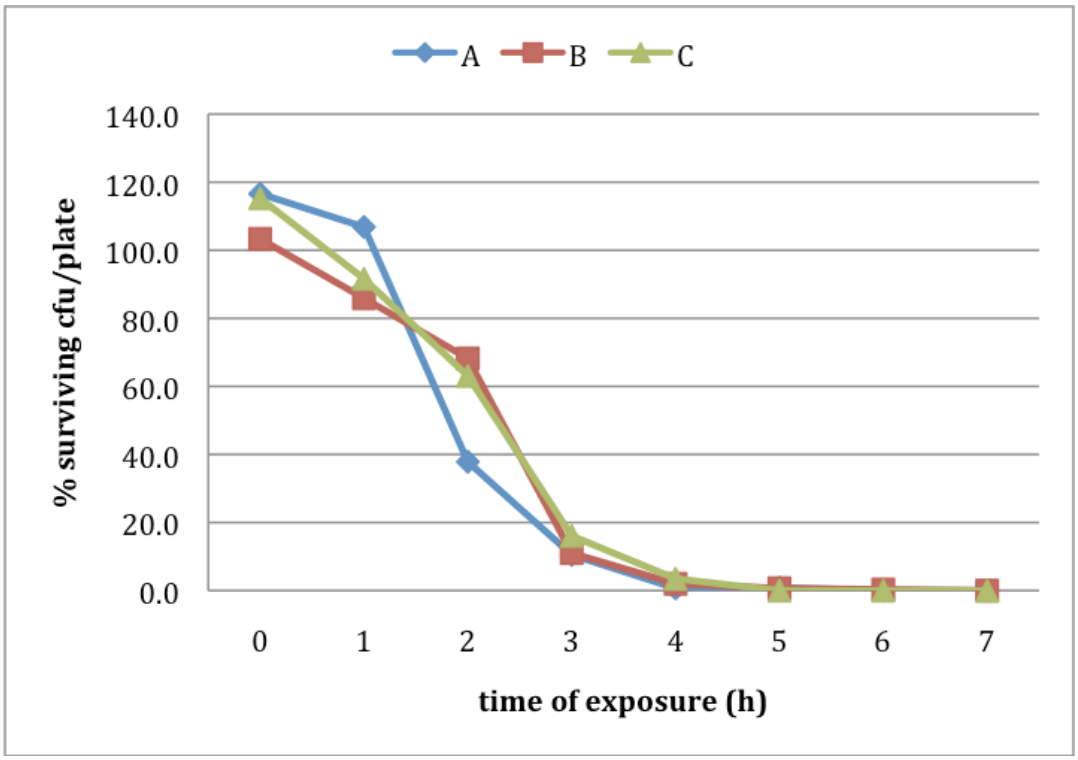


Figure 4.12: *Combined results of P. aeruginosa experiments, demonstrating the effect of HINS-light EDS exposure on P. aeruginosa seeded on agar surfaces, with bacterial cfu counts expressed as a % of the control count at each hour.*

4.8 Summary of all five species

Results obtained following exposure of all five clinical isolates to the HINS-light EDS whilst seeded on agar surfaces are summarised in the table below.

Table 4.2: *Summary of the time to achieve significant kill using the HINS-light EDS, based on raw data and % reductions.*

Species	Time to significant kill based on raw data (h)	p-value	Time to significant kill based on % reductions (h)	p-value
MSSA	2	0.006	2	0.002
MRSA	2	<0.001	1	0.008
S. pyogenes	1	0.038	1	0.020
MDR-*A. baumannii*	3	0.007	1	0.005
P. aeruginosa	3	0.005	2	0.010

The mean inactivation curves for each clinical isolate are shown in Figure 4.13, with results represented as the % surviving cfu/plate as compared to the control samples. It can be seen that the curves produced by all five organisms tested are remarkably similar, with an initial rapid decrease, halving the number of colonies by between 2 h and 3 h. This is followed by a gradual tailing effect, with two or three colonies remaining for up to 7 h. The results of this graph seem to suggest that *A. baumannii* is the most susceptible organism, displaying more than 30% reduction after just one hour. Conversely, the only other Gram-negative organism tested, *P. aeruginosa*, was reduced by just 5% after the first hour. The organism that appeared to be least susceptible was MSSA. This required 6-7 h to achieve a 90% reduction in bacterial colony count. These results must be interpreted with caution, however, due to the relatively small starting populations used. This will be further explored in the discussion. When these variations are taken into account, the similarities between the different species are even more striking, with similar kinetics shown throughout. In short, all organisms were significantly reduced by between 1 and 3 h, based on raw data, or 1 h to 2 h if % reductions are considered (which will reduce the impact of variation in starting populations). A 50% kill was observed at between 2 h and 4 h, and 90% kill observed at between 3 h and 7 h.

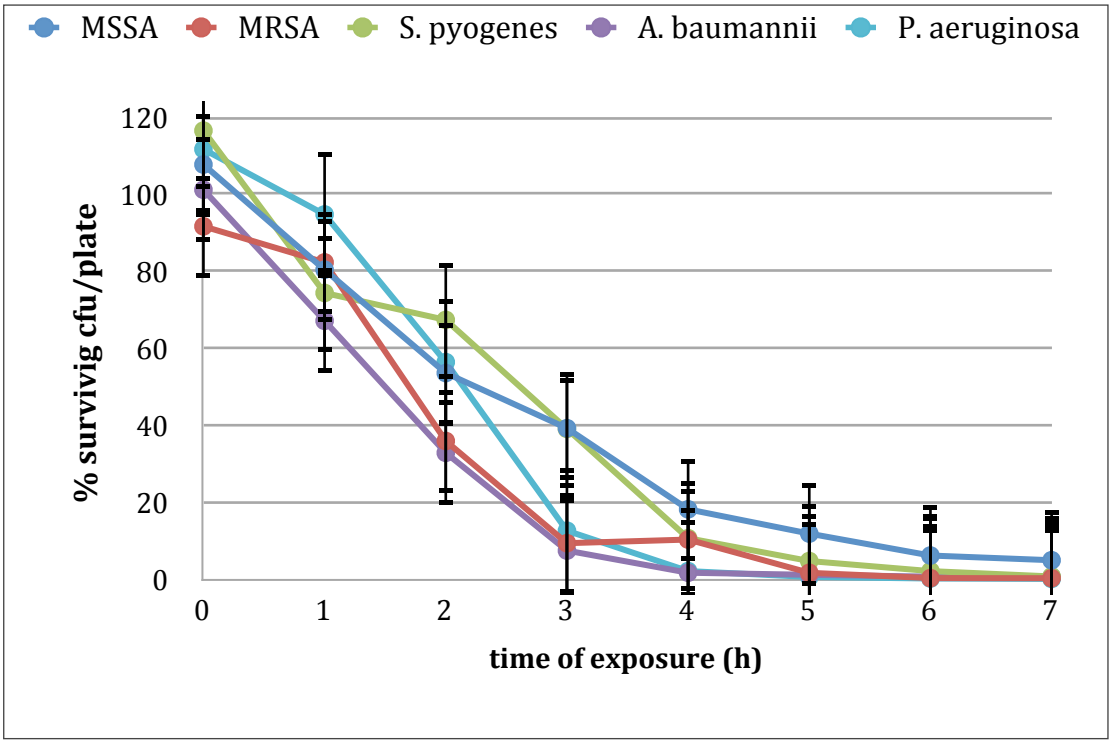


Figure 4.13: *Mean results of the inactivation of five clinical isolates seeded on agar surfaces using the HINS-light EDS. Results are expressed as the % surviving cfu/plate as compared to the control count at each hour, with standard error bars.*

4.9 Inactivation of *Pseudomonas stutzeri* in liquid suspension using a high-intensity single HINS-light LED array

This section describes methods used for the preparation of a liquid suspension of clinically isolated *P. stutzeri*, and the results obtained following exposure to a 405 nm LED array, in a similar manner to that described by Maclean et al.[2] This experiment was carried out to ascertain if *P. stutzeri* could be successfully inactivated by HINS-light exposure. In contrast to the previously described experiments using the HINS-light EDS, this experiment investigated the inactivation of high-density liquid suspensions of *P. stutzeri* using a single LED array at much higher irradiances. The aim was to determine if high population densities of the organism could be successfully inactivated. Results could then be compared to inactivation rates of other organisms that had been exposed to a similar system, and also compared to the inactivation rates of the surface seeded bacteria by the HINS-light EDS (as in the previous section).

Background to P. stutzeri

P. stutzeri is a Gram-negative rod-shaped flagellated bacterium. It generally lives in the soil, and although opportunistic pathogenicity is possible, it is rare. *P. stutzeri* was chosen to recreate the experiments of Maclean et al as it was an organism that had not previously been assessed for its susceptibility to 405 nm HINS-light.[2] *P. stutzeri* was isolated from an environmental sample taken using TSA contact plates during an outpatient clinic study (Chapter 6). The organism was identified using the tests described in Section 3.3. Briefly this comprised of: appearance on TSA (pale orange filamentous umbrate colonies); Gram-stain and microscopy (Gram-negative bacilli); oxidase test (positive); and API 20 NE (96% likely to be *P. stutzeri*).

Methods of exposure of *P. stutzeri* in liquid suspension to the HINS-light LED array

Suspensions of *P. stutzeri* were prepared using the methods described in Section 3.3. In summary, *P. stutzeri* was cultured in 100 ml TSB at 37°C for 18 h under rotary conditions. The broth was then centrifuged and the resultant cell pellet was re-suspended in 100 ml PBS. A 10^{-5} dilution of this suspension was then prepared: this provided a population density of 10^4 cfu/ml. For light exposure, 3 ml volumes of bacterial suspension were pipetted into the central well of a 12-well multi-dish (Nunc, Denmark). The 405 nm LED array (as described in Section 3.5) was mounted in a PVC housing designed to hold the LED array in position directly above the sample-containing central well of the multi-dish. Using this arrangement, the LED array was at a distance of 2cm from the bacterial sample. The input current and voltage used to power the LED array were 0.8 A and 12.55 V, respectively. These settings provided an output irradiance of approximately 50 mW/cm^2 at 2 cm from the LED array, measured using a

radiant power meter and photodiode detector (Oriel Instruments, Stratford CT, USA). The experimental arrangement is shown in Figures 4.14 and 4.15.

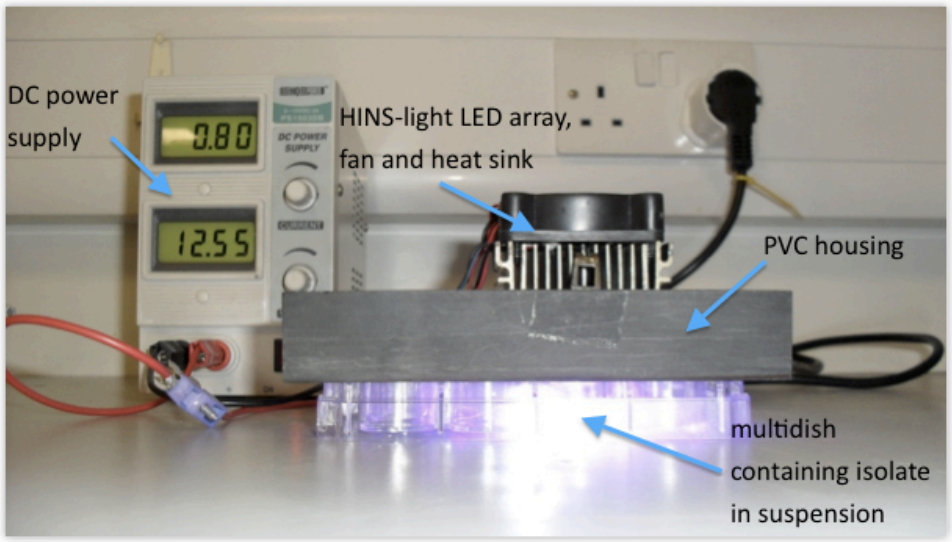


Figure 4.14: *Experimental arrangement for exposure of liquid suspensions of P. stutzeri to a single 405 nm HINS-light LED array.*

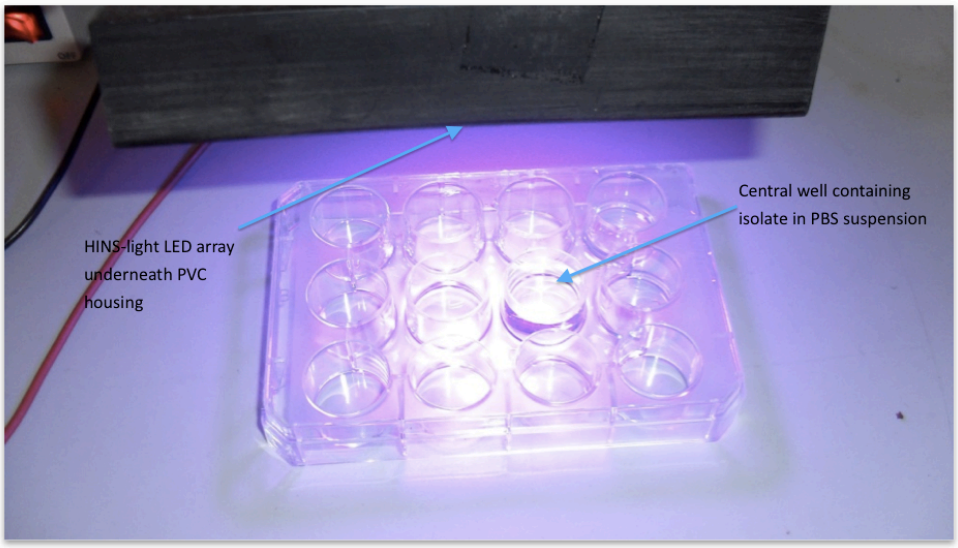


Figure 4.15: *View of multidish, with PBS suspension of P. stutzeri in one of the central wells, underneath the HINS-light LED array.*

Once the bacterial sample was in the well, and the LED array had been positioned above the well, bacterial samples were then exposed to increasing durations of 405 nm HINS-light treatments. The exposure times used in this experiment were 0, 20, 40, 60 and 80 min. Control samples were also set-up for each exposure period: these were samples prepared in the same way and remained in the same room, but were not exposed to HINS-light.

To determine the bactericidal effect of 405 nm HINS-light on the P. stutzeri suspension, the bacterial population was monitored before and after the light exposures:

- To determine the experimental starting population (0 min samples), duplicate samples were taken from each newly prepared suspension, and plated onto 90mm TSA agar plates;
- Following exposure to the HINS-light LED array, an appropriate volume of the suspension was plated onto 90mm TSA plates. This provided a count of the bacterial population surviving exposure to the HINS-light LED array;
- Samples were also taken from the non-exposed control suspensions and plated onto 90 mm TSA plates. This provided a count of the bacterial population surviving after no HINS-light exposure.

Duplicate test and control samples were plated for each exposure time, and each exposure time was repeated twice. The plating methods used were described in Section 3.4. Following overnight incubation at 37°C, the plates were enumerated, using appropriate counting methods as detailed in Section 3.4.

Results of exposure of *P. stutzeri* in liquid suspension to the HINS-light LED array

The results of exposure of liquid suspensions of *P. stutzeri* to the HINS-light LED array are summarised in Table 4.3. Total inactivation (~4-$\log_{10}$ cfu/ml reduction) of the bacteria took place after an 80 min exposure period.

These results are represented graphically as $\log_{10}$-transformed data in Figure 4.16. The results demonstrated no inactivation was achieved in the first 20 min, following which time, linear kill was shown. A 3.2 $\log_{10}$ reduction was shown by one hour, and after 80 min exposure time, all bacteria were killed.

Table 4.3: *Exposure of P. stutzeri in suspension to the HINS-light LED array, expressed as mean raw data.*

Exposure time (min)	Dose (J/cm^2)	Mean control population (bacterial cfu/ml)	Mean test population (bacterial cfu/ml)
0	0	2.76×10^4	2.76×10^4
20	60	2.53×10^4	2.53×10^4
40	120	2.50×10^4	3.10×10^2
60	180	3.23×10^4	5.95×10^1
80	240	1.70×10^4	0.0

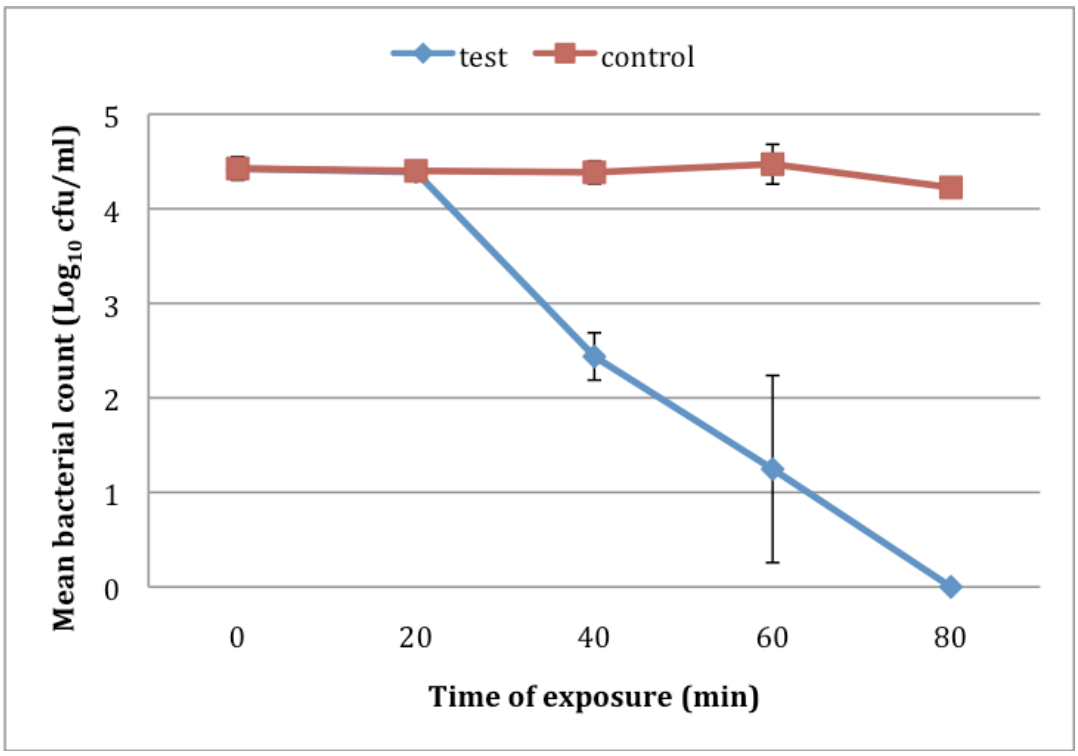


Figure 4.16: *Exposure of P. stutzeri in suspension to the HINS-light LED array, expressed as log_{10}-transformed data, with standard deviations indicated as bars.*

Based on log_{10}-transformed data, one-way ANOVA demonstrated that there was a significant difference achieved between the test and control bacterial cfu/ml counts after a 40 min exposure period ($p<0.0001$).

4.10 Discussion and conclusions

Overview and aims

The experiments detailed in this chapter provide evidence for the inactivation of clinical bacterial isolates by exposure to 405 nm wavelength light. This was carried out through the use of two distinct experimental models:

- Firstly, a replication of the hospital environment using a ceiling-mounted HINS-light EDS to inactivate relatively low densities of five clinical isolates of bacteria seeded on agar surfaces was performed;
- Secondly, the inactivation of high-density liquid suspensions of *P. stutzeri* by a high intensity HINS-light LED array was described.

Exposure of agar-seeded bacteria to a HINS-light EDS

The experiments involving laboratory testing of the HINS-light EDS for the inactivation of known bacterial isolates were designed to simulate a similar situation to that which will be occurring in clinical studies detailed in Chapters 5 and 6. This was a novel experimental arrangement to those previously reported. By performing the experiments in controlled laboratory conditions, with pre-determined starting populations of known bacteria, the generation of inactivation kinetics for bacteria treated by the EDS in a clinical environment was estimated. The EDS was arranged 1.5 m from the plate, which represented a typical distance between the ceiling-mounted EDS and the frequently touched contact surfaces (such as table tops and bed rails) assessed in the clinical studies. The HINS-light EDS used in these experiments delivered light at the same intensity as those used in the clinical studies.

In a further attempt to mimic the hospital environment, all isolates used in this study were isolated from the burns unit environment or patients, rather than from a national repository and included species with antimicrobial resistance. The density of bacteria found on surfaces around the hospital was also replicated. Scoping work in the burns unit had revealed populations of between 20 and 200 cfu/plate were isolated on contact agar plates. For these experiments, a bacterial density of 100-200 cfu/plate was therefore sought. The bacteria were seeded onto a solid agar surface, permitting some duplication of the environmental effects bacteria are subject to when on inanimate surfaces in patients' rooms.

Each of the five species tested demonstrated similar inactivation kinetics over the course of three independent experimental runs. This provides supportive evidence for the efficacy of the HINS-light EDS on a range of pathogenic bacteria in the burns unit environment. Each inactivation curve showed sharp reductions in populations between

0 - 2 h, followed by a more gradual reduction. Significant % reductions were achieved for all species tested at between 1 h and 2 h exposure, with complete or near-complete kill by 7 h.

Of note, the % survival of MRSA was significantly reduced by just 1 h compared with the 2 h required for MSSA. It may therefore seem that MRSA was more susceptible to the effects of the HINS-light EDS than MSSA. However, these species are the same in all but their antimicrobial resistance, and experiments using higher-densities populations have shown them to be equally susceptible.[2] This highlights the potential for experimental error when using lower-density populations as the % reduction in MRSA achieved at 1 h was actually less than the reduction in MSSA (17.7% compared with 19.6% reduction respectively). The other Gram-positive bacterium, *S. pyogenes*, displayed similar susceptibility to both MSSA and MRSA when raw data or % reductions were considered. Analysis of raw data alone suggests that the two Gram-negative bacteria were killed more slowly than the three Gram-positive bacteria. However, this is contradicted when comparing % survival per hour. Rather, Gram-negatives *P. aeruginosa* and MDR-*A. baumannii* were significantly reduced by 1 h, whereas MRSA and *S. pyogenes* took 2 h. However, once again, this is probably a reflection of the low starting populations used during these experiments. If fact, it is likely that all five species demonstrated very similar susceptibility in these conditions, which is in contrast to findings from experiments on high density liquid suspensions of bacteria to 405 nm light, as will be discussed later.[2]

Considering dose

Throughout this chapter exposure time, rather than dose, has been the reported variable. This enabled clinically relevant (time-dependent) conclusions to be drawn. In order to compare the results of this study directly with the results obtained by other authors, the dose, rather than exposure time has been reported for one MRSA study (Figure 4.5c). Figure 4.17 displays the inactivation curve for MRSA with dose as the independent variable. Simultaneous examination of this alongside Enwemeka et al's results reveal almost identically shaped curves: a rapid initial descent, followed by a slow tailing.[262] However, Enwemeka et al were using a high intensity 405 nm light source, producing an irradiance of 100 mW/cm^2, in comparison with 0.5 mW/cm^2 used here. Interestingly, the experimental arrangement described here seems to produce greater kill at much lower doses (complete kill is achieved with 10 J/cm^2 compared with 60 J/cm^2 in the Enwemeka et al model). Possible reasons for enhanced kill over longer exposure times is discussed below.

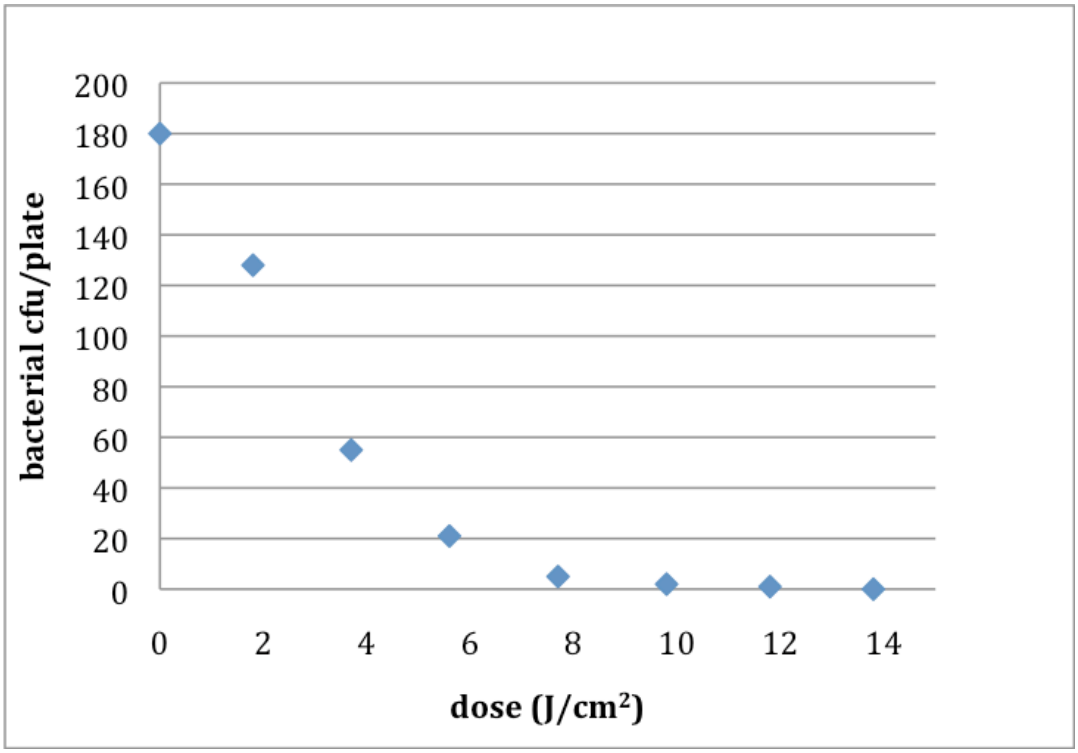


Figure 4.17: *Exposure of MRSA on agar surfaces to the HINS-light EDS. Bacterial cfu/ plate is reported as a function of dose to allow comparison to previous work by Enwemeka et al*[262]*.*

Germicidal efficiencies

In order to compare the $\log_{10}$ reduction per unit dose for each species, germicidal efficiencies (GE) were also calculated. A high GE represents a high susceptibility to the light. The germicidal efficiency (ŋ) (magnitude of $\log_{10}$ reduction of a bacterial population by inactivation per unit dose in cm^2/J) was calculated as follows:[355]

$$ŋ = \log_{10} (N/N0)\ (cm^2/J)$$

Where: N0 = bacterial starting population; N = the final bacterial population after exposure.

Equation 4.3: *Germicidal efficiency*

The germicidal efficiency for the five species tested following 1h and 4 h of exposure was reported, as was mean GE for the total 4 h (the average of the GE values for 1, 2, 3 and 4 h exposures). Both are reported in Table 4.4. In summary, although at 1 h a faster rate of kill is observed in Gram-positive species, by 4 h a faster rate is observed in Gram-negative species. However, similar mean GE are shown for all five species over the total 4 h exposure.

Table 4.4: *Germicidal efficiency (GE) following 1 h and 4 h exposures to HINS-light EDS for each of five species. Also reported is the overall mean GE following 1 h to 4 h exposure.*

Organism	GE at 1 h $\eta = \log_{10}(N/N0)$ (cm^2/J)	GE at 4 h $\eta = \log_{10}(N/N0)$ (cm^2/J)	Mean GE per hour between 1h and 4h $\eta = \log_{10}(N/N0)$ (cm^2/J)
MSSA	0.08	0.10	0.08
MRSA	0.06	0.14	0.13
S. pyogenes	0.07	0.18	0.10
MDR-*A. baumannii*	0.05	0.26	0.16
P. aeruginosa	0.02	0.24	0.13

One must be cautious about over-emphasising these conclusions, when only five species were tested and the GE are so similar. Further work on a wider range of bacteria is warranted. Of note, during studies by Maclean et al using high intensity LED arrays to inactivate over 10 different bacterial species in liquid suspension, Gram-positive organisms were shown to be more susceptible to HINS-light than Gram-negatives, but *A. baumaunnii* and *P. aeruginosa* were the most susceptible Gram-negatives tested, which may explain why Gram-positives and Gram-negatives appear almost equally susceptible here.[2]

Environmental stressors

These studies used a very different experimental arrangement to that used in the experiments by Maclean *et al*[2], which was based on log-transformed counts of high-density populations. A further complication when attempting to compare the two experimental arrangements is that it requires comparison of bacteria exposed in liquid versus those exposed on a solid agar surface. Although agar has a large water content, there would be some element of desiccation as the lids were not on the agar plates during exposure, which may have contributed to the inactivation of the bacteria at much lower doses than those used by Maclean *et al*.[2] Bacteria survive for variable lengths of time following dispersal into the inanimate environment, with Gram-negatives in general being more susceptible to the effects of desiccation.[356] Lengths of survival for the species tested are summarised in Table 4.5.

Although examination of the control populations for each experiment reveals no significant decrease in bacterial cfu due to the desiccation effect alone, it may be that this extra stress, in combination with the 405 nm light meant that Gram-negatives were inactivated at least as quickly as the Gram-positive bacteria in this work. Further studies looking at different species could explore this presumption. In support of this theory, Murdoch *et al* exposed both *Salmonella enterica* and *Listeria monocytogenes* to a high irradiance 405 nm LED array, in liquid suspension, seeded onto an agar surface, and

nebulised onto acrylic and PVC.[264] The results are summarised in Table 4.5. While the Gram-positive *L. monocytogenes* had a higher GE than the Gram-negative *S. enterica* when exposed in suspension, and a slightly higher GE when exposed on agar surfaces, the reverse was true when the organisms were seeded onto PVC and acrylic. In other words, although Gram-positive organisms were inactivated easily on liquid or agar surfaces, Gram-negatives are inactivated more easily in a dried state on inert surfaces.[264]

Table 4.5: *Survival of different species tested on inanimate surfaces. Adapted from[356].*

Species	Survival (range)	Reference(s)
S. aureus, including MRSA	7 days to 7 months	93, 366-369
S. pyogenes	Up to 4 weeks	370
Acinetobacter sp.	3 days to 5 months	93,125,371-374
P. aeruginosa	6 h to 16 months; 5 weeks on dry floor	93,367,368,375

Table 4.6: *Summary of experiments described by Murdoch demonstrating Gram-positives are more easily killed by HINS-light in liquid suspension, but Gram-negatives are more easily killed when dried onto solid surfaces.*[264]

Preparation	Dose (J/cm^2)	*S. enterica* (Gram-negative)		*L. monocytogenes* (Gram-positive)	
		log_{10} reduction	GE	log_{10} reduction	GE
liquid	288	3.50	0.012	5.00	0.017
agar	180	1.14	0.006	2.25	0.012
PVC	45	2.19	0.048	0.90	0.020
acrylic	60	1.63	0.027	0.42	0.007

The studies by Murdoch *et al* used a much higher irradiance (110 mW/cm^2) than that used in the surface exposure experiments in this study, so exposure times were vastly shorter. More desiccation is likely to take place during the course of the 7 h exposures reported here, compared with Murdoch *et al*'s 45 min exposures.[264] This may explain why in the current studies, although the bacteria were on agar, they acted similarly to Murdoch's findings on solid surfaces, with Gram-negatives being at least as susceptible to inactivation as Gram-positives. Of note, % survival of test populations were calculated from the relative control counts at each time interval, not starting populations, so any natural cell-death due to desiccation was accounted for.

Other groups have carried out similar laboratory work, using various experimental arrangements into the effect of visible blue light on bacterial counts. This has demonstrated that multiple pathogenic bacteria can be inactivated using visible wavelengths around 400-420 nm, with peak inactivation at 405 nm, in the absence of exogenous photo synthesisers.[2,257-260,262-266] In general much higher light intensities

have been used in published work, compared with the current experiments using the HINS-light EDS. As a result of using irradiance levels of the order of 0.5 mW/cm^2, rather than between 10 and 110 mW/cm^2 as used in other studies, much longer exposure times were required to achieve bacterial inactivation, although the lethal doses were found to be much lower.

Inter-species variation

Possible reasons for inter-species variation in susceptibility to the effects of visible light have been discussed in Section 2.9. In the absence of exogenous photosensitisers, differences between species is most likely to be due to variations between intra-cellular porphyrin levels, types or wavelength absorption maxima.[2,264,376] As previously mentioned, studies by Nitzan *et al* demonstrated the predominant porphyrin in the Gram-positive staphylococcal strains to be coproporphyrin (CP), which was two to three times higher than that found in Gram-negative strains, which produced no one predominant porphyrin.[269] However, other studies have shown that even when levels of porphyrin accumulation are comparable between Gram-positives and Gram-negatives, differences in photosensitivity may still exist.[260] This was attributed to differences in the type of porphyrin produced.

Differences between laboratory and clinical conditions

Although the studies using the HINS-light EDS for inactivation of agar-seeded bacteria attempted to replicate the hospital environment, certain differences remained which may have an impact on the rate of decontamination in the clinical environment. The bacteria used in these experiments are less stressed than those occurring in the clinical environment. They have been cultured in a nutritious broth and incubated at their optimal temperature before being prepared for exposure on a nutritious and moist agar surface. In comparison, bacteria on hospital surfaces travel as aerosolized particles or carried on flakes of skin or other human tissues. Following airborne travel or direct contact with a surface, they are precipitated onto non-nutritious surfaces where they gradually desiccate. It is likely that the bacteria that naturally occur on hospital surfaces are highly stressed and therefore more susceptible to the inactivation effects of the HINS-light EDS than those exposed in the laboratory. This phenomenon has been confirmed in previous laboratory work that has demonstrated that environmentally stressed *S. aureus* is significantly more susceptible to HINS-light inactivation than optimally cultured *S. aureus*.[377] It is therefore possible that the time taken to achieve a significant reduction in hospital surface bacterial contamination is less than the times reported in these experiments.

Exposure of a high intensity light to a high-density liquid suspension

With regards to the inactivation of high density bacteria in liquid suspension, an experiment using high intensity 405 nm light to inactivate *P. stutzeri* in suspension was designed in order to replicate Maclean *et al*'s methods on an untested species.[2] Results showed that a 4.4-$\log_{10}$ reduction was achieved following a dose of 240 J/cm^2. The results were comparable to those reported by Maclean et al who demonstrated 180 J/ cm^2 was required to achieve a 4.2-$\log_{10}$ reduction in *P. aeruginosa*.[2] The shape of the inactivation curve produced (Figure 4.16) closely resembles those from laboratory studies by Maclean *et al*, where little reduction is seen initially, followed by a sharp decrease in population.[2,255] This initial plateau is thought to be due to the mechanism of endogenous porphyrins producing reactive oxygen species (ROS), predominantly singlet oxygen. It is hypothesised that singlet oxygen must reach sufficient quantities before a detrimental effect on the bacteria is seen.

This curve, with its initial plateau phase, is in sharp contrast to those produced using the lower intensity EDS on agar seeded bacteria. However, as discussed, those results are similar to other studies into the inactivation of bacteria on surfaces rather than in suspension.[262] It would appear that there is a fundamental difference in the inactivation kinetics of bacteria exposed to light in a liquid suspension (slow initial kill followed by rapid kill at higher doses), and those plated onto agar surfaces (fast initial kill followed by gradual decrease in rate of inactivation at higher doses). This is likely to be an artifact of the low population densities used, and the exposure time points selected for use in the different experiment protocols. More frequent sampling periods may have made the initial plateau become apparent, even during studies on agar-plated bacteria.

The irradiance levels used in the liquid exposure experiments was 100 times that used in the agar-seeded experiments (50 mW/cm^2 vs. 0.5 mW/cm^2). The main finding when comparing the two study protocols is that when using higher irradiance levels, faster inactivation rates can be achieved. This suggests a dose-dependent response, whereby equivalent levels of inactivation may be demonstrated using high irradiance over short exposure times, or low irradiance over longer times. As an indication of the major differences between the two experimental models used in this study, a summary of the doses and log-transformed results at each exposure time tested for *P. aeruginosa* on agar, and *P. stutzeri* in liquid suspension are given in Table 4.6.

Table 4.7: *Summary of doses (expressed as J/cm^2) and bacterial counts (expressed as log-transformed data) of two different experimental models exposing Pseudomonas sp. to differing intensities of HINS-light, under different conditions.*

P. aeruginosa **on agar surface exposed to HINS-light EDS (approximate irradiance 0.5mW/cm^2)**			***P. stutzeri*** **in liquid suspension exposed to HINS-light LED array (approximate irradiance 50mW/cm^2)**		
Time (h)	**Dose (J/cm^2)**	**log10 cfu/ plate**	**Time (min)**	**Dose (J/cm^2)**	**log10-cfu/ml**
0	0.0	2.40	0	0	4.43
1	1.8	2.40	20	60	4.39
2	3.6	1.99	40	120	2.44
3	5.4	1.40	60	180	1.25
4	7.2	0.30	80	240	0.00
5	9.0	0.30	-	-	-
6	10.8	0.00	-	-	-
7	12.6	0.00	-	-	-

This table serves to demonstrate further, the varying susceptibilities of bacteria in different environmental conditions to inactivation through 405 nm light exposure. Although starting populations of bacteria in liquid suspensions are much higher than those on agar, it is still obvious that much greater doses are required to inactivate Pseudomonas sp. in liquid than are required when they are spread onto an agar surface. 60 J/cm^2 is required to achieve a 1.95-log$_{10}$ reduction at one phase of the experiment in liquid suspension (between 20 and 40 min). However, only a 3.6 J/cm^2 dose is required to achieve a 1.99-log$_{10}$ reduction after 2 h exposure on an agar surface. Caution should be taken when comparing these two very different experimental arrangements, but it certainly highlights the differing levels of dose required for the inactivation of bacteria under differing conditions.

Summary

Overall, this chapter has shown that clinical bacterial isolates from the burns unit and seeded onto agar surfaces can be successfully inactivated using HINS-light technology. It also appeared that there seems to be little difference between the susceptibility of Gram-positive and Gram-negative organisms, although this may be due to the low population densities or the particular Gram-negative organisms used. The use of known population densities of identified bacterial isolates in these experiments has enabled the generation of inactivation kinetics, and hence an understanding of the efficacy of the HINS-light EDS. The HINS-light EDS has been shown to inactivate a diverse range of bacterial species, and this, coupled with the previous work on 405 nm light bacterial inactivation demonstrates the non-selective decontamination effect exerted by the system.[2,264]

P. stutzeri, an organism which has never before been tested for its susceptibility to HINS-light, was used to demonstrate the effect of using a single 405 nm LED array to inactivate high population suspensions of the organism. This work successfully demonstrated the susceptibility of *P. stutzeri*, and also demonstrated the fact that high irradiance light can be used to rapidly inactivate high-density bacterial populations.

Knowledge of the decontamination range of the system, with regards to both the wide range of species that are susceptible, and the time taken for inactivation when using these low irradiance levels, has confirmed that the HINS-light EDS has potential to be used in the clinical environment for environmental decontamination applications: this will be investigated in the following chapters.

Chapter 5

Inpatient studies

5.0 Outline

The previous chapter described laboratory-based studies that tested the efficacy of the HINS-light EDS for inactivation of bacterial isolates from the burns unit. These experiments demonstrated that in controlled conditions, known starting populations of bacteria were significantly reduced following exposure to the HINS-light EDS. This chapter progresses to detail clinical studies carried out in the inpatient setting of the burns unit at Glasgow Royal Infirmary (GRI) using the HINS-light EDS. The focus was on reducing bacterial contamination of the inanimate environment around a burns inpatient.

Numerous studies were carried out investigating different applications of the HINS-light EDS within inpatient isolation rooms; these were designed to determine:

- The optimal time of day for environmental sampling of the isolation room;
- The decontamination effect of differing durations of HINS-light EDS;
- The use of one versus two HINS-light EDS units per isolation room, and;
- The relationship between HINS-light irradiance and the decontamination effect

The methods and results of these investigations are discussed in this chapter.

5.1 Background

This section describes the current practice of managing burns inpatients and examines the evidence for environmental contamination in the inpatient setting.

Introduction

Burns patients have traditionally been managed separately to other hospital inpatients; historically due to the suppurating burns smell causing offence, rather than concerns about infection control. In 1833 GRI made separate provision for the treatment of burns, followed by the opening of the burns unit in 1873.[378] Burns patients were managed conservatively, with long convalescent periods and inevitable bacterial colonisation of the wounds. Later, a recognition that cross-contamination should be prevented where possible, led to the design of burns units with single-patient isolation rooms and air conditioning systems.[133]

Most of the major advances in burn care have occurred within the last six decades, and in general, length of hospital stays for burns patients are decreasing.[7] During the ten-year period from 2002 to 2011, the average length of stay declined from 10.8 to 8.4 days, according to the American Burn Association (ABA) national burn repository.[379] However, as patients are surviving ever more severe injuries, albeit with protracted recoveries, some burns patients still require prolonged admissions, increasing their risk of acquiring nosocomial infection. Outbreaks of hospital-acquired infection remain a major challenge in burns units, and isolation and barrier techniques are applied vigilantly to prevent inpatients cross-contaminating one another.

Criteria for management as an inpatient

The burns unit at GRI receives patients from all over the west of Scotland. It is an adult unit, with patients younger than 14 years being treated at the nearby children's hospital. Practitioners may refer patients from any emergency department, medical centre or hospital ward. Patients can also be admitted directly to the general intensive care unit, under the joint care of the burns team and anaesthetists, and may be discharged from there to the burns unit.

Determining whether a patient requires admission to the burns unit follows consideration of the size of the burn, depth of the burn, location of the burn and co-existing medical problems, as outlined in Section 2.1. National and international guidelines exist to determine what is appropriate for referral to a burns unit, including those published by the ABA (summarised in Table 5.1) and the NHS National Network

for Burn Care (NNBC).[380,381] In Scotland, similar guidelines are produced by the Care Of Burns in Scotland (COBIS) managed clinical network.[382]

Table 5.1: *ABA Selection criteria for referral to a burns unit for admission, adapted from*[380].

Partial thickness burn greater than 10% Total Body Surface Area (%TBSA)
Face, hands, feet or genital burns
Full thickness burns
Electrical and lightning burns
Chemical burns
Inhalation injury
Burns in patients with coexisting trauma or medical conditions complicating recovery
Burn injuries in patients who will require special social, emotional or rehabilitation care

It is important to note that these are only guides and that each patient should be considered on an individual basis to determine their ability to cope at home with a new burn injury. If any doubt exists as to whether the patient should be admitted to the unit or not, they are usually seen that day on the burns unit where a decision is made about whether they should be admitted as an inpatient or treated as an outpatient. This is summarised in the flow diagram in Figure 5.1, adapted from the COBIS website.

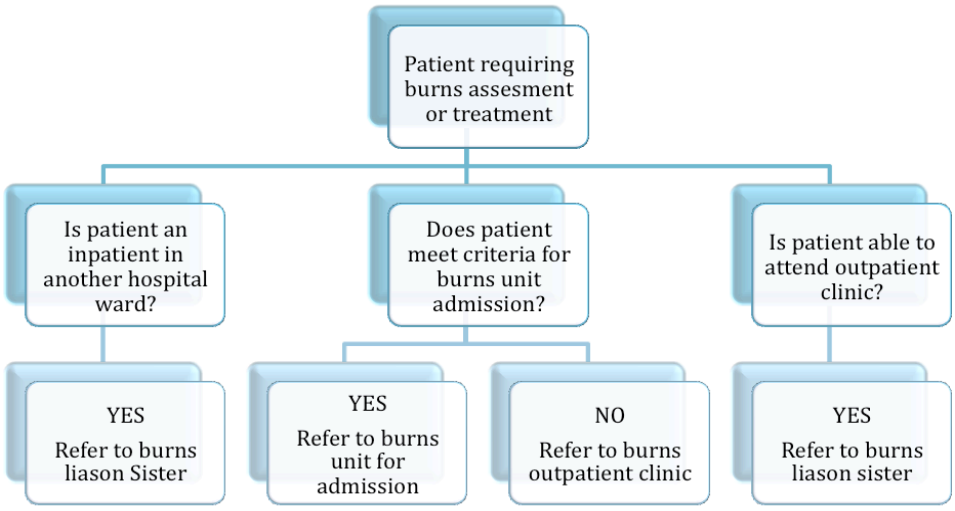


Figure 5.1: *Flow diagram to indicate referral pathway for burns patients, adapted from COBIS guidelines.*[382]

The role of the burns unit as an inpatient facility

The majority of the burns unit is dedicated to the management of burns inpatients. These patients remain in the ward from a day to several months depending on the severity of their injury and complications of their recovery. During their stay in the burns unit patients may have one or more operations, see several different medical and allied health care specialties and undergo investigations. The majority will have unhealed wounds that need dressing changes between every day and every five days, depending on the stage in healing. Patients with severe injuries will undergo a period of rehabilitation, with the physiotherapists, doctors, staff nurses, dieticians, social workers, psychiatrists and psychologists all providing input and help with recovery and return to life outside the unit. The healing of wounds is just one facet of care delivered by the burns unit team.

The burn wound is under constant review. Progress is monitored by regular examination, with photographs and wound swabs. On admission to the unit, all patients have swabs of the burn sent for cultures and sensitivities, and routine MRSA-specific swabs of the nose, throat and groin. After this, burns are swabbed approximately twice weekly or more frequently if clinically indicated. Once the wound is manageable in the community, the patient is discharged home to return as an outpatient, or to be reviewed by the burns liaison sister.

The focus for prevention of cross-contamination between patients has always been on the inpatient setting of the burns units, as long stays and multiple dressing changes increase the chance of nosocomial spread. Thus it was an important area to study the effects of the HINS-light EDS.

Previous studies using the HINS-light EDS in the inpatient setting

A series of studies carried out by Maclean *et al* evaluated the performance of the HINS-light EDS in inpatient hospital isolation rooms.[3] Performance efficacy was assessed by Baird Parker Agar (BPA) contact plate sampling and enumeration of staphylococcal bacteria. Ceiling-mounted HINS-light EDS were installed into inpatient isolation rooms and switched on during the day alongside normal room lighting. The studies investigated the effect of the HINS-light EDS in three scenarios: A) in an unoccupied room; B) in an occupied room with the HINS-light EDS operated for an extended period; and C) in an occupied room with and without the HINS-light EDS intervention.

Studies B and C provided the most useful information, as the main advantage to the HINS-light EDS is that it can safely be used continuously in the presence of patients. Study B was performed in a room occupied by an MRSA-positive patient with 25%TBSA burns. Samples were taken from 100 sites (i) before the HINS-light EDS was switched

on; (ii) after the HINS-light EDS had been on for two days; and (iii) after a further three days of HINS-light EDS use. Samples were taken from the same 100 sites during each of the three sample collection phases, and all samples were collected at 0730 h. The results of this study yielded a 56% reduction in environmental contamination after two days use, and a further 30% reduction following a further three days' exposure.

During Study C in the paper by Maclean *et al*, an MRSA-positive patient with 35%TBSA burns occupied the room.[3] Environmental samples were collected (i) before (ii) during and (iii) after use of the HINS-light EDS Samples from 70 sites were collected twice during each phase, with a minimum of two days between sampling. Again, all samples were collected at 0730h. The results demonstrated a mean percentage reduction in environmental contamination of 62%, with a mean percentage increase of 126% when the HINS-light EDS was switched off again in the *post-HINS* phase.

Study C was used as the basic model for the inpatient studies performed in this chapter.

5.2 Materials and general methods

This section describes the methods employed throughout assessment of the effect of the HINS-light EDS in inpatient rooms on the burns unit.

Setting

GRI has a purpose-built burns unit that opened in April 2003. The inpatient facility consists of six single-patient isolation rooms (Rooms 1 to 6), one three-patient shared room (Room 7), and one four-patient shared room (Room 8). There is a communal day room at the entrance to the ward, and two bathrooms, one of which has a small gymnasium adjacent to it. There is a central staff base that is open. The medication preparation and storage room, kitchen, linen storage and staff room are all behind this. Staff changing rooms and toilets are located on the far side of the unit, next to a further storage room.

Isolation rooms are reserved for patients with complex medical problems, large burns or from whose wounds nosocomial pathogens such as MRSA have been isolated. Unstable patients tend to be nursed in Rooms 5 and 6 where possible, due to their location opposite the staff base. Room 7 is often used to house sick patients who do not need to be isolated, for example those who have recently been admitted and have not yet had surgery. Room 8 is for relatively well patients, such as those with small burns who are stable. A schematic diagram of the burns unit is given (Figure 5.2).

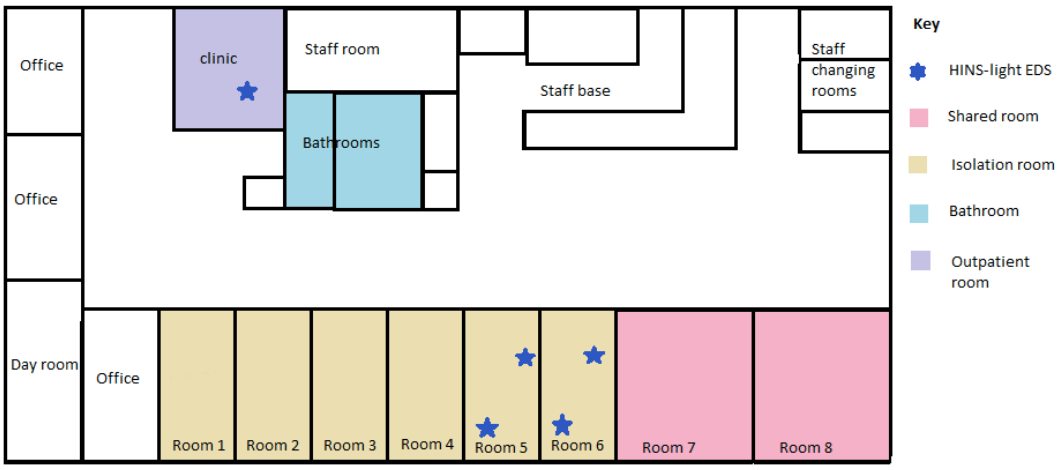


Figure 5.2: *Schematic diagram demonstrating the layout of the burns unit at GRI.*

Cleaning and infection control practices

Before entering a patient's room, healthcare workers (HCW) decontaminate their hands with alcohol gel or soap and water. Personal protective equipment (PPE), usually disposable gloves and aprons are then donned before approaching the patient. This must happen if the patient's wounds are undressed, or if staff are to have any contact with the patient or their immediate environment. Following interaction with the patient and their environment, PPE are removed and hands decontaminated. Regular audits of hand hygiene and the environment are carried out. Visiting relatives and friends are asked to observe hand hygiene rules. Occasionally disposable full-length gowns are worn when entering isolation rooms on the advice of the hospital microbiology department. This is usually to reduce the risk of an outbreak developing if a particularly virulent or problematic microorganism, such as *Acinetobacter baumannii,* has been isolated from that patient.

Environmental decontamination was carried out in line with the Greater Glasgow and Clyde standard operating procedures for patient isolation rooms.[383] Rooms are cleaned daily by domestic staff, or twice daily if the patient is MRSA positive. Floors are cleaned using Indur Top chlorine based detergent, while surfaces of lockers, table tops, sinks, toilets and ledges are cleaned using Brial Top detergent, and toilets are cleaned using Into Top (all ECOLAB Ltd, England). Following meals, tables and locker tops are wiped by HCW using Tuffie hard surface disinfectant wipes (Vernacare Ltd, England). Following patient discharge and vacation of the room, all surfaces, including the bed, mattress, locker and table are cleaned using Tuffie wipes, and domestic staff clean the floor and hard surfaces using chlorine based detergent (1000ppm).

The bathrooms are cleaned daily by domestic staff using the above products. All dressings in the bathrooms are kept in a closed cupboard. Following hydrotherapy, nursing staff clean all surfaces, the hoist and the bath using Actichlor disinfectant tablets (ECOLAB Ltd, England). The bath is allowed to air dry.

Air conditioning

All air entering through the hospital air conditioning system passes through High Efficiency Particulate Absorbing (HEPA) filters. Scottish guidelines state that there should be at least ten air changes per hour, with a room air flow of 158 l/s.[384] The individual isolation rooms on the burns unit are kept at a negative pressure to prevent contamination of the surrounding ward. The pressure is not regularly monitored unless there is a problem with the air conditioning.

HINS-light EDS installation

Two HINS-light EDS units were installed into the ceilings of both inpatient isolation Rooms 5 and 6. They were always positioned away from the head of the patient, and as centrally as possible. The EDS units were connected to a power supply contained in a secure box in the corner of the room. An example of HINS-light EDS *in situ* is given in Figure 5.3.

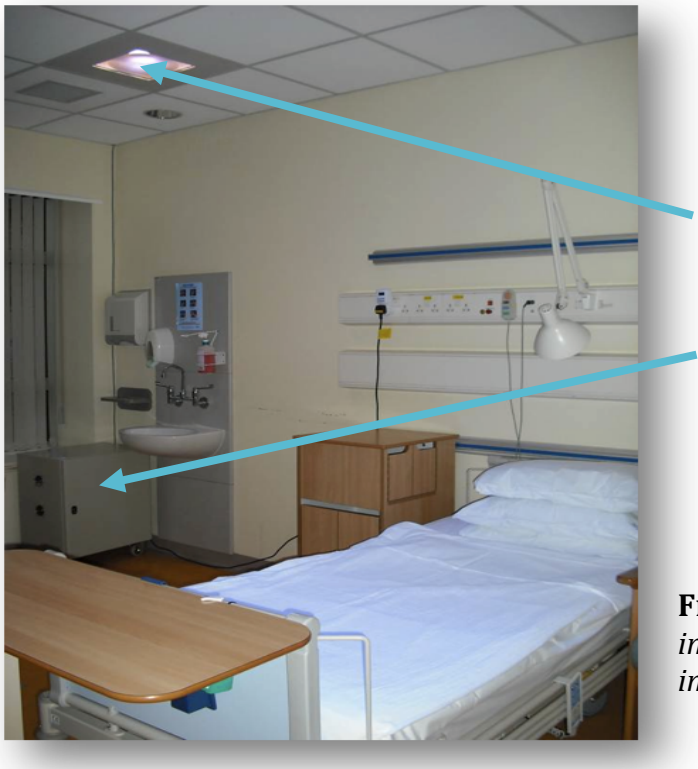


Figure 5.3: *HINS-light EDS in situ in the burn unit inpatient isolation Room 6.*

The HINS-light EDS units were connected to mains electricity and switched on and off at the wall socket. Minimal staff training was required and there was no disruption of the normal hospital routine. Lights were programmed by a timer to switch on at 0800h and switch off at 2200h, alongside normal room lighting: they were always off at night so they did not disturb patient sleep.

Bacteriological methods

Between 40 and 70 sampling sites were selected for each study. In general, early studies used 40 sites, but a greater number of sampling sites were used in later experiments to increase the power of the study. The sites chosen were evenly distributed throughout the room, on contact surfaces frequently touched by the patient and staff. All sites were sampled using BPA contact plates (Cherwell Laboratories Ltd., Bicester, UK). The rationale for using BPA contact agar plates for sampling was discussed in Sections 2.2 and 3.1. BPA was previously found to yield high but countable numbers of

staphylococcal-type organisms likely to be from a human source. Contact plates were pressed against the sampling site for 1-2 sec, then covered and transported to the laboratory for incubation at 37˚C for 48 h before enumeration.

Study protocols

All studies took place in single patient isolation rooms. All hospital standard cleaning and infection control practices continued as usual. The same sites were sampled during every sampling period for any one study, with a minimum of two days between each sampling period:

- ***Pre-HINS*** samples were taken before the EDS was used;
- ***During-HINS*** samples were taken when the EDS had been used for a pre-determined length of time; and
- ***Post-HINS*** samples were taken a period after the EDS had been switched off.

The *pre-HINS* samples gave a baseline from which the effect of the EDS could be measured, as each patient disperses different amounts of bacteria, depending on their wound size, bacterial colonisation and mobility. The *during-HINS* samples demonstrated the decontamination effect of the HINS-light EDS: a decrease in environmental contamination was expected. The *post-HINS* samples were a final control to demonstrate that any decrease seen in the *during-HINS* sampling period was due to the effect of the HINS-light EDS, rather than a general trend (for example, due to wound healing). A rise demonstrated that any decrease seen in the *during-HINS* phase was likely to be due to the EDS.

The basic sampling protocol used for inpatient studies is represented in Figure 5.4.

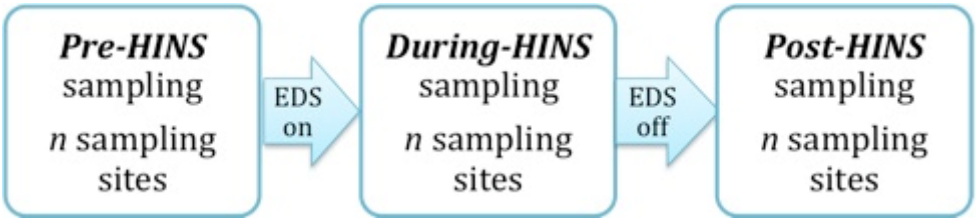


Figure 5.4: *Flow diagram to show basic protocol for inpatient HINS-light EDS studies.*

Statistics

Each sampling period produced 40 and 70 results per session. The mean number of bacterial colony forming units per plate (cfu/plate) was reported for each sampling period. The inpatient studies followed an A-B-A model, whereby the same patient remained in the same room throughout the study. Therefore the *pre-HINS* and *post-HINS* sampling periods acted as controls for each *during-HINS* sampling period. The only intervention was the use of the HINS-light EDS throughout the *during-HINS* phase.

Comparisons were made between mean cfu/plate *pre-HINS* and *during-HINS* periods to determine the percentage reduction in bacterial contamination following the use of the HINS-light EDS. These were calculated as follows in Equation 5.1:

$$1 - \frac{\text{mean cfu/plate } \textit{during-HINS}}{\text{mean cfu/plate } \textit{pre-HINS}} \times 100$$

Equation 5.1: *% reduction using EDS*

Comparisons were also made between the mean cfu/plate *during-HINS* and *post-HINS* sampling periods, to demonstrate the percentage increase in bacterial contamination that occurred after the HINS-light EDS was switched off. This was calculated as follows:

$$1 - \frac{\text{mean cfu/plate } \textit{post-HINS}}{\text{mean cfu/plate } \textit{during-HINS}} \times 100$$

Equation 5.2: *% increase without EDS*

For the purposes of publication, Prof George Gettinby, from the Department of Mathematics and Statistics at The University of Strathclyde, advised on and oversaw all statistical analysis.[385] Statistical software (Minitab version 16) was used and a log-transformation was found to normalise data and equalise variances when analysing cfu data. Analysis of variance (ANOVA) and Tukey pair-wise comparisons or a Dunnet test was performed, depending on the number of sampling periods in each study. A 95% confidence interval (CI) was calculated for the differences obtained between the means of the sampling periods. Results were displayed using least square mean (LSM) values and statistical testing was carried out at the 5% significance level ($p \leq 0.05$). Where available, the statistical significance based on log-transformed data has also been quoted.

In order to establish the correlation coefficient between two variables in Section 5.7 Pearson's correlation was determined using Minitab Version 16.

5.3 Two-day use of the HINS-light EDS: the effect of sampling at different times of day

This section outlines initial inpatient studies to determine the best sampling time for monitoring the effect of the HINS-light EDS on levels of environmental contamination.

Background

All environmental sampling carried out by Maclean *et al* in rooms occupied by patients was performed at 0730h, when the patient had been asleep and minimal activity had taken place in the room for the preceding ten hours.[3] It was possible that collecting samples at different times of day may influence any variation observed, due to activities within the room (such as dressing changes and bed sheet changes) affecting bacterial dispersal. This variable baseline could obscure the effects gained by the use of the HINS-light EDS. A study was therefore planned to determine the effect of sampling the environment at three different times of day, over a three week period, when the same patient was in the same room. This would establish a protocol for future studies of the impact of the HINS-light EDS on levels of environmental contamination, based on the most consistent sampling time.

Methods

The study was carried out in inpatient isolation Room 5 containing Patient A, a 49 year-old with 45%TBSA mixed deep partial and full thickness flame burns to the face, neck, upper limbs and thighs with inhalation injury following an indoor gas explosion. The study began one month after admission following grafting to both upper limbs. Patient A had MRSA, *P. aeruginosa* and coliforms isolated from wound swabs. Initially they had a tracheostomy, although at the time of the study they were not ventilated. Their mobility was limited and they required assistance to move from the bed to the chair. Dressing changes were carried out on alternate days, at varied times between 0800h and 1400h each day. The layout of the room is illustrated in Figure 5.5. For this investigation, three studies were performed to assess the effect of collecting environmental samples at three different times of day: 0800h, 1500h and 2200h.

Figure 5.5: *Schematic diagram of the layout of inpatient Room 5.*

Forty sampling sites (*n*=40) were identified around the room, with the same sampling sites being used each time (Table 5.2).

Table 5.2: *Sites selected for environmental sampling during the studies investigating the effect of collecting samples at different times of day*

Sampling site	No. samples
bed sheet	4
locker top	2
ledge	6
table	4
foot of bed rail	3
drip stand	2
patient chair	2
light switches	2
door handles	3
air con supply	2
waste bins	4
sink area	4
TOTAL	**40**

Environmental sampling was carried out as described above using BPA contact plates. *Pre-HINS* samples were collected from the 40 sites, after which two HINS-light EDS were switched on during daylight hours for two days. Following this two-day exposure period, *during-HINS* samples were collected from the same sites and the EDS units were then switched off. After a further two days (without the use of the HINS-light EDS), *post-HINS* samples were collected from the same 40 sites.

The study was carried out three times over three consecutive weeks, with the same patient in the same room. Sampling was performed at 0800 h during week one; 1500 h during week two; and 2200 h during week three (Figure 5.6).

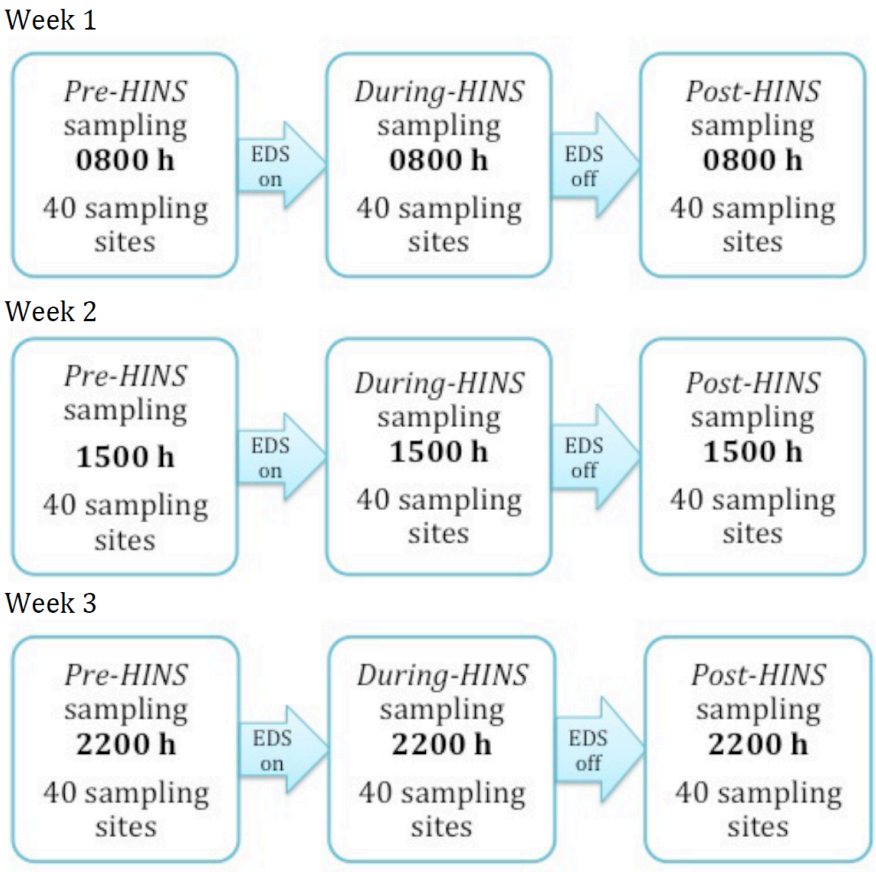


Figure 5.6: *Flow diagram to illustrate the study protocol for investigation of the two-day use of the HINS-light EDS, with samples collected at three different times of day. Patient A was occupying the room during all three studies.*

Results

After enumeration, the 40 contact plate counts for each of the study phases (*pre-HINS, during-HINS, post-HINS*) were pooled and mean values were calculated. Results are given in Table 5.3. For each part of the study the mean cfu/plate count is reported. A decrease was seen in environmental contamination when the HINS-light EDS had been on for two days. A subsequent rise in bacterial contamination when use of the HINS-light EDS was discontinued is also demonstrated, indicating that the effect seen is due to the HINS-light EDS. This was true of each of the three studies when samples were routinely collected at 0800h, 1500h and 2200h. The raw data of the bacterial cfu/plate counts for each of the 40 contact plates collected before, during and after use of the HINS-light EDS can be found in Appendix B, Table B.1.

Table 5.3: *Summary of two-day exposure study with samples taken at three times of day. Mean cfu/plate count and % increase or decrease is given.*

Sampling time	0800h	1500h	2200h
Pre-HINS mean cfu/plate	220.7	179.6	153
During-HINS mean cfu/plate	131.5	110.9	101.8
Mean cfu decrease with HINS use	89.2	68.7	51.2
% decrease with HINS use	**40**	**38**	**34**
Post-HINS mean cfu/plate	204.2	128.2	165.6
Mean cfu increase without HINS use	72.7	59.5	63.8
% increase without HINS	**55**	**16**	**63**

A 'V-shaped' curve was demonstrated in each of the three parts of the study, as shown in Figure 5.7. This illustrates a reduction in the mean cfu/plate across the room as a result of use of the HINS-light EDS of between 34% and 40%. The subsequent increase in the mean cfu/plate of between 16% and 63% indicates that the decrease in environmental contamination *during-HINS* was due to the HINS-light EDS intervention.

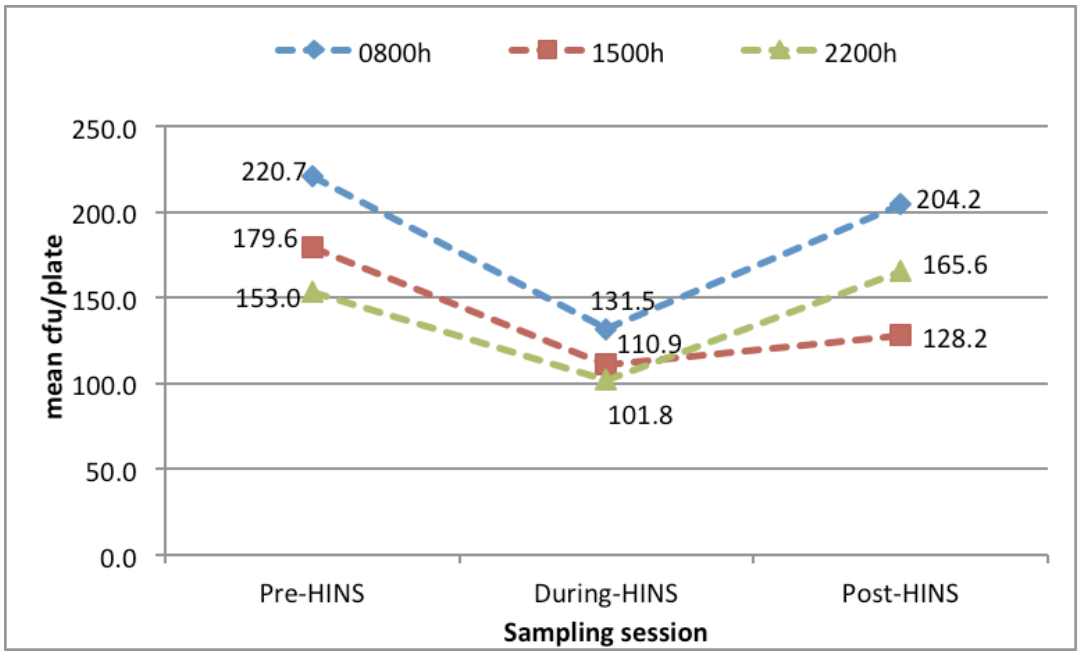


Figure 5.7: *Graph illustrating mean cfu/plate across the room for each pre-, during- and post-HINS sampling period, for each of the three sampling times investigated (n=40).*

Statistical analysis published is quoted in Table 5.4.[385] This demonstrates a significant 43% decrease in mean bacterial cfu following a two-day exposure when sampling took place at 0800h (p=0.043), based on log–transformed data. Although reductions of 45% and 39% were observed at 1500h and 2200h, these interactions were not significant

(p=0.252 and p=0.054). Increases in mean cfu/plate were observed following cessation of the HINS-light EDS use during all three sampling times tested, with significant increases of 48% (p=0.040) at 0800h and 60% (p=0.005) at 2200h. The increase at 1500h was not significant.

Table 5.4: *Published log-transformed statistical analysis of raw data, during two-day exposure studies, where samples were taken at different times of day during each study. Means expressed as least square means (LSM), and standard errors (SE) are provided. P-values are given to demonstrate whether each increase and decrease seen is significant.*[385]

Sampling time	**0800h**	**1500h**	**2200h**
Pre-HINS LSM cfu/plate	206.7	165.4	132.1
(SE mean)	(29.5)	(28.2)	(25.4)
During-HINS LSM cfu/plate	117.8	90.6	80.9
(SE mean)	(29.5)	(28.2)	(25.4)
% decrease with HINS use based on LSM	**43**	**45**	**39**
P value	0.043	0.252	0.054
significant	yes	no	no
Post-HINS LSM cfu/plate	173.8	107.8	129.6
(SE mean)	(29.5)	(28.2)	(25.4)
% increase without HINS use based on LSM	**48**	**19**	**60**
P value	0.04	0.149	0.005
significant	yes	no	yes

Discussion

This study concurred with previous published work, and demonstrated the significant reduction of environmental contamination achieved when using two HINS-light EDS for decontamination of a single patient isolation room. A reduction in contamination of between 34% and 40% was demonstrated after two days' exposure for 14 h a day. This was over and above what was achieved by standard hospital cleaning, which was maintained throughout. Statistical analysis from the accompanying publication showed that while the same 'V-shaped' curve was seen for each sampling time tested, significant reductions were only shown at 0800 h.[385] Crucially, this may be a Type 2 statistical error (no statistical difference demonstrated when it does in fact exist) as only 40 sampling sites were used, a number that was increased in later studies, increasing the power of the test.

Differences in the levels of bacterial contamination during daylight hours – likely due to direct contamination by patients or HCW, or cleaning by domestic staff – is reflected by the results of sampling at 1500h and 2200h, when there was much more variability of

activity within the room. There is no logical reason to suspect that the HINS-light EDS would be any less effective at these times of day than at 0800h: Indeed it might be expected that 0800h sampling would produce the least dramatic reduction in contamination levels as the HINS-light EDS had been off for 10 h prior to samples being taken. The main advantage to sampling at 0800h is that the activity levels in the room had been relatively constant overnight, as the patient was asleep in bed and staff had minimal input, which prevented large surges or reductions in bacterial deposition. This allowed a steady level of bacteria and a reliable estimate of contamination levels to be achieved. Although a similar pattern of reduction was demonstrated at the other times of day, variability in staff and patient activity was thought to obscure the HINS-light EDS effect.

For future studies 0800h sampling was used to achieve the most reproducible conditions possible so that the effect of the intervention can be seen.

5.4 Two-day use of the HINS-light EDS: confirmation of the effect using two further patients

This section describes two further studies undertaken using the same study protocol, with samples collected at 0800h, with two different patients in isolation rooms. As discussed in Chapter 2, bacterial contamination from any one patient depends on a variety of factors including wound size, movement and activity. Therefore different patients are likely to disperse different amounts of bacterial contamination into the surrounding environment. These experiments were performed to establish the reproducibility of the decontamination results under different experimental conditions (i.e. different rooms and different patients) despite varying starting levels of contamination.

Methods

The study was repeated in Room 5 while occupied by Patient B, a 35 year-old with 25%TBSA mixed deep dermal and full thickness flame burns. Routine wound swabs had isolated MRSA and coliforms. A further study was undertaken in Room 6, containing Patient C, a 55 year-old with 40%TBSA full thickness burns to the neck, chest and upper limbs. Routine wound swabs had isolated MRSA and *P. aeruginosa*. Room 6 had the same layout as Room 5, but in a mirror image (see Figure 5.5). Sampling for both studies took place at 0800h. The number of sampling sites was increased to 50 to include the bedside rails, as these were thought to be potentially important sites for cross-contamination (n=50). Five samples were taken from each of the two bed rails and the remaining 40 sample sites were the same as those used for the previous study (Table 5.2).

Environmental sampling was performed as before using BPA contact plates. *Pre-HINS* sampling took place, after which two HINS-light EDS were switched on during daylight hours for two days. Following this, *during-HINS* sampling took place, with samples collected from the same 50 sites, and the EDS were then switched off. After a further two days, *post-HINS* sampling took place, again using the same 50 sites. Each study was performed once for each patient (Figure 5.8).

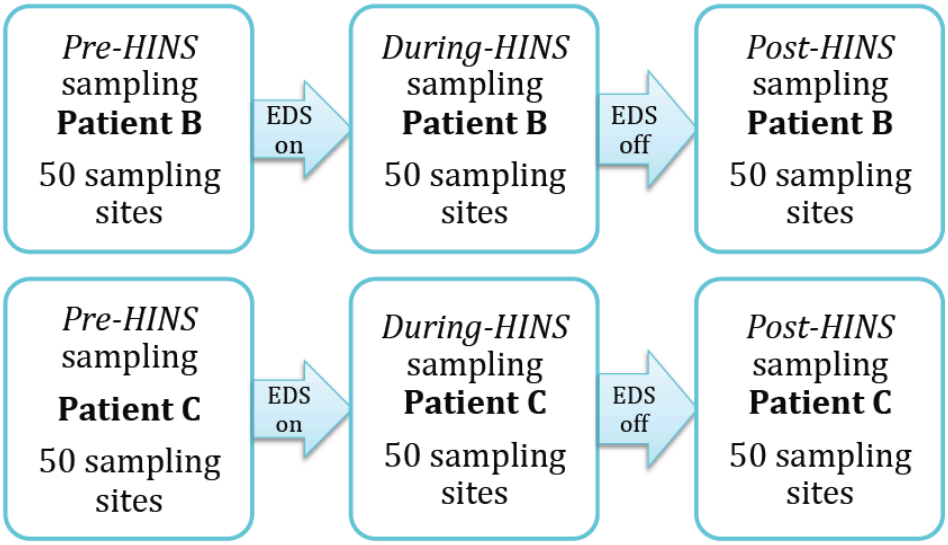


Figure 5.8: *Flow diagram to illustrate the study protocol investigating the two-day use of the HINS-light EDS, with two further inpatients.*

Results

The mean cfu/plate for each sampling period are given in Table 5.5. Raw data for the bacterial cfu/plate counts for each of the contact plates collected before, during and after use of the HINS-light EDS can be found in Appendix B, Table B.2. During the study in Patient C's room, ANOVA of the sites demonstrated a grossly contaminated sampling site at the sink (349 cfu on a single plate). This was thought to arise from direct contamination by a HCW immediately prior to sampling. During statistical analysis, both samples taken at the sink were therefore excluded on statistical grounds during the three sampling periods, thus n=48.

A decrease was seen in environmental contamination when the HINS-light EDS units had been on for two days in both studies. A subsequent rise when use of the HINS-light EDS was discontinued was also demonstrated in Patient B's study, although this could not be demonstrated in Patient C's study.

Table 5.5: *Summary of the results of using the HINS-light EDS for two days, with samples taken at 0800h in rooms containing two further patients. Mean cfu/plate count and % increase or decrease is given.*

Patient	B (n=50)	C (n=48)
Pre-HINS mean cfu/plate	19.4	31.4
During-HINS mean cfu/plate	2.5	24.6
Mean cfu decrease with HINS use	16.9	6.8
% decrease with HINS use	**87**	**22**
Post-HINS mean cfu/plate	7	23.3
Mean cfu increase without HINS use	4.5	-1.3
% increase without HINS	**180**	**-5**

A 'V-shaped' curve was demonstrated in the study carried out in Patient B's room. Although a decrease in the mean cfu/plate was achieved in the *during-HINS* phase in Patient C's room, there was no subsequent p*ost-HINS* rise observed. These are displayed in Figure 5.9. This graph illustrates a reduction in the mean cfu/plate in the presence of the HINS-light EDS of between 22% and 87%, after two days' exposure.

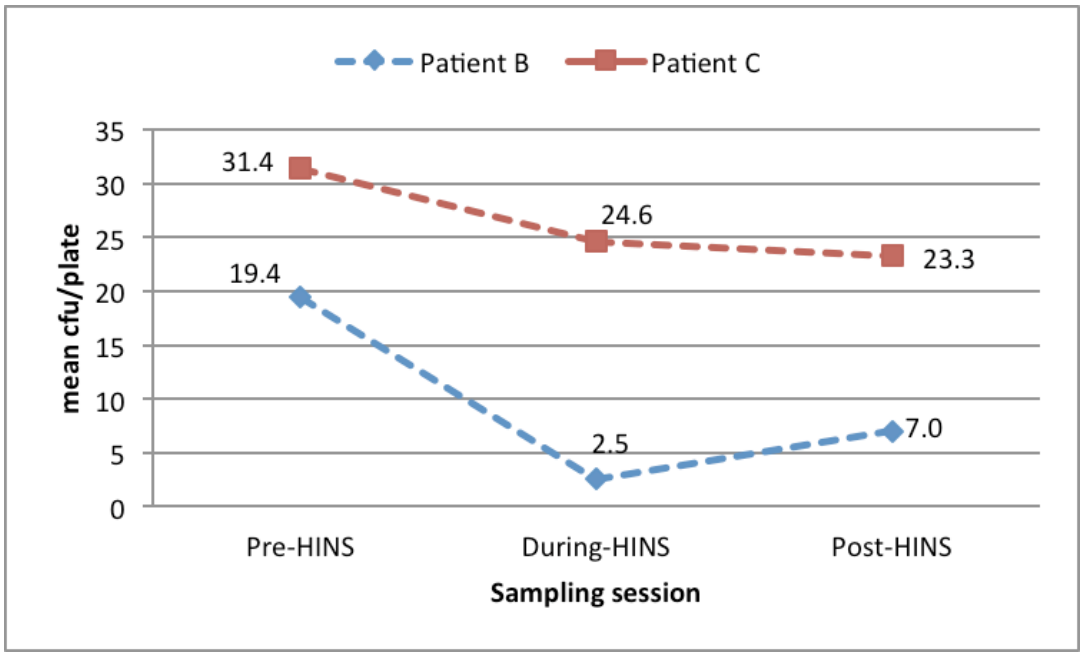


Figure 5.9: *Graph illustrating mean cfu/plate for each pre-, during- and post-HINS sampling period in isolation rooms containing two further inpatients when using the HINS-light EDS for a two day period. Results are represented as the mean cfu/plate count from contact plate samples collected from across the isolation room (Patient B study: n=50; Patient C study: n=48).*

As before, ANOVA and Tukey pair-wise comparisons were undertaken on log-transformed data and these are quoted in Table 5.6. A significant 27% and 75% decrease in mean bacterial cfu was observed the two patients ($p<0.001$ and $p=0.022$). Although a significant rise was seen in the *post-HINS* period in the study on Patient B, a small decrease was seen in the mean cfu/plate in the study on Patient C that was not significant.

Table 5.6: *Published log-transformed statistical analysis of raw data, during two further two-day studies using the HINS-light EDS in isolation rooms with two different patients. Means are expressed as least square means (LSM), and standard errors (SE) provided.*[385]

Patient	B	C
Pre-HINS LSM cfu/plate	22.5	25.3
(SE mean)	(3.4)	(8.1)
During-HINS LSM cfu/plate	5.6	18.5
(SE mean)	(3.4)	(8.1)
% decrease with HINS use based on LSM	**75**	**27**
P value	<0.001	0.022
significant	yes	yes
Post-HINS LSM cfu/plate	10.1	17.2
(SE mean)	(3.4)	(8.1)
% increase without HINS use based on LSM	**80**	**-7**
P value	<0.001	0.692
significant	yes	no

Summary of the two-day use of the HINS-light EDS in isolation rooms containing different patients

The two further studies carried out using a two day exposure period and two HINS-light EDS in an inpatient isolation room supported the results of the preceding study. Despite very different *pre-HINS* bacterial starting populations on contact surfaces around the room, a decrease was observed around the room of all three patients following the use of the HINS-light EDS. Again, the 'V shaped' curve observed during the study with Patient A was seen in the study in Patient B's room. Although *post-HINS* bacterial levels did not rise in the study in Patient C's room, there was a decrease seen during the use of the HINS-light EDS. Statistical analysis showed significant reductions were achieved with the use of the EDS in all three studies. The study in Patient B's room produced a significantly high reduction of 87% when compared with results from the other two-day studies. Although not observed at the time, there may be some explanation such as an unrecorded extra clean of the room. Either that, or the particularly low starting populations of 19.4 cfu/plate meant that even a relatively small decrease of 16.9 cfu/

plate generated an 87% change. In contrast, the 51.2 cfu/plate decrease seen in the study in Patient A's room only produced a 34% decrease in contamination.

Variability in bacterial deposition was clearly demonstrated by the three inpatients. Patient A produced high levels of environmental contamination, with mean *pre-HINS* levels of 220.7 cfu/plate. Patients B and C had mean starting populations of 19.4 and 31.4 cfu/plate respectively. There are several possible explanations for this: Patient A was ambulant around the room with assistance. Furthermore, they had loose motions on several occasions during the study, and although no infective cause for this was found, had to use the en-suite bathroom several times a day. They had the highest %TBSA burns, although comparable with Patient C. Patient A was also noted to have very dry flaky skin and hair, and was consequently likely to be a relatively heavy shedder of squames when compared to other patients. Nonetheless, despite these differences in starting populations, a significant decrease in the number of environmental bacteria around the isolation rooms was demonstrated following two days' continuous use of the HINS-light EDS, during daylight hours, in all three patient rooms.

5.5 Extended use of the HINS-light EDS: five, six and seven day exposure times

The previous studies were limited in only examining the effect of the HINS-light EDS for a relatively short period of 14 h a day on two consecutive days. It was unknown whether extending the period of use would augment the effect by continuing to reduce the levels of environmental bacterial contamination; have the same effect due to the establishment of equilibrium between inactivation and bacterial release; or decrease the effect as the bacterial release overwhelmed the effect of the light. This section outlines work undertaken using a similar study protocol to that used previously, but the use of the HINS-light EDS was extended to five, six and seven consecutive days, in three different studies.

Methods

This section consisted of three studies using the HINS-light EDS for increasing time periods: a five day exposure period; a six day exposure period; and a seven day exposure period. Each study took place in an inpatient isolation room (either Room 5 or Room 6), containing the same patient for the duration of the study. Three different patients were in the isolation room during each of the three different studies (Patient D, Patient E, Patient F). During each study the same protocol was intended:

- *Pre-HINS* samples were collected from 60-70 frequently touched sites around the room;
- the HINS-light EDS was switched on for between five and seven consecutive days, during which time two sets of *during-HINS* samples would be taken from the same 60-70 sites; and finally,
- *Post-HINS* samples were taken two days after the HINS-light EDS had been discontinued.

Occasionally the studies had to be adapted due to availability of contact agar plates, or early discharge of a patient.

Five-day use of the HINS-light EDS

This was carried out in Room 6 containing a 58 year-old, Patient D, with 10%TBSA mixed superficial and deep partial thickness flash burns to the hands and face, that did not require operative intervention. The study began five days after admission. Dressings took place on each sampling day, after the environmental samples had been taken. The

room was laid out in a mirror image to Room 5, illustrated in Figure 5.5. Seventy sampling sites (*n*=70) were identified around the room, with the same sampling sites being used for each sampling session detailed in Table 5.7.

Table 5.7: *Sampling sites used for five and six day HINS-light EDS use studies.*

Sampling	No. samples
door	2
light switch	2
chair	2
HINS power supply 1	4
drip stand	4
left bed rail	6
right bed rail	6
bottom bed rail	4
patient TV	2
locker top	4
ledges	10
table top	6
toilet light switch	1
toilet door	2
HINS power supply 2	4
waste bins	4
sink area	4
window ledge	3
TOTAL	**70**

Environmental sampling was carried out as before using BPA contact plates, with samples collected at 0800h. *Pre-HINS* sampling was performed, after which the HINS-light EDS were switched on during daylight hours. The HINS-light EDS units were in operation for a 5-day period, with environmental samples collected after Day 2, and again after Day 5 (termed '*during-HINS 1'* and '*during-HINS 2'*, respectively). After the *during-HINS 2* samples were taken, the EDS units were then switched off. The patient was discharged home at that time, so no *post-HINS* samples could be obtained. The protocol used for the five day study is summarised in Figure 5.10.

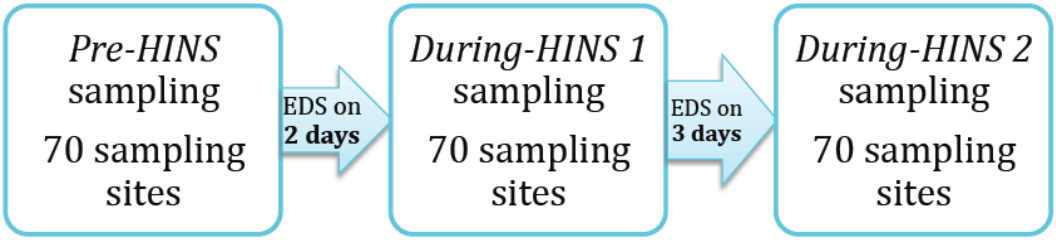


Figure 5.10: *Flow diagram to illustrate the protocol for the study investigating the use of the HINS-light EDS for a five-day period.*

Six-day use of the HINS-light EDS

This study was carried out in Room 6 containing 38 year-old Patient E. They had sustained 25%TBSA mixed deep partial and full thickness flame burns to the hands, upper limbs, chest, thighs and back, and a hypoxic brain injury. The study began 60 days after admission. Burns to the arms and chest had been debrided and grafted, with the use of Integra. There were still areas of unhealed wounds to the upper limbs, chest and thighs. The back had been treated with flammacerium and had developed a hard eschar. A tracheostomy was in use, although they were self-ventilating. Dressings took place on alternate days, and not on sampling days. Routine wound swabs had isolated *S. aureus*, *Pseudomonas* sp., and coliforms, with yeast in blood cultures. The room was laid out in a mirror image to Room 5, illustrated in Figure 5.5. The same seventy sampling sites (n=70) were used as for the five day study (Table 5.7).

Environmental sampling was carried out as described above using BPA contact plates, with all sampling carried out at 0800h. *Pre-HINS* sampling was performed, and the HINS-light EDS units were then switched on. The EDS units were in operation for a 6-day period with samples collected at Day 4 and again at Day 6 (termed '*during-HINS 1*' and '*during-HINS 2*', respectively). After the '*during-HINS 2*' samples were taken, the EDS were switched off and *post-HINS* samples were taken three days later. The protocol used for this study is summarised in Figure 5.11.

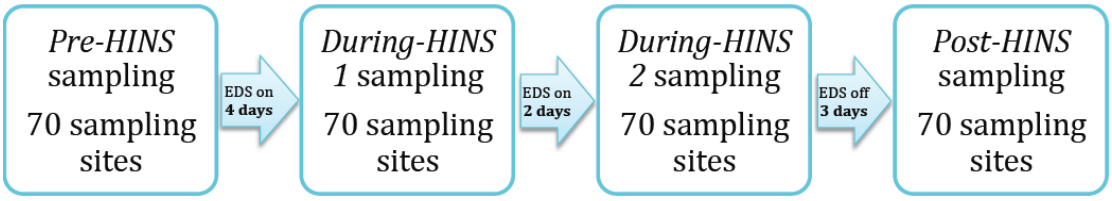


Figure 5.11: *Flow diagram to illustrate the protocol for the study investigating the use of the HINS-light EDS for a six-day exposure period.*

Seven-day use of the HINS-light EDS

This study was carried out in Room 5 containing 55 year-old Patient F who had sustained 23%TBSA full thickness flame burns to the face, neck, chest and upper limbs. The study began 22 days after admission. The burns to her arms and chest had been debrided and grafted, or managed with Integra. There were still areas of unhealed wounds to the neck, chest and upper limbs. A tracheostomy was in use, although the patient was self-ventilating. Dressings took place on alternate days, and not on sampling days. Routine wound swabs had isolated *Enterobacter cloacae*, and coliforms. The room was laid out as in Figure 5.5. Sixty sampling sites were used due to a shortage of contact agar plates. The same sixty sampling sites (*n*=60) were used for each sampling period (Table 5.8).

Environmental sampling was carried out as before using BPA contact plates, with all samples collected at 0800h. *Pre-HINS* sampling took place, and the HINS-light EDS units were then switched on and operated for 7-days. *During-HINS* samples were then collected at Day 4 and again at Day 7 (termed '*during-HINS 1'* and '*during-HINS 2'*, respectively). After the '*during-HINS 2'* samples were taken, the EDS units were switched off. *Post-HINS* samples were taken one day later. The protocol used for this study is summarised in Figure 5.12.

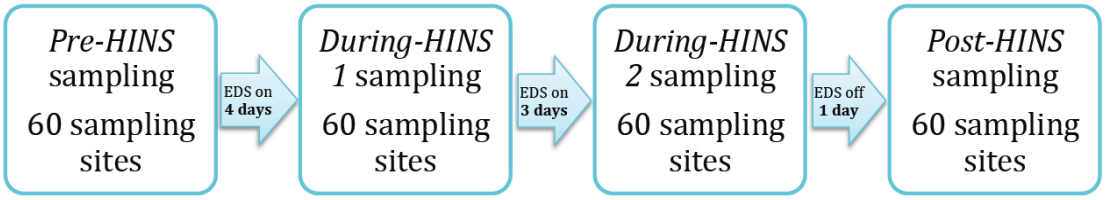


Figure 5.12: *Flow diagram to illustrate the protocol for the study investigating the use of the HINS-light EDS for a seven-day exposure period.*

Table 5.8: *Sampling sites used for the seven day HINS-light EDS use study.*

Sampling site	No. samples
light switch	1
HINS power supply 1	4
top bed rail	5
bottom bed rail	5
patient TV	2
locker top	4
ledges	12
table top	6
lamp	1
drip stand	2
toilet door	2
toilet light switch	1
HINS power supply 2	4
waste bins	4
sink area	4
window ledge	3
TOTAL	**60**

Results

The mean cfu/plate count for across the room is reported in Table 5.9 for each study. Raw data for the bacterial cfu/plate counts for each of the contact plates collected before, during and after use of the HINS-light EDS can be found in Appendix B.

A decrease in environmental contamination was seen when the HINS-light EDS had been on for two or four days. A further decrease is seen when the EDS was used for a further two or three days (five to seven day total exposure).

The results of all three studies are displayed as a scatter graph in Figure 5.12, to show the mean cfu/plate across the room during each day of sampling. The filled data points are *during-HINS* treatments and the non-filled points are *before* or *after-HINS* treatment (Figure 5.13). It is immediately clear that the filled data points are lower than the non-filled points (i.e there is a general trend towards lower cfu counts *during-HINS* compared with *before* or *after-HINS*). It is also apparent that for each study the mean cfu/plate is lower during the second *during-HINS* point than it is during the first *during-HINS* data point (i.e. the light has a cumulative effect up to at least seven days).

Table 5.9: *Summary of extended HINS exposure studies. Mean cfu/plate count and % increase or decrease is given.*

Study	5-day (n=70)	6-day (n=70)	7-day (n=60)
Pre-HINS mean cfu/plate	127.8	48.5	64.1
During-HINS 1 mean cfu/plate (no. days' exposure)	55.2 (2)	26.0 (4)	15.7 (4)
During-HINS 2 mean cfu/plate (no. days' exposure)	31.3 (5)	14.8 (6)	7.1 (7)
Mean cfu decrease with HINS use - session 1	72.6	22.5	48.4
Mean cfu decrease with HINS use - session 2	96.5	33.7	57.0
% decrease with HINS use – session 1	**57**	**46**	**76**
% decrease with HINS use – session 2	**76**	**70**	**89**
Post-HINS mean cfu/plate	n/a	80.1	67.8
Mean cfu increase without HINS use	n/a	65.3	60.7
% increase without HINS	**n/a**	**441**	**854**

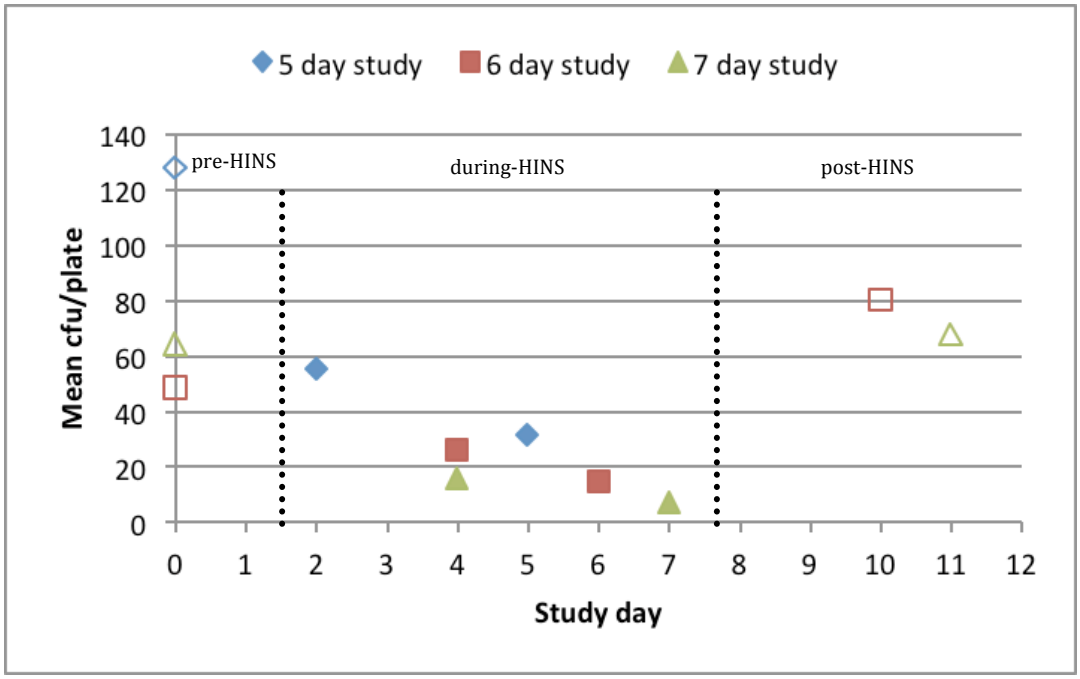


Figure 5.13: *Graph illustrating mean cfu/plate across the room for three extended HINS-light EDS studies. Non-filled data points indicate pre-HINS and post-HINS values. During-HINS* samples are indicated by filled data points. (n=70 for 5-day and 6-day studies; n=60 for 7-day study)

To summarise this data clearly, the mean cfu/plate after each complete extended-HINS study (i.e. *during-HINS 2* result) has been plotted in Figure 5.14.

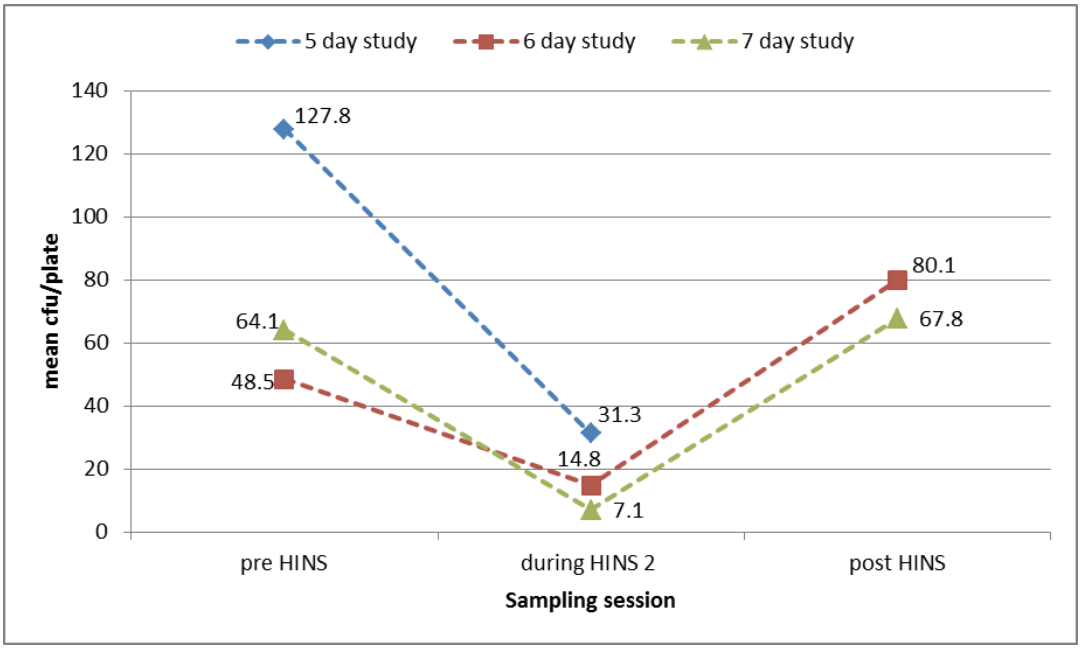


Figure 5.14: *Graph illustrating mean cfu/plate across all sampled surfaces in the isolation room for three extended HINS-light EDS studies, where the 'during HINS' samples were collected after 5, 6 and 7 days use of the HINS-light EDS.*

Statistical analysis

As before, ANOVA and Tukey pair-wise comparisons were undertaken based on log-transformed counts, under the supervision of Prof George Gettinby, for presentation purposes. The results of these are quoted in Table 5.10.[386,387] Results demonstrate a significant decrease in mean bacterial cfu/plate was produced following each of five, six and seven day exposures ($p<0.0001$).

Table 5.10: *Log-transformed statistical analysis of data for studies carried out using 5, 6 and 7 days use of the HINS-light EDS.*[386,387] *Means expressed as least square means (LSM) and standard errors (SE) are provided.*

Study	5-day	6-day	7-day
Pre-HINS LSM cfu/plate	111.2	48.8	66.6
(SE mean)	(18.5)	(7.7)	(9.9)
During-HINS 2 LSM cfu/plate	14.7	15.1	9.6
(SE mean)	(18.5)	(7.7)	(9.9)
% decrease with HINS use based on LSM	**87**	**69**	**86**
P value	<0.001	<0.001	<0.001
significant	yes	yes	yes
Post-HINS LSM cfu/plate	n/a	80.4	70.3
(SE mean)		(7.7)	(9.9)
% increase without HINS use based on LSM	**n/a**	**432**	**632**
P value	n/a	<0.001	<0.001
significant	n/a	yes	yes

Summary

These studies investigating the extended use of the HINS-light EDS have demonstrated that statistically significant reductions in mean environmental cfu counts were achieved following use of the systems for five, six and seven days. Furthermore, the effect has been shown to be cumulative; the longer the period of treatment, the greater the decontamination effect. Considering the previous two day studies, reductions of between 22% and 34% (excluding the anomalous study on Patient C) were observed. In contrast to this, with the extended decontamination times of five, six and seven days, 76%, 70% and 89% decreases in environmental contamination were achieved. Comparisons between studies on different patients can be problematic, as discussed in Section 5.4, due to variations in starting populations (*pre-HINS* counts). However, to demonstrate the hypothesis that extended exposure times result in enhanced decontamination, one can consider two exposure times on the same patient. Comparing the two *during-HINS* bacterial cfu counts in the seven day study, an initial decrease of 76% after four days becomes a final decrease of 89% after seven days. In all three studies the *during-HINS 1* count is higher than the *during-HINS 2* count.

The simplest explanation for the correlation between decontamination and length of exposure is that the dose is increased, as dose is a function of irradiance and time. As the next section will show however, while dose is proportional to amount of bacterial kill in a laboratory situation, the relationship between the dose received on a surface and the amount of kill achieved at that surface is less evident. It is also important to remember that in each of these studies the light was off for 10 h in each 24 h period, yet as an

accumulative effect is seen over an extended number of days, this does not appear to be sufficient time for the bacterial levels to recover.

When dealing with prolonged exposure to any form of decontamination, the question of bacterial resistance will invariably arise. Resistance would be highly unlikely to arise during a week; hence the need for extended studies. However, even without such studies, it is believed that resistance to the HINS-light EDS is unlikely. As discussed in Chapter 2, the main mechanism of HINS-light inactivation is believed to be oxidative damage via reactive oxygen species generated by the excitation of endogenous porphyrins upon exposure to 405 nm wavelength light.[260,268] Inactivation via oxidative damage is non-selective, and bacterial resistance to oxidative damage has not been previously reported. Conversely, inactivation by UV-light is a result of the formation of DNA mutations, which can be passed on and confer resistance to subsequent bacterial progeny.[152,191] Furthermore, resistance is more likely to occur on replicating cells, and the desiccated cells on environmental surfaces are not able to undergo replication.

5.6 Comparison of the decontamination effect using one HINS-light EDS versus two

The next section of this chapter considers the effect of using a single HINS-light EDS per room, rather than two, over an extended exposure time, in order to assess whether comparable decontamination effects can be achieved.

Background

In all previous inpatient studies, two HINS-light EDS units had been used per isolation room. One of the most important considerations when introducing the HINS-light EDS to a hospital ward or unit will be the installation and running costs of the systems. To date, the University of Strathclyde has produced each HINS-light EDS as a prototype unit for research purposes, so the cost of a mass-produced unit is difficult to estimate. However, it is self-evident that if a comparable decontamination effect could be achieved with fewer units per room, the cost would be considerably reduced. Furthermore, as the unit is based on LED lighting, it is relatively efficient to run as compared with incandescent or fluorescent lighting systems; a cost that would be further reduced if fewer units were required to achieve the same overall decontamination effect. As dose is a function of irradiance and time, it may be that comparable doses can be achieved as long as the EDS is used for a sufficient amount of time. Due to the high clinical relevance of answering these questions, a study was designed to investigate the effect of extended periods of a single HINS-light EDS exposure in an occupied isolation room, in a similar format to before.

Methods

Three studies with the HINS-light EDS in operation for 7-days were performed, each with samples collected pre-HINS, during-HINS and post-HINS, as before. Each study took place in an inpatient isolation room (either Room 5 or Room 6), containing the same patient for the duration of the study. Three different patients were in the isolation room for the duration of each of the three different studies (Patient G, Patient H, Patient I). During each study the same protocol was used: *Pre-HINS* samples were collected from 70 frequently touched sites around the room; the HINS-light EDS was switched on for seven consecutive days, during which time between one and three sets of *during-HINS* samples would be taken from the same 70 sites; and finally *post-HINS* samples were taken two or three days after the HINS-light EDS had been discontinued. The number of *during-HINS* samples and time between samples depended on contact plate availability and activities within the room.

The same sampling sites were used for each of the three studies, either in Room 5 (Figure 5.5), or Room 6. The seventy sampling sites (n=70) were on frequently touched

surfaces, and were unchanged from those used during the extended-HINS-light EDS studies in the previous section, and are listed in Table 5.7. Environmental sampling was carried out as before using BPA contact plates, with sampling at 0800h, before any dressing changes took place.

Single HINS-light EDS Study in Patient G's room

Patient G was a 65 year-old with a 19%TBSA flame burn to the back, forearm and head. The burn had been managed conservatively and much of it had healed, with only approximately 11%TBSA still unhealed at the time of the study. The study was carried out in Room 6, and only the HINS-light EDS near the window was used. Samples were collected (i) before the EDS was in operation (*pre-HINS*), (ii) at days 2, 4 and 7 during the 7-day use of the HINS-light EDS, and (iii) after the EDS has been switched off for 2 days. The protocol for sample collection is detailed in Figure 5.15.

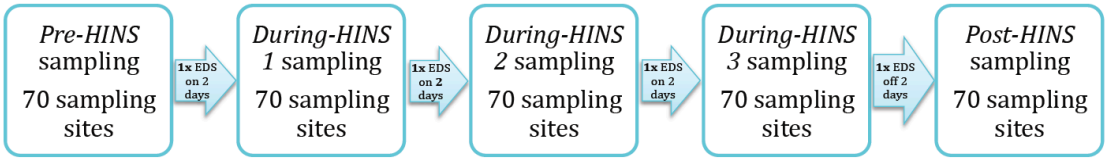


Figure 5.15: *Flow diagram to illustrate the protocol for the study investigating the use of a single HINS-light EDS for a seven-day exposure period in the isolation room with Patient G.*

Patient H

Patient H was a 38 year-old with a 50%TBSA flame burn to the chest, upper limbs, back and abdomen a month after admission. About 40%TBSA had been excised and replaced with Interga or skin graft. At the time of the study the patient was receiving treatment for a chest sepsis. The study was carried out in Room 6, with the light nearer to the door being used alone. During the study in Patient H's room, samples were taken (i) before the HINS-light EDS was switched on, (ii) at days 2 and 7 during the 7-day use of the HINS-light EDS, and (iii) after the EDS has been switched off for 3 days. This is summarised in the diagram in Figure 5.16.

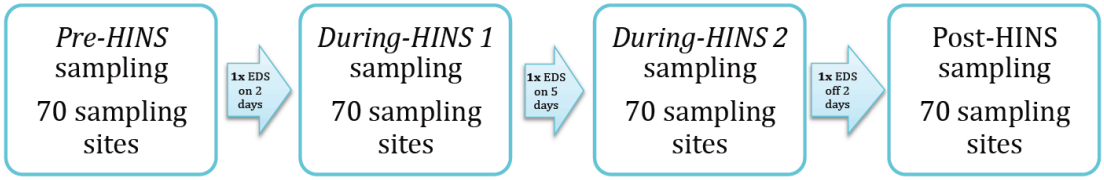


Figure 5.16: *Flow diagram to illustrate the protocol for the study investigating the use of a single HINS-light EDS for a seven-day exposure period in the isolation room with Patient H.*

Patient I

Patient I was a 48 year-old with a 12%TBSA scald to the feet, legs and buttocks. He had had a protracted stay of two months due to respiratory infections and large parts of his wounds were healed. The study was carried out in Room 6, with only the light near the door used throughout. Samples were collected (i) before the HINS-light EDS was switched on, (ii) after the 7-day use of the HINS-light EDS, and (iii) after the EDS has been switched off for 3 days. This is summarised in the diagram in Figure 5.17.

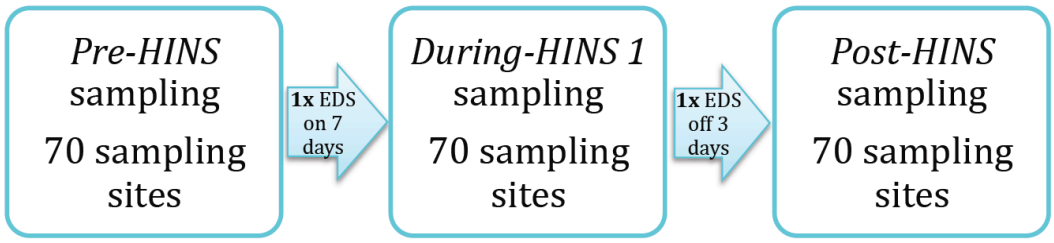


Figure 5.17: *Flow diagram to illustrate the protocol for the study investigating the use of a single HINS-light EDS for a seven-day exposure period in the isolation room with Patient I.*

Results

Raw data for the bacterial cfu/plate counts for each of the contact plates collected before, during and after use of the HINS-light EDS can be found in Appendix B. The mean cfu/plate count for all samples across the room is reported in Table 5.11 for each of the three studies.

When looking at mean counts across the isolation room, a decrease was seen in environmental contamination when a single HINS-light EDS has been on for any time

between two and seven days, although this is small in the study on Patient I. A subsequent increase was seen in all studies when the EDS was switched off again.

Table 5.11: *Summary of the results for the seven-day use of a single HINS-light EDS in three different patient's rooms. Mean cfu/plate and % change is given.*

Sampling session	Patient G	Patient H	Patient I
Pre-HINS mean cfu/plate	44.2	145.1	33.0
During-HINS 1 mean cfu/plate (no. days' exposure)	20.9 (2)	96.2 (4)	25.8 (7)
During-HINS 2 mean cfu/plate (no. days' exposure)	13.8 (4)	38.2 (7)	n/a
During-HINS 3 mean cfu/plate (no. days' exposure)	6.2 (7)	n/a	n/a
Mean cfu decrease with HINS use – session 1	23.3	48.9	7.2
Mean cfu decrease with HINS use – session 2	30.4	106.9	n/a
Mean cfu decrease with HINS use – session 3	38.0	n/a	n/a
% decrease with HINS use – session 1	**53**	**34**	**22**
% decrease with HINS use – session 2	**69**	**74**	**n/a**
% decrease with HINS use – session 3	**86**	**n/a**	**n/a**
Post-HINS mean cfu/plate	25.4	68.1	56.9
Mean cfu increase without HINS use	19.2	29.9	31.3
% increase without HINS	**309**	**78**	**120**

All three studies are displayed as graphs below, to show the mean cfu/plate during each sampling session (Figure 5.18-5.20). It can be seen that the *during-HINS* sampling sessions are lower than the *pre-* and *post-HINS* sessions for each study. It is also apparent that for any one study, the mean cfu/plate is reduced as the exposure time increases: this is particularly apparent in the study in Patient G's room.

The results of the use of a single HINS-light EDS unit for a 7-day period in Patient G's room are shown in Figure 5.18; results of the use of a single HINS-light EDS unit for a 7-day period in Patient H's room are shown in Figure 5.19; and results of the use of a single HINS-light EDS unit for a 7-day period in Patient I's room are shown in Figure 5.20.

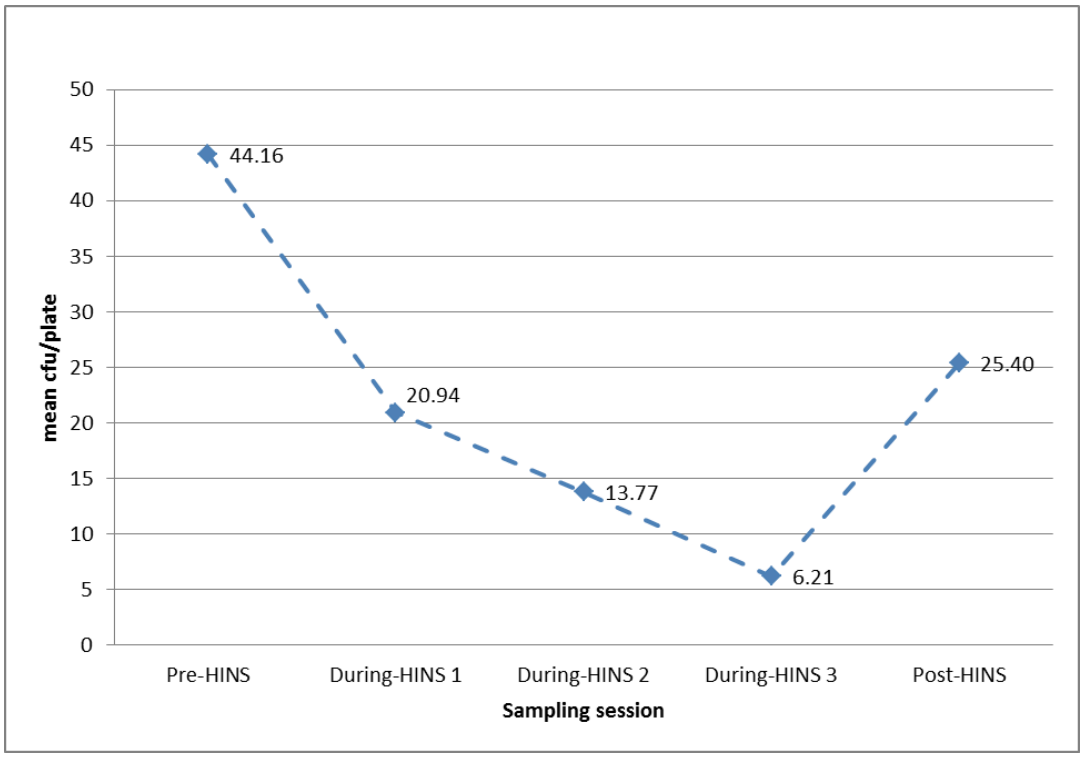


Figure 5.18: *Graph illustrating the mean bacterial counts around the room before, during & after a 7-day use of a single HINS-light EDS unit in the isolation room with Patient G. During-HINS 1, 2 and 3 samples were taken after 2, 4 and 7 days use of the EDS, respectively (n=70).*

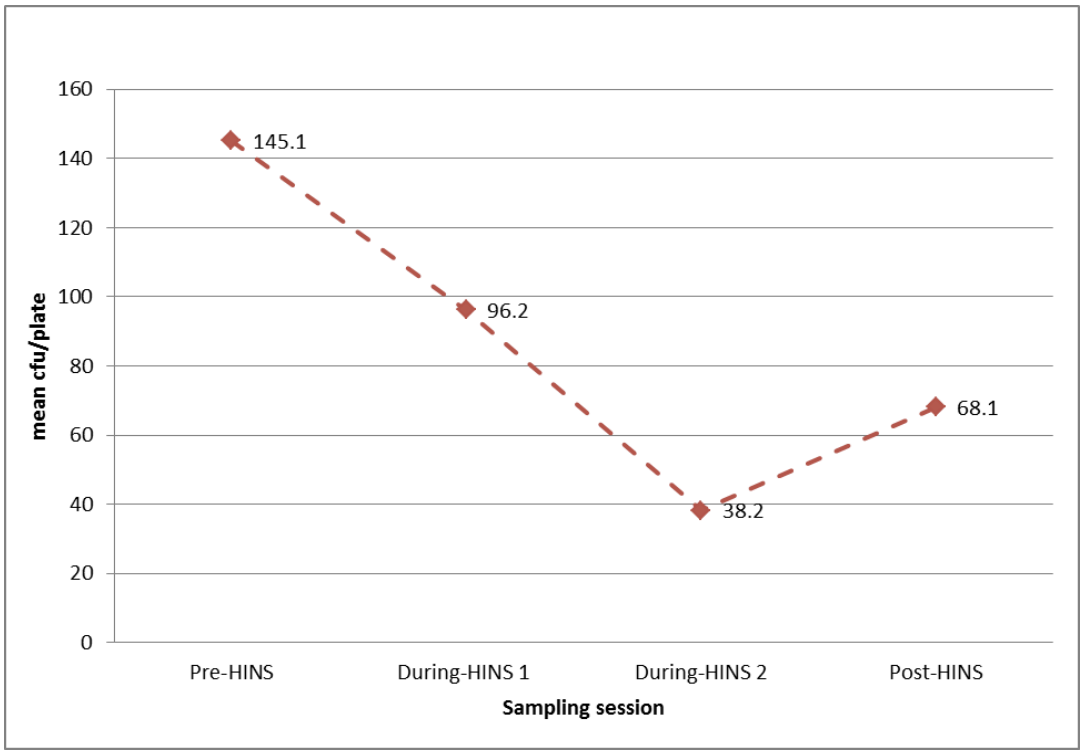


Figure 5.19: *Graph illustrating the mean bacterial counts around the room before, during & after a 7-day use of a single HINS-light EDS unit in the isolation room with Patient H. During-HINS 1 and 2 samples were taken after 2 and 7 days use of the EDS, respectively (n=70).*

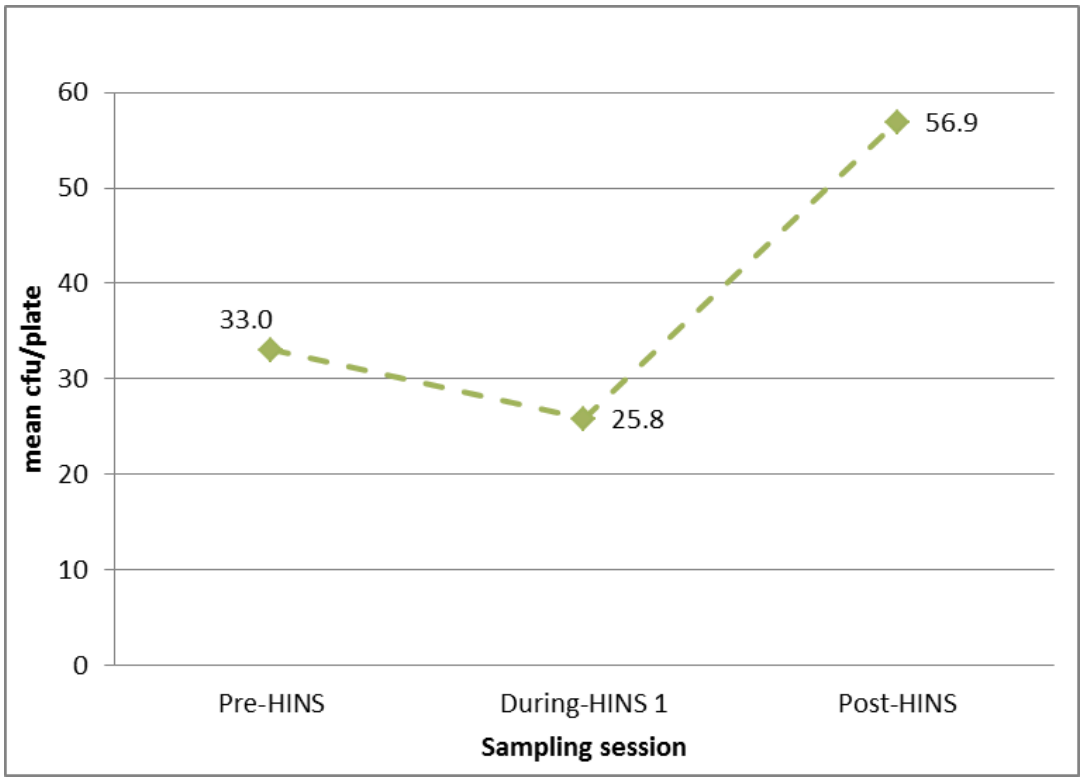


Figure 5.20: *Graph illustrating the mean bacterial counts before, during & after a 7-day use of a single HINS-light EDS unit in the isolation room with Patient I. During-HINS 1 samples were taken after 7 days use of the EDS (n=70).*

Statistical analysis

ANOVA and Dunnet comparisons undertaken on log-transformed counts were performed under the supervision of Prof George Gettinby to examine for significant differences between *pre-HINS* and each of the *during-HINS* periods for each study, and between *post-HINS* and the final *during-HINS* period for each study. These demonstrated that significant decreases in mean bacterial cfu were produced when a single HINS-light EDS was used during the first two studies (in the rooms of Patients G and H), and significant increases were demonstrated when EDS use was discontinued in all three studies (Table 5.12).

Summary

These studies which investigated the use of a single HINS-light EDS within isolation rooms demonstrated statistically significant reductions in the mean environmental contamination following between two and seven days' exposure. Importantly, these studies have shown that the reductions in environmental contamination which were observed with use of two HINS-light EDS units per isolation room can also be achieved using a single HINS-light EDS in the isolation rooms. This may be a result of the

decontamination effect plateauing: the bacterial contamination may only require a certain exposure for the inactivation effect to be seen and application of increased irradiance may not induce any further significant effects.

Table 5.12: *Statistical analysis, based on log-transformed data, for the 7-day use of a single HINS-light EDS in three different patient's rooms. Means are expressed as least square means (LSM) and standard errors (SE) are provided.*

Study	Patient G	Patient H	Patient I
Pre-HINS LSM cfu/plate (SE mean)	43.2(5.8)	146.4 (12.2)	26.2 (4.4)
During-HINS 1 LSM cfu/plate (SE mean)	20.0 (5.8)	97.5 (12.2)	25.9 (4.4)
During-HINS 2 LSM cfu/plate (SE mean)	12.9 (5.8)	39.6 (12.2)	n/a
During-HINS 3 LSM cfu/plate (SE mean)	5.3 (5.8)	n/a	n/a
% decrease with HINS use based on LSM -session 1	**54**	**33**	**1**
% decrease with HINS use based on LSM – session 2	**70**	**73**	**n/a**
% decrease with HINS use based on LSM – session 3	**88**	**n/a**	**n/a**
P value – session 1	<0.001	0.014	0.999
P value – session 2	<0.001	<0.001	n/a
P value – session 3	<0.01	n/a	n/a
significant	yes	yes	no
Post-HINS LSM cfu/plate (SE mean)	24.5 (5.8)	69.5 (12.2)	57.0 (4.4)
% increase without HINS use based on LSM	**362**	**76**	**120**
P value	<0.001	0.036	<0.001
significant	yes	yes	yes

Furthermore, the effect has been shown to be cumulative: the longer the period of treatment, the greater the decontamination effect seen during the course of any one study. As discussed in the previous section, accurate comparisons between studies on different patients are difficult. However, once again when multiple *during-HINS* samples were taken from the same patient (e.g. Patient G), a 53% decrease is seen after 2 days; 69% decrease after four days; and 86% after seven days. In this particular study, the decrease after seven days is comparable with those achieved using two HINS-light EDS in the extended HINS studies in Section 5.5.

The study on Patient H also achieved similar reductions to those demonstrated in seven day studies using two lights, although the study on Patient I did not. This study only demonstrated a 22% decrease in environmental contamination after seven days, which translated as a 1% decrease based on LSM: not a statistically significant reduction. This is somewhat incongruous result when compared with the other data from this chapter. When one considers the *post-HINS* bacterial cfu counts, they are considerably higher than both the *during-HINS* and *pre-HINS* mean counts. In fact a 120% increase is shown

following cessation of the EDS use. In light of the effect of the EDS that has been demonstrated repeatedly during previous inpatient studies, the most plausible explanation is that the *pre-HINS* levels were falsely low. Were this true, the *post-HINS* mean count of 56.9 cfu/plate would be a more accurate baseline level than the *pre-HINS* count of 33.0 cfu/plate. An explanation for the low starting levels is not available from the information gathered at the time of the study. The most likely scenarios are that either an extra clean was preformed prior to the *pre-HINS* session, or the patient mobility and activity around the room surged following the *pre-HINS* sampling session. No extra cleaning sessions could be identified retrospectively, but the patient was more mobile during the course of the study, and began to wander around the room at his own free will over the course of the week. In such a situation the EDS may have halted the surge in bacteria that would accompany this increase in activity: an effect that was only apparent when its use was discontinued.

The implications of a single EDS having comparable effects to two lights are significant. In equipping a burns unit with the HINS-light EDS, the cost is immediately halved if half the number of lights can be used. Caution must be taken however, before assuming that this is the case. Only three studies have been performed to date. Compared with the plethora of inpatient studies using two lights per isolation room during this research and that by Maclean *et al*, there is still a relative paucity of evidence for the use of a single light.[3] The reason two lights were always used is based on calculations performed by the engineers who designed the HINS-light EDS units. These were based on the area of irradiance that a single EDS unit could cover. However, these calculations were based on the presumption that the main effect of the light took place on the bacterial contamination on surfaces. If significant decontamination of airborne bacteria also occurs then these bacteria are inactivated in the air before precipitation onto surfaces. If this is the case then the area of irradiance is less important. Furthermore, these studies have shown that dose is an important factor for decontamination, with studies that involved multiple *during-HINS* sample collections demonstrating that there is a cumulative decontamination effect over time (and dose is proportional to time). Therefore, as long as the HINS-light EDS is in operation for a sufficient period, a sufficient dose can be achieved, even with low irradiance levels.

5.7 Determination of correlation between irradiance and decontamination

This section of this chapter will attempt to address the relationship between the irradiance received on a sampling site, and the level of decontamination achieved at that site. This will begin to determine whether a uniform decontamination effect is seen around the room, or whether the amount of decontamination achieved is dependent on the irradiance of the HINS-light EDS at that site.

Background

One of the uncertainties regarding the action of the HINS-light EDS is where the majority of the decontamination effect takes place. Two main types of inactivation can occur:

- Inactivation of bacterial contamination on the inanimate surfaces around the hospital environment, and
- Inactivation of airborne bacterial contamination.

To date, all environmental measurements of the effect of the HINS-light EDS have been by contact plate sampling of contact surfaces around the treated rooms, and these have shown a decrease in the levels of contamination during the operation of the EDS. Environmental sampling has also indicated that the surfaces around a patient are heavily contaminated, making them an important target in the cycle of cross-contamination.

It is likely that, in addition to inactivation of surface contamination, airborne bacterial contamination will also be affected by the HINS-light EDS. Particles released from burns patients have been shown to be relatively small, making them airborne for substantial periods of time.[54,388] Many airborne particles will be in closer proximity to the ceiling-mounted EDS units, and therefore more exposed to the EDS treatment, than bacteria on a surfaces.

Methods

A hypothesis was proposed that if the main decontamination effect of the HINS-light EDS was on static bacteria on surfaces, the irradiance received on any one surface would be proportional to the amount of kill achieved at that surface. However, if the decontamination effect reduced airborne bacteria, which were then precipitated at random, the amount of kill achieved at any surface would have little correlation with the amount of irradiance received at that surface.

In order to address this, a radiant power meter and photodiode detector (Oriel Instruments, Stratford CT, USA) was used to measure the irradiance, in mW/cm^2, received at each of the sampling sites around the isolation room. This was performed during the study on Patient G, described in Section 5.6. Irradiance measurements around the room were taken with the blue-light LED element of the single HINS-light EDS near the window switched on, and all other light in the room eliminated. The level of bacterial decontamination achieved at each of the sampling sites during use of the HINS-light EDS was then compared to the level of irradiance received at each site.

Statistical analysis

The 70 contact plate samples were divided into 18 sample sites (e.g. bedside table, six samples) as shown in Table 5.7. For each site the mean % reduction achieved following two, four and seven days' exposure to the HINS-light EDS was calculated. A scatter graph was produced to determine whether there was any relationship between the irradiance (x axis) and mean % reduction after a 7-day exposure period (y axis) at each sampling site.

Statistical analysis was carried out on Minitab V16 to determine Pearson's correlation coefficients for the interaction between irradiance and % reduction achieved at two, four and seven days.

Results

An example scatter graph produced from the seven-day study is shown in Figure 5.21. Examination of the scatter graph shown in Figure 5.21 demonstrates that there is no real correlation between irradiance and the mean % bacterial reduction following seven days' use of the HINS-light EDS. If anything, there appears to be a fairly constant relationship, with between a 50% and 100% reduction achieved, whatever the irradiance received at that site. Nonetheless, statistical analysis was performed to ascertain the correlation coefficient for the two variables.

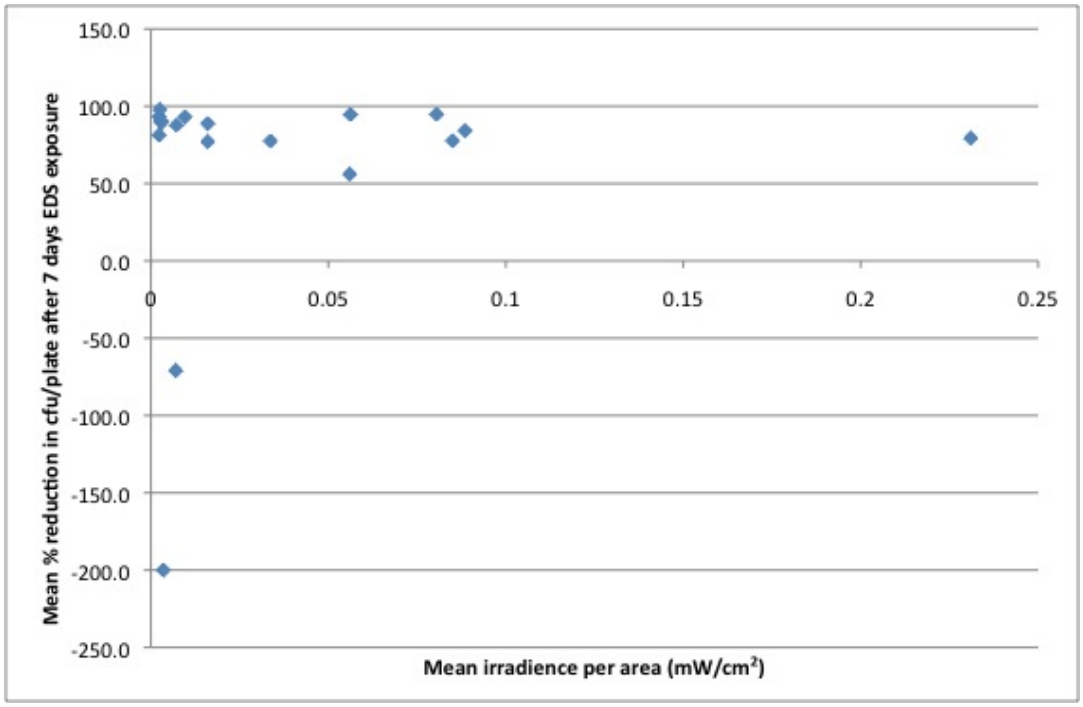


Figure 5.21: *Scatter graph illustrating the mean % reduction following seven days' exposure to the HINS-light EDS at each sampling site, correlated with the mean irradiance at each sampling site.*

The results for the mean % bacterial reduction on each site at each of the three exposure times are summarised in Table 5.13.

The very weak correlation between % reduction in bacterial cfu and amount of irradiance received at any one site, and high p-values demonstrate that there is little to no correlation between the irradiance at a site and the decontamination effect at that site.

Discussion

This single study provides the evidence for the first time that the HINS-light EDS has a significant effect on airborne, in addition to surface-precipitated bacteria. Simultaneous evaluation of % bacterial reduction and the irradiance at each sampling site demonstrated that no correlation was found between the two. This is perhaps unsurprising, as the variation in irradiance is small (between 0.0000023 W/cm^2 and 0.000231 W/cm^2). Conversely, the variation in exposure time (in seconds) is much greater during studies that take place over several days.

Table 5.13: *Mean irradiance and % reduction following 2, 4, and 7-day uses of a single HINS-light EDS, at each sampling site.*

Area	*Mean irradiance (mW/cm^2)*	*Mean % reduction after 2 days*	*Mean % reduction after 4 days*	*Mean % reduction after 7 days*
Door area	0.0030	86.4	75.8	89.4
EDS power supply box near door	0.0023	-5.2	39.0	93.5
Chair	0.0070	-1258.2	-205.5	-70.9
Upper ledge near light	0.0023	63.6	67.1	81.4
Upper ledge far from light	0.0160	75.4	65.1	88.8
Lower ledge near light	0.0027	52.9	69.3	90.8
Lower ledge far from light	0.0337	81.0	76.9	77.6
TV	0.0035	66.7	-233.3	-200.0
Left bed rail	0.0096	90.8	91.2	93.2
Right bed rail	0.0562	84.5	94.8	94.7
Top bed rail	0.0160	45.7	77.1	77.1
Locker top	0.2310	70.1	66.0	79.4
Bedside table	0.0072	82.6	57.0	87.8
Drip	0.0025	87.5	83.3	97.9
Toilet door	0.0885	84.2	82.5	84.2
EDS power supply box near window	0.0805	76.7	79.1	94.8
Bins	0.0850	52.1	-4.1	77.7
SInk	0.0560	51.5	27.3	56.1
Mean % reduction		**-6.2**	**33.8**	**60.7**
Pearson correlation of mean irradiance and mean % reduction		**0.146**	**0.177**	**0.171**
P value (significant)		**0.562 (no)**	**0.482 (no)**	**0.497 (no)**

The fact that a uniform decontamination effect is demonstrated on surfaces throughout the room supports the theory of airborne inactivation of bacteria creating a decreased airborne density and hence less recoverable bacteria are precipitated onto surfaces. The irradiance received by bacteria suspended in the air, in closer proximity to the HINS-light EDS than those bacteria on surfaces, also increases the dose. Moreover, the small particle size of airborne bacteria in such circumstances means they remain airborne for a sufficient time for decontamination to occur. The time taken for particles to precipitate onto surfaces following dispersal into the environment has been categorised as follows:

- particles of 1-3μm diameters remain suspended almost indefinitely;
- particles of 10 μm remain suspended for 17 min;
- particles of 20 μm remain suspended for 4 min;
- and particles of 100 μm take just 10 sec to fall to the floor.[388]

The median particle size dispersed by burns patients has been found to be between 3.5 μm and 5.6 μm, indicating a great potential for airborne spread of microorganisms from burns patients.[54] Airborne particles that precipitate onto surrounding environmental surfaces may then be mechanically disturbed again and made airborne, or picked up by direct contact, by HCW or patients. Otherwise they will remain on the surface due to greater attractive forces and low airflow following sedimentation. This would explain why even if the effect was airborne, it is demonstrated by contact plate sampling of surfaces.

There are multiple implications from the demonstration that the HINS-light EDS elicits a significant decontamination effect on airborne bacteria, causing fairly uniform decontamination on surfaces around the room. It helps to explain the similar decontamination effects seen in the studies using a single-HINS light EDS versus two EDS units in the previous section, where theoretically only one side of the room was 'directly' illuminated but the decontamination effect was evident throughout the room. The low correlation between the bacterial reductions and irradiance received at each sampling site also suggests that the position of the light may not be so crucial, as long as there is air circulating throughout the room. There is also the potential for periods of 'boosted' HINS-light treatment during high-risk activities (such as bed-making and dressing changes), where large amounts of airborne bacteria are dispersed and precipitated onto surfaces and HCW are present. These aspects will be discussed further in Chapter 7.

5.8 Site to site variation

Throughout this chapter, results have been reported as the mean cfu/plate, with the raw cfu counts from each contact plate being averaged to give the mean effect across the room during each sampling phase. The results from each study tended to be represented by a 'V' shaped curve seen over the course of *pre-, during-* and *post-HINS* sampling, with an overall mean decrease in bacterial contamination observed across the room during use of the HINS-light EDS. In addition to assessing the mean effect around the isolation room, another aspect to be investigated was whether this decontamination effect was evident on each of the sampled surfaces around the room. To do this, the results from each sampled surface were averaged to get the level of bacterial contamination on each surface before, during and after use of the HINS-light EDS.

Table 5.14: *Results for 18 sampling areas pre-, during-, and post-HINS following use of a single HINS-light EDS for seven days.*

Area	*Pre-HINS* mean cfu/ plate	*During-HINS* 7 days mean cfu/plate	*Post-HINS* mean cfu/plate
Door area	33.0	3.5	2.8
Power supply box near door	19.3	1.3	6.5
Chair	13.8	23.5	143.8
Upper ledge near light	46.7	8.7	11.0
Upper ledge far from light	42.0	4.7	21.0
Lower ledge near light	46.7	4.3	37.0
Lower ledge far from light	49.0	11.0	15.6
TV	1.5	4.5	6.5
Left bed rail	93.6	6.4	55.4
Right bed rail	168.8	9.0	22.4
Top bed rail	17.5	4.0	45.5
Locker top	24.3	5.0	11.8
Bedside table	54.7	6.7	7.2
Drip	24.0	0.5	31.5
Toilet door	14.3	2.3	8.3
Power supply box near window	43.0	2.3	9.5
Bins	30.0	6.8	13.3
Sink	11.0	4.8	8.5

For illustrative purposes, an example has been given, using the results from the 7-day study carried out using a single HINS-light EDS, reported in Section 5.6. As previously described, all 70 sampling sites were grouped into 18 sampling areas, outlined in Table 5.13. The mean cfu/plate for each of the 18 sampling sites is reported in Table 5.14.

The results of this are displayed in graphical form in Figure 5.22. Here it is evident that the majority of the 18 sampling sites display the familiar 'V' shaped curve that has been

observed previously when a mean of all samples is displayed. However, the variation between sampling sites is also seen, with some outlying results (e.g. 'right bed rail') observed. These variations between sampling sites are taken into account when carrying out statistical analysis, and when taking the mean bacterial cfu/plate for all 70 sampling sites.

Overall, these results display the site to site variation in bacterial contamination across the isolation room, but also demonstrate that the successful decontamination effect of the HINS-light EDS is achieved when looking at each of the different sampled surfaces around the room.

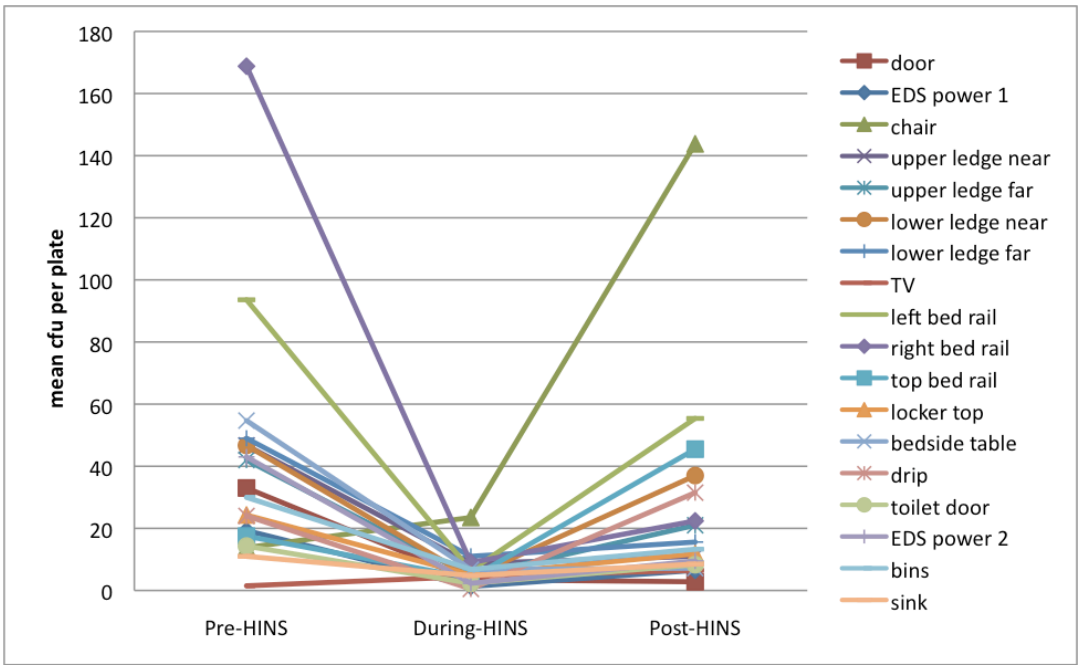


Figure 5.22: *Scatter graph illustrating the mean cfu/plate on all 18 different sampling areas pre-, during- and post HINS.*

5.9 Conclusions

This chapter has examined the effect of the HINS-light EDS in the clinical environment of a burns unit inpatient facility. Considering what is known about high rates of transfer and cross-contamination between burns inpatients, it is important to identify the HINS-light EDS as a reproducible, dependable and workable adjunct to current infection control measures for reducing environmental contamination amongst these highly vulnerable patients. Studies began by identifying the most suitable time for the collection of samples as being early morning, around 0800h. This was thought to be due to the preceding period of rest overnight, when bacteria around the room were able to settle following the activity of the day before. The study was repeated on two further patients, with similar results achieved, addressing reproducibility.

The study protocol was then developed to investigate the effect of extending the operation period from two consecutive days (14 h a day), to up to seven consecutive days. This demonstrated a cumulative decontamination effect when environmental samples were collected at multiple points throughout the period of EDS operation in a patient's room. Further development of the study protocol showed that somewhat surprisingly, similar levels of decontamination were achieved when one EDS was used in an isolation room, rather than two as had been used previously. This raised the possibility that the decontamination effect plateaus, and even with use of two EDS units, no more inactivation is seen with use of two units compared to one. Also, the irradiance received at any one sampling site was found to not have a direct bearing on the level of decontamination achieved at that site. This theory was addressed in the final section, when the irradiance at each sampling site was recorded alongside the % reduction in bacterial contamination achieved at that site. No correlation was found between the two. Rather, a constant effect was seen whatever the irradiance at that sampling site. This suggests a uniform effect, possibly taking place on airborne bacteria, which are then dispersed evenly throughout the room, may be taking place.

The results of the studies are summarised in the Table 5.14.

Table 5.14: *Summary of HINS-light EDS inpatient studies % reductions in environmental contamination achieved.*

Section	Study	No of EDS & period of operation	% Reduction	Significant effect	Conclusion
5.3	0800 h sampling (Patient A)	2 days, 2 EDS	40%	yes	The best time for consistent sampling is 0800 h
5.3	1500 h sampling (Patient A)	2 days, 2 EDS	38%	no	
5.3	2200 h sampling (Patient A)	2 days, 2 EDS	34%	no	
5.4	2 day study (Patient B)	2 days, 2 EDS	87%	yes	Reproducible results can be achieved with different patients in different rooms
5.4	2-day study (Patient C)	2 days, 2 EDS	22%	no	
5.5	5 day study (Patient D)	5 days, 2 EDS	76%	yes	Longer exposure periods create greater decontamination effects
5.5	6 day study (Patient E)	6 days, 2 EDS	70%	yes	
5.5	7 day study (Patient F)	7 days, 2 EDS	89%	yes	
5.6	Single light study (Patient G)	7 days, 1 EDS	86%	yes	Comparable results can be achieved with a single EDS as with two
5.6	Single light study (Patient H)	7 days, 1 EDS	74%	yes	
5.6	Single light study (Patient I)	7 days, 1 EDS	22%	no	

Of note, this work took place over a three year period, and involved nine inpatients who were exposed to the HINS-light EDS for a minimum of two days, as well as the HCW caring for them. Although no formal assessment of acceptance was carried out, there was a positive reception by both staff and patients when the principles of the HINS-light EDS technology, and the available results were explained to them. Most patients found the light to be soothing and non-obtrusive, and some asked for its use to be continued after the study was complete, as they preferred it to the usual white-hued room lighting. Two patients asked for a study to be terminated, which was done immediately. Both times were during periods of prolonged recovery and emotional upset. One of these patients claimed the EDS caused her to have headaches, although the light had been on for several days before she mentioned this.

The main limitation of a study using a visible light is the impossibility to blind the trial in any way. When a study is being carried out the light is on for all to see, and although domestic staff and HCW were encouraged to act as usual, there is the possibility that they carried out more frequent or thorough cleaning, through awareness of being observed, even subconsciously. Similarly, when entering a room, it is possible that the blue light served as a visual reminder to visitors and staff to carry out decontamination

techniques and be vigilant with infection control procedures. This possibility cannot really be overcome, although the light was quickly accepted as part of the normal burns unit routine, and one may expect that effect to dwindle as time progresses. The studies are described in this chapter roughly in the chronological order in which they were carried out, and no general decrease in % reductions is seen as time goes on. Furthermore, as this work formed a PhD, all collection and enumeration of environmental samples was performed by SEB, who was not blinded. An independent practitioner, who was not aware of the sampling session from which samples were taken, enumerated previous studies by Maclean *et al*.[3] During these studies, similar results were achieved, validating the effect of the HINS-light EDS in the clinical situation.

Maclean *et al*'s paper, upon whose methods the initial studies in this chapter were based, described a 56% and 62% after a two-day HINS-light EDS exposure.[3] Unsurprisingly, as the systems used were the same to those, this is within the broad range of % reductions achieved following two-day exposures in this chapter (22% to 87%). As a greater number of patients and scenarios were tested in this work, a much broader range was demonstrated over the five two-day studies carried out on three separate patients. In fact, it highlights the variation in both initial contamination levels and response to EDS exposure seen between experiments on different patients. Maclean *et al* demonstrated an increase in decontamination effect seen when exposure time was prolonged by a further three days: an effect that is corroborated by this work.[3] They found a further 30% reduction, producing a total % reduction of 86%. This is in a similar range to the results demonstrated in the 5, 6, and 7-day extended HINS studies here (70% to 89% decrease).

The large variation in % reduction achieved is a result of the huge variation in starting populations found in different studies of different patients in different rooms. For this reason, all experiments adapted an A-B-A model, whereby the same patient remained in the same room throughout, with as similar conditions as possible, except for the intervention of the HINS-light EDS use. Naturally, this limited the duration of exposure that was possible, as it was difficult to guarantee that patients would not change room, be discharged, or undergo a significant alteration in bacterial contamination (e.g. due to wound healing), over a course of more than nine days (the longest continuous examination period.) However, although these studies were limited by these factors, in real life they would be used continuously during daylight hours for an unlimited period of time, thus maintaining low levels of environmental contamination.

Comparison with the other environmental decontamination technologies discussed in Section 2.6, is difficult, due to the different experimental methods used. Clinical studies using gaseous hydrogen peroxide reduced the number of contaminated sites from 39% to 2% of those tested.[155-163] A further study reported a greater than 99% reduction in environmental contamination following a 3 h fogging treatment.[165] These figures

demonstrate that hydrogen peroxide vapour (HPV) is much more efficient than HINS-light at decontamination of the clinical environment. However, as noted in Section 2.6, HPV has the disadvantage of not being useful in the presence of patients, and therefore bring unable to maintain low levels of environmental bacteria. Clinical studies have not been carried out examining environmental using chlorine dioxide and ozone decontamination technologies, due to their toxic by-products making them unpractical in the hospital environment.

Technologies that do have the potential for continuous use include the Inov8 (hydroxyl radical disinfection unit) and essential oil (EO) vapours. The Inov8 system was used in an inpatient isolation room, with levels of airborne, rather than surface-precipitated bacteria being quantified. A reduction of 55% (range -24% to 86%) in the mean air total viable count per plate was found when the Inov8 was used, compared to when it was not used, over eight paired repetitions.[171] Results were similar when settle plates were enumerated. This followed at least 18 h of continuous use of the system before sampling took place. A further study using the Inov8 system demonstrated a reduction in mean colony count on agar settle plates from 99.8 cfu/plate without the Innov8 to 28.3 cfu/ plate with the Innov8 in use for an unstated period of time.[172] These studies demonstrate comparable results to those in this chapter, although there is much less experimental data available at this time. Interestingly, the authors have chosen to focus on enumerating the airborne counts, rather than surface bacteria. As has been previously discussed, it may be that a high proportion of decontamination activity takes place on airborne bacteria, but we would still favour the contact plate method of sampling precipitated bacteria. The authors commented on the large variability between counts when using the air sampler, which they contributed partly to human activity within the room, but partly due to their sampling methods allowing the microbacterial quality of the air to vary with individual events. Of note, the correlation between settle plates and air sample results was high ($R = 0.79$).[172] Studies using EO vapours are similarly in an early stage, and no clinical trials have been carried out at this stage, although air sampling in an unoccupied office showed an 89% reduction in airborne bacteria after 15 h of use. There was no control period, and no activity within the room during the study.[185]

Throughout these studies, BPA contact plates were used, isolating staphylococcal-type bacterial likely to originate from a human source. However, Chapter 4 has illustrated, that while the isolation of staphylococci is an acceptable marker for bacterial levels within the clinical environment, the HINS-light EDS has a decontamination effect on a range of both Gram-positive and Gram-negative bacteria. Although similar work could have been carried out using different agars, the availability of contact plates is generally limited to BPA, TSA and blood agar. BPA was preferred over the others, as blood agar and TSA yielded such high counts as to render them uncountable. For comparison, TSA

was used in some subsequent outpatient studies (see Chapter 6), but, as it was found to correlate highly with the number of cfu isolated on BPA, conferred no real advantage to using BPA. Indeed, it was felt that as BPA was selective, results obtained through its use were more likely to reflect activity on relevant pathogens, rather than non-pathogenic environmental commensals. The final concern was that attempted examination of more specific organisms, such as MRSA using a selective agar that supports the growth of that organism would yield relatively low counts, conferring a low statistical power of the study.

This chapter has demonstrated repeatedly the effect of the HINS-light EDS on the environmental reservoir of bacteria surrounding the burns patient. However, no matter how many studies are performed to demonstrate the efficacy of the HINS-light EDS on environmental cleanliness, a major question is whether this reduction in environmental contamination translates into a reduction in colonisation and infection of burns patients. Difficulties in proving altered infection rates account for the paucity of evidence that other established infection control methods and disinfection technologies have achieved reductions in infection rates. Rather, a logical and pragmatic approach has been adopted that a cleaner environment and cleaner hands are likely to result in the transfer of fewer numbers of bacteria to patients, and thus generate fewer infections. The impact of surface disinfection in hospitals cannot be dismissed due to the lack of outcome trials, as hospital acquired infection as an outcome has reasonably low frequency, so any potential trial would suffer from low statistical power.[11,150] The huge numbers of variables in the patients, burns and treatment administered, make obtaining reliable numbers of hospital acquired infections difficult in itself. A chosen 'marker' for nosocomial infection, such as MRSA rates could be used, but even this would probably take several years to obtain statistical significance. A large multi-centre or cross-over trial in two wards, comparable in terms of their size and patient population would be ideal.

Chapter 6

Outpatient clinic studies

6.0 Outline

The previous chapter detailed studies that demonstrated the efficacy of the HINS-light EDS in inpatient single-bed isolation rooms on the burns unit. In these conditions, the HINS-light EDS was shown to significantly reduce levels of environmental contamination around the room. It was decided, following the success of these studies, that attention should be turned to the burns outpatient clinic. This environment has significant differences compared to the inpatient isolation room, not least because of the greater frequency of patients in the room, where up to fifteen burn patients can be seen in the outpatient clinic throughout the course of a day. This high turnover of patients also means that the advantages of a continuous decontamination technology are of particular relevance, because the room cannot be physically cleaned with chemicals between each new patient, as is the case with inpatient rooms. This chapter investigates and discusses the use of the HINS-light EDS technology in the Glasgow Royal Infirmary burns unit outpatient clinic setting.

6.1 Background

This section describes the current practice of management of burns patients as outpatients, and examines the evidence for environmental contamination in the outpatient setting.

Outpatient management of the burn patient

Figures from the US estimate that while there are more than one million burn injuries annually, only approximately 50,000 of these require hospitalisation. This indicates that most burns are minor, and between 80% and 95% of patients can be treated on an outpatient basis, under the auspices of a burn unit, an emergency department or family practice.[389-391] While the volume of burns admissions has remained quiescent for several years, the number of visits to burns outpatient clinics has increased. This has been attributed to a variety of reasons including pressure to decrease healthcare costs, leading to the development of more outpatient-based facilities, including clinics, pre-operative assessment clinics and day-case operations.[389]

Changing attitudes to the management of burns means that increasing numbers of patients are deemed to be fit to be managed as outpatients from the outset, and patients who are admitted are discharged home early with outpatient clinic follow-up. Early discharge reduces the risk of hospital-associated complications, including the development of nosocomial infections and venous thrombo-embolism. Furthermore, the psychological benefits of being home in a familiar environment as soon as possible have been demonstrated.[389] Early discharge gives the added benefit of reducing the number of beds in use in overstretched NHS facilities. As burns clinics become more established, referrals are made directly to the clinic from general practitioners or nurse practitioners, as well as local emergency departments. The burns outpatient clinic involves a multi-disciplinary team of specialist nurses, physiotherapists, psychologists, dietitians, occupational therapists and doctors.

Criteria for management as an outpatient

Following a primary survey, the burn wound is evaluated according to its size, depth, causative mechanism, and site. The decision to treat the patient on an outpatient basis depends on the severity of the burn, pre-existing co-morbidities or trauma sustained, and the home circumstances of the patient, including the concern of psychiatric illness, or non-accidental injury.[389,392] Any suspicion of possible airway injury or smoke inhalation must be excluded.[393] Typical selection criteria include those listed in Table 6.1.

Table 6.1: *Selection criteria for patients suitable for outpatient treatment, adapted from: Sheridan, 2005.*[393]

No question of airway compromise
Wound <10% TBSA in children or 15% TBSA in adults so fluid resuscitation is unnecessary
Patient must be able to take adequate oral fluids
Patient should not have serious burns of face, ears, hands, genitals or feet
Patient should have no circumferential burn
The patient and family must have adequate resources to support an outpatient care plan
Patient should have available transportation to clinic
No suspicion of abuse or self-harm
No surgery for full-thickness burn thought to be necessary

Infection control in the outpatient clinic

The risk of nosocomial infection spread exists in the outpatient setting although to a lesser extent than in the inpatient setting. The high turnover of patients during a clinic means that although some surfaces are wiped down, the room is not physically decontaminated between patients, as it would be following the discharge of a patient from an inpatient bed. This may contribute to an environmental reservoir of pathogenic bacteria. As has been discussed, and will be covered in more detail in Chapter 8, the removal of dressings liberates bacteria into the air and onto surfaces.[52] The majority of burns patients coming to a burns outpatient clinic will require dressing removal for assessment and toilet of their wound, with each patient liberating their own unique mixture of commensal and pathogenic microbes into the environment.

Despite burns patients being some of the most potent dispersers of bacteria and susceptible to infections, little data exists on the risk of cross-contamination in burn outpatient clinics between consecutive patients. Studies carried out in other medical disciplines have highlighted the potential for cross-infection between outpatients and the need for infection control policies to be in place, as they are in the inpatient setting.[393]

Outpatient clinic environments have been shown to harbour bacteria, with environmental swabbing in one outpatient clinic yielding between 44 and 1140 cfu/swab.[188] There have been concerns raised about links to antibiotic resistant organisms and the outpatient clinic environment. Studies focusing on environmental MRSA levels have reported between 7% and 19% of surfaces sampled in emergency departments and outpatient clinics testing positive for MRSA.[98,395] These were cultured from communal sites frequently touched by HCW, including keyboards, chairs and telephones. VRE dispersal in the outpatient setting has also been studied. It has been shown to be dispersed in outpatient clinics by asymptomatic VRE-colonised patients,

resulting in environmental contamination during between 29% and 58% of encounters.[396,397]

6.2 Overview of the burns outpatient clinic

This section describes the burn unit outpatient clinic at GRI and explains the rationale behind studying this particular setting.

The role of the burn outpatient clinic at GRI

Just within the main entrance to the burns unit at GRI, is the burn outpatient clinic (see Figure 5.2 in Chapter 5). This consists of a reception room and office, a nurse practitioner clinic room, an open waiting area with adjacent lavatory, and the outpatient clinic room itself. The primary function of the burn unit outpatient clinic at GRI is of daily nurse-led clinics. Newly referred burns are cleaned and assessed before dressings are applied. Nurses educate patients in the management of their wound or scar including likely healing time. These patients are seen in the clinic until they are healed, or they can self-care. A further role is the continuing management and dressing changes of post-operative patients who have been discharged from the inpatient burns unit following more severe burn injuries. Patients with infected wounds may be managed in the outpatient setting until they are free of infection and suitable for operative intervention.

Patients requiring physiotherapy will also be seen in the clinic, while having a dressing change. The long term follow up of the severely burned patient is imperative.[392] Monthly multidisciplinary team clinics are held with burns surgeons, physiotherapists, psychologists and nurse specialists as appropriate for the assessment and management of the long term sequelae of burn injuries, including uneven pigmentation, hypertrophic scars and contractures. Further management can be provided, including the supply of pressure garments and advice on camouflage make up and elective surgical procedures.

Rationale for studying the outpatient clinic

It became apparent during research into the effect of the HINS-light EDS in the inpatient setting that the outpatient clinic room represented an important potential area for further studies. Not only was it a good example of a communal room in the burns unit, with a constant flow of new patients into and out of the room, but all patients seen in the room would have their wound dressings removed, thus potentially releasing organisms into the surrounding environment, and possibly becoming contaminated with environmental bacteria.

6.3 Materials and general methods

This section describes the methods used during the assessment of the effect of the HINS-light EDS during an outpatient clinic on the burns unit.

Setting

The focus of these studies was on the daily nurse led clinic, carried out in the outpatient clinic room. There is usually a morning or an afternoon clinic each day, with a full-day clinic common on Monday and Thursday. These are run by one of two specialist nurse practitioners. The number of patients seen varies significantly depending on the number of referrals.

The clinic room was laid out as demonstrated in the schematic diagram in Figure 6.1.

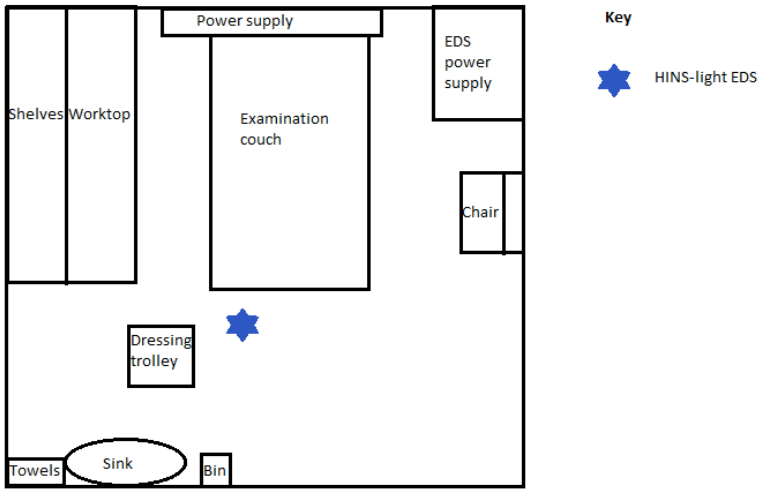


Figure 6.1: *Schematic diagram of the outpatient clinic room*

Cleaning and infection control practices

On entering the clinic room, staff must decontaminate their hands with alcohol gel or soap and water. Disposable gloves and plastic aprons are then donned before approaching the patient. This happens before any contact is made with the patient. Following any interaction with the patient and their environment, personal protective equipment must be removed and hands decontaminated.

The outpatient clinic room is thoroughly cleaned by the domestic staff at the start of the day, before any patients are seen. All surfaces are wiped using Brial Top detergent and

the floor is mopped using Indur Top chlorine based detergent (both ECOLAB Ltd, England). In between patients, the counter top, table, couch and anything else that may have been contaminated are wiped down using Tuffie hard surface disinfectant wipes (Vernacare Ltd, England) by the nurse running the clinic. The dressings and equipment used by the nurses are left out on shelves above the level of the patient couch.

Environmental sampling

As with the studies in inpatient rooms, Baird Parker agar (BPA) contact plates were used for the majority of studies, as this agar yielded countable cfu counts of staphylococcal-type organisms likely to have originated from a human source. The studies in the outpatient clinic room were also repeated using contact plates of Tryptone Soya agar (TSA), a non-selective agar that permits the growth of the total bacterial bioburden collected from the environmental surfaces. This was useful as it enabled the efficacy of the HINS-light EDS against total bacterial contamination levels to be assessed. A limitation of the use of TSA plates was that they can often yield very high, often uncountable, results. This is because TSA permits the growth of all viable bacterial contamination, not just the staphylococcal-type bacteria which are collected with the BPA plates. Because of this the TSA plates will be collecting both pathogenic and non-pathogenic environmental bacteria, therefore although these results provide a good indication of the overall decontamination capability of the HINS-light EDS, these results may be less relevant to a study of cross-contamination between burns patients.

During studies in the clinic room, fifty sampling sites were identified on frequently touched surfaces around the clinic room (n=50), and these are listed in Table 6.2. Studies were carried out using either BPA or TSA contact plates.

Table 6.2: *Outpatient clinic sampling sites*

Sampling Site	No. samples
waste bin	4
apron dispenser	4
glove dispenser	2
basin area	6
dressings trolley	4
dressings shelves	8
worktop	6
lamp	2
examination couch	6
patient chair	4
power supply	2
light switch	1
door handle	1
TOTAL	50

HINS-light EDS installation

The HINS-light EDS was installed into the ceiling of the outpatient clinic room, as centrally as possible, allowing for the service pipes and wires above the ceiling tiles. Cables were fed along the wall and connected to a power supply in the corner of the room. The EDS was switched on and off at the power supply. The HINS-light EDS *in situ* can be seen below in Figure 6.2.

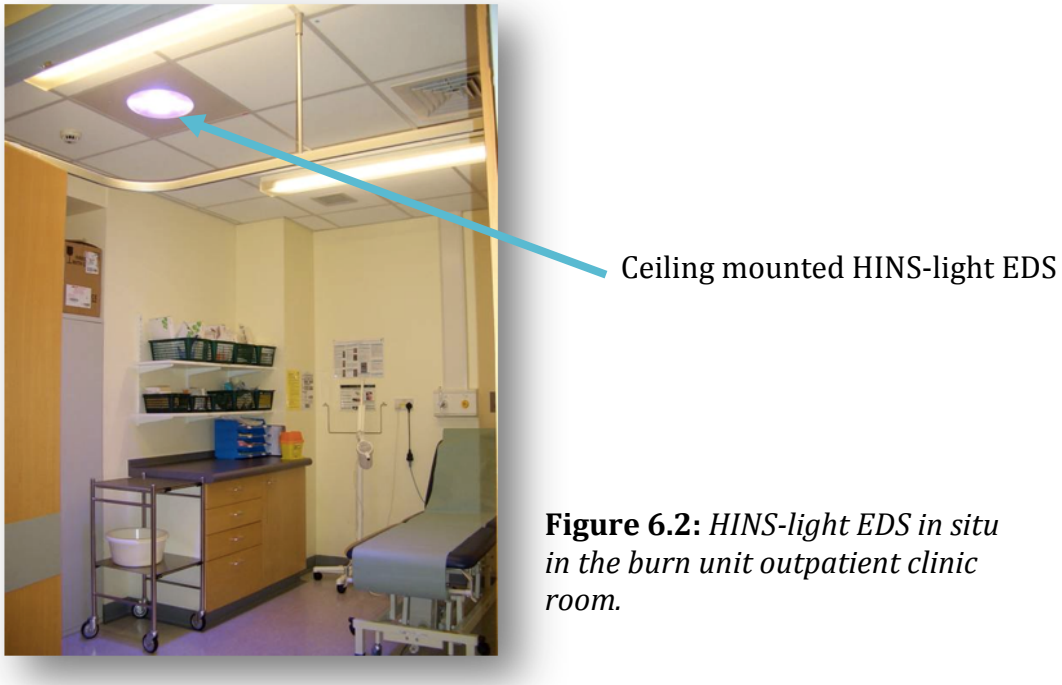


Figure 6.2: *HINS-light EDS in situ in the burn unit outpatient clinic room.*

General methods

Clinics were described as:

- 'control' clinics, where the HINS-light EDS was OFF during the clinic, or
- 'intervention' clinics, where the HINS-light EDS was ON during the clinic.

A member of the domestic staff, as described, cleaned the outpatient clinic room before the start of each study previously. This usually took place around 0730h. *Before clinic* sampling took place between 0800h and 0830h, from the 50 sites detailed above using contact agar plates. When *before-clinic* samples had been taken, the HINS-light EDS was switched on, if it was being used ('intervention' clinics). Following this, a nurse-led burns clinic took place with the first patient seen around 0900h. Between seeing patients, the clinic nurse would disinfect the worktop, dressings trolley, examination couch and any grossly contaminated areas using 'Tuffie' wipes. The nurse adhered to NHS Greater Glasgow and Clyde hand hygiene guidelines.

To ensure fair comparisons could be made between clinics, inclusion criteria were set. These were:

- nurse-led burns clinics of between 7 and 12 patients;
- clinics taking place exclusively in the outpatient clinic room;
- clinics running throughout the whole day from 0900h to 1600h;
- the same domestic assistant cleaning the room prior to the clinic at 0730h ± one hour.

Exclusion criteria were also set and these were:

- clinics with too many or too few patients;
- clinics where appointments were not evenly distributed throughout the day;
- non-burns patients were seen at the burns clinic.

Each patient would be seen individually by the nurse, with or without a family member of friend. The patient would have their dressings removed, their wound examined, usually washed and then redressed.

Clinics usually finished around 1600h. At approximately 1630h *after clinic* samples would then be taken, immediately adjacent to the same fifty sample sites used at the start of the day. Following collection of *after-clinic* samples, the HINS-light EDS was switched off if it had been used. This meant that in 'intervention' clinics the light was switched on continuously for between eight and nine hours. In 'control' studies the

HINS-light EDS remained switched off. Contact plates were incubated at 37°C for the appropriate time and total number of bacterial cfu/plate enumerated.

Clinic protocols

Three separate clinic protocols were followed to compare the increase in environmental contamination seen during the course of a clinic with and without use of a HINS-light EDS. Each 'control' clinic and 'intervention' clinic was carried out twice using BPA plates, and once using TSA plates. The 'no-patient' clinic was carried out once using both BPA and once using TSA contact plates. Thus a total of 8 separate outpatient clinics were studied. The protocols for each type of clinic were as follows:

- **'Control' clinics**: These were carried out to determine the change in levels of environmental contamination (calculated as mean cfu/plate) throughout the course of a typical burn outpatient clinic, without use of the HINS-light EDS. *Before clinic* samples were taken from the 50 sites after the room was cleaned; the clinic was carried out as usual; and *after clinic* samples were then taken. The 'control' clinic was carried out three times in total (BPA 'control' clinics A and B, and TSA 'control' clinic).

- **'HINS intervention' clinics:** These were carried out to determine the change in levels of environmental contamination (calculated as mean cfu/plate) throughout the course of a burn outpatient clinic with the HINS-light EDS switched on. *Before clinic* samples were taken from the 50 sites after the room was cleaned; the clinic was carried out as usual with the HINS-light EDS switched on throughout; and *after clinic* samples were then taken. The 'HINS intervention' clinic was carried out three times in total (BPA 'HINS intervention' clinics A and B, and TSA 'HINS intervention' clinic).

- **'No-patient' clinics**: These were carried out to determine the change in levels of environmental contamination (calculated as mean cfu/plate) during the same time period as a clinic, but without any activity in the room. *Before clinic* samples were taken from the 50 sites after the room was cleaned; the room was closed, and no activity took place within it; and *after clinic* samples were then taken at 1630h. The 'no-patient' clinics were carried out twice in total (BPA 'no-patient' clinic and TSA 'no-patient' clinic).

The sampling method used for outpatient clinic studies is represented diagrammatically below (Figure 6.3).

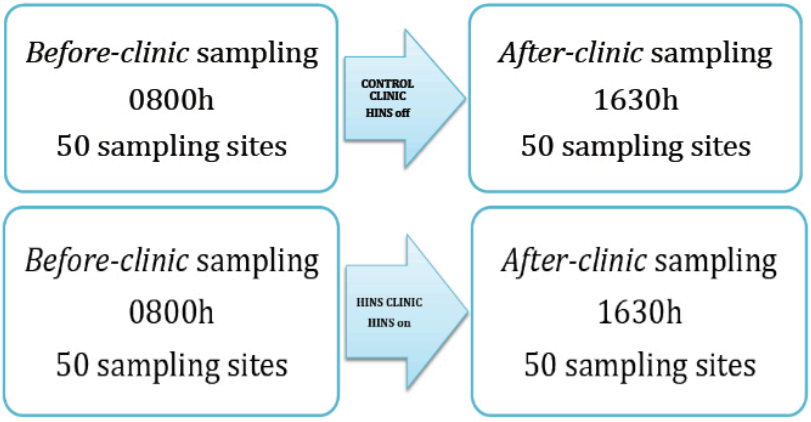


Figure 6.3: *Flow diagram to illustrate the model for outpatient HINS-light EDS studies.*

Statistics

Data was reported as total number of bacterial cfu/plate *before clinic* and *after clinic* for each clinic. The total number of bacterial cfu (i.e. the total number of cfu on all 50 contact plates) and the mean number of bacterial cfu/plate was calculated for each clinic. Increase in environmental contamination observed during the course of a clinic was calculated as follows:

After clinic mean bacterial cfu/plate – *before clinic* mean bacterial cfu/plate

Following this, comparisons were made between the increase in environmental contamination (reported as increase in mean number of bacterial cfu/plate) observed during the course of different clinics. These comparisons were examined with the aim of showing that the increase in mean number of bacterial cfu/plate (i.e. the environmental contamination) of the room produced during the course of the clinic was less when the HINS-light EDS was switched on ('intervention' clinics) than when the HINS-light EDS was switched off ('control' clinic). Comparisons were made as follows:

- Comparison of increase in mean cfu/plate observed during BPA 'control' clinic A with BPA 'HINS intervention' clinic A.
- Comparison of increase in mean cfu/plate observed during BPA 'control' clinic B with BPA 'HINS intervention' clinic B.
- Comparison of combined mean increase in mean cfu/plate during both BPA 'control' clinics A and B and both BPA 'HINS intervention' clinics A and B.
- Comparison of increase in mean cfu/plate during TSA 'control' clinic with TSA 'intervention' clinic.

These comparisons were expressed as the amount of environmental contamination increase observed during the course of a 'HINS intervention' clinic compared with the amount of environmental contamination increase observed during the course of a 'control' clinic. This demonstrated any decrease in the amount of environmental contamination (mean bacterial cfu/plate) produced during the course of a 'HINS intervention' clinic (i.e in the presence of the HINS-light EDS). This was calculated as follows:

increase in mean cfu during 'control' clinic - increase in mean cfu during 'HINS' clinic

Equation 6.1: *Environmental contamination produced during clinic*

These comparisons enabled the % efficacy of the HINS-light EDS for reducing the amount of environmental contamination to be calculated as follows:

$$1- \frac{\text{increase in mean cfu/plate during- 'HINS' clinic}}{\text{increase in mean cfu/plate during 'control' clinic}} \times 100$$

Equation 6.2: *% Efficacy of EDS*

Examination of the 'no-patient' clinic was carried out to demonstrate that any increase in environmental contamination observed throughout the course of a clinic was due to the activity from the patients and nurse in the clinic, and not due to any outside influence such as faulty ventilation systems.

For the purposes of publication, statistical analysis on the studies using BPA contact plates was carried out by Prof George Gettinby.[385] Where available, the statistical significance based on log-transformed data has been quoted as published.

Energy calculations

In order to determine if the power at each site correlated with the reduction in environmental contamination seen at that site, the irradiance the HINS-light EDS received at each site was measured. Irradiance measurements of the 405 nm output of the HINS-light EDS were recorded in mW/cm^2 at each of the 50 sampling sites as illustrated in Figure 6.7. The irradiance received at each sampling site was plotted against the reduction in environmental contamination (measured as bacterial cfu/plate) achieved at that site during an 'intervention' clinic (i.e with the HINS-light EDS on), and examined for evidence of correlation.

6.4 Baird Parker agar (BPA) outpatient clinic studies

This section outlines the methods and results of studies using BPA as a selective medium for studies in the burns outpatient clinic.

BPA methods

As described above, environmental sampling was carried out using BPA contact plates in five separate clinics. BPA 'control' clinics and 'intervention' clinics were carried out twice, and a 'no-patient' clinic was carried out once. Statistical analysis was performed as described above, with additional parametric analysis of variance (ANOVA) of log-transformed data [calculated by Prof Gettinby] for publication purposes are also detailed.

BPA clinic results

All five BPA clinic studies are summarised in the table below, alongside the number of patients seen at each clinic.

Table 6.3: *Summary of outpatient clinic studies performed using BPA contact plates.*

BPA clinic	No. patients seen	HINS-light EDS
BPA 'control' clinic A	7	off
BPA 'control' clinic B	11	off
BPA 'intervention' clinic A	7	on
BPA 'intervention' clinic B	9	on
BPA 'no-patient' clinic	0	off

Mean results from across the fifty sampling sites *before clinic* and *after clinic* for each of the five clinics are summarised in Table 6.4.

The raw counts on the BPA contact plates collected before and after each clinic on each of the 50 sampling sites can be found detailed in Appendix B

Table 6.4: *Five studies using BPA to measure environmental contamination (measured as bacterial cfu/plate) produced during a burns outpatient clinic. 'Control' clinics were with HINS-light EDS off, 'intervention' clinics were with HINS-light EDS on, and the 'no-patient' clinic was with the HINS-light EDS off and no patients.*

Clinic	BPA 'Control' clinic A	BPA 'Control' clinic B	BPA 'Intervention' clinic A	BPA 'Intervention' clinic B	BPA 'No patient' clinic
Before-clinic mean cfu/plate	8.0	8.3	2.2	10.8	8.1
After-clinic mean cfu/plate	21.6	22.8	6.5	17.5	6.8
Mean cfu increase during clinic	13.6	14.5	4.3	6.7	-1.3

The mean increase in bacterial cfu/plate observed across the 50 sampling sites during the course of each clinic was calculated and comparisons between BPA 'control' clinics and BPA 'intervention' clinics were made. These comparisons are briefly discussed below.

1. Comparison of increase in mean bacterial cfu/plate observed during the course of BPA 'control' clinic A and BPA 'intervention' clinic A

The increase in environmental contamination that occurred during the course of BPA 'control' clinic A, measured as mean bacterial cfu/plate, was 13.6 cfu/plate. By comparison, the increase in mean cfu/plate arising during the course of BPA 'intervention' clinic A was 4.3 cfu/plate. The overall efficacy of the HINS-light EDS in reducing the average cfu count during a clinic was therefore 9.3 cfu/plate compared with 13.6 cfu/plate, which is an efficacy of 68.4%. This was found to be significant on analysis for publication ($p=0.015$).[385]

These interactions can clearly be seen on interaction plots comparing the mean number of bacterial cfu isolated during BPA 'control' clinic A and BPA 'intervention' clinic A (Figure 6.4).

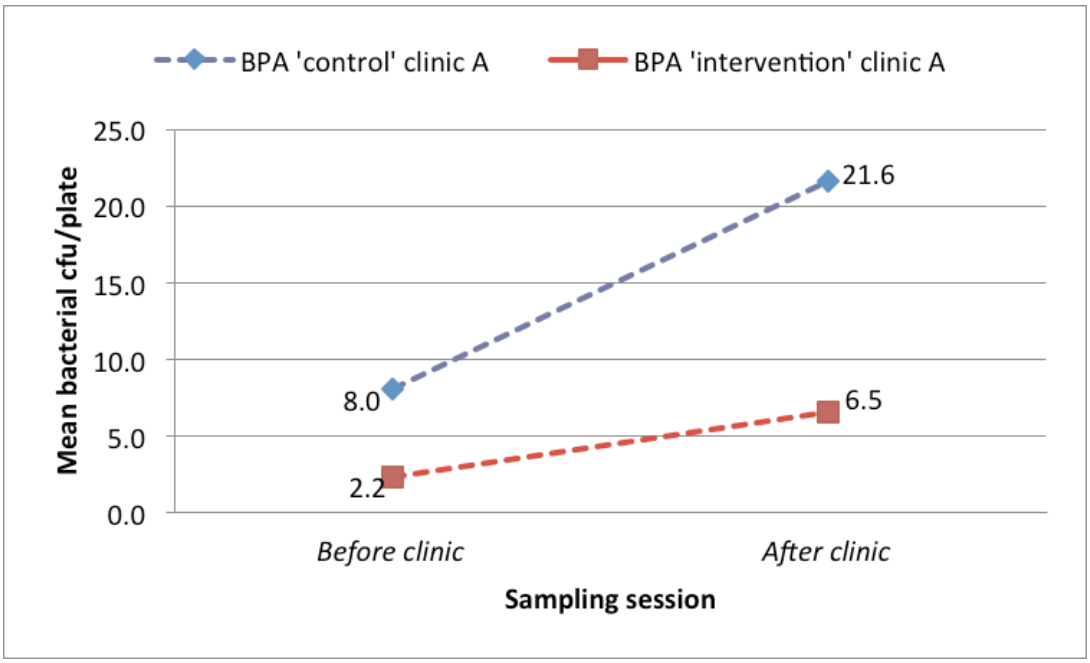


Figure 6.4: *Interaction plot based on the mean bacterial cfu/plate collected on BPA contact plates before and after an outpatient clinic, with (intervention clinic A) and without (control clinic A) use of the HINS-light EDS.*

2. Comparison of increase in mean bacterial cfu/plate observed during the course of BPA 'control' clinic B and BPA 'intervention' clinic B

The increase in environmental contamination that occurred during the course of BPA 'control' clinic B measured as mean bacterial cfu/plate, was 14.5 cfu/plate. By comparison, the increase in mean bacterial cfu/plate arising during the course of BPA 'intervention' clinic B was 6.9 cfu/plate. The overall efficacy of the HINS-light EDS in reducing the average bacterial cfu count during a clinic was therefore 7.6 cfu/plate compared with 14.5 cfu/plate, which is an efficacy of 52.4%. This was found not to be significant (p=0.212) on statistical analysis for publication.[385]

These interactions can be seen on interaction plots comparing bacterial cfu isolated during BPA 'control' clinic B and BPA 'intervention' clinic B (Figure 6.5).

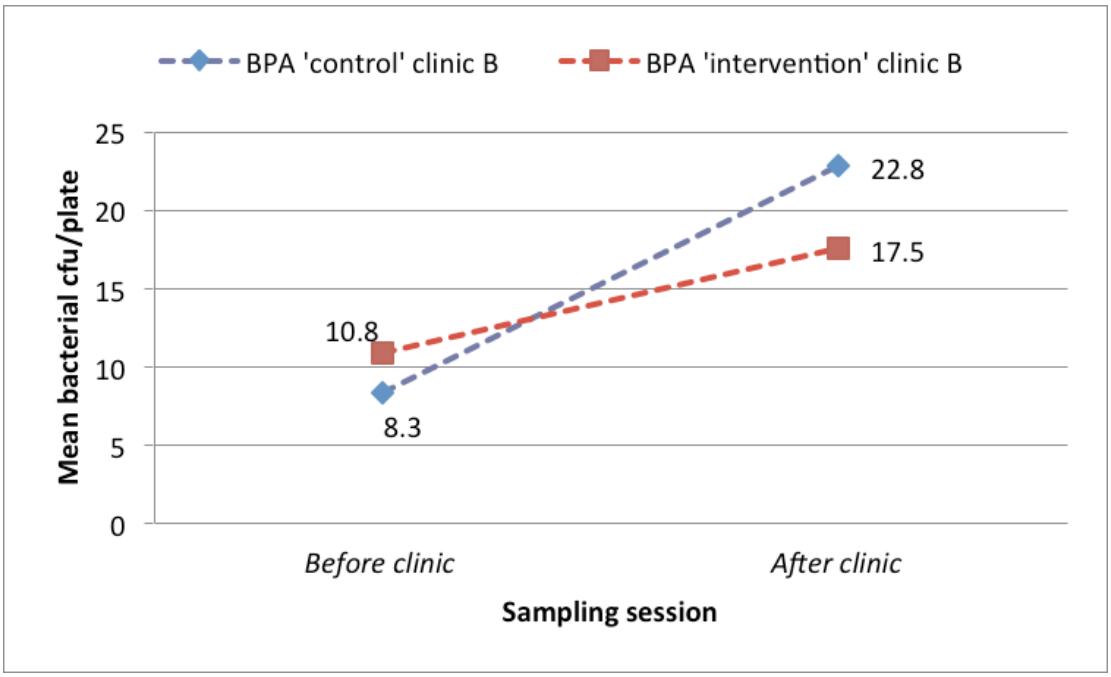


Figure 6.5: *Interaction plot based on the mean bacterial cfu/plate collected on BPA contact plates before and after an outpatient clinic, with (intervention clinic B) and without (control clinic B) use of the HINS-light EDS.*

3. Comparison of combined average increase in mean bacterial cfu/plate during both BPA 'control' clinics A and B, and both BPA 'intervention' clinics A and B

The mean increase in environmental contamination that occurred during the course of both BPA 'control' clinics A and B was 14.0 cfu/plate. By comparison, the average increase in mean bacterial cfu/plate arising during the courses of BPA 'intervention' clinics A and B was 5.5 cfu/plate. The overall efficacy of the HINS-light EDS in reducing the average count in a comparison between the BPA 'control' clinics and BPA 'intervention' clinics was therefore 8.5 cfu/plate compared with 14.0 cfu/plate, which is an efficacy of 60.7%. This was found to be significant on statistical analysis for publication ($p=0.020$).[385]

These interactions can clearly be seen on interaction plots comparing the mean bacterial cfu/plate released during BPA 'control' clinics A and B, and BPA 'intervention' clinics A and B (Figure 6.6).

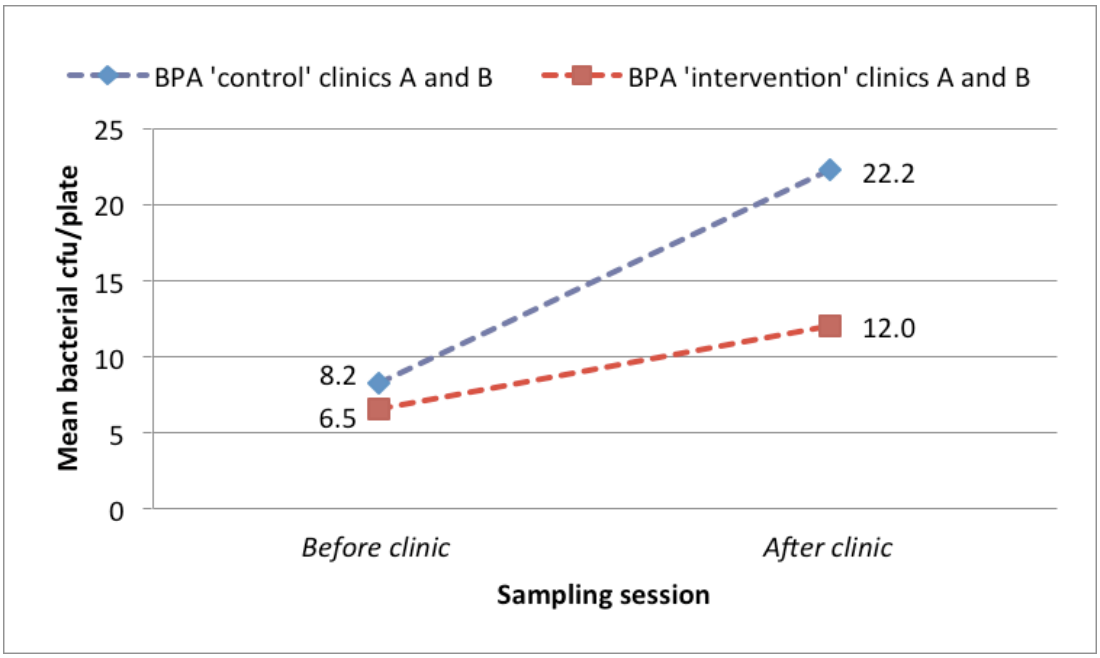


Figure 6.6: *Interaction plot based on mean bacterial cfu/plate collected on BPA contact plates before and after an outpatient clinic, with (intervention clinic) and without (control clinic) use of the HINS-light EDS. Results represent the combined results of Clinics A & B.*

4. Comparison of mean bacterial cfu/plate *before clinic* and *after clinic* in the BPA 'no-patient' clinic

A slight decrease in environmental contamination was observed during the course of the 'no patient' clinic, with a reduction in the mean bacterial count of 1.3 cfu/plate. On analysis for publication, this was found to not be significant (p=0.066).[385]

5. Correlation between the reduction in mean bacterial cfu/plate during 'intervention' clinics and dose received at each sampling site

For both BPA 'intervention' studies A and B, a scatter graph was produced in order to determine if there was any correlation between dose and the level of decontamination achieved at each of the 50 sampling sites, and this is shown in Figure 6.7. Results demonstrated there was poor correlation between the irradiance received at any one site, and the level of decontamination achieved at that site, by the HINS-light EDS.

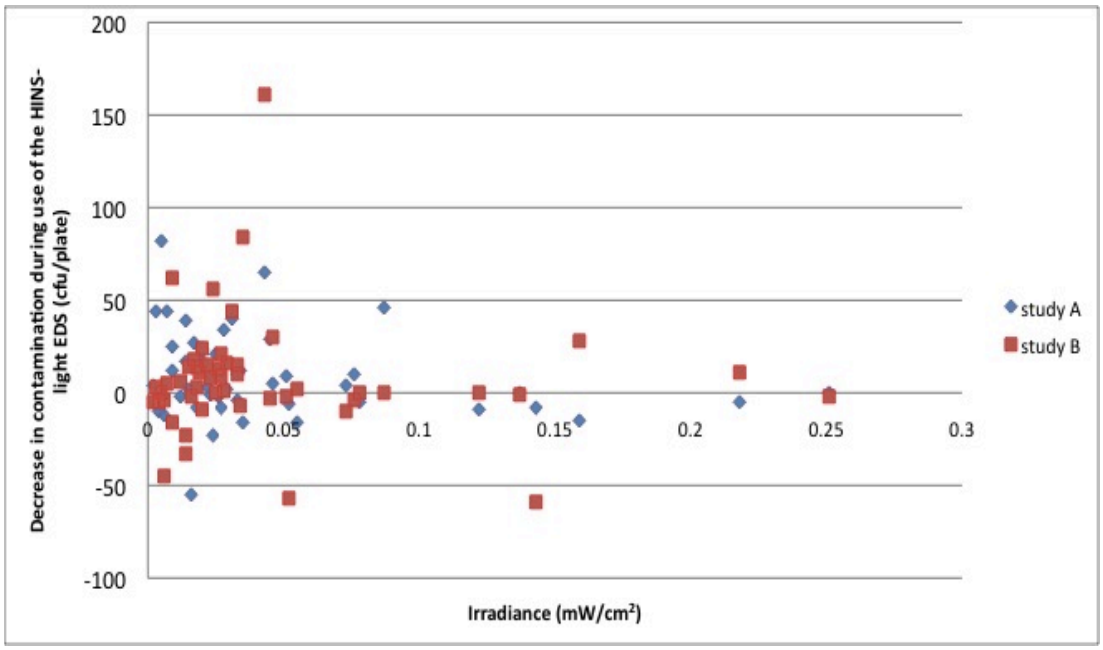


Figure 6.7: *Scatter graph showing the reduction in environmental staphylococcal-type contamination at each of 50 sampling sites as a function of dose, and reduction in environmental contamination at that site during BPA 'intervention' periods A and B (y axis).*

Summary of BPA outpatient clinic results

A summary of all results and calculations for outpatient studies using BPA contact plates is found in the table below.

Table 6.5: *Summary of results of BPA studies in the outpatient clinic. Results are expressed as mean bacterial cfu/plate, with changes in environmental contamination produced during the course of each clinic calculated. BPA 'control' and 'intervention' clinics are compared to show the effect of the HINS-light EDS in terms of mean cfu/plate and % efficacy.*

Clinics	Reduction in cfu increase during 'intervention' clinic	% Efficacy of HINS-light EDS reducing cfu increase
BPA 'control' clinic A vs BPA 'intervention' clinic A	9.3	68.4
BPA 'control' clinic B vs BPA 'intervention' clinic B	7.6	52.4
BPA 'control' clinics A and B vs BPA 'intervention' clinics A and B	8.5	60.7

6.5 Tryptone soya agar (TSA) outpatient clinic studies

This section outlines the methods and results of studies using TSA as a broad-spectrum bacterial medium for studies in the burns outpatient clinic.

TSA methods

As described earlier, the environmental contamination of three separate clinics was assessed using TSA contact plates. A TSA 'control' clinic, a 'HINS intervention' clinic, and a 'no-patient' clinic were carried out once each. Statistical analysis was performed as described above with no additional parametric ANOVA carried out for publication purposes.

TSA clinic results

All three TSA studies are summarised in the table below, alongside the number of patients seen at each clinic.

Table 6.6: *Summary of outpatient clinic studies performed using TSA-contact plates*

TSA clinic	No. patients seen	HINS-light EDS
TSA 'control' clinic	12	off
TSA 'intervention' clinic	10	on
TSA 'no-patient' clinic	0	off

Mean results of the bacterial contamination from across the fifty sampling sites *before clinic* and *after clinic* for each of the three clinics are summarised in Table 6.6.

The raw counts on the BPA contact plates collected before and after each clinic on each of the 50 sampling sites can be found detailed in Appendix B.

The mean increase in bacterial cfu/plate observed during the course of each clinic was calculated and comparisons between TSA 'control' clinics and TSA 'intervention' clinics were made. These comparisons are briefly discussed below.

Table 6.7: *Five studies using TSA to measure environmental contamination (measured as bacterial cfu/plate) produced during a burns outpatient clinic. 'Control' clinics were with HINS-light EDS off, 'intervention' clinics were with HINS-light EDS on, and the 'no-patient' clinic was with the HINS-light EDS off and no patients.*

Clinic	TSA 'Control' clinic	TSA 'Intervention' clinic	TSA 'No patient' clinic
Before-clinic mean cfu/plate	14.1	20.3	14.5
After-clinic mean cfu/ plate	22.0	14.8	12.4
Mean cfu increase during clinic	7.9	-5.5	-2.1

1. Comparison of increase in mean bacterial cfu/plate observed during the course of TSA 'control' clinic and TSA 'intervention' clinic

The increase in environmental contamination that occurred during the course of the 'control' clinic, measured as mean bacterial cfu/plate, was 7.9 cfu/plate. In contrast, a decrease in mean bacterial cfu/plate of -5.5 cfu/plate was observed during the course of the 'intervention' clinic. The overall efficacy of the HINS-light EDS in reducing the average total bacterial contamination count in a comparison between TSA 'control' and 'intervention' clinics was therefore 13.4 cfu/plate compared with 7.9 cfu/plate, which is an efficacy of 170%.

These interactions can clearly be seen on interaction plots (Figure 6.8) comparing the mean bacterial cfu/plate released during the TSA 'control' clinic and TSA 'intervention' clinic.

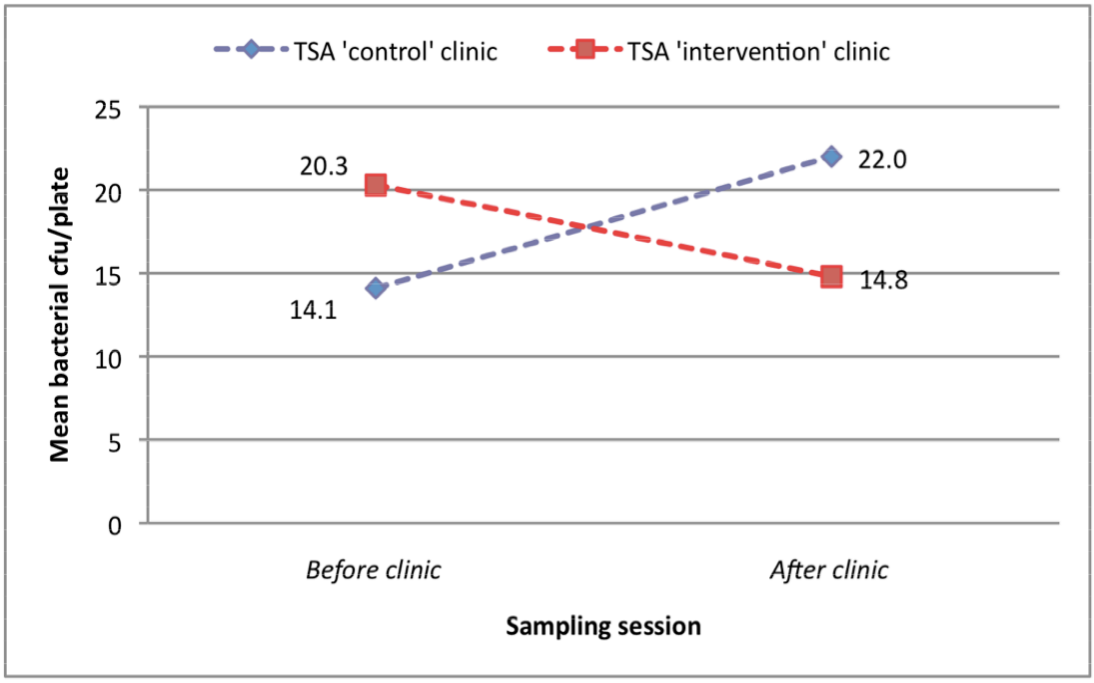


Figure 6.8: *Interaction plot based on the mean bacterial cfu/plate collected on TSA contact plates before and after an outpatient clinic, with (intervention clinic) and without (control clinic) use of the HINS-light EDS.*

2. Comparison of mean bacterial cfu/plate *before clinic* and *after clinic* in the TSA 'no-patient' clinic

A slight decrease was seen in the total environmental bacterial contamination around the room during the course of the 'no-patient' clinic, with a mean bacterial reduction of -2.1 cfu/plate.

A summary of all results and calculations for outpatient studies using TSA contact plates is found in Table 6.8.

Table 6.8: *Summary of results of TSA studies in the outpatient clinic. Results are expressed as mean bacterial cfu/plate, with changes in environmental contamination produced during the course of each clinic calculated. TSA 'control' and 'intervention' clinics are compared to show the effect o the HINS-light EDS in terms of mean cfu/plate and % efficacy.*

Clinics	Reduction in cfu increase during 'intervention' clinic	% Efficacy of HINS-light EDS reducing cfu increase
TSA 'control' clinic vs TSA 'intervention' clinic	13.4	170

6.6 Discussion and conclusions

Although there are no burns-specific outpatient clinic studies, results of work carried out in other outpatient clinics has highlighted the potential for cross-contamination to occur between consecutive patients, with the environment acting as a reservoir of infection.[98,188,394-397] High turnover of patients compared to the inpatient setting means that physical disinfection of all surfaces within the room is impractical given the ever-increasing volumes of patients seen during a single clinic. The continuous environmental decontamination provided by the HINS-light EDS is of particular value in this instance.

As inpatient clinical studies on the burns unit had taken place over the course of several consecutive days, it was unclear whether an eight-hour HINS-light treatment period would be sufficient to achieve enhanced environmental cleanliness. Furthermore, any decontamination effect would have to be achieved over and above that conferred by the disinfection of a limited number of surfaces between each patient that is carried out by the clinic nurse, which continued throughout all studies.

BPA was the main agar used for these studies, based on previous work in the inpatient setting. The outpatient clinic room had a limited number of sampling sites on frequently touched surfaces. Fifty sampling sites were chosen, on a range of surfaces both directly and indirectly exposed to the HINS-light EDS. Results showed that there was no correlation between the irradiance received at any one sampling site, and the amount of decontamination achieved at that site. This suggests a uniform decontamination effect, with a role for both airborne and surface decontamination, as highlighted in Chapter 5.

It was expected that the clinic room would be more heavily contaminated at the end of the clinic (*after clinic* samples) than it had been at the start of the clinic (*before clinic* samples). Indeed when BPA was used as the sampling media, the mean number of bacterial cfu/plate rose from around 7 cfu/plate to around 22 cfu/plate during the 'control' clinics - an approximately 300% increase. While the HINS-light EDS did not stop this increase in contamination altogether, it certainly reduced it. The 'intervention' clinics had an average increase from 8 bacterial cfu/plate to 12 cfu/plate - an approximately 50% increase.

The first two clinics compared were BPA 'control' clinic A and BPA 'intervention' clinic A. As expected, for both clinics there was an increase in levels of environmental contamination produced during the course of the clinic, but this was significantly less during the 'intervention' clinic, when the HINS-light EDS was in use.

Fifty sampling sites was a relatively low number, and this may have meant the study was somewhat underpowered. This may explain why the amount of environmental

contamination produced throughout the course of BPA 'intervention' clinic B was not significantly less than that produced during the course of BPA 'control' clinic B when statistical tests were carried out for publication. Despite this, there was clearly less of an increase in the amount of environmental contamination produced during the 'intervention' clinic (with the HINS-light EDS on). In fact, even though the room had more environmental contamination before the 'intervention' clinic than it did before the 'control' clinic, it had less environmental contamination after the 'intervention' clinic than it did after the 'control' clinic. The most likely explanation for this in light of the other studies is that a Type 2 statistical error occurred (i.e. although there was a difference, there were not enough samples to prove this statistically).

The 'no-patient' clinics were carried out to demonstrate that the increase in environmental contamination produced during the course of the clinic was due to dispersal from the patient or nurse and not due to outside influences, such as a faulty air-conditioning unit. There was a small decrease in mean bacterial cfu/plate before the clinic compared with those after the clinic, which may be due to the natural death of desiccated and stressed bacteria on environmental surfaces. As no increase was seen throughout the 'no-patient' clinic, this was not repeated with the HINS-light EDS switched on.

Similar trends were seen when the clinic studies carried out using BPA were repeated using TSA contact plates. TSA contact plates had not been used in the inpatient studies due to the frequency of high, uncountable results on these plates, however due to the level of environmental contamination in the clinic expected to be lower than that of the inpatient isolation rooms, one set of 'control' and 'intervention' clinics were carried out using TSA to sample the environment. Numbers of bacterial cfu isolated on the TSA plates in the clinic studies were of similar levels to those collected on the BPA plates, however, it was felt that as BPA-isolated organisms were staphylococcal type, and therefore more likely to originate from a human source, this continued to be the preferred medium throughout. Although BPA was the preferred sampling medium, the use of TSA proved the principle that the HINS-light EDS reduces the level of total viable bacterial contamination, not just the staphylococcal-type bacteria. As previously discussed in Chapter 5, the HINS-light EDS has been shown to be effective against a range of organisms in laboratory conditions, but for sampling purposes, BPA was used.

The nature of burns clinics means that comparable studies to those described in Chapter 5 are not possible. There is no means of carrying out the same clinic with exactly the same patients three times. Often patients are seen very sporadically at clinic, or discharged after their first visit. It has been the primary aim throughout this work to examine the effect of the HINS-light EDS in as similar circumstances as may be found on the burns unit as possible. Any sort of mock clinic for the purposes of replication would have gone against this principle. For this reason, the model had to be adapted, with two

different clinics during each study, containing comparable groups of patients, and the HINS-light EDS being the intervention. Studying in an outpatient clinic was also complicated due to the high activity levels creating a generalised increase in levels of environmental bacteria as the day progresses: each patient may be expected to release bacteria into the air and environment so the levels on environmental surfaces increase throughout the course of the day. This is in direct contrast to the inpatient studies detailed in Chapter 5. Here, samples (that take place at the same time of day for any one study) are expected to have reached a plateau as the same patient has always been in that room for a minimum of two days before the study starts. Indeed the *pre-HINS*; *during-HINS*; *post-HINS* protocol relies on the assumption that there is a reasonably constant level of bacteria in a room without the HINS-light intervention. The opposite is true for outpatient clinics, which, during 'control' clinics have displayed an increase in environmental contamination levels throughout the course of the clinic. Instead, the % efficacy of the EDS in reducing this increase is examined. When compared with the % reductions achieved in Chapter 5, the results in the outpatient clinic support those described in Chapter 5, with between 52% and 170% efficacy demonstrated in reducing the expected increased environmental contamination. This is all the more notable for being achieved within 8 h, compared with the inpatient studies taking place over several days.

Previous studies of outpatient clinics have usually examined for specific bacteria. They have revealed relatively low levels of environmental contamination: 0% contamination rate for MRSA in an HIV clinic;[395] 4% contamination rate with *Staphylococcus aureus* in a dental clinic;[398] 29% rate of samples positive for vancomycin resistant enterococcus (VRE) in a cancer clinic;[396] between 36% and 48% of chair and couch cultures being positive for VRE in mock outpatient consultations.[397] No studies were found detailing the rate of environmental contamination in a burns outpatient clinic, nor any studies of an environmental decontamination method in an outpatient setting. These studies are the first using the HINS-light EDS in an outpatient setting. Of note, in this work, examination of the two burns clinics held without the addition of the HINS-light EDS, revealed that just 5/100 (0.5%) samples had no growth, indicating the high level of environmental contamination produced when caring for burns patients compared with other groups.

As the HINS-light EDS unit installed was a prototype, and newer models were being created during the course of the study, no official data was recorded on the opinion of the patients about the presence or aesthetic effect of the HINS-light EDS during clinic. Informal discussion with the clinic revealed that none of the 56 patients seen at clinic during the studies commented on its presence at all. The nurses did not find any problem with having the HINS-light EDS switched on, and found it easy to use. One nurse preferred to switch it off while she assessed the depth of the burn, due to

concerns that her perception of colour may be impaired, but she did not think this was a problem as the unit was easy to use. Although studies finished over a year ago at the time of writing, the clinic nurses have continued to use the HINS-light EDS due to the successful results obtained.

In conclusion, the studies performed in the outpatient burns clinic demonstrated that the addition of a HINS-light EDS throughout the course of a burn outpatient clinic reduced the amount of environmental contamination produced from activity within the clinic. This was over and above that achieved by current infection control practise.

Chapter 7

Contribution of nursing 'events' to the cycle of cross-contamination

7.0 Outline

An essential component when evaluating a decontamination system for use in the burns unit is the thorough understanding of the cycle of cross-contamination between patients. Established principles have been discussed in Chapter 2, but ambiguities remain. In order to address these, experiments were performed to demonstrate both airborne and direct contamination of healthcare workers (HCW) during pertinent high risk 'events' that take place on a daily basis within any burns unit. The main events highlighted were dressing changes, often in combination with bed sheet changes.

This chapter is divided into sections that aim to help gain a further understanding of the cross-contamination cycle. Firstly the development of a model for quantifying levels of airborne bacteria released during events is outlined. This is followed by a series of 'control charts' to demonstrate periods of significantly increased levels of airborne bacteria. Investigations then progress to determine the direct contamination received by HCW performing dressing/bed changes on burns patients, and a mathematical model is developed to predict the level of contamination a HCW is likely to receive when partaking in these events. The effect of carrying out the events in the presence of the HINS-light EDS is then considered. Finally a telephone questionnaire of the current practices of UK burns units is reported before the chapter conclusion.

7.1 Background

As discussed in Section 2.4, bacteria can be transferred from patient to patient via the air, the environment or via contaminated HCW. This led to the concept of a cycle of cross-contamination, shown in Figure 7.1. At each stage of the cycle, questions have been raised. Some are answered sufficiently in the current literature. Others have yet to be explored. Where evidence was felt to be lacking, an attempt has been made to rectify this through novel experimental work.

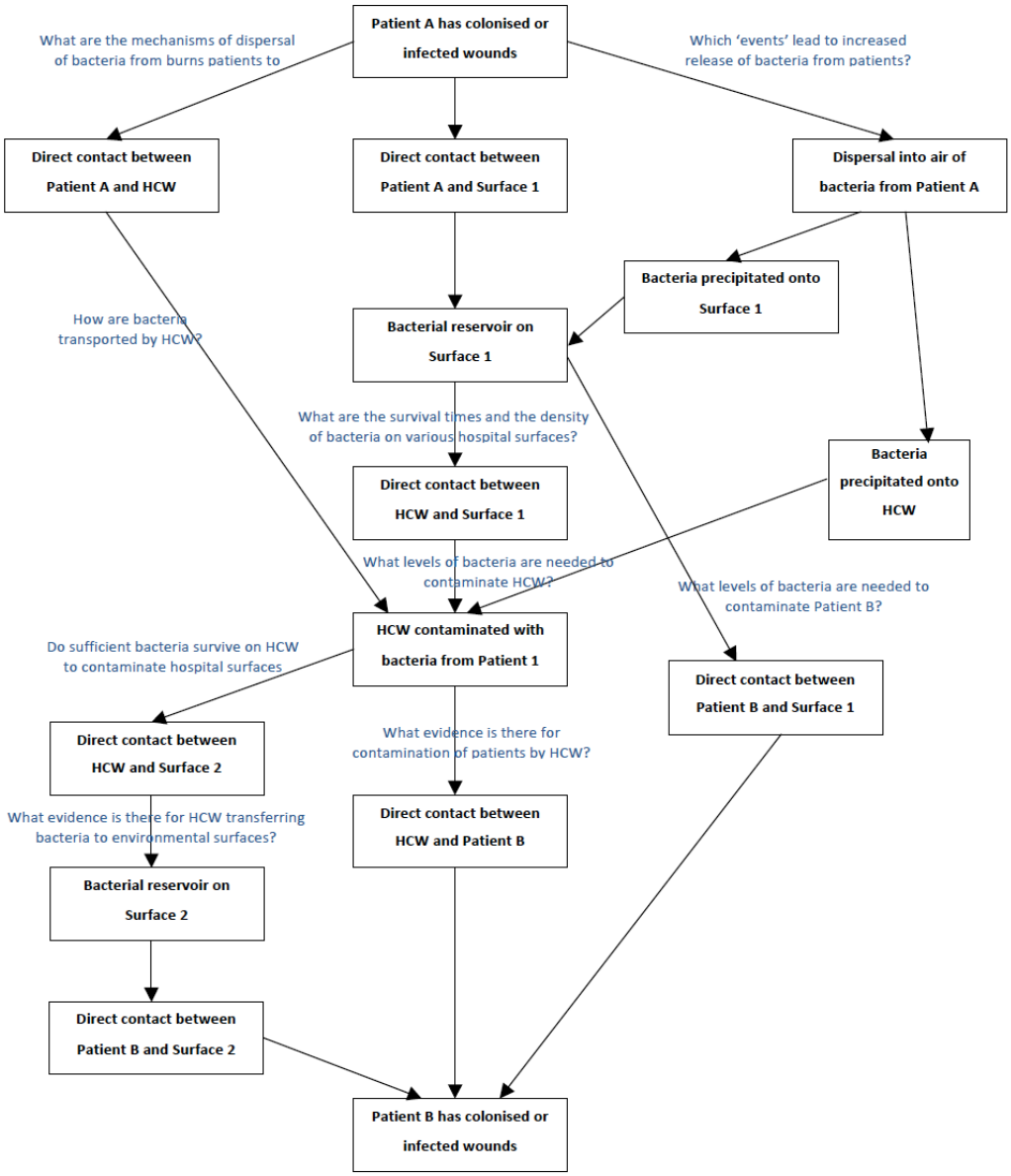


Figure 7.1: *Diagram of the cycle of cross-contamination between burns patients*

7.2 Preliminary airborne contamination studies: developing a model

Introduction

Dressing changes on small non-burn wounds increase airborne dispersal of bacteria.[52] Bed sheet changes have also been shown to liberate bacteria into the air.[51] Studies carried out in the rooms of medical and surgical patient carriers of MRSA yielded MRSA-positive airborne samples in 33%, and MRSA-positive settle plates in 57%, of samples.[66] The aeroisolisation of MRSA has been demonstrated during dressing changes on burns patients.[53] Numerous studies have compared the relative contributions to airborne bacteria made by both nasal carriers and patients with wounds infected or colonised with staphylococci: all emphasised the importance of friction on the skin and agitation caused by bed making.[51,54,57-61] The airborne route is significant in the cycle of cross-contamination, with reports existing of healthy *S. aureus* dispersers causing wound infections in nearby patients.[68,132,399,400] The contribution of the airborne route to cross-contamination between burns patients was discussed in Section 2.4.

Near ubiquitous colonization of burns wounds means that burns patients may be expected to release higher levels of airborne bacteria than non-burns patients. In the 1970s, attempts were made to link the size of a burn and the airborne dispersal of *S. aureus* during a dressing change: they demonstrated a correlation between the size of the burn and the number of bacteria precipitated onto settle plates over a period of days.[54] More recently, it was shown that 32% (11/35) of dressing changes on MRSA-colonised burns patients liberated the organism into the air by the means of a laminar flow air sampler.[53]

These two studies begin to tackle the issue of airborne dispersal of bacteria during dressing changes, however they have significant limitations. Settle plates left exposed for several days have the potential to collect bacteria from a plethora of sources. Furthermore their use is limited due to the agar in the settle plates drying out when uncovered for prolonged periods.[54] An air sampler is therefore a preferred sampling tool. However, the authors of the paper using the laminar flow air sampler did not report the point at which sampling was started, nor how long after the dressing change was complete that post-dressing change samples were taken.[53] The following experimental methods were therefore developed to more accurately evaluate the airborne bacterial dispersal during dressing/bed changes.

General methods

To assess the levels of airborne bacteria around isolation rooms during events such as bed and dressing changing, an SAS Super 180 air sampler, described in Section3.2 was used.

For collection of airborne samples, the air sampler was positioned at the foot of the bed of a bed-bound patient, or within 1 m of a mobile patient, in a closed room prior to the start of the 'event'. Samples were collected on 90 mm TSA agar plates, and as previously described, the air sampler enabled the collection of different volumes of air.

Two samples of 200 L and 500 L were taken at 5 min intervals throughout the course of the event. When each new sample was collected, a note was made of the activity taking place at that time. The main focus was on dressing changes, although for larger burns this would usually incorporate a bed sheet change while rolling the patient to apply bandages (hereafter termed dressing/bed change). It was not possible to separate the dressing and bed change components of the activity, as the bed sheet change was often integrated into the dressing change when the patient was rolled for application of bandages. The intention was to mimic real-life situations as much as possible and not to inconvenience the patient or HCW, by carrying out separate dressing changes and bed changes during what can be a distressing and uncomfortable time. The events examined were divided into three broad activity categories.

- **Minimal activity ('min'):** included the patient sitting or lying down. They may be eating, talking, reading, watching television etc. Nursing input was minimal, such as feeding, measuring observations, attending drips, giving medications, brushing teeth and washing the patient with a damp cloth. This category excluded any bed sheet disturbance or changing and the removal or application of dressings.

- **Bed sheet activity ('sheet'):** included shaking out or rearranging the bed sheets and blankets, removing soiled laundry, replacing with clean laundry and making the bed.

- **Dressing change activity ('dress'):** included removing dressings, cleaning wounds and reapplying fresh dressings and splints.

Following collection of air samples throughout an event, the agar plates were incubated. These were then enumerated and converted to probable cfu counts per 1000L (i.e. cfu/ m^3), as detailed in Section 3.2, with the cfu counts from the plate with the higher sampling volume (500L) used when possible. During preliminary studies this data was used to create graphs that demonstrate the probable cfu/m^3 at each point in time, with the activity at the time noted.

Preliminary air contamination studies

Preliminary studies using these methods were performed to develop a model for measuring airborne contamination during an event. One study was carried out in a burns outpatient clinic during dressing changes on four consecutive patients with <5% Total Body Surface Area (%TBSA) burns. Four further studies were carried out in inpatient isolation rooms when dressing/bed changes were performed on patients with larger burns wounds.

Study 1: Four patients with < 5%TBSA burns in an outpatient clinic

Airborne bacteria were collected at 5 min intervals as described above during an outpatient clinic where four patients were reviewed. Two events were observed: minimal and dressing change activity.

- **Patient 1:** A 24 year old with a six day old 2%TBSA scald to the chest
- **Patient 2:** A 26 year old with a six day old 2%TBSA scald to the abdomen
- **Patient 3:** A 44 year old with a 10 day old 2%TBSA flame burn to the arm and axilla
- **Patient 4:** A 19 year old with a <1%TBSA cigarette burn to the hand

None of the patients had contaminated wounds, and none had required operative intervention. Figure 7.2 shows the results of airborne measurements during the clinic.

The graph demonstrates an obvious correlation between activity levels in the room and the amount of airborne bacteria. Bacterial counts during the first minimal activity period are between 86 cfu/m^3 and 118 cfu/m^3. During the first two dressing changes, they rise to between 218 cfu/m^3 and 1325 cfu/m^3. Counts plateau during a second brief minimal activity period before rising again to a maximum of 550 cfu/m^3 during the final two dressing changes. A gradual decline in levels is seen following cessation of all activity.

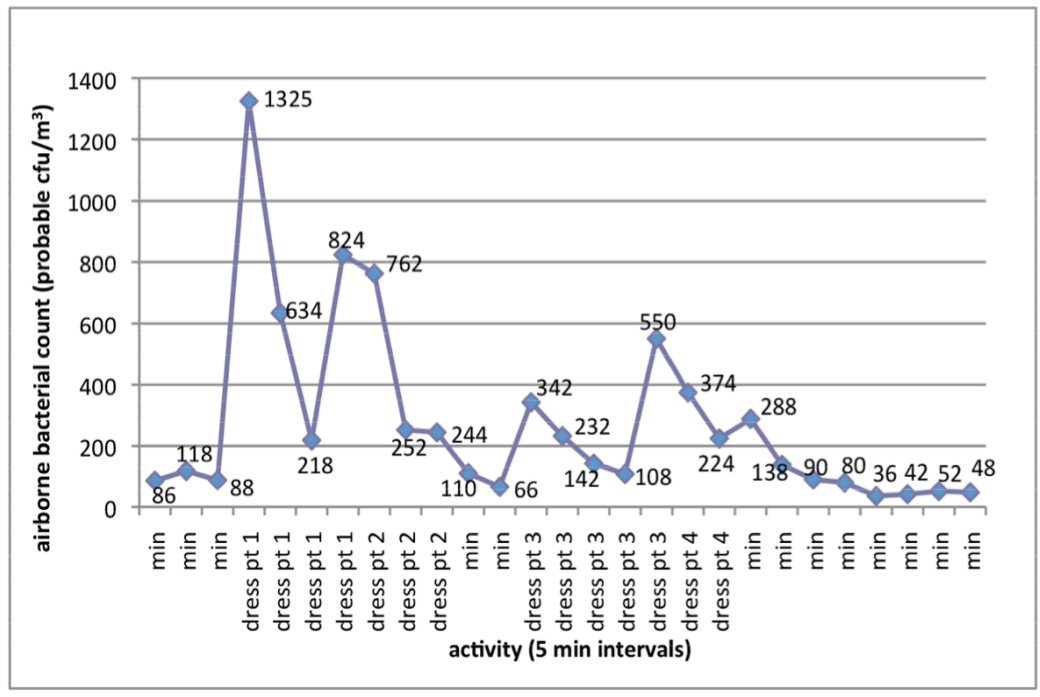


Figure 7.2: *Chart of probable airborne bacterial count (cfu/m³) from air samples taken at 5 min intervals during an outpatient clinic of four consecutive patients (Pt 1 to Pt 4). The event occurring during each measurement is stated along the x-axis (min = minimal activity; dress = dressing change).*

Study 2: Patient with 6%TBSA burn in an inpatient isolation room

The following study was carried out on Patient 5 in Room 3 in the Burns Unit.

- **Patient 5:** A 33 year old with nine day old 6%TBSA flame burns to both feet and legs. The burn had been surgically debrided and covered with a split skin graft, producing a further 6%TBSA donor site. Wound swabs isolated *S. aureus*; group G *Streptococcus*; coliforms and *Bacillus* sp. The patient was awake and breathing spontaneously.

The events observed were minimal activity and a dressing change. Results are shown in Figure 7.3. Again, an increase in airborne bacteria is demonstrated during a dressing change, with a sharp rise from less than 36 cfu/m³ to 250 cfu/m³.

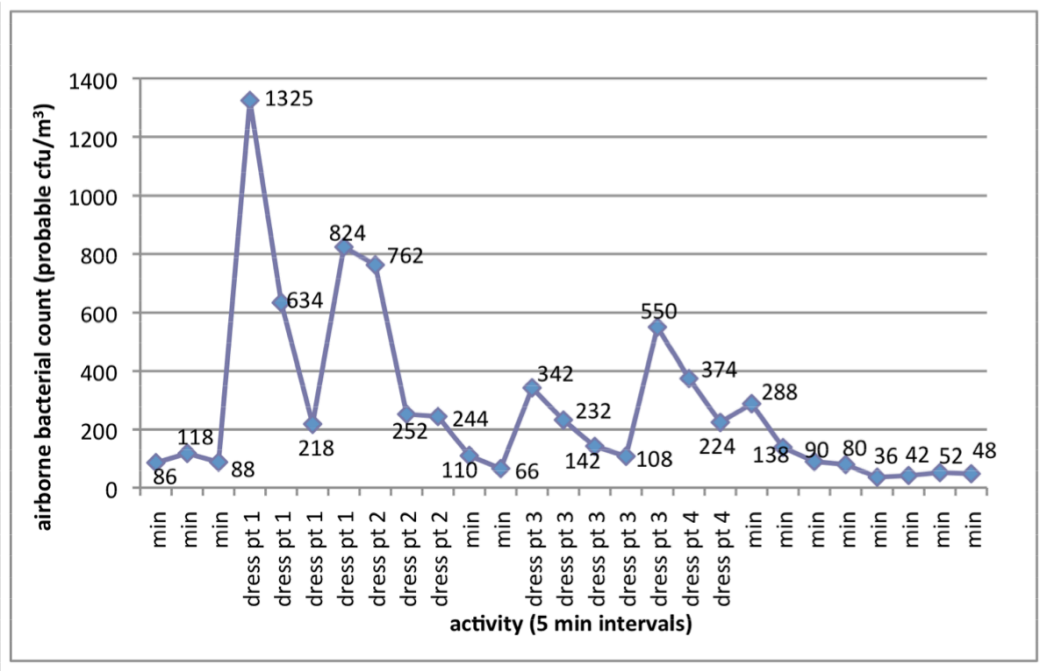


Figure 7.3: *Probable airborne bacterial count (cfu/m³) from air samples taken at 5 min intervals during a dressing change event in an inpatient isolation room containing a patient with 6%TBSA burns. The event occurring during each measurement is stated along the x-axis (min = minimal activity; dress = dressing change).*

Study 3: Patient with 10%TBSA burn in an inpatient isolation room

The following study was carried out on Patient 6 in Room 4 in the Burns Unit.

- **Patient 6:** A 74 year old with a 30 day old 10%TBSA flame burns to shoulder and chest. The burn had not been surgically debrided due to co-morbidities. Wound swabs had isolated *S. aureus*; coliforms and *P. aeruginosa*. The patient was awake and breathing spontaneously.

The events observed were minimal activity, a dressing change and a bed sheet change. Results, given in Figure 7.4, again demonstrate an increase in airborne bacteria during this dressing change on a burns patient, although more bacteria are liberated during the bed sheet change. An increase from 156 cfu/m^3 to 1038 cfu/m^3 occurred during the dressing change, then a further increase to 2614 cfu/m^3 was shown when the bed change takes place.

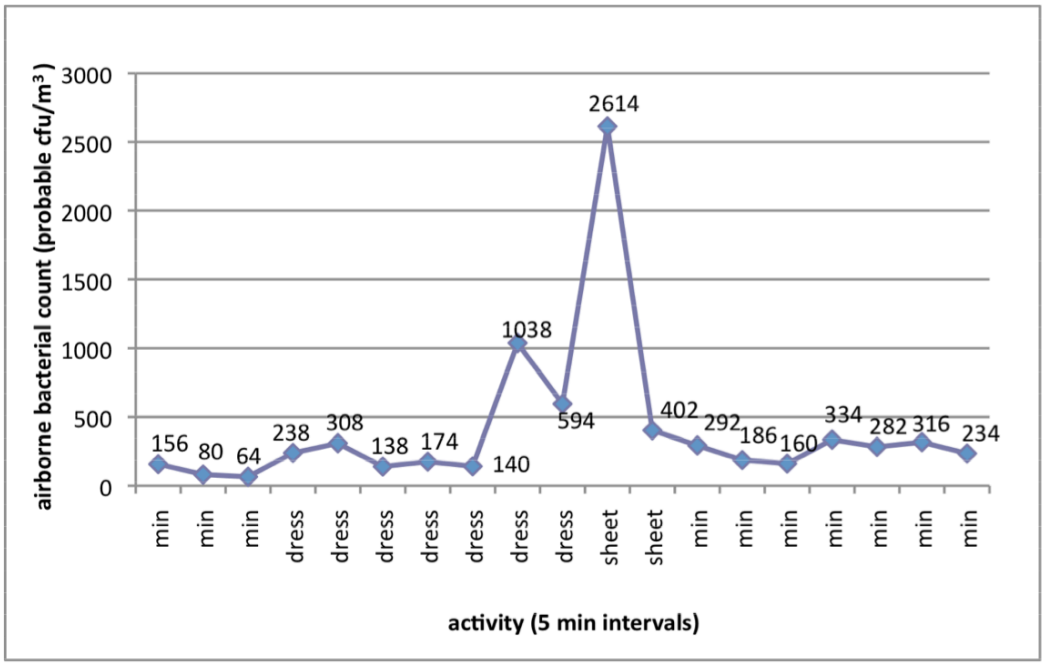


Figure 7.4: *Probable airborne bacterial count (cfu/m³) from air samples taken at 5 min intervals during a dressing and bed change event in an inpatient isolation room containing a patient with 10%TBSA burns. The event occurring during each measurement is stated along the x-axis (min = minimal activity; dress = dressing change; sheet = bed sheet change).*

Study 4: Patient with 15%TBSA burn in an inpatient isolation room

The following study was carried out on Patient 7 in Room 7 on the Intensive Care Unit.

- **Patient 7:** A 45 year old with a six day old 15%TBSA full thickness flame burn to the face, chest, left arm and forearm and hand. The burn had been debrided and grafted the preceding day, producing a further 9% TBSA donor site. Wound swabs isolated *S. aureus*, *Bacillus* sp., and *Clostridium perfringens.* The patient was sedated and mechanically ventilated.

The events in this study consisted of minimal activity, a dressing change and a bed sheet change. The results are shown in Figure 7.5. This study provides further evidence that bacteria are liberated into the air during a dressing/bed change on a burns patient. An increased bacterial count from less than 43 cfu/m^3 to 1060 cfu/m^3 during the dressing change is shown. Furthermore, the bed sheet change produced a secondary surge in airborne bacteria, up to 740 cfu/m^3.

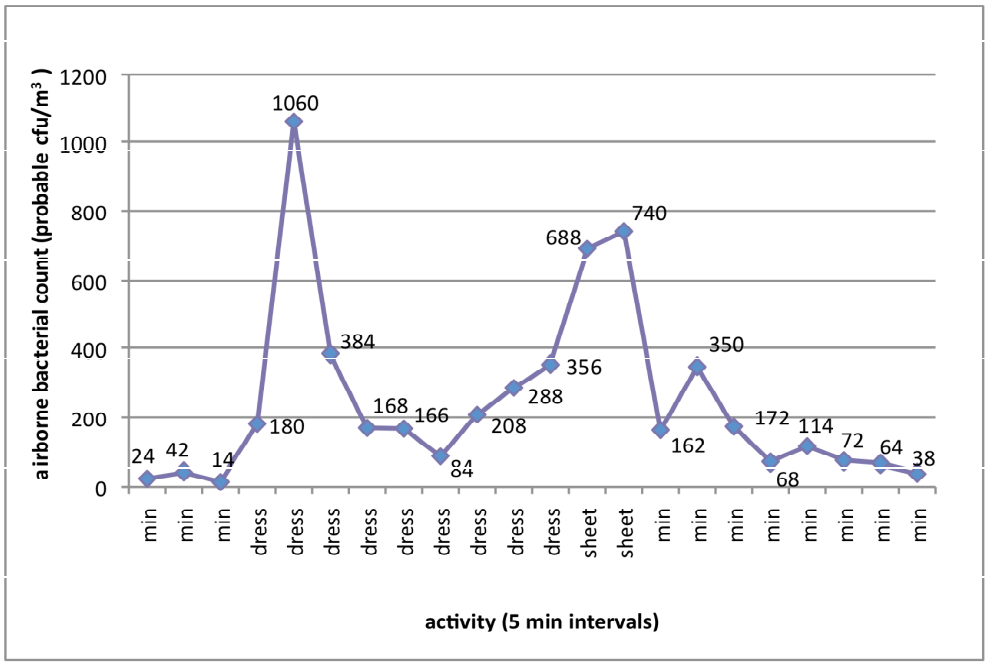


Figure 7.5: *Probable airborne bacterial count (cfu/m³) from air samples taken at 5 min intervals during a dressing and bed change event in an inpatient isolation room containing a patient with 15%TBSA burns. The event occurring during each measurement is stated along the x-axis (min = minimal activity; dress = dressing change; sheet = bed sheet change).*

Study 5: Patient with 30%TBSA burns in an inpatient isolation room

The following study was carried out on Patient 8 in Room 11 on the Intensive Care Unit.

- **Patient 8:** A 46 year old with a six day old 30%TBSA full thickness flame burn to both upper limbs and lower limbs, hands, back and buttocks. The burn had not been surgically debrided due to the poor health of the patient, but had been treated with flammacerium. Wound swabs had isolated *S. aureus*, *Bacillus* sp, and streptococci. The patient was sedated and mechanically ventilated, with a severe inhalation injury and respiratory infection.

The events in this study consisted of minimal activity, a dressing change and a bed sheet change. The results are given in Figure 7.6. This study provides further evidence that bacteria are liberated into the air during a dressing/bed change on a burns patient. The probable cfu/m^3 increased from less than 34 cfu/m^3 to 168 cfu/m^3 during the dressing change. A further surge in airborne bacteria, of up to 284 cfu/m^3 was created by the bed sheet change.

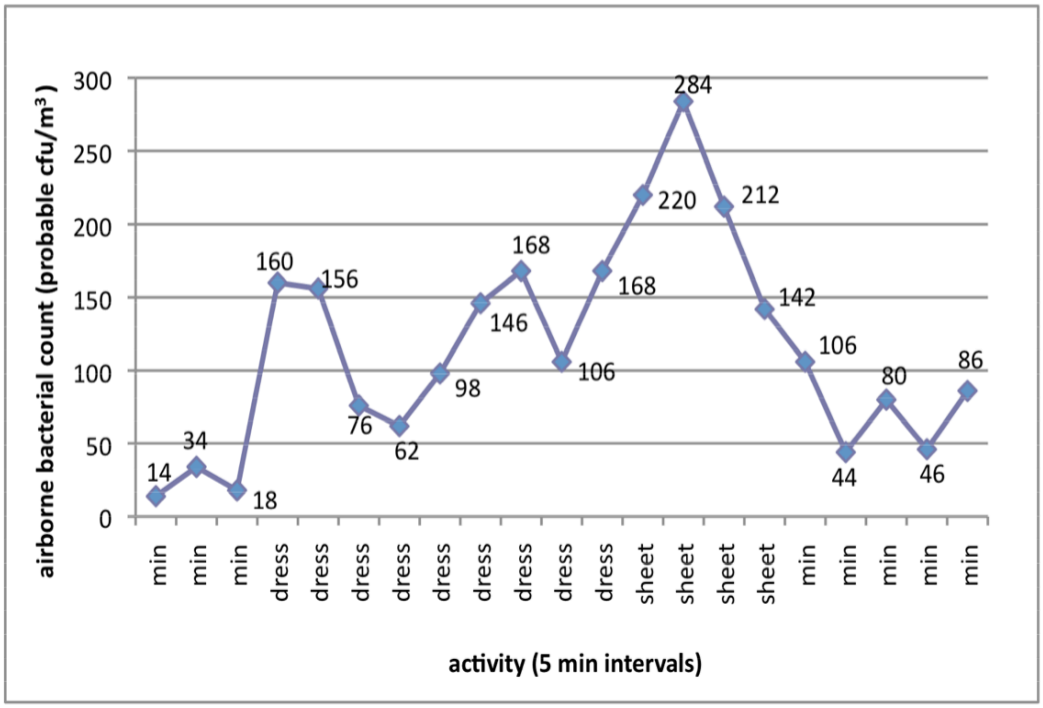


Figure 7.6: *Probable airborne bacterial count (cfu/m³) from air samples taken at 5 min intervals during a dressing and bed change event in an inpatient isolation room containing a patient with 30%TBSA burns. The event occurring during each measurement is stated along the x-axis (min = minimal activity; dress = dressing change; sheet = bed sheet change).*

Discussion

These five studies on eight patients provide evidence that airborne release of bacteria occurs during dressing/bed changes on burns patients.[53] Although this has been suggested by previous studies, this work using samples taken at 5 min intervals provides a more accurate illustration of the dispersal occurring. The most comprehensive study to be carried out on airborne release during dressing changes on burns patients previously, used a Burkhead air sampler with 90 mm blood agar plates exposed within the air sampler for 2 min at a rate of 10 L/min. Samples were taken from four points around the room, and one point outside the room before, during and after the dressing change.[53] The results demonstrated that all air sample cultures carried during a dressing change on an MRSA positive burns patients, were positive for MRSA. Conversely, air samples taken in the presence of patients who were negative for MRSA, or non-burns patients who were positive for MRSA, were always negative, although these were not taken during a dressing change.[53]

Much smaller volumes of air were sampled during these studies, compared with the current study (20 L compared with 200 L or 500 L samples that were adjusted to produce 1000 L estimates). Thus the number of colonies in the previously reported study was in the range of 1 to 9 cfu per 20 L air, whereas they ranged form 8 to 2614 cfu per 1000 L air in the present studies.[53] Although the previous study indicated the importance of the airborne route of transmission of MRSA, and the authors went on to redesign their burns unit based on these findings, the studies outlined here give vastly more detail about the link between airborne bacterial levels and activity levels. A minimum of nine consecutive samples was taken during dressing or bed sheet change activity (compared with five simultaneous samples), with the activity taking place noted to enable correlation between the two. Furthermore, samples took place at 5 min intervals continuously throughout the study period (including the minimal activity phases before and after the dressing/sheet change. This enabled the rate at which bacterial levels in the air increase before the activity, and decrease after it, to be demonstrated.

Although there is no statistical analysis of these preliminary studies, several useful points may be noted which had a bearing on future studies. The most striking conclusion is that all studies showed increased airborne bacterial counts when a dressing change was performed, compared to levels when there was minimal activity in the room. Furthermore, this high level of airborne bacteria was sustained and sometimes surpassed by a bed sheet change in the same room. This highlights sheet changes as another 'high-risk' event during the cycle of cross contamination. The link between bed sheet changes and airborne bacterial levels has previously been demonstrated.[51] The authors used MSO agar (salt egg-yolk agar containing oxacillin, for detection of MRSA) and an Andersen cascade air sampler, which had the advantage of allowing particles to be separated according to their size, but the disadvantage that it is cumbersome and impractical to use in the burn unit environment during a dressing change, when space is limited. In the study 283 L of air were collected using the air sampler before (resting period), during, and 15 min, 30 min and 60 min after bed making.[51] Thirteen patients were studied, three times each. Ten were infected and three colonised with MRSA. Mean MRSA cfu counts collected in the rooms of infected patients rose from 4.7 cfu/m^3 during the resting period, to 116 cfu/m^3 during bed making. This rise was sustained for 15 min after the bed making finished (mean 29.6 MRSA cfu/m^3), but was not significantly higher than resting levels after 30 min (mean 4.9 MRSA cfu/m^3). Of course, as the authors were only isolating MRSA, counts were much lower than those reported in the current study. However, a single set of samples during the rest period, taken using TSA, showed total populations during the resting period of 163 cfu/m^3.[51] These are notably higher than the populations during rest reported here, although comparisons between different units are difficult due to variations in air conditioning, patient population, cleaning routines and other factors. The current studies have

augmented the knowledge about the duration of an increased bacterial airborne bioburden by increasing the number of samples taken after the bed change has finished. However, further work to determine the length of time the bacteria remained at elevated levels is addressed in the next section.

Although burns of different sizes were examined, there was little correlation between the size of the burn and the maximum levels of airborne bacteria found during the event in question. In fact the highest value recorded was 1325 cfu/m^3, which was recorded during the dressing change on one of the smallest burns: Patient 1. In contrast, dressing and bed-change of Patient 8, who had the largest burn, produced a maximum count of 284 cfu/m^3. These discrepancies suggest that the size of the burn is not the only factor that must be considered, but that several other contributing factors must be taken into account. For example, Patient 1 entered the outpatient clinic room from outside the hospital. They wore outdoor garments that were removed during the dressing change period: an activity that is likely to have dispersed large numbers of bacteria into the air. In contrast, Patient 8 had been managed in the intensive care unit for several days beforehand and was wearing hospital gowns and bed sheets that were changed daily. Furthermore, Patient 8's wounds had been treated topically with flammacerium (which hardens and dries the burn eschar) may decrease levels of bacteria harboured on the skin.

The aforementioned discrepancies make it difficult to compare one patient with another. It was therefore decided that for future studies the patient would act as their own control, to enable statistical analysis by the production of control charts. This process is described in the following section.

7.3 Control charts of airborne contamination on the burns unit during events

Introduction

The previous section described preliminary studies to demonstrate increased levels of airborne contamination during a dressing/bed change. To create statistical control charts, several changes were made to the experimental model and are detailed below.

General methods

For the creation of control charts, the following changes were made:

- Narrower intervals between air samples (3 min intervals)
- More samples taken during first minimal activity phase to enable the estimation of mean and standard deviations (minimum 12 samples)
- More samples taken following the event to observe a return to pre-event levels

With the exception of these changes the methods detailed in Section 7.2 were used and the events examined were divided into the same three broad activity categories: minimal activity ('min'); bed sheet change ('sheet'); and dressing change ('dress').

Statistical analysis and establishing a control chart

Microsoft Excel and Minitab Version 16 were used throughout. Following TSA plate enumeration, the counts were converted to probable cfu per 1000 L (i.e. cfu/m^3) as previously described. The data was input into Excel and raw probable cfu/m^3 counts were converted to log-transformed counts. The data was inserted into Minitab. Control charts (I charts) were produced based on the raw probable cfu/m^3 counts. Mean (of raw date and the mean of the log transformed data) and standard deviation were estimated by analysing samples from the first period of minimal activity (usually the first 12-18 samples). Once these were calculated, they were applied to the subsequent data. Four internationally accepted tests were applied to the chart. Results that failed to pass any of these tests were indicated in red, with a number to denote the statistical test failed, such that the overall probability of obtaining a false special cause is circa 1 in 100.[401] The tests for establishing a control chart were:

1. One point > three standard deviations from centre line
2. Nine points in a row on the same side of the centre line
3. Six points in a row all increasing or decreasing

4. 14 points in a row, all alternating up or down

Initial analysis was carried out on raw data to create clinically relevant charts. However, the tests were also repeated on log-transformed data, as a more widely accepted method of analysing bacterial cfu counts.

Control Chart 1: Patient with 35%TBSA burns in an inpatient isolation room

The following study was carried out on Patient 9 in Room 12 on the intensive care unit.

- **Patient 9:** A 55 year old with a four day old 35%TBSA superficial partial thickness flame burn to both upper limbs and lower limbs and chest. The burn had not been surgically debrided as it was mainly superficial, but had been treated with flammacine. Wound swabs had isolated MRSA. The patient was sedated and mechanically ventilated, with an inhalation injury.

The events in this study consisted of minimal, dressing and sheet changing activities. Control charts are shown in Figure 7.7. Analysis of the raw data shows that the first minimal activity stage has a mean of 121 cfu/m^3 and standard deviation of 44.66 cfu/m^3: a mean of 4.63 Log_{cfu/m^3+1} and standard deviation of 0.39 Log_{cfu/m^3+1} on log-transformed data. At the beginning of the dressing change, the data points are immediately flagged as failing Test 1 (values are > 3 SD above the centre). This is sustained for the first six readings during the dressing change activity: a period of 18 min. The chart appears to return to the control mean, only to go 'out of control' again during the sheet change activity. Levels of airborne bacteria remain 'out of control' for the duration of the second minimal activity period (24 min).

In conclusion, the dressing change on a patient with 35%TBSA burns created significantly increased levels of airborne bacteria. The bed sheet change also increased airborne bacterial levels, with prolonged high levels following the termination of this activity suggesting that the effects of a sheet change may continue for at least 24 min. This raises the possibility that protective clothing may be needed by anyone entering the room for a period of time during and *after* a dressing/bed change.

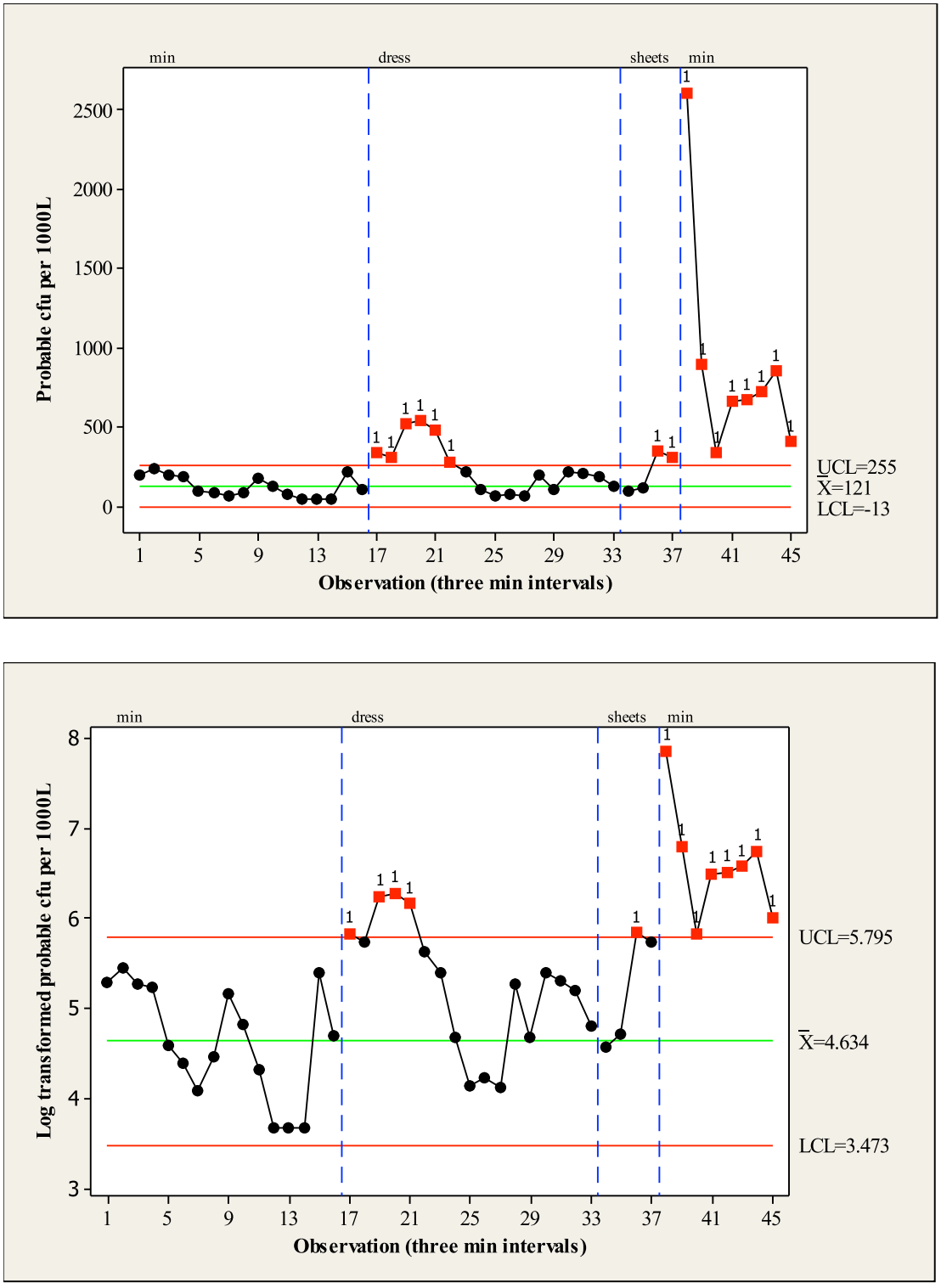


Figure 7.7: *Control charts (Minitab v16) based on raw data (above) and log-transformed data (below), demonstrating levels of airborne bacteria during events involving a patient with 35%TBSA burns. Probable cfu per 1000 L (i.e. cfu/m^3) from air samples taken at 3 min intervals are given. The event has been divided into stages according to the activities taking place (min = minimal activity; dress = dressing change; sheet = bed sheet change). 'Out of control' data points are flagged in red.*

Control Chart 2: Patient with 45%TBSA burns in an inpatient isolation room

The following study was carried out on Patient 10 in Room 7 on the intensive care unit.

- **Patient 10:** A 43 year old with a two day old 45%TBSA deep partial and full thickness flame burn to both upper limbs and lower limbs, chest, back, face and neck. Part of the burn, amounting to 19%TBSA had been surgically debrided the day before, with 1%TBSA covered with skin grafts and 18%TBSA covered with Integra. No wound swabs had isolated organisms at this point. The patient was sedated and mechanically ventilated, with an inhalation injury.

The events in this study consisted of minimal activity, a dressing change and a bed sheet change. Control charts are given in Figure 7.8.

Analysis of the raw data shows that the first minimal activity stage has a mean of 21.5 cfu/m^3 and standard deviation of 11.86 cfu/m^3: a mean of 2.94 Log_{cfu/m^3+1} and standard deviation of 0.56 Log_{cfu/m^3+1} for log-transformed data. At the beginning of the dressing change, the data points are immediately flagged as failing Test 1 (values are > 3 SD above the centre). An 'out of control' status is sustained almost continuously throughout the dressing change, sheet change, and for at least 54 min following the event, due to failing Test 2 (more than nine values in a row the same side of the centre line).

This provides evidence that levels of airborne bacteria are significantly increased during a dressing/bed change of a patient with a 45%TBSA burn. Again, prolonged 'out of control' counts after the activity ceases suggests that protective clothing may be needed by anyone entering the room during and for at least 54 min *after* a dressing/bed change.

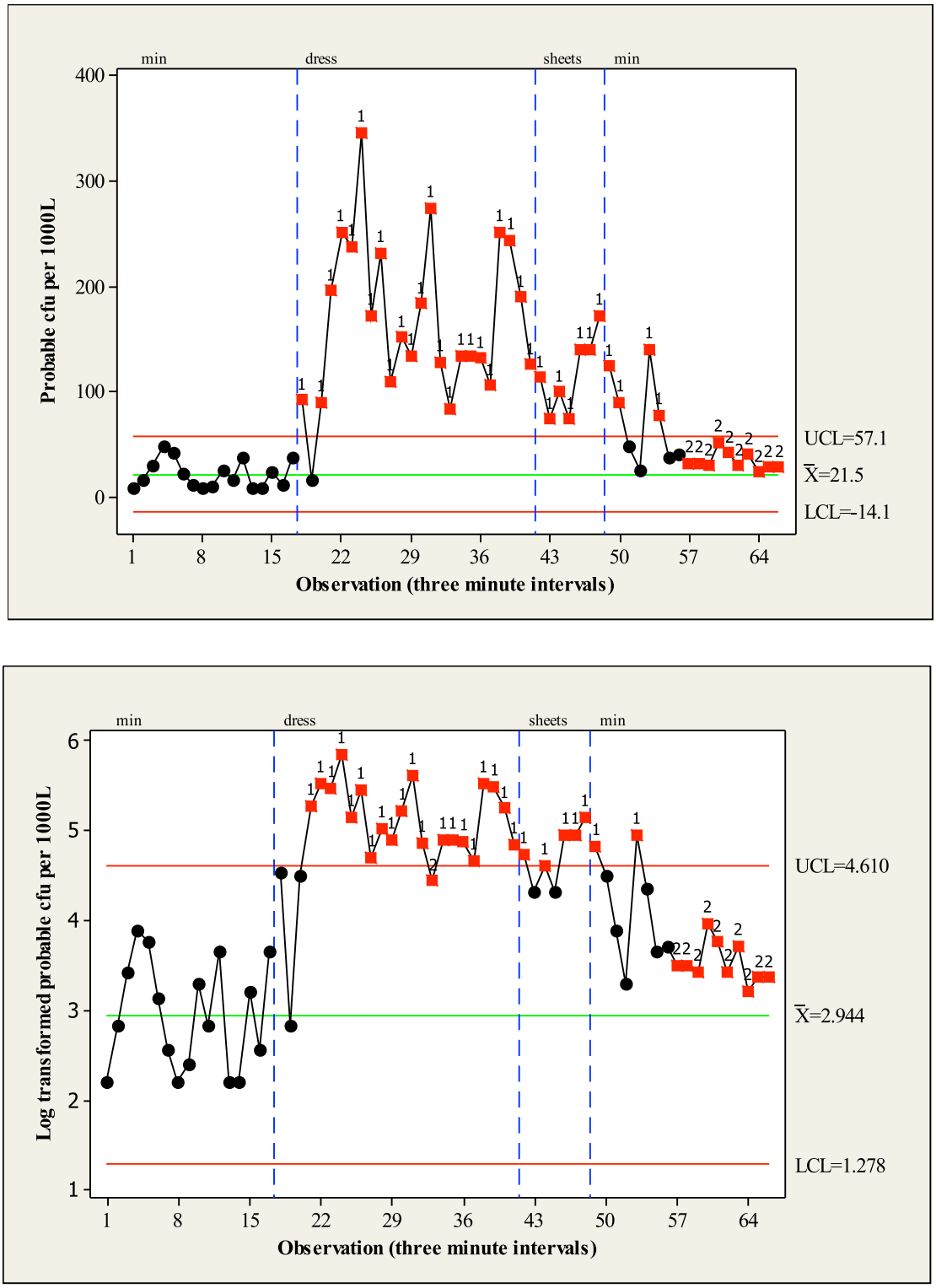


Figure 7.8: *Control charts (Minitab v16) based on raw data (above) and log-transformed data (below), demonstrating levels of airborne bacteria during events involving a patient with 45%TBSA burns. Probable cfu per 1000 L (i.e. cfu/m³) from air samples taken at 3 min intervals are given. The event has been divided into stages according to the activities taking place (min = minimal activity; dress = dressing change; sheet = bed sheet change). 'Out of control' data points are flagged in red.*

Control Chart 3: Patient with 51%TBSA burns in an inpatient isolation room

The following study was carried out on Patient 11 in Room 11 in the intensive care unit.

- **Patient 11:** A 40 year old with a six day-old 51%TBSA mixed deep and superficial partial thickness flame burn to both upper limbs and lower limbs, trunk, face and neck. The burn was surgically debrided two days earlier, producing a further 2%TBSA donor site. Coverage was provided by Integra for 32%TBSA of the burn to the trunk and upper limbs. Wound swabs had isolated *Enterobacter cloacae*. The patient was sedated and mechanically ventilated, with an inhalation injury.

The events in this study consisted of minimal, dressing and sheet changing activities. Control charts are given in Figure 7.9.

Analysis of the raw data shows that the first minimal activity stage has a mean of 7.0 cfu/m^3 and standard deviation of 6.43 cfu/m^3: a mean of 1.785 Log_{cfu/m^3+1} and standard deviation of 0.8556 Log_{cfu/m^3+1} for log-transformed data. From the beginning of the dressing change, data points are flagged as failing Test 1 (values are > 3 SD above the centre). This is sustained throughout the dressing and sheet change activities. Airborne bacterial counts remain 'out of control' for a minimum of 30 min after these activities stop.

This study demonstrates significantly increased levels of airborne bacteria during and for at least 30 min after, a dressing/bed change on a patient with a six day old 51%TBSA mixed depth burn.

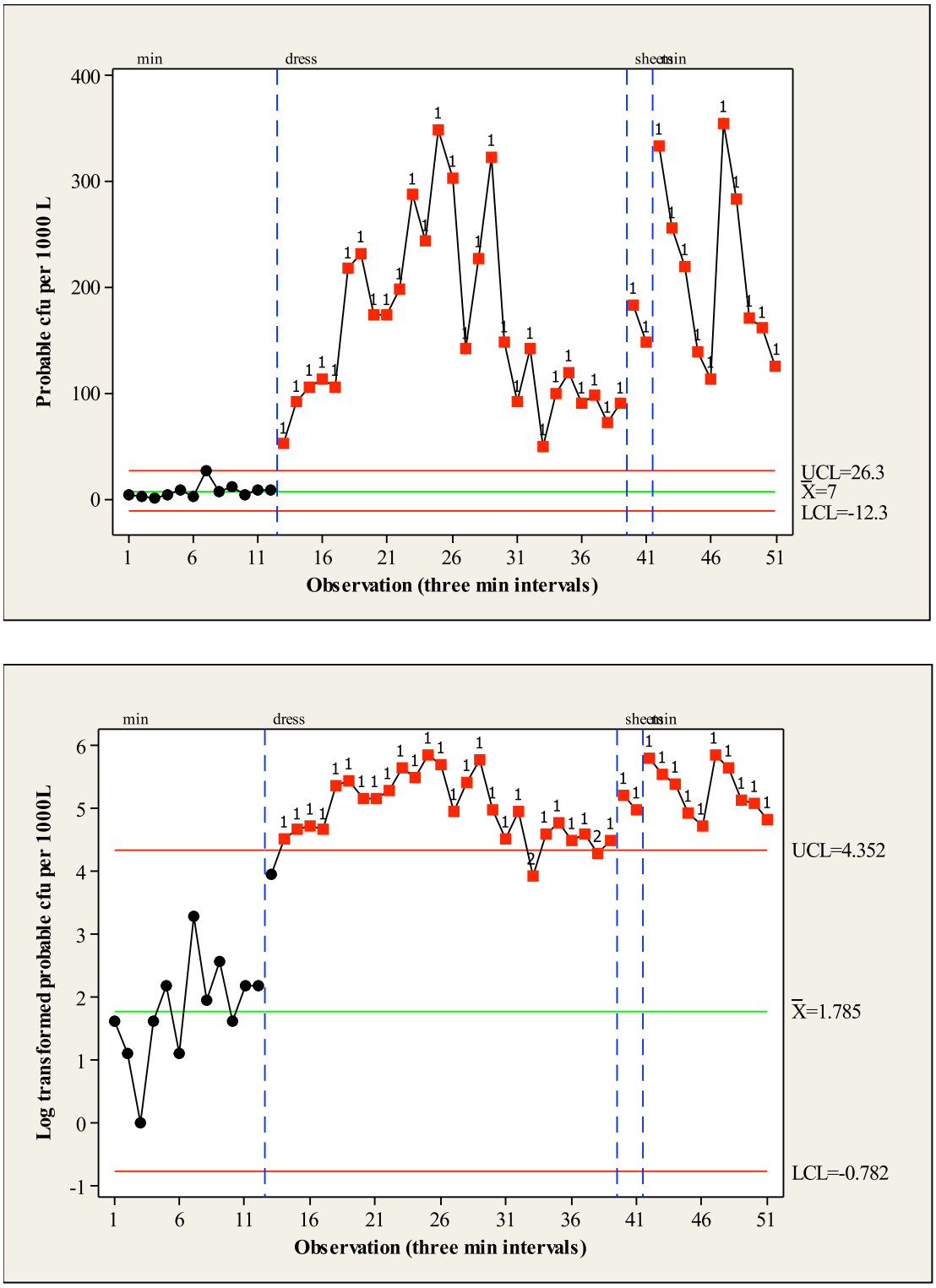


Figure 7.9: *Control charts (Minitab v16) based on raw data (above) and log-transformed data (below), demonstrating levels of airborne bacteria during events involving a patient with 51%TBSA burns at an early point of care. Probable cfu per 1000 L (i.e. cfu/m³) from air samples taken at 3 min intervals are given. The event has been divided into stages according to the activities taking place (min = minimal activity; dress = dressing change; sheet = bed sheet change). 'Out of control' data points are flagged in red.*

Control Chart 4: A further study on a patient with 51% TBSA burns in an inpatient isolation room

The following study was also carried out on Patient 11, when they had been moved to Room 5 on the burns unit.

- **Patient 11:** a 40 year old with a 28 day old 51%TBSA mixed depth (40%TBSA deep) flame burn to both upper limbs and lower limbs, trunk, face and neck. The burn been surgically debrided several times by this stage, producing a further 20%TBSA donor site. Coverage was provided by Integra or graft to 32%TBSA of the burn to the trunk and upper limbs. Recent wound swabs had isolated *P. aeruginosa,* MRSA, *S. aureus*, and coliforms. The patient was alert and breathing spontaneously.

The events in this study consisted of minimal activities, dressing changes and sheet changes. The control chart based on raw data is shown below in Figure 7.10.

Analysis of the raw data shows that the first minimal activity stage has a mean of 7.1 cfu/m^3 and standard deviation of 5.133 cfu/m^3: a mean of 1.609 Log_{cfu/m^3+1} and standard deviation of 0.926 Log_{cfu/m^3+1} for log-transformed data. Of note there were two sheet change activities during this study. This was due to the patient requiring a bedpan, an activity that necessitates in rolling the patient and moving sheets on and off the bed. This is the first time that a sheet change activity has preceded a dressing change activity, and provides evidence that sheet changes in themselves create increases in airborne bacteria. The dressing change and further sheet change activity produced high levels of airborne bacteria that were also 'out of control' when compared to the first period of minimal activity. For the first time a return to 'control' bacterial levels is achieved, 45 min after the dressing/bed change activities finish.

This provides evidence that a dressing/bed change carried out on a patient with 28 day old 51%TBSA mixed depth burns significantly increased airborne bacterial levels, and that the effects of the dressing/bed change remain for a considerable amount of time following their cessation.

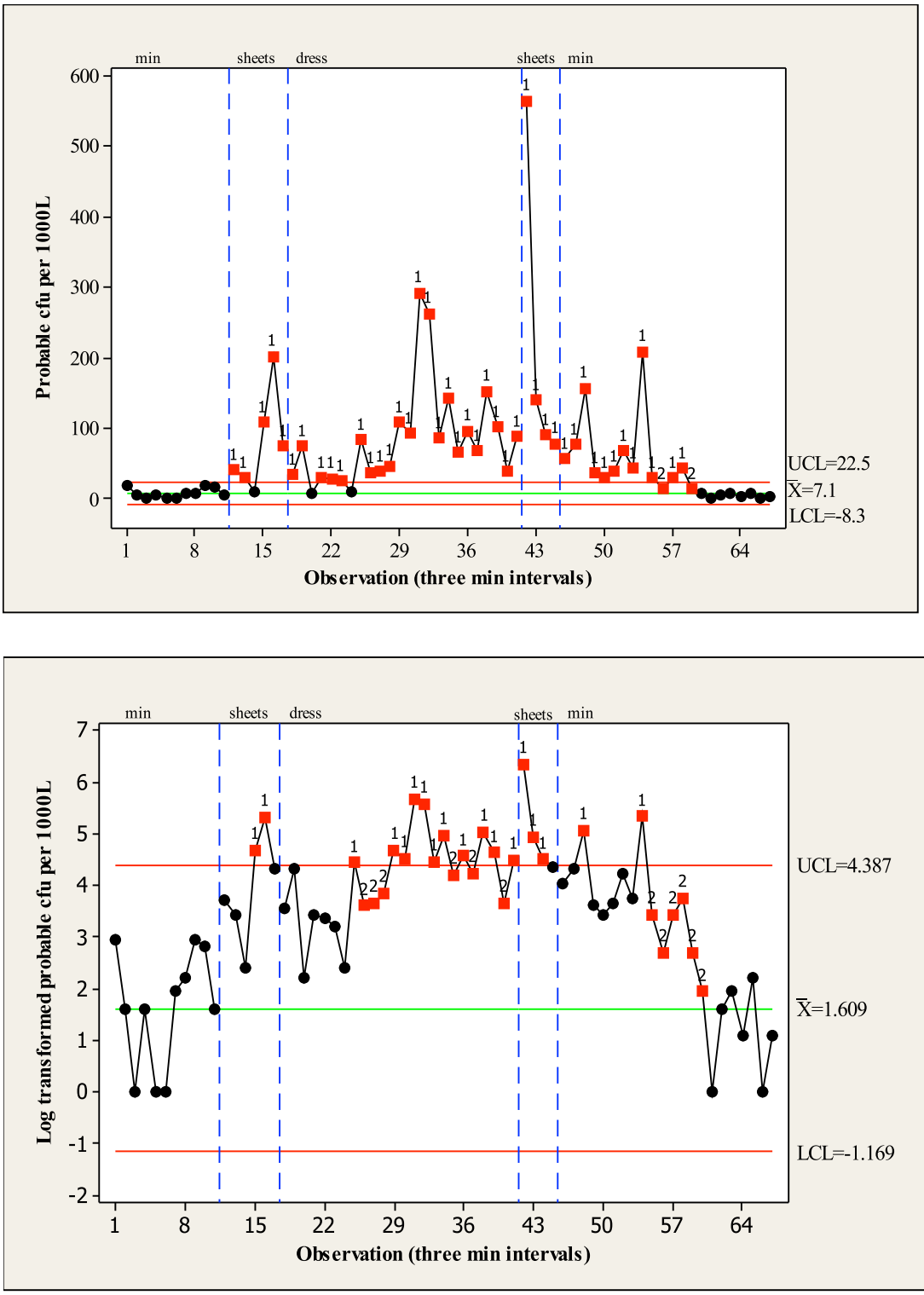


Figure 7.10: *Control charts (Minitab v16) based on raw data (above) and log-transformed data (below), demonstrating levels of airborne bacteria during events involving a patient with 51%TBSA burns at a later stage of care. Probable cfu per 1000 L (i.e. cfu/m^3) from air samples taken at 3 min intervals are given. The event has been divided into stages according to the activities taking place (min = minimal activity; dress = dressing change; sheet = bed sheet change). 'Out of control' data points are flagged in red.*

Discussion

The four control charts presented demonstrate that the levels of airborne bacteria created during a dressing/bed change are significantly greater than those before the event began. As pilot studies had indicated that inter-patient comparisons were difficult due to numerous variables, the patients acted as their own controls. A minimal activity period at the start of each study established 'control' airborne bacterial counts bacteria for that patient in that room at that time. These baseline levels were seen to significantly increase during dressing/bed changes. Furthermore, the effects lasted for approximately 45 min to 60 min after the dressing/bed change ceased. Studies were carried out on patients with burns from 35%TBSA to 51%TBSA, and on burns between two and 28 days old.

These control charts are a unique way of quantifying airborne levels of bacteria during activities within a burns unit, and have not been used before. Direct comparison with other studies is therefore not possible. The studies mentioned in the previous section are the most comparable available to date,[51,51] but as was discussed, they have far fewer sampling periods, so an accurate picture of what is taking place during the hour or so it takes to complete a bed/sheet change, and the period beyond, is not apparent. The control charts here produce an accurate reflection of the level of airborne bacteria throughout the period before, during and after such events, with samples taken every 3 min, and up to 60 samples per study.

These findings implicate the airborne route in the cycle of cross-contamination between burns patients. Logically, all airborne bacteria will precipitate eventually, onto environmental surfaces or persons present or be inhaled. It is a characteristic of the airborne route that the possibility of aerial transfer also raises the possibility of transfer by other routes.[57] The precipitation of airborne bacteria has previously been demonstrated in a study of opened sterile operating trays.[65] The instruments inside became contaminated without being touched within an hour, suggesting an airborne route of contamination.[65] Further studies have demonstrated contamination of wounds by airborne bacteria.[68] Combined with the information from the control charts, this suggests that any person entering the room during a dressing change or bed sheet change needs to don adequate personal protective equipment (PPE), regardless of whether they are actively participating in the activity or not. It also highlights the risk of contamination, not just of the parts of the body of a HCW such as the hands and abdomen that come into contact with burns patients and their surroundings, but other areas where airborne bacteria may land (such as the hair). This provides an argument for HCW also wearing protective caps and visors. When airborne bacteria ultimately land on surfaces around the room, they will contribute to the reservoir for infection. This highlights the need for a thorough cleaning of the whole room after a dressing/bed change, rather than just areas that have been physically touched by the patient or HCW.

As the airborne bacterial counts remain 'out of control' for a period of up to an hour or more after a dressing/bed change, this cleaning should be delayed until the maximum number of bacteria have precipitated out of the air and settled onto surfaces. This may also be regarded as a 'high-risk' time, during when anyone entering the room should don adequate PPE.

The importance of bed sheet changing as an independent event leading to airborne dispersal of bacteria has been highlighted, indicating that the same degree of protective clothing may be required for sheet changes as is required for dressing changes. The impact of bed sheet changes on the level of bacteria in the air has previously been demonstrated.[51] The authors simultaneously carried out surface sampling using MSO (MRSA isolating) agar stamps directly onto the floor (x3), sheet, table and patient clothing before and 60 min after the bed sheet change. They found that the levels on surfaces were the same or lower after bed making than before.[51] There are several possible explanations for this. Firstly a relatively low number of samples were taken, three repetitions from six sites each time. Secondly, the authors did not take into account the fact that the bed sheet had been changed, so that sampling from a dirty bed sheet before the sheet change was bound to yield a higher bacterial count than that from a fresh sheet after the change. Similarly, they did not detail any cleaning that took place in the room at around the sampling time, although cleaning would often coincide with a bed sheet change. Finally, their 'post sheet change' sample was only taken once, 60 min after the end of the bed sheet change. No attempt was made to determine whether this was adequate time to allow for settling of airborne particles onto surfaces.[51] They concluded that the airborne route was a significant means of cross contamination of MRSA between patients via inhalation, direct patient and staff contamination and environmental contamination.[51] Samples of the environment were not taken during the production of the control charts, but the effects of activities within the patient's room have previously been highlighted in Chapter 5. The variation in environmental bacterial contamination at different times of day led to the use of an early morning sampling period for all future inpatient studies.

Control charts were used as a statistically validated method to demonstrate that the level of contamination significantly increased during a dressing/bed change compared with levels before that event began. It is also useful to consider the levels found in relation to recommended guidelines for acceptable levels of airborne contamination. Guidelines state that there should be fewer than 35 cfu/m^3 in an empty operating theatre, and fewer than 180 cfu/m^3 during an operation.[402-404] These parameters change to 1 cfu/m^3 and 10 cfu/m^3 respectively in an 'ultra-clean' theatre (usually reserved for patients undergoing joint replacement surgery).[405] In reality, reported levels of airborne bacteria in operating theatres range from 1-500 cfu/m^3.[68,400,406] Levels in a medical intensive care unit were found to be on average 447 cfu/m^3.[78] One study on a burns unit

identified a maximum airborne dispersal of 36 cfu/m^3 during a routine nursing period.[88] The same authors found up to 339 airborne *S. aureus* cfu/m^3 during the early treatment of a burn.[54] As previously discussed, a further study on a burns unit identified levels of 1-9 MRSA cfu per 20 L (50-450 MRSA cfu/m^3).[53] In the four studies presented here, mean levels during minimal activity 'control' periods before the dressing/bed change commenced ranged from 7.0 cfu/m^3 to 212.0 cfu/m^3: similar levels to those previously reported. During the dressing/bed changes, the maximum levels recorded for each of the four studies was between 346 cfu/m^3 and 2614 cfu/m^3: clearly higher than any of the recommended levels for operating theatres, although no recommendations for burns units exist. This supports the theory that burns patients are potent dispersers of airborne bacteria, particularly during high levels of activity, and that this route is a significant method of cross-contamination on the burns unit.[53]

This study is limited and a greater number of repetitions would be needed to enable definitive conclusions about airborne bacterial dispersal from burns patients or inter-patient comparisons to be made. Previous work has claimed a correlation between burn size and amount of bacteria dispersed into the air and onto settle plates.[54] This was not in relation to any particular activity, such as dressing changes, and the settle plates were exposed tor 3-5 days. It is unclear how settle plates exposed for this amount of time would not have completely desiccated, and how the wide range of exposure times did not affect results.[54] In the more precise measurements outlined here, where air quantity was measured for 3 min intervals, no such correlation could be found between patients. This may be due to the amount of dispersal changes as the burn wound ages: the authors from the same paper also claimed that the number of bacteria precipitated by the same patient increased during the first two weeks, after which time it began to plateau.[54] However, this paper was from the era of exposed wounds and before the practice of early excision and grafting, so results are not comparable with a modern burns unit.[54] No such attempt has been made since to link the size of the burn and the amount of bacteria released. Airborne bacterial counts are likely to depend on a multitude of factors such as the burn size, age of burn, patient co-morbidities, the use of assisted ventilation, infections of the respiratory tract and wound contamination or infection. What these studies have achieved is to develop a sampling method for future work and to indicate the potential implications for the burns unit.

Further work may involve the use of an Anderson air sampler, in order to stratify the organisms collected according to their size. This would help determine how long the bacteria are likely to be suspended in the air, as the size of a particle is known to be inversely proportional to the length of time it is airborne.[388] The median particle size dispersed by burns patients has been shown to be between 3.5 μm and 5.6 μm (indicating an airborne suspension time of between 17 min and indefinite), thus highlighting the great potential for airborne spread of microorganisms from burns

patients.[54] Such work may enable the creation of evidence-based guidelines for the PPE worn by HCW carrying out both dressing changes and bed sheet changes, as well as anyone entering a room for a period of time during and after a dressing/bed change has taken place. In fact, these studies have already led to a policy review in the burns unit at GRI.

Considering the HINS-light EDS, these findings point to exciting avenues of research, as they clearly identify dressing/bed changes as periods of increased bacterial dispersal. It may be that carrying out these activities in a room where the HINS-light EDS has been in use for several days beforehand reduces the amount of bacteria on the bed sheets to such an extent that fewer are dispersed during sheet changes. Alternatively a 'boosted' HINS-light irradiance during these events may expedite decontamination of the airborne bacteria during these 'high-risk' activities. Certainly, the studies in Chapter 5, using a HINS-light EDS while measuring irradiance suggest that a significant proportion of the HINS-light EDS effect takes place on airborne, rather than surface bacteria. The control charts suggest that the bacteria released during a dressing/bed change are airborne for at least 45 min: sufficient time to enable the HINS-light EDS to have a decontamination effect, particularly in a desiccated and stressed state. This is an area that would be of great interest in future work involving the HINS-light EDS, however unfortunately, due to time restraints, was unable to be investigated in the present body of work.

7.4 Healthcare worker (HCW) contamination during an event

Introduction

HCW uniforms are reservoirs of infection,[407-409] and their contamination can be directly attributed to patients.[79-81] Not only can bacteria be transferred from burns patients to uniforms during dressing changes, but also laboratory simulations have demonstrated that these bacteria can then be transferred from the uniform to other patients.[88,410] Despite this, there is little consensus for the appropriate PPE to be worn by HCW carrying out dressing changes on burns patients. In a survey of US burn units, only 24% of units required full protective coverage on entering a patient's room and changing a dressing.[43] UK guidelines are similarly vague and not burns-specific.[138,140,411] Quantitative data on key issues surrounding the contamination received by a HCW caring for a burns patient may help in their development.

Bacteriological sampling methods and preliminary study results

To investigate the levels of contamination received by HCWs during events such as dressing and bed sheet changes, BPA contact plates were used to collect bacterial samples from HCW during these events. To ensure that samples were taken from a standardised baseline, HCW were asked to don sterile, impermeable, disposable full-body gowns over their uniforms prior to performing dressing/bed changes. This was done to eliminate natural variations in bacterial contamination between different HCW before the start of the dressing/bed change. It also provided a consistent sampling material, which was preferable to sampling from a variety of textures and surfaces including cotton and skin. Gowns were thus worn by the HCW to facilitate the study design and sampling objectives. Usually, disposable non-sterile plastic aprons would be worn over uniforms for the execution of routine dressing/bed changes. All HCW maintained standard hand hygiene by decontaminating hands and putting on fresh disposable gloves before entering the patient's room to carry out the nursing activity. Thereafter, with the exception of wearing gowns rather than aprons over their uniforms, the HCW carried out the dressing/bed change in the usual manner. Gowns were removed and hands washed following the dressing/bed change and gown sampling, before leaving the room.

To investigate both the total viable bacterial contamination and the staphylococcal-type contamination received by HCWs, initial studies were carried out using both TSA and BPA contact plates, with samples being collected simultaneously from adjacent sampling sites on the HCW gown.

For initial studies using both the selective and non-selective contact plates, two nurses were sampled each time during two different dressing changes. The first was on a

patient with 35%TBSA flame burns, and the second on a patient with 51%TBSA flame burns. Samples were taken from each of the 20 sampling sites shown on the left of Figure 7.11 using both TSA and BPA contact agar plates, immediately adjacent to each other at each site. The contact agar plates were incubated and enumerated.

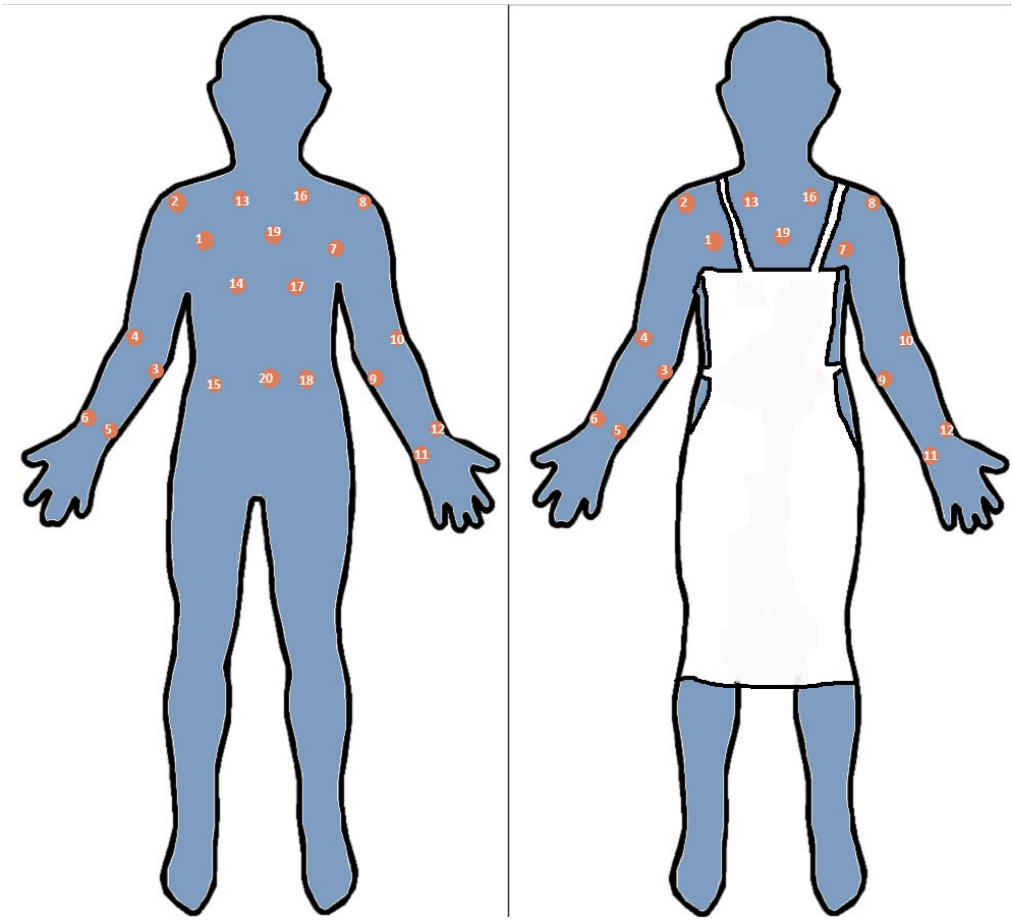


Figure 7.11: *Diagram to demonstrate sampling sites on the front of HCW gowns. The image on the left shows the positions of all 20 sampling sites (termed 'no apron' sites). The image on the right highlights the 15 sampling sites theoretically left exposed if the HCW had been wearing an apron (termed 'with apron' sites). The two sets of samples were analysed separately.*

Figure 7.12 shows an example of the correlation between bacterial contamination detected using BPA and TSA contact plates at each individual sampling site on the gown of one HCW.

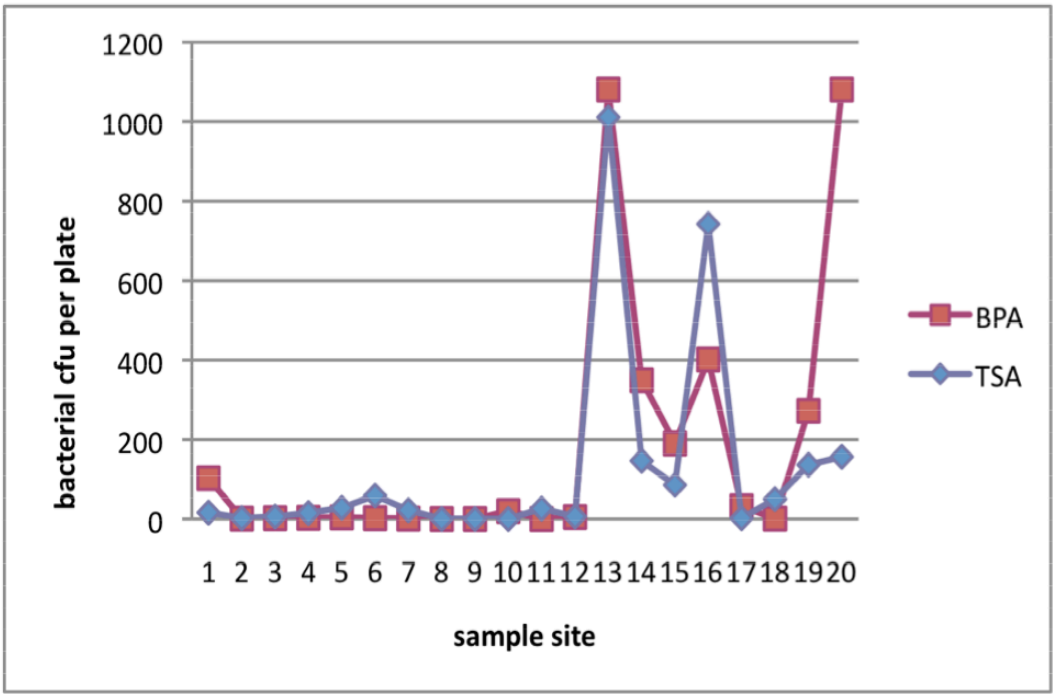


Figure 7.12: *Bacterial cfu/plate collected using TSA and BPA contact plates from 20 sampling sites on one HCW. At each sampling site, TSA and BPA contact agar plates were used immediately adjacent to each other.*

This particular study showed good correlation at almost every sampling site between the two types of agar, and this was seen to some extent in all four preliminary studies. For comparison, an average for all 20 sample sites for both agar types was taken from each study, and the results found when using TSA were compared to those seen when using BPA, as shown in Figure 7.13.

These four preliminary studies helped to confirm that either agar would show comparable trends, indicating that it is likely that the majority of the contamination deposited on HCWs during dressing and bed sheet changes are staphylococcal-type bacteria. BPA was therefore selected for use in all future studies as it had been used for all previous environmental sampling, and would be more likely to isolate bacteria from a human source.

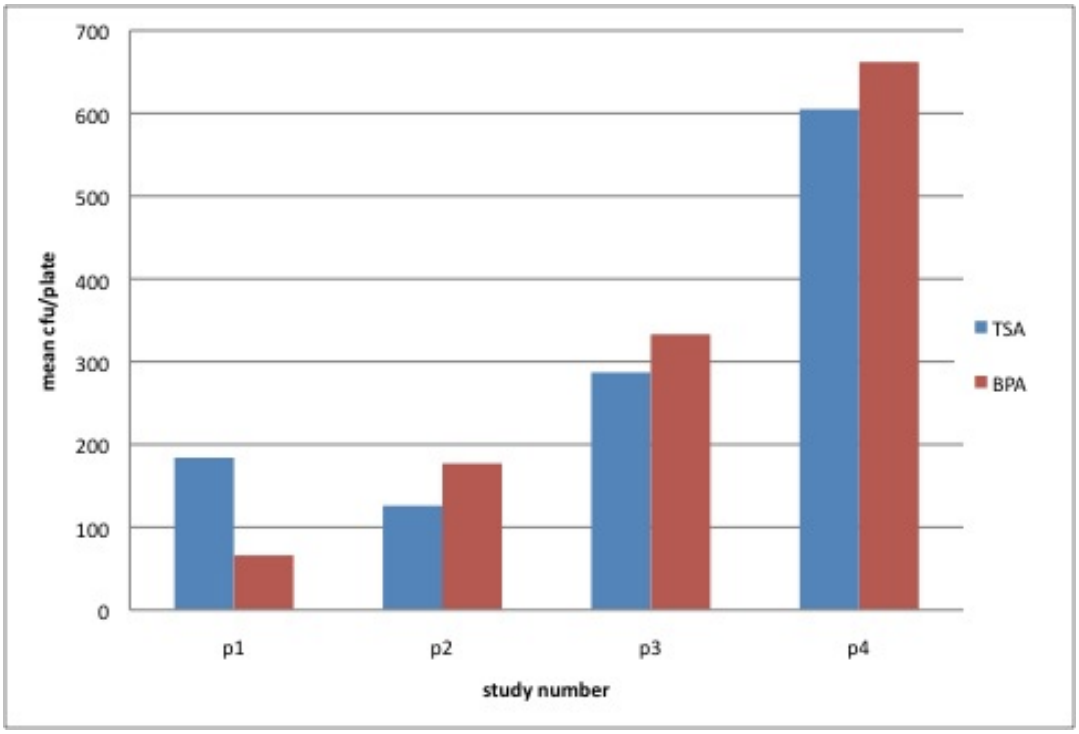


Figure 7.13: *Preliminary studies comparing the bacterial contamination collected from the gowns of four healthcare workers after a dressing/bed change. Results represent the mean cfu/plate from the 20 sampling sites across the HCW gown.*

Confirmed study protocol

With the contact plate media selected, studies progressed using the BPA contact plates to sample the HCW gowns. During studies, samples were taken from the two most 'involved' HCW carrying out the dressing change, each of whom would usually stand either side of the bed and simultaneously carry out undressing followed by redressing of wounds. For smaller burns, one HCW often carried out the dressing change alone, and only one set of samples was obtained. Sampling during dressing/bed changes on any one patient was carried out once only. The HCW gown was sampled following the dressing/bed change, while the HCW was still wearing the disposable gown, and remained in the patient's room. Samples were collected by firmly pressing the contact plate on the gown surface for approximately 2 sec.

The 20 sampling sites used are illustrated in Figure 7.11 (left image). These 20 selected sites were located across the front of the gown, as it is more likely that the front than the back of a HCW will come into contact with a patient or hospital equipment when carrying out nursing tasks, and it was the aim of the study to collect samples from areas that were likely to become most contaminated during dressing/bed changes. These 20 sites were used to represent the level of bacterial contamination that HCW would

receive if not wearing an apron during dressing and bed changing. These were therefore termed '*no apron*' sites.

In order to estimate the protection afforded had a disposable plastic apron been worn, a subset of 15 sites outwith the zone which would typically be protected by use of an apron were analysed separately. These excluded the five sampling sites on the chest and abdomen that would normally be covered by a disposable apron, as also demonstrated on the right of Figure 7.11. These 15 sites were termed '*with apron*' sites. These 'no apron' and 'with apron' sites are described anatomically in Table 7.1.

Table 7.1: *Twenty sampling sites on gowns worn by HCW. Sites that would be covered by disposable plastic aprons, had these been worn, are identified in the final column.*

Sample number	Anatomical site	Potentially covered by apron?
1	Right anterior shoulder	No
2	Right posterior shoulder	No
3	Right anterior elbow	No
4	Right posterior elbow	No
5	Right anterior forearm	No
6	Right posterior forearm	No
7	Left anterior shoulder	No
8	Left posterior shoulder	No
9	Left anterior elbow	No
10	Left posterior elbow	No
11	Left anterior forearm	No
12	Left posterior forearm	No
13	Right clavicle	No
14	Right chest	Yes
15	Right waist	Yes
16	Left clavicle	No
17	Left chest	Yes
18	Left waist	Yes
19	Sternum	No
20	Umbilicus	No

For analysis, the mean bacterial cfu/plate count was calculated. The mean value for the full 20 sites across the HCW gown was used if analysing the 'no apron' sites, and the mean value of the 15 subset sites was used if analysing 'with apron' sites.

'Minimal activity' controls

To determine the sterility of the gowns, and show levels of bacterial contamination produced by merely donning the gown and wearing it in a patient room, a volunteer donned a gown and stood in an inpatient isolation room containing a patient with 30%TBSA burns for 30 min before a dressing change started. A further volunteer nurse donned a gown to carry out her usual duties (checking monitoring, adjusting drug pump

rates, entering data into computer etc.) within the room for 30 min before the start of the dressing change. Both gowns were sampled from the same 20 sampling sites used for the other studies. This provided two 'minimal activity' controls.

Time parameters

The time taken for the dressing/bed change to take place was measured from when the HCW entered the patient's room to commence the dressing/bed change (the point at which they would usually don a plastic apron). It finished at the point when the dressing and bed sheet change (if that was also being carried out) was completed, when they would usually remove their apron and gloves prior to leaving the room. At this point the gown was sampled. Any further activities, including tidying the room, assisting with feeding, or brushing the patients' hair or teeth were not included in the time taken for dressing/bed change. The gown was sampled before these extra activities took place. This meant that only the contamination received during the dressing/bed change was measured.

Statistical analysis

For the purposes of publication, statistical analysis was carried out under the supervision of Prof. George Gettinby. HCW bacterial contamination was expressed as mean number of bacterial cfu per $25cm^2$ agar plate, or mean cfu/plate. Statistical analysis was carried out using NCSS Windows Version 7 software. Relationships between three variables were examined:

- %TBSA and time taken for the dressing/bed change;
- %TBSA and HCW contamination;
- time taken for the dressing/bed change and HCW contamination.

Separate analysis was carried out on all 20 'no apron' sites, and on the 15 'with apron' sampling sites. Mathematical modelling was used to identify equations which best described the three relationships. These were used to predict the contamination a HCW would receive during dressing/bed change of a burn patient by %TSBA. The coefficient of determination, R^2, was used to measure how well the model fitted to the observed data and $p < 0.05$ was considered significant.[413]

Results: Patient demographics

Samples were collected from the gowns of 24 HCW carrying out dressing changes on 15 different patients, with a mean burn size of 19%TBSA (range 1%TBSA to 51%TBSA).

Mean age of patient was 39 years (range 19 years to 85 years). Samples were taken a mean of 6.4 days after the burn injury (range 2 days to 10 days). Mean time taken for the dressing change was 45 min (range 10 min to 90 min). The most common organism identified on routine wound swabs was *Staphylococcus aureus. Bacillus* sp., coliforms, and *Streptococcus* sp. were also commonly isolated. Results are summarised in Table 7.2.[413]

Study no	Patient	Pt age (years)	% TBSA burn	Depth of burn	Age of burn (days)	% TBSA donor site harvested	% TBSA covered in skin or substitute	Wound swab results	Dress-ing change	Bed sheet change	Time taken (min)	Mean cfu/ plate 20 sites	Mean cfu/ plate 15 sites
1	A	19	1	DPT	6	0	0	Not taken	Yes	No	10	23	18
2	B	24	2	SPT	6	0	0	Not taken	Yes	No	25	12	9
3	C	26	2	SPT	6	0	0	Not taken	Yes	No	10	14	5
4	D	44	2	SPT	10	0	0	Not taken	Yes	No	20	13	4
5	E	34	6	DPT/ FT	8	6	6	*Staphylococcus aureus*, *Bacillus* sp.	Yes	No	40	40	27
6	E	34	6	DPT/ FT	8	6	6	*S. aureus, Bacillus* sp.	Yes	No	40	13	5
7	F	33	6	DPT	9	6	6	coliforms, *S. aureus*, Gp G *Streptococcus*, *Bacillus* sp.	Yes	No	50	1	1
8	G	22	7	SPT	8	0	0	coliforms, *S. aureus*, Gp A *Streptococcus*, *Bacillus* sp.	Yes	No	20	50	22
9	H	45	15	FT	6	9	15	*S. aureus*, , *Bacillus* sp., *Clostridium perfringens*	Yes	Yes	55	54	41
10	H	45	15	FT	6	9	15	*S. aureus*, , *Bacillus* sp., *C. perfringens*	Yes	Yes	55	50	21
11	I	85	16	DPT/ FT	120	0	0	*S. aureus, Bacillus* sp.	Yes	Yes	25	101	90
12	I	85	16	DPT/ FT	120	0	0	*S. aureus*, , *Bacillus* sp.	Yes	Yes	25	20	20
13	J	39	30	DPT/ FT	7	9	15	*S. aureus, Streptococcus pneumoniae*	Yes	Yes	50	108	118
14	J	39	30	DPT/ FT	7	9	15	*S. aureus, S. pneumoniae*	Yes	Yes	50	97	52
15	K	46	30	DPT/ FT	6	0	0	*S. aureus, Streptococcus* sp., *Bacillus* sp.	Yes	Yes	55	28	7
16	K	46	30	DPT/ FT	6	0	0	*S. aureus, Streptococcus* sp., *Bacillus* sp.	Yes	Yes	55	25	26
17	L	55	35	DPT/ FT	4	0	0	Methicillin resistant *S. aureus* (MRSA)	Yes	Yes	60	177	126
18	L	55	35	DPT/ FT	4	0	0	MRSA	Yes	Yes	60	66	71
19	M	29	41	FT	8	18	18	coliforms, *S. aureus, S. pneumoniae, Bacillus* sp.	Yes	Yes	90	142	96
20	M	29	41	FT	8	18	18	coliforms, *S. aureus, S. pneumoniae, Bacillus* sp.	Yes	Yes	90	294	233
21	N	40	51	FT	6	4	32	*Enterococcus cloacae*	Yes	Yes	78	662	569
22	N	40	51	FT	6	4	32	*E. cloacae*	Yes	Yes	78	333	569
23	O	45	43	FT	2	1	18	No growth	Yes	Yes	85	287	259
24	O	45	43	FT	2	1	18	No growth	Yes	Yes	85	420	341

Table 7.2: *Summary of all 24 studies of HCW carrying out dressing/bed changes on 15 patients. Information collected included: size of burn (% TBSA); depth of burn (SPT= superficial partial thickness; DPT= deep partial thickness; FT= full thickness); age of burn in days; the %TBSA that had been harvested as a split thickness skin graft; the %TBSA that has been covered by autograft or dermal substitute; recent wound swabs; whether a dressing change and bed change took place; time taken for the dressing/bed change; and the mean cfu per plate for all 20 'no apron' sites, and the 15 'with apron' sites. Adapted from* [413].

Results: 'minimal activity' controls

The gown of the volunteer standing still in the inpatient room for 30 min before the start of the dressing change yielded a mean of 1.4 cfu/plate. The gown worn by the nurse carrying out routine activities for 30 min before the start of the dressing change, yielded a mean of 2.6 cfu/plate. Most bacteria were isolated from across the central abdomen, supporting the theory that this area tends to come into contact with contaminated surfaces such as tables and cot sides more than any other when caring for a patient.

Results: Relationship between time taken for dressing/bed change and %TBSA

A relationship was demonstrated between the time taken for the dressing/bed change to take place and the size of the burn (%TBSA). This was explained by a linear correlation (coefficient of determination, R^2=0.76; p<0.001) and is demonstrated in Figure 7.14.

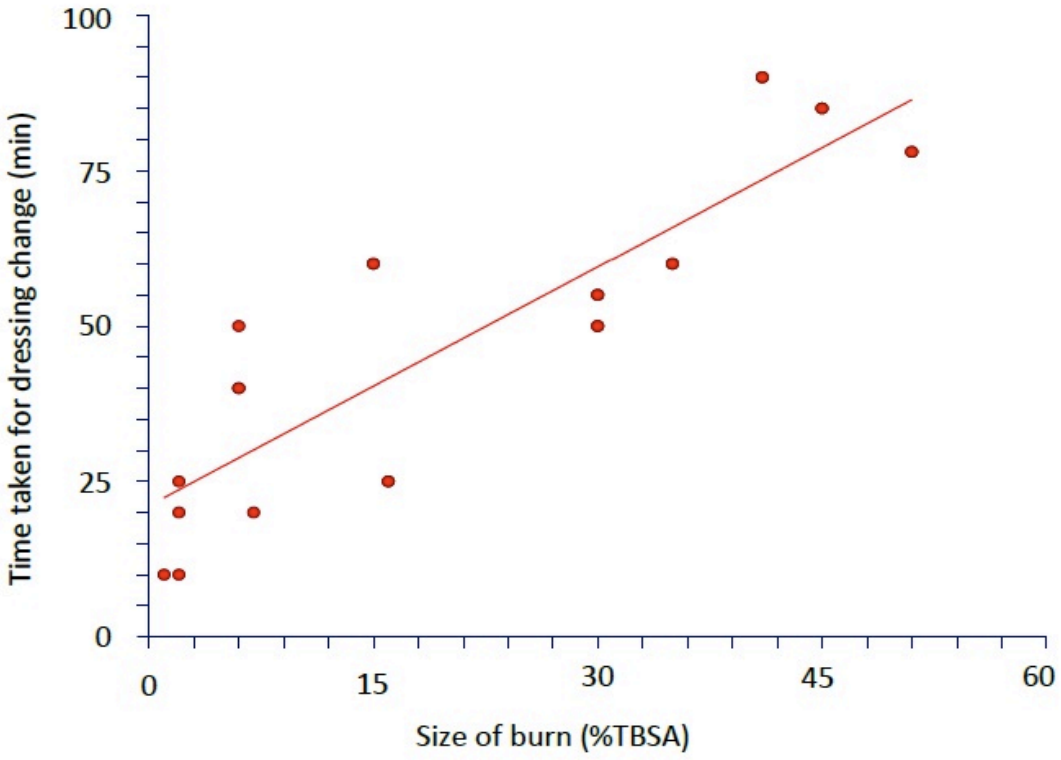


Figure 7.14: *Chart (NCSS Windows V7) demonstrating linear relationship between %TBSA of the burn, and time taken in min to complete the dressing/bed change. Adapted from* [413].

Results: Analysis of 20 'no apron' sites

The variation in contamination received by a HCW during a dressing/bed change when 20 'no apron' sampling sites were analysed was examined in relation to %TBSA of the burn and time taken for the dressing/bed change. Exponential models explained both relationships. These were as follows:

Relationship between HCW contamination and %TBSA (coefficient of determination, R^2=0.82; p<0.001):

Mean cfu/plate = 8.59 $Exp^{(0.080 \times \%TBSA)}$

Relationship between HCW contamination and time taken in min for dressing/bed change (coefficient of determination, R^2=0.52; p<0.002):

Mean cfu/plate = 17.44 $Exp^{(0.034 \times \text{time taken in min})}$

These curves are illustrated in Figure 7.15. Both charts demonstrate an exponential relationship between the variable (%TBSA or time taken for the dressing/bed change to take place) and the contamination received by the HCW. However, although they are both significant relationships, time taken correlates less strongly than %TBSA as shown by the lower R^2. Therefore, %TBSA is a more accurate predictor of HCW contamination than time taken for the dressing/bed change to take place.

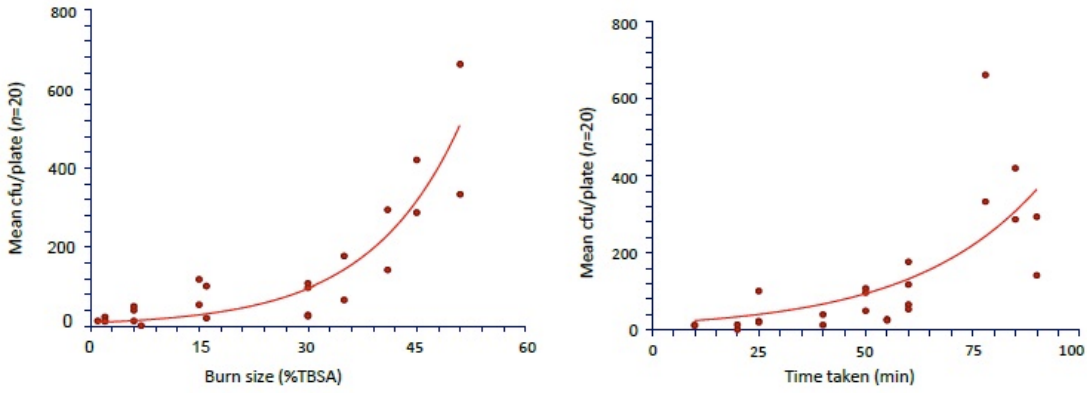


Figure 7.15: *Charts demonstrating exponential relationships between %TBSA and mean cfu/plate (left) and time taken in minutes for dressing change and mean cfu/plate (right) when all 20 'no apron' sampling sites on a HCW gown are analysed.* Adapted from[413].

Results: Analysis of 15 'with apron' sites

The variation in contamination received by a HCW during a dressing/bed change when 15 'with apron' sampling sites was examined in relation to %TBSA of the burn and time taken for the dressing/bed change. Exponential models explained both relationships. These were as follows:

Relationship between HCW contamination and %TBSA (coefficient of determination, R^2=0.86; p<0.001):

Mean cfu/plate = 2.05 $Exp^{(0.110 \times \%TBSA)}$

Relationship between HCW contamination and time taken in min for dressing/bed change (coefficient of determination, R^2=0.44; p=0.007):

Mean cfu/plate = 15.98 $Exp^{(0.034 \times \text{time taken in min})}$

These curves are illustrated in Figure 7.16. Again, both charts demonstrate an exponential relationship between the variable (%TBSA or time taken for the dressing/ bed change to take place) and the contamination received by the HCW. However, although they are both significant relationships, time taken correlates less strongly than %TBSA as shown by the lower R^2. %TBSA is a more accurate predictor of HCW contamination than time taken for the dressing/bed change to take place.

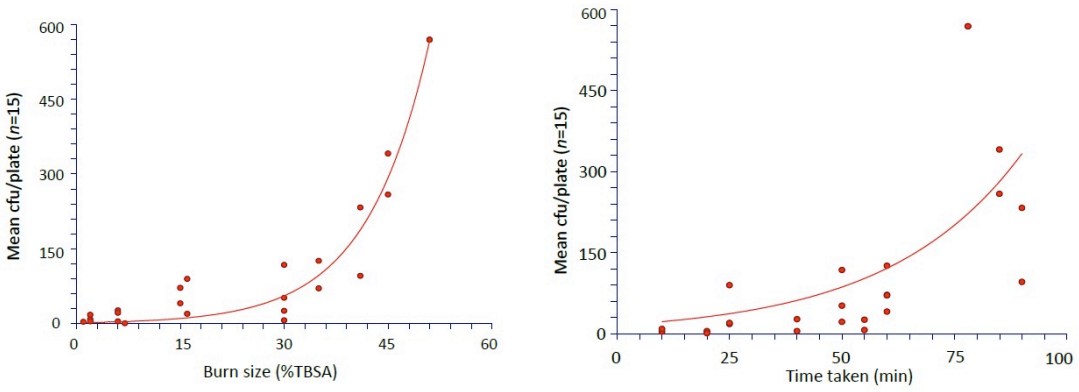


Figure 7.16: *Charts demonstrating exponential relationships between %TBSA and mean cfu/plate (left) and time taken in minutes for dressing change and mean cfu/plate (right) when 15 'with apron' sampling sites on a HCW gown are analysed.* Adapted from [413].

Results: Predicted contamination of HCW

Using the above statistical models, the expected mean number of bacterial cfu per 25cm^2 plate from a HCW performing a burns dressing/bed change can be predicted. This was produced from both data sets for all 20 'no apron' sites and the 15 'with apron' sites. These values are summarised in Table 7.3. It was found that for every 9%TBSA increase in burn size, the mean number of cfu/plate doubled when all 20 sites were analysed. This was true for every 6%TBSA increase in burn size when 15 'with apron' sites were analysed.

Table 7.3: *Predicted mean contamination received by HCW performing a burn dressing/ bed change. All 20 'no apron' sites, and the 15 'with apron' sites that would be left exposed if the HCW donned a plastic apron, are analysed separately for comparison. Results are expressed as mean bacterial cfu per 25cm^2 agar plate.*

% TBSA	Predicted mean cfu per 25cm^2 plate for 20 'no apron' sites	Predicted mean cfu per 25cm^2 plate for 15 'with apron' sites
5	13	4
10	19	6
15	29	11
20	43	18
25	64	32
30	95	56
35	141	97
40	211	168
45	314	292
50	469	507

Discussion

The potential for HCW and their uniforms to become contaminated with microorganisms has long been known.[80,86,87,407,409] This study highlights high levels of HCW contamination following a dressing/bed change and quantifies levels of bacterial contamination received for the first time. A HCW who has become contaminated by carrying out a dressing change will subsequently contact other patients or environmental surfaces, dispersing organisms, where they can survive for several weeks and form an environmental reservoir.[93,413] The environment may then contaminate another patient directly or indirectly via the hands or uniform of a HCW acting as a carrier for nosocomial infection.[31,70,86]

Previous studies have examined the degree of contamination of nurses' uniforms and protective clothing in a variety of wards and using different sampling methods. A Cassella slit sampler, sampling 150 L of air was used to 'vacuum' an area of uniform,

from the belt to the hem of 57 HCW.[408] Prior to the commencement of duty, 39% of uniforms were positive for MRSA, *Clostridium difficile,* and VRE, with levels ranging from 1 to 100 cfu/150 L air sample. At the end of duty, 54% uniforms were positive for one or more of the three organisms, although on one medical ward, levels were 92%.[408] While providing valuable results, there was no record of what activity the HCW were carrying out, nor for how long the HCW was 'on duty'. The staff worked on different wards and presumably played different roles. Furthermore, there was no standardised baseline: some staff members even confessed to wearing dirty uniforms at the start of their duty. The possibility of staff members contaminating their own uniforms throughout the course of the day by shedding skin cells was not adequately addressed: the authors sampled one uniform that had not been worn before duty commenced and found levels to be the same as those on uniforms that had been worn. From this they concluded that no self-contamination took place. This did not take into account contamination occurring from the HCW throughout the day. Although the authors claimed that the vacuum sampling method yielded higher counts than contact plating, contact plating was chosen for the current studies. As a single-stage sampling method that samples directly from a surface (the HCW gown), it was felt that the vacuum method may also collect contamination from other sources. Furthermore, controlling the area that is sampled is not possible with the vacuum method as it is with contact plate sampling, as the same size area is sampled each time.[408]

It is true however that a higher number of bacteria are isolated from the gown of a HCW using a vacuum method than by the two other main methods used: sweep plate or contact plate sampling.[408] A study into the contamination of the uniforms of burns nurses compared the three and found that about 10 times as many *S. aureus* cfu were isolated using the vacuum method than with the other two.[88] The authors went on to develop a 'wash method' of bacterial sampling, which isolated even more bacteria, whereby the gown was removed and washed, and the wash water used to inoculate agar plates with bacteria.[88] Again, the obvious concern with this method, and the reason it was not chosen for the current studies was the great potential for contamination of the uniform with the HCW own organisms. It was felt that for these reasons contact plating was the most reproducible method of quantifying only the staphylococcal-type bacteria originating during the course of the specific activity (bed/sheet change).

Notwithstanding any personal reservations about the wash method, these authors examined in a great deal of depth the amount of contamination received by the staff of a burns unit, and the transfer of such contamination to burns patients.[88,410,414] The situations were broader than the specific scenarios studied here. Uniforms were sampled after a morning of nursing activity, with colony counts of 4×10^3 cfu of *S. aureus* isolated by the wash method, and 1×10^2 cfu of *S. aureus* by the vacuum method.[88] The authors adopted the wash method, multiplying the total number of colonies by a

number to estimate the total number of *S. aureus* per gown. This was based on their estimation of 38% of the total number of *S. aureus* being recovered by the wash method.[88] The complexity of these methods, and estimations required was something that was avoided in the studies here, by carrying out a simple one-stage procedure to estimate the mean number of cfu per 25cm^2 BPA plate. The authors did show via a model experiment that the bacteria from the nurses' uniforms were transferred to a model patient in a mock nursing situation.[88,410] A transfer rate of approximately 1% from mock nurse to mock patient was claimed, although this involved several different people donning gowns and uniforms, with a great potential for cross contamination at each stage of the experiment.[88] Furthermore, this very false situation may or may not correlate with real life: something that was avoided here by keeping everything the same as it would be during any dressing/sheet change, bar the use of gowns. It was intentional during this work that the focus be on the areas of high contamination that are likely to come into contact with another surface or patient (arms, chest and abdomen), rather than the total count for the whole gown.

Other authors have used a 'sweep plate' method, whereby an agar plate is rubbed 3-4 times over four sites on the surface of cotton dresses worn by HCW working in a general surgical ward.[80] *S. aureus* was found to be isolated from 145/736 (20%) plates. The average number of cfu was low (2.8 cfu/plate). This may represent the sampling method being less efficient at recovering bacteria than the contact plate method, or the lower levels of contamination on a non-burns ward, making the study difficult to compare with the present work. Alternatively, cultures have been taken using a moistened cotton-tipped applicator.[8=79,81,409] The first study, examining MDR *A. baumannii* transfer, found an 11% frequency of contamination of the HCW gown after an interaction with an infected patient.[79] A further paper using a moistened swab to sample HCW uniforms described swabbing a 'W' on the beltline using a twirling motion.[81] However, the size of the 'W' and the amount of twirling involved can alter the collection rate of bacteria considerably. The authors used selective agar to examine for MRSA and VRE. A 19% rate of transfer of MRSA, and a 9% transfer of VRE was observed on HCW gowns and gloves following interaction with infected patients. Again, this is a valuable indicator of the transfer of bacteria from patients to HCW during routine nursing activities, but the methods, patients and activities studied are not really comparable with the present study.

The final paper using this method, described sampling nurses' uniforms at the beginning, middle and end of a shift using a moistened swab that was applied to a blood agar plate.[409] Wide individual variation was found between staff members, with a mean of 55 cfu/plate on HCW uniforms at the start of the shift. This was not significantly higher by the end of the shift (59 cfu/plate). The use of plastic aprons did not appear to reduce the colony count of the underlying uniforms.[401] These findings highlighted the

important contribution of staff members themselves to the contamination of their own uniforms, as levels were high to begin with. For this reason, sterile disposable full-body gowns were worn for the current study to enable sampling from a surface that was known to be sterile prior to the nursing activities. Standard practice on the burns unit at GRI is for disposable non-sterile plastic aprons to be worn for most dressing and bed changes, excluding those taking place in the intensive care unit or on known heavily contaminated patients.

With regards to quantifying the level of contamination received by a HCW in relation to the size of the burn surface area, this has not been attempted previously. As mentioned in Section 7.3, attempts were made in the 1970s to quantify the airborne release of bacteria in relation to the size of the burn.[54] The methods used are fairly crude however; with settle plates being exposed for up to five days, and only five plates being used each time.[54] In contrast, the current study measures specifically what is of interest: the amount of contamination received by a HCW carrying out a single task.

Guidelines on the use of protective clothing for HCW during burns dressing/bed changes are limited and not burns-specific. As will be seen in Section 7.6, this has led to a variety of practices amongst UK burns units. Based on the results of this study, guidelines may require to be revised with consideration of the amount of contamination received by HCW during performance of these routine nursing activities. The use of gloves and meticulous hand hygiene for all dressing changes is accepted practice and not examined here.[81,84] Of note, WHO recommend a '5 moments for hand hygiene' approach whereby hands should be cleaned before and after all procedures and contact with patient surroundings.[139] It may be argued that the HCW in this study should have been encouraged to wash their hands several times during the activity, rather than just at the beginning and end. However as they were in constant contact with the environment, patient, and open wounds throughout the duration of the activity, dividing the dressing/ bed change into distinct 'moments for hand hygiene' was difficult, and a significant change from current practice in our unit. The compliance with these recommendations is, however, unlikely to affect the levels of bacteria found on the gowns as they concern only hand hygiene. The results of this study have led to a review of clinical practice within the unit, and revised guidelines on protective attire worn by HCW.

The mathematical models produced indicate that a HCW performing a dressing change on a patient with a 15%TBSA burn could expect to become contaminated with a mean of 29 bacterial cfu per 25cm^2 if they wore no protective clothing, and 11 bacterial cfu per 25cm^2 if a plastic apron was worn, supposing absolute protection is afforded by the apron. For large burns, prediction of levels of contamination when a HCW wears or does not wear an apron highlights the limitation of relying only on the apron as a means of prevention of HCW contamination. For example, a 50% TBSA burn is estimated to

produce 469 cfu/plate when a HCW is wearing 'no apron', compared to 507 cfu/plate when they are 'with apron'. The majority of samples were collected from the forearms, arms, shoulders and chest: areas of skin and uniform which would not be protected or cleaned during hand washing and may come into contact with other patients or equipment. Before the study was initiated, HCW were encouraged to act exactly as they would do were they wearing an apron. Whilst this was the agreed intention, it is nevertheless possible that they may have been less careful than usual, knowing they were covered by a gown, or more careful as they were conscious they were part of a study. Regardless of this possible effect, the results highlight the need for a review of protective guidelines for HCW.

Burns between two and ten days old were examined, although numerous factors such whether debridement had taken place, donor site size, comorbidities and bacteria isolated from the wound were unable to be controlled. Despite the inclusion criteria being fairly broad, %TBSA was still shown to be an important predictor of HCW contamination. Future studies would be useful to monitor the change in HCW contamination as a burn progresses towards healing, or as the patient becomes colonised with increasingly resistant organisms. Furthermore, BPA was used throughout to monitor staphylococcal-type bacteria, but other selective media may be used in the future to identify other organisms that colonise burns wounds, such as Gram-negatives, which may show different transfer characteristics between patients and HCW. Were the studies to be repeated on a larger sample size, quantitative analysis of wound contamination may be attempted, although this would only be an estimate. However this would not be helpful in predicting contamination and thus guiding HCW on which protective attire to wear due to the results being unknown until after the dressing/bed change had taken place.

Despite the relatively small sample size, an excellent correlation of 82% was demonstrated, enabling the production of mathematical models. The largest burn studied was 51%TBSA so extrapolation to predict contamination from larger burns was not attempted. Although further studies may help to show the contamination produced by much bigger burns, at the upper limits of %TBSA tested, many agar plates were very heavily contaminated, and much more contamination would probably render the number of bacterial cfu uncountable. Suffice to say any burns over 50%TBSA would cause very heavy contamination of > 500 cfu/plate. It is important to note that all results are reported as cfu per 25cm^2 plate, and the total contamination across a whole gown wound be many times this figure. What is not known is what constitutes a 'significant number' of bacteria. Further work would need to be carried out to determine the transfer rate from the HCW to another surface or patient. As previously discussed in Section 2.4, the transmission of bacteria from HCW hands and uniforms to patient bedclothes has been demonstrated during bed making and when touching surfaces

around a patient room.[89,90] Alternatively, an arbitrary figure may be assigned as a pre-determined cut off point above which full-body protection should be worn. The cost of full body protection must also be considered and weighed up against the perceived risk of transfer from a HCW.

It is logical to assume that a larger burn will take longer to dress, and indeed this was shown by a direct relationship between %TBSA and total time taken. Although time taken was related to the level of HCW contamination, it explained less of the variation than burn size, with a lower coefficient of determination, R^2. Furthermore, as the time taken for the dressing change will not be known until after the event, and may depend on HCW experience, %TBSA was preferentially considered to predict HCW contamination. A rough guide is that for every 6-9%TBSA increase in burn size, bacterial contamination doubles.

This study increases knowledge of the transfer of bacteria from burns patients to HCW. It highlights the need for guidelines on protective clothing worn by HCW to be developed, as burns patients have been shown to disperse high levels of bacteria onto HCW. For the first time, a quantitative analysis of bacterial contamination received by HCW performing burns dressing and bed changes has been performed. The risks of HCW contamination must be balanced against the cost of protective measures and resources available to burns units.

7.5 The effect of the HINS-light EDS on HCW contamintion during an event

Introduction

Section 7.4 described the production of a mathematical model to establish for the first time a clear correlation between the %TBSA of a burn and the bacterial contamination received by a HCW carrying out a dressing/bed change. This section outlines preliminary studies carried out to assess the effect of use of the HINS-light EDS in isolation rooms on the bacterial contamination received by HCW carrying out bed/ dressing changes.

Methods

As described in Section 7.4, sampling with BPA contact plates from the surface of a sterile disposable gown worn by a HCW has been shown to be a robust and reproducible method of measuring contamination received during an event.

To study the effect of the HINS-light EDS on the amount of contamination received by HCW during dressing/bed changing, these same sampling methods were used. Two consecutive studies were carried in an inpatient isolation room to investigate the difference in levels of bacterial contamination received by HCW after dressing/bed changes in the room with and without use of the HINS-light EDS. These studies were set-up in a similar manner to those carried out in Chapter 5, with the study being broken up into *pre-HINS*, *during-HINS*, and *post-HINS* sampling sessions, but with samples being taken from the HCW gowns rather than the environmental surfaces.

The consecutive studies were carried out over a total of 11 days, during which time two separate studies were performed (Study 1 and Study 2). The same patient remained in the inpatient isolation room throughout the course of the studies to minimise the variation in bacterial release. The patient was a 64 year old who sustained 20%TBSA flame burns to their scalp, upper limbs and back 27 days previously. The burn had been surgically debrided, and a 9% TBSA donor site had been produced. Approximately 10%TBSA of the burn had been treated with flammacerium, and the rest had been covered with graft or dermal replacement. Of the grafted area, approximately 80% had 'taken' and was beginning to heal. Wound swabs yielded *S. aureus, Bacillus* sp. and coliforms. Each event took approximately 60 (±10) min and consisted of a dressing change followed by a bed sheet change.

The protocol for these studies is summarised in Figure 7.17. Day 1 of the study begun by sampling HCW after they had carried out a dressing/bed change in a room with the HINS-light EDS off: this was termed *pre-HINS 1* sampling. The HINS-light EDS was then

switched on for two days from 0800h to 2200h each day. On Day 3, after the patient's dressing and bed sheet change, *during-HINS 1* sampling of HCW was performed. The HINS-light EDS was then switched off for four days. On day 7, HCW samples were collected again: these samples simultaneously provided contamination levels received by HCW during dressing and bed-changing that represented both *post-HINS.1* and *pre-HINS.2* sampling periods. When HCW sampling was complete, the HINS-light EDS was switched on for a further two days and *during-HINS 2* samples were then taken from HCW following the patient's dressing and bed sheet change on Day 9. The HINS-light EDS was then switched off and *post-HINS 2* HCW samples were taken on Day 11, completing Study 2.

Two HCW carried out the dressing/bed change each time, and 20 samples were taken from each HCW, as demonstrated on the left of Figure 7.11, giving a total of 40 samples collected after each dressing and bed change. As in the previous section, samples were taken while the HCW was wearing the gown and still in the patient's room. Usual hand hygiene and infection control practices were observed.

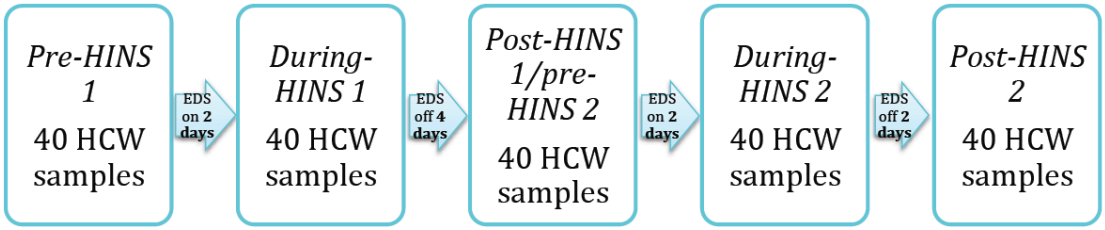


Figure 7.17: *Schematic diagram to outline the sampling periods during a study into the effect of two-day HINS-light EDS exposure on level of HCW contamination when carrying out a bed/dressing change*

Results: HCW contamination during events with and without HINS-light EDS exposure

The results of two days' HINS-light EDS treatment of a room on the levels of contamination received by HCW are summarised in Table 7.4.

These results of the study are illustrated in the following graph (Figure 7.18). A clear reduction in levels of contamination of HCW is seen following the two periods of HINS-light EDS treatment of the room in the preceding two days. When the EDS is switched off in the middle of the study and at the end of the study, the levels of bacteria precipitated onto a HCW carrying out a dressing change can be seen to rise.

Table 7.4: *Summary of two consecutive studies of the effects of HINS-light EDS treatment for two days on the level of HCW contamination received during a dressing/bed change*

Sampling period	*Pre-HINS 1*	*During-HINS 1*	*Post-HINS 1 / Pre-HINS 2*	*During-HINS 2*	*Post-HINS 2*
Mean cfu per plate	298.3	159.6	255.7	114.8	164.7
Median cfu per plate	198	86.5	46.5	20.0	74.5
% decrease in mean cfu following 2 days' HINS-light EDS treatment	-	46.5%	-	55.1%	-

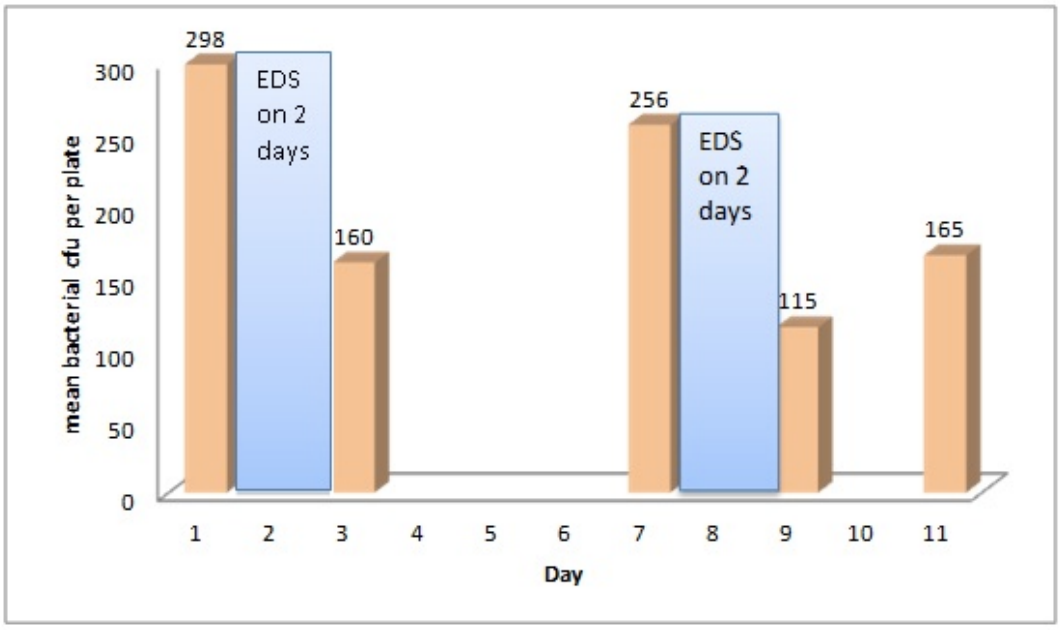


Figure 7.18: *Graph to illustrate the effect of a 2-day use of the HINS-light EDS on the mean bacterial cfu per gown on HCW undertaking dressing/bed changes on a patient with a 20%TBSA burn. Blue blocks illustrate periods of EDS treatment.*

Overall, the reduction shown in the level of HCW contamination received by dressing/ bed changing during the '*during-HINS*' phase (compared to the equivalent in pre-HINS phase) demonstrated the successful decontamination effect by use of the HINS-light EDS. The subsequent increase in HCW contamination received by dressing/bed changing during the '*post-HINS*' phase (compared to the equivalent in during-HINS phase) demonstrated that any observed reduction in contamination had been due to the effect of the HINS-light EDS alone.

This 'A-B-A' model meant that the patient and room acted as the control, and any difference in HCW contamination seen in the *during-HINS* phase was not due to a generalised decrease in bacterial release from the patient's wounds as a result of healing. The fact that this result was achieved at two separate during-HINS dressing/ bed changes reinforces the successful decontamination effect of the HINS-light EDS,

suggesting that its use not only reduces environmental contamination around the room, but reduces the amount of contamination that HCWs become contaminated with during routine nursing events such as dressing and bed sheet changing.

Discussion

A preliminary study into the possible effects of the HINS-light EDS in the burns unit has been described. The results suggest that the EDS may play a significant role not only in reducing environmental contamination, but also in reducing direct HCW contamination during 'high-risk' events such as dressing/bed changes. This chapter has served to highlight the significant contribution made by these events to contamination of the air and HCW, with this section indicating a possible way in which the HINS-light EDS may begin to address this risk.

Due to time restraints, only two studies were carried out. However, the results shown are certainly encouraging, and point the way for future work looking specifically at the effect of the HINS-light EDS on HCW themselves. It certainly suggests that a HCW carrying out a dressing change in a room where the HINS-light EDS has been in use for two days will receive approximately 50% less contamination than a HCW carrying out a dressing in a room without an EDS in use. It is likely that this effect is due to an overall reduction in bacterial contamination of the room and environmental surfaces achieved over the previous two days. This would mean that the agitation caused by carrying out a dressing/bed change would liberate fewer bacteria, as there are fewer bacteria to be liberated on the sheets and environmental surfaces.

Although this seems to be the most likely explanation, others must be considered. These include the possibility that the HINS-light EDS takes effect during the hour when a HCW is carrying out the dressing change and that bacteria landing on the HCW during this time are inactivated *in situ*. From experimental studies detailed in Chapter 4, we know that it takes approximately two hours to achieve a 50% reduction of *S. aureus* on agar exposed to the EDS. This suggests that either the reduction is quicker in the clinical environment, as the bacteria are more stressed and desiccated, or there is another explanation. It may be that the majority of bacteria are inactivated when airborne. However, as the contamination received by a HCW is a mixture of direct contact and airborne contamination, it is probably a combination of both of these mechanisms that produce the effect seen.

These studies indicate a highly interesting avenue for future studies. The effect of the HINS-light EDS on a HCW carrying out 'high-risk' tasks may have significant implications, particularly if reduced contamination similar to that achieved by wearing PPE could be demonstrated. Further studies may examine the consequences of having

the EDS on for more than two consecutive days, particularly as the studies described in Chapter 5 indicate that the effect of the HINS-light EDS is cumulative, and greater environmental decontamination is achieved following longer exposure periods, up to exposures of seven consecutive days. In order to ascertain whether the effect demonstrated is due to an overall reduction in environmental bacteria within that room, or the effect of the EDS at the time of the dressing/bed change, studies could be carried out where the EDS is switched on only for the duration of the dressing/bed change. Furthermore, as the intensity of 405 nm light delivered by the HINS-light EDS may be adjusted, if the effect was seen mainly during the dressing/bed change, a 'booster-light' may be used during this 'high-risk' event. Studies in Section 7.3 demonstrated that airborne levels of bacteria during a dressing/bed change persist for approximately 40-60 min after the activity has ceased, and this may be another time when the 'booster-light' may be used. The use of an increased intensity light would be subject to further safety analysis.

7.6 A telephone questionnaire of UK burns units about the use of PPE for dressing changes

Introduction

This chapter has dealt at length with the risks to HCW carrying out dressing changes on burns patients. In particular, Section 7.4 has highlighted the likelihood of contamination received by HCW carrying out dressing/bed changes. The current paucity of burns-specific guidelines on PPE has been previously discussed: NICE and SIGN guidelines are vague and not specific to the unique infection control requirements of burns patients.[138,140,411] For example, NICE guidelines state "Disposable plastic aprons should be worn when there is a risk that clothing may be exposed to blood, body fluids, secretions or excretions, with the exception of sweat," and "full-body fluid-repellent gowns must be worn where there is a risk of extensive splashing of blood, body fluids, secretions or excretions, with the exception of sweat onto the skin or clothing of healthcare practitioners."[138] It is unlikely when carrying out a dressing/bed change that "extensive splashing" of body fluids would occur, and yet due to their high propensity to disperse bacteria, burns patients have been shown to liberate high numbers of bacteria onto the HCW caring for them.

In light of these inadequate guidelines a telephone survey was conducted to ascertain current UK practice.

Methods

A telephone survey was conducted of all 16 adult burns units and facilities registered with the British Burns Association in 2011. The nurse in charge was asked to answer questions on the demographics of the unit, infection control policies, and the use of PPE by HCW carrying out dressing changes. The survey was designed by SEB and the survey completed by RM and SY (medical students at GRI) following a script written by SEB in preparation for a poster presentation at the ISBI 2012.[415] The questions asked are detailed below (Figure 7.19).

Draft telephone questionnaire for British Burns Units – FINAL VERSION

Date	
Burns Unit	
Contact phone number	
Name of person asking questions	
Name of person answering questions	
Job title	

Q.	About your unit		
1	Does the unit only admit ADULT BURNS patients?	Yes >q3	No >q2
2	What other patients are admitted? (children, plastics patients, other specialities)		
3	How many isolation room beds are in the unit?		
4	How many beds in open bays or wards are in the unit?		

Q.	General infection control considerations		
5	What is your policy on swabbing burn wounds?		
6	When, if ever, are burn wounds biopsied to diagnose infection?		
7	Where do the majority of your dressing changes take place?		
8	Is there a unit policy about personal protective equipment (PPE) for staff?	Yes >9	No > 13

9 What should staff wear for a dressing change on an ADULT with the following size burns in an isolation room in your unit?

% TBSA	N/A	Hand protection		Body protection				Head/face protection	
		No gloves	Gloves	Usual Uniform	Plastic apron	Gown	Fresh scrubs	Hat	Mask/visor
<5									
5-10									
10-15									
15-20									
>20									

10 Are there any exceptions to these rules? Eg Hep C patient / ICU patient?

11 Would your policy be different if the patient was having the dressing change in an open / shared bay (ONLY ask if they put a number in Q 4)?

12 would you wear the same PPE for a bed / sheet change?

13 how do you decide what to wear for a dressing change(ONLY ask if no to Q7)?

Figure 7.19: *Proforma of telephone questionnaire carried out in 2011.*[415]

Results of telephone questionnaire

Fifteen units took part in the survey. Twelve units were adult only, and three cared for adults and children. Several units were shared with other specialities, including plastic surgery, maxillofacial surgery, and trauma and orthopaedics, although seven were exclusively burns units. There was a mixture of isolation rooms and shared patient bays as demonstrated in Figure 7.20.

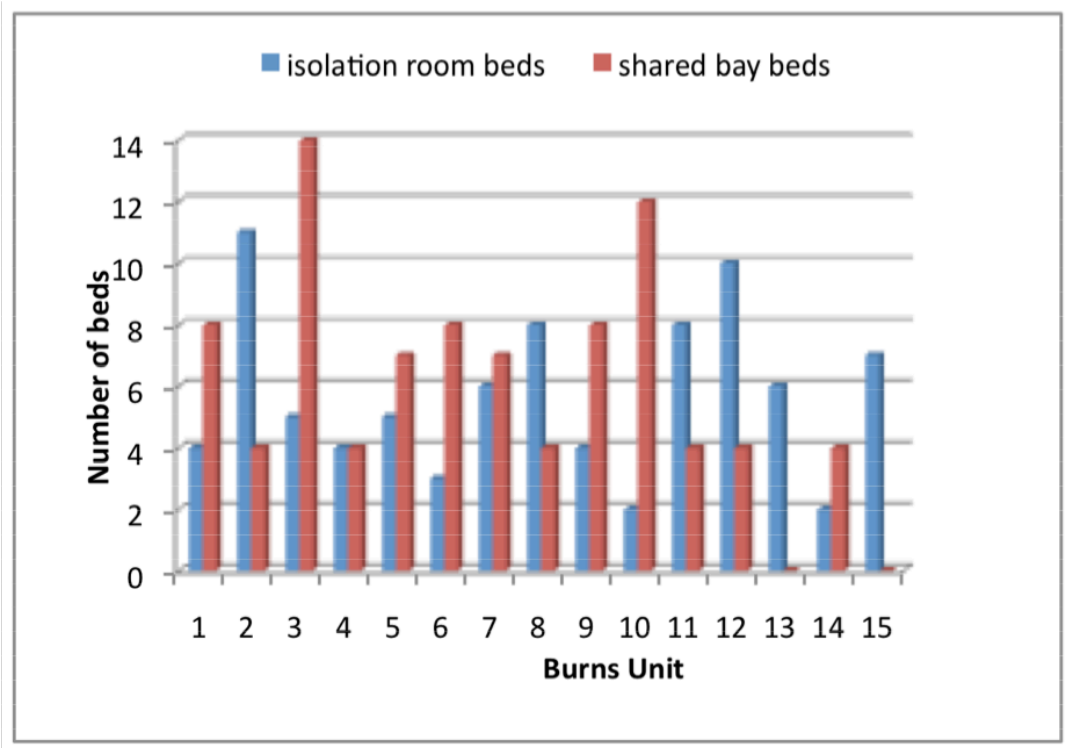


Figure 7.20: *Distribution of beds in isolation rooms and shared patient rooms in 15 UK burns units.*

The frequency at which routine wound swabs were carried out and the most common location to carry out a dressing change were distributed as follows in Figure 7.21:

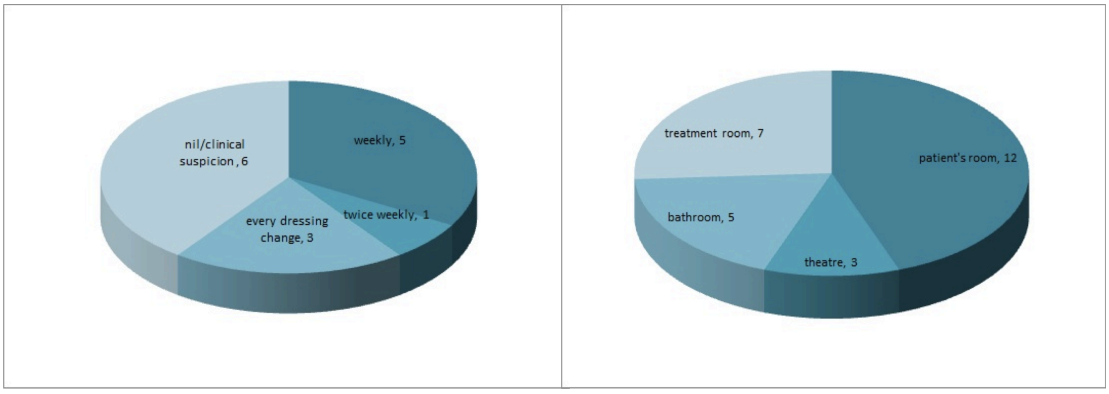


Figure 7.21: *Frequency of routine wound swabs (left) and the usual location for dressing changes to be carried out (right) as reported by 15 UK burns units.*

Only one unit had a burns-unit specific policy on the use of PPE. The rest used general hospital or national guidelines of the use of PPE when caring for patients. All units reported the use of routine hand hygiene measures and the use of gloves for all dressing changes. Only three units stated they would wear different PPE if a burn was over 15%TBSA. More commonly, units stated that a higher level of protection of PPE would be used if a burn would was deemed to be 'infected'. These findings are illustrated in Figure 7.22.

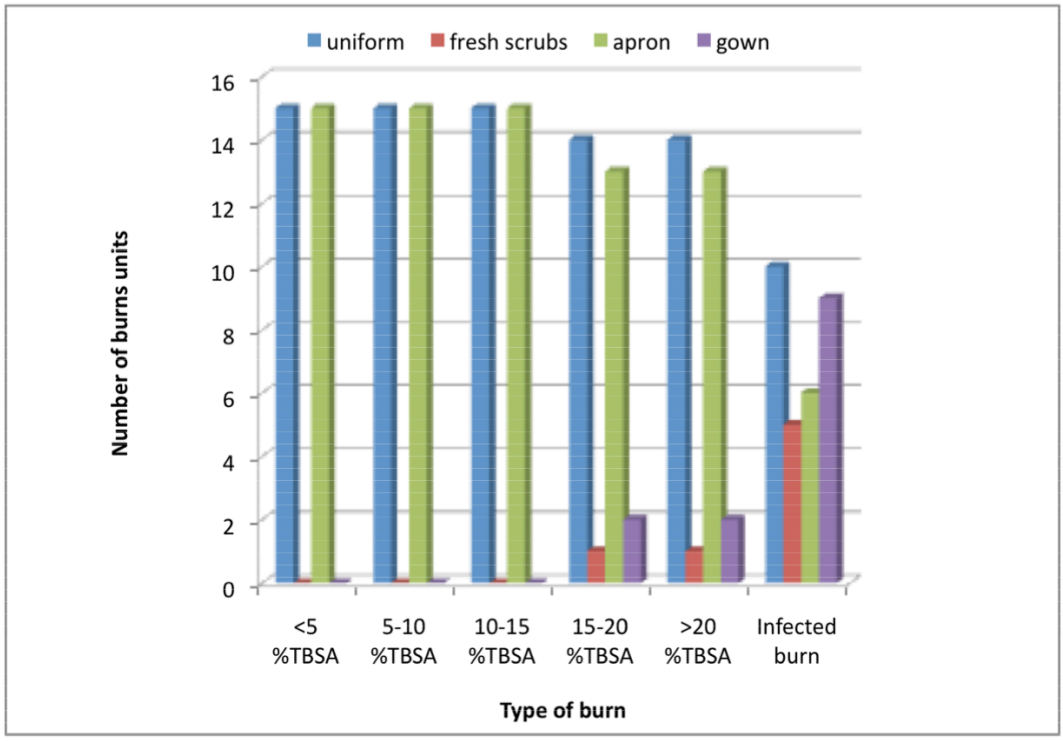


Figure 7.22: *PPE worn by HCW caring for patients with different burns as reported to telephone questionnaire of 15 UK burns units.*

With regards to protective headwear, one unit stated they would wear a hat to carry out dressing changes on patients with burns greater than 15%TBSA, one unit stated they would wear a hat if a burn was infected, and three stated they would wear a visor if a burn was infected.

Discussion

This brief questionnaire really demonstrates the lack of clarity and consistency with regards to the PPE that should be worn by HCW working on burns units in the UK. A similar study has not previously been carried out in the UK. A questionnaire performed in the USA, however, highlighted similar differences between burns units and lack of unity.[43] Of note, in the UK survey, only one unit had burns-specific guidelines, whereas 93/104 units in the US survey had burns-specific infection control guidelines.[43] In the US 22 % units routinely required 'full protective apparel' to be worn when entering a patient's room, undergoing a dressing change or hydrotherapy.[43] However, 'full protective apparel' was not described, and may mean either a full body gown, or an apron, so cannot be compared with the UK study. In the US, 30% of units took 'routine wound swabs', although unlike in the present study, the frequency was not defined.[43] Wound biopsy was also rare, with just 15% of units routinely performing it for bacteriological purposes.[43]

Despite the evidence in this chapter that larger burns create more bacterial contamination, only two units considered the size of the burn when deciding on the PPE that should be worn for these 'high-risk' activities. It was more common for units to increase the level of protection if a burn was deemed to be infected. However, burns wounds are not like other wounds, and the mere presence of bacteria in the wound does not necessarily mean it is infected. Most burns wounds after a few days will almost certainly be colonised with bacteria, and the relevance may not immediately be apparent. Wound swab results will not be known in NHS practice for two or three days to allow for incubation, and the clinical picture of wound infection may lag behind a high bacterial count on the wound surface. In GRI, a quantitative bacterial count is not routinely reported beyond an arbitrary division into 'scanty' or 'heavy' growth. This does not actually give a definitive diagnosis of a wound infection. Therefore, at the time of the dressing change, declaring a wound to be infected at that point is almost impossible.

This survey, together with the results in Section 7.4 suggest an overhaul of the way HCW select PPE prior to carrying out dressing changes on burns patients is needed. Current guidelines and practises are inconsistent and not evidence-based. The size of the burn has been shown to be a significant factor in the contamination of HCW and should be considered first and foremost when choosing PPE for a dressing change on a burns patient.

7.7 Discussion

At the beginning of the chapter, Figure 7.1 outlined the concept of a 'cycle of cross contamination. Some stages were already established, with sufficient evidence in the literature to support their existence; others were not, and novel experimental methods were designed to address this. At the beginning of the cycle is the concept of Patient A dispersing bacteria into the surrounding environment and onto a HCW. Development of this included the examination of 'events' that may increase bacterial dispersal from a patient and lead to a surge of bacteria. It is already well known that burn patients harbour large numbers of bacteria due to their large wounds, with an eschar that is a protein–rich barteria-harbouring environment.[11,36,37] Logically therefore, burns patients are potent dispersers of bacteria, particularly during any activity that causes friction to this wound, and the subsequent dissipation of skin cells or droplets of pus bearing bacteria, either into the air or onto surrounding surfaces.[54,57,61,89]

In order to address the issue of airborne release of bacteria during the two main 'events' identified (dressing changes and bed sheet changes), a series of studies was designed to examine the relationship between dressing/sheet changes and airborne bacteria, by means of repetitive air sampling using a sieve impaction air sampler. This demonstrated peaks in airborne bacterial counts during dressing changes and showed that bed sheet changes could produce peaks that were just as high as dressing changes. This suggested that the same level of PPE might be required by HCW carrying out a dressing change as those carrying out a bed sheet change, in order to prevent their contamination. Further work developed 'control charts' which proved that the peaks seen during dressing/ sheet changes were statistically greater than pre-event levels. Furthermore, they showed a lag time of approximately 40-60 min after the activity had finished when airborne levels remained out of control. This has implications for the use of PPE by HCW entering the room for a considerable time after any dressing/bed change activity has ceased; a new concept for many burns units. Although previous authors had examined these dressing and bed sheet changes, it was with the intention of merely proving the existence of a link between them and levels of airborne bacteria: the present work clarified more precisely what takes place during and after dressing and sheet changes on burns patients.[51-53]

Following confirmation of significantly increased levels of bacteria during and after a dressing/sheet change, attention was turned to the direct transfer of bacteria from burns patient to HCW themselves. Again, studies had identified this as a potential pathway of cross-contamination, but had not quantified this contamination in relation to the size of the burn.[79-81,88,408,409] The only similar study to examine the relationship between burn size and bacterial release had been one of airborne bacteria, using a settle

plate method over several days.[54] Novel methods were established to sample HCW from a sterile baseline of disposable full-length gowns, using a preferred method of contact plate sampling. Despite testing a range of burns of different ages, with different bacteria isolated on the wounds themselves, the link between burn size and HCW contamination was obvious and highly significant. Arguably the most valuable section of this chapter is that where the contamination received by HCW carrying out a dressing change is shown to have a significant and high correlation with the %TBSA of the burn in question. This is an important point to establish in the cycle of cross-contamination and allowed the likely level of contamination to be predicted from burn size. While no attempt has been made to create guidelines for the use of PPE for different sizes of burns here, the results have been submitted to international journals and presented at an international forum, and it is hoped that they will contribute to the development of future guidelines. Current guidelines are scant and not burns-specific, creating inconsistency of practices between different burns units.[43,415]

With regards to the rest of the cycle of cross-contamination, it was felt that extensive work had already been carried out into the survival of organisms in a desiccated state on surfaces, or on various fabrics.[93,122-126] MRSA have been demonstrated to survive for up to 38 weeks on inanimate surfaces, and other organisms, including *Acinetobacter* sp for 60 days, with the same strain being recoverable from the environment at two points, six months apart.[122,126] Similarly, the fact that bacteria can be transferred from HCW hands and uniforms to environmental surfaces and other patients has previously been demonstrated.[88,89] Outbreaks of nosocomial infections in hospital wards have been directly attributed to HCW.[73-81] Experimental models included an excellent study where the transfer of VRE from one contaminated site to another non-contaminated site took place via the hands of HCW.[90] In order to monitor the cross-contamination, false situations and mock patients and nurses were employed to create models.[88-90] These had successfully achieved the desired aim of reproducing the conditions for cross-contamination as closely as possible, but were out with the remit of the current research, which was clinically-based, and attempted to disrupt the normal pattern of events and day to day running of the burns unit as little as possible. An overriding principle was to work the studies around the staff and patients on the unit not vice versa. For these reasons, and the fact that HCW act as vectors of transmission of infections between patients is so well established, this part of the cycle of cross contamination was not re-examined. Beyond the scope of this research was the number of bacteria that may contaminate a burns patient's wounds. No figure can be given as these patients are severely immunosuppressed, with large open wounds, and in such conditions even a single bacterium has the potential to replicate exponentially.

Having completed the cycle of cross contamination, the potential uses for the HINS-light EDS in interrupting the cycle were explored. As discussed in Chapter 5, it is likely that a

significant proportion of the effect of the HINS-light EDS takes place on airborne particles. It was not possible during the course of this research to study the effect of the HINS-light EDS on levels of airborne bacteria during dressing/bed changes and the time afterwards when levels of bacteria remain 'out of control'. However, the 'control charts' established here provide a useful basis for the future study into the effect of the light on airborne bacteria. The evidence of significant accumulation of bacteria onto HCW performing dressing changes provides another avenue for future research, with encouraging preliminary results. Although guidelines for the protection of HCW carrying out dressing/bed changes would always include the use of PPE, co-existing use of the HINS-light EDS may reduce contamination to such an extent that only very large burns may necessitate the use of gowns. Certainly the preliminary studies contained in Section 7.5 suggest that the HINS-light EDS may reduce the amount of contamination received by a HCW carrying out a dressing/bed change by a half. As the cost to run an EDS unit would be much less than the cost of using sterile disposable gowns, this may be a cost-efficient way of addressing the transfer of bacteria from patient to HCW during 'high-risk' activities.

Chapter 8

Conclusions and future work

8.0 Outline

The initial aim of this study was to investigate the effect of a patient-safe continuous visible light disinfection system, the HINS-light EDS, on environmental bacterial levels in the burns unit setting. Throughout the course of the work however, several questions about the cycle of cross-contamination of infection between patients arose, and these were answered wherever possible. Furthermore, the primary aim was broadened to develop valuable studies into the most efficient way of delivering this supplementary disinfection within the confines of the burns unit. This final chapter summarises the conclusions gathered from the work completed and makes recommendations for areas of future study.

8.1 Conclusions

This section summarises the findings of each distinct area of study in turn.

Laboratory inactivation of bacterial isolates from the burns unit

In Chapter 4, five species of bacteria, all isolated from the burns unit environment or patients, and including those shown to have antimicrobial resistance, were exposed to the HINS-light EDS under laboratory conditions. The five organisms chosen were methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes*, multi-drug resistant *Acinetobacter baumannii* (MDR-*A. baumannii*), and *Pseudomonas aeruginosa*. The experiments aimed to replicate the clinical environment, but in laboratory conditions to enable standardisation and repetition. This was achieved by using an identical ceiling-mounted HINS-light EDS, at a similar distance from the exposed surfaces to those used in the hospital setting. The bacteria were exposed on solid agar surfaces and in similar densities to those found in the clinical environment, and the intensity of 405 nm light used was comparable to that used in the hospital.

Inactivation curves produced for each species demonstrated significant % reductions of all species at between 1 h and 2 h exposure time, and complete or near complete kill by 7 h. Similar inactivation curves were demonstrated for MSSA and MRSA, as one may expect due to the only difference between these strains being that MRSA is antibiotic resistant. *S. pyogenes* proved to be a difficult organism to cultivate, with widely varying starting populations. The organism did, however, demonstrate similar susceptibility to *S. aureus.* The two Gram-negative species were also found to have similar inactivation rates to the Gram-positives tested. Calculation of germicidal efficiency showed similar results for all species tested.

The finding that similar rates of inactivation were seen for all five species was contrary to previous findings that Gram-negatives are less susceptible to HINS-light, and require a higher dose to become inactivated than Gram-positives. This may be because two particularly susceptible Gram-negatives were tested: both had previously been observed to act almost like Gram-positives upon exposure to HINS-light.[2] Alternatively, it may be because these were experiments were carried out with the bacteria seeded onto solid agar surfaces, rather than in liquid suspension as had been previously performed, therefore making them more exposed to environmental stresses such as desiccation.[2] Although, the agar plates have a high water content, the organisms are seeded on the surface and potential desiccation effects may have affected the Gram-negative bacteria to a greater extent than the Gram-positive bacteria, rendering them

highly stressed and thus more susceptible to the effects of photodynamic inactivation than they otherwise would be: thus causing them to have a similar susceptibility to Gram-positives. Other reasons for inter-species variation were discussed in Chapter 4, as was the potential difference between high intensity exposures for a short period of time versus lower intensity exposures for a longer period of time (as performed here). In summary, although the dose may be the same, other effects (such as experimental set-up, irradiance, exposure time, slight desiccation) may produce different inactivation curves when the two experimental protocols are compared. These differences may be explored to demonstrate the more effective way to achieve inactivation: high intensity for short periods, or low-intensity for longer periods.

Inpatient studies

Chapter 5 examined the effect of the HINS-light EDS in the inpatient isolation rooms of the burns unit, allowing the most effective way of delivering the light to be refined. This was achieved through a series of studies that evolved with each conclusion formed. The initial studies demonstrated that, in a similar manner to clinical studies previously reported, significant reductions in levels of environmental bacteria were achieved, when the EDS was on for 2 days, in excess of those produced by standard infection control and cleaning methods. These were reproducible throughout studies of the rooms of three different patients, whereby each patient acted as their own control before and after HINS-light exposure. A sampling time of 0800 h was shown to be the most consistent for sample collection, probably due to limited variation in activity within the room at that time, therefore 0800 h was used as a standardised sampling time. An average reduction of between 22% and 87% was achieved following two consecutive days' exposure, with the light on for 14 h a day. The wide range in the decontamination effects achieved was believed to be due to variations in starting populations ('*pre-HINS*' counts) of bacteria between different patients. This enforced the requirement for an A-B-A style model whereby the same patient remained in the same room for the duration of a single study, with the use of the EDS being the only intervention. This model provided both before and after controls, without the complications associated with comparing two different patients in different rooms, and their inherently different bacterial loads.

The success following 2 day exposure periods led to a progression to 5, 6 and 7 day exposure periods in three further experiments. At this time the number of samples taken during each session was increased from 40 to 70, thus improving the statistical power of the studies. Greater and more consistent levels of kill were achieved following these longer exposure periods, up to 89% reduction. This provided further evidence that the HINS-light EDS could prove a valuable weapon in the armamentarium of the burns unit against infection. This continuous method of environmental decontamination

was proving to be acceptable to both patients and staff, and had the potential to make a significant contribution to environmental cleanliness in burns units worldwide. This led to considerations of potential limitations and objections to its usage, one of which may be cost. A simple method of reducing cost would be to use fewer lights, and the practicality of this was tested in three further studies, where a single light was used per room, rather than two, as had been used in previous work.

Results using a single HINS-light EDS unit were encouraging. Decreases in environmental bacterial load of 86% and 74% were achieved in the first two studies. While the third study only demonstrated a 22% reduction, it was believed that this was due to spuriously low *pre-HINS* levels, as *post-HINS* levels were much higher, and probably indicative of the true baseline environmental bacterial load in the presence of that particular patient. In short, although single EDS studies are limited, these early results indicate that comparable effects may be achieved using a single unit per isolation room. While the same volume of evidence has not yet been established for the use of one light as it has for two, the cost implications are so significant that this is sure to be an area of future development.

The final arm of the inpatient studies was to use the established model to explore the mechanism through which the HINS-light EDS decontaminates the environment. The intention was to determine whether the decontamination effect mainly took place on bacteria precipitated onto surfaces or suspended in the air. The irradiance received at each sampling site around the room was therefore measured during a study using a single-HINS-light EDS. These irradiance levels were compared with the % reduction in contamination achieved at that site. No correlation was found between irradiance and % reduction in bacteria. Rather, a steady effect was achieved throughout the room at each of the three exposure times tested (2 days, 4 days and 7 days). That effect increased with time of exposure, but did not increase with intensity of light. This suggested a uniform effect, such as one might expect to be exerted on airborne bacteria, rather than a site-specific one. This supports a theory that bacteria are inactivated both on surfaces and when airborne and then precipitated at random onto environmental surfaces.

Overall, the work produced in the inpatient setting gave encouragement that fewer lights may be used, and that their placement in the ceiling may not be as crucial as initially thought. Inactivation of bacteria is likely to take place in the air and on surfaces. Furthermore, longer exposure times demonstrate greater kill, although this has only been tested up to 7 days, and should be explored further.

Outpatient clinic studies

Work performed in the outpatient clinic in Chapter 6 highlighted a previously untested use of the HINS-light EDS: during a clinic where several patients pass through the room

in any given day. Despite the relatively short exposure times, of the order of hours rather than days, significant reductions in the usual increases in environmental bacteria precipitated onto surfaces around the clinic were shown. This was relevant in this situation as thorough cleaning of the room is not possible in the time allowed between patients, as it would be following the discharge of an inpatient. Furthermore, bacteria from burns patients are airborne for significant periods of time, so surface cleaning alone immediately following the exit of a patient will not remove bacteria in the air, which may be then precipitated onto surfaces. The continuous contribution of the EDS means it continues to take effect throughout the course of the clinic, not just at specific points in time, as happens with surface wiping.

While clinics without the use of the EDS demonstrated an approximate 300% increase in surface bacteria during the course of the clinic, those with the EDS in use demonstrated only a 50% increase. This was significantly less than the increase produced without the use of the HINS-light. Another important factor to consider during the course of the outpatient studies was that 56 patients experienced the HINS-light EDS. Not a single patient commented on the presence of the light until it was pointed out to them, and none of them experienced any adverse effects.

The contribution of nursing 'events' to the cycle of cross contamination

Preliminary studies using an air sampler were the first to demonstrate a clear surge in airborne bacteria during specific nursing events in Chapter 7: namely dressing changes and bed sheet changes. Air samples collected every 5 min provided evidence for an immediate increase in bacterial dispersal into the air as a consequence of a dressing/ sheet change. In order to determine whether this was statistically significant, a technique of establishing control charts was developed for the purpose. This was a novel use of statistical control charts. The charts demonstrated that both dressing and bed sheet changes (i.e 'events') created an increase in airborne bacteria that was deemed to be 'out of control' when compared with pre-event levels.

Four control charts established that during dressing/bed changes taking place on patients with between 35%TBSA and 52%TBSA burns, the 'events' observed caused levels of airborne bacteria to become significantly 'out of control'. Furthermore, the effects lasted for approximately 45 min to 60 min after the dressing/bed change ceased. This has significant implications for healthcare workers (HCW) selecting which personal protective equipment (PPE) should be worn not only during nursing tasks, but also on entering the room for up to an hour after a dressing/bed change has finished. The significance of bacteria remaining suspended in the air for so long after the event has not been appreciated in previous studies on burns patients. These studies highlight an important area for application of the HINS-light EDS, particularly in view of the fact that a large proportion of the effect of the EDS is likely to take place on airborne bacteria.

A further study was carried out to quantify the levels of bacteria a HCW carrying out a dressing/bed change would become contaminated with. A significant relationship between the %TBSA of a burn and the level of resulting contamination was shown. This was so highly correlated (fitting an exponential model) that a mathematical model could be used to predict the expected level of contamination a HCW would receive during a dressing change. Given the current paucity of evidence, and inconsistencies between burns units about which PPE should be worn when caring for burns of different sizes, this information may well help to guide HCW in these respects in the future. A preliminary study using the HINS-light EDS certainly suggested that dressing/bed changes carried out in inpatient isolation rooms that had been treated with the HINS-light EDS for the preceding two days, created less contamination of the HCW performing them than would otherwise have been expected. Further work is needed in this area.

8.2 Recommendations for future work

Laboratory inactivation of bacterial isolates from the burns unit

As discussed, the laboratory work performed in Chapter 4 contradicted previous studies that showed Gram-negative bacteria to be less susceptible to HINS-light than Gram-positives. This may be due to the different experimental arrangement used in the current body of work, whereby the bacteria are spread onto a solid agar surface (rather than suspended in liquid medium) and allowed to desiccate to some extent. In order to determine whether Gram-negatives do exhibit similar susceptibility to photodynamic inactivation as Gram-positives in these conditions, further work could be carried out examining the effect of desiccation on different species.

A study may be completed with desiccation of the species tested carried out prior to exposure to the HINS-light EDS. This may demonstrate that Gram-negatives that had undergone prior desiccation, say for several hours before EDS exposure, exhibit greater susceptibility to the HINS-light photodynamic inactivation than Gram-positives that tolerate desiccation relatively well.

Further methods of stressing bacteria, such as exposing them on less nutritious surfaces, including pillow slips or nebulised onto plastic or metal surfaces such as those seen around the hospital, would establish inter-species variation in the effects of different environmental stressors. It may be expected that Gram-positives and Gram-negatives react differently to these stressors, but to exaggerate this difference, Gram-negatives that are known to be particularly resistance to the effects of the EDS may be tested. It had been noted that both *P. aeruginosa* and *A. baumannii* are among the most susceptible Gram-negatives tested during previous work.

Inpatient studies

As highlighted in Chapter 5, while the HINS-light EDS has been shown to significantly decrease levels of environmental bacteria, the over riding aim wound be to demonstrate that this translates to a decrease in infection rates amongst burns patients. Various studies have been suggested to further refine the use of the light, such as prolonged exposure for several weeks or months, and consideration of the least number of lights that can be used throughout a burns unit, all with levels of environmental contamination as an end point. However it is essentially a decrease in nosocomial infection rate that would ultimately persuade any funding board that the initial cost of installing EDS throughout a hospital was cost effective.

The obstacles to proving the effect of any infection control policy have been discussed, and the previous adoption of a pragmatic stance to the effects of infection control

policies, such as carrying out surface disinfection and hand washing, has been noted. However, the current financial restraints of the NHS and a requirement to validate the cost-effectiveness and longevity of new treatments afford a need to attempt a patient outcome based trial, albeit at considerable expense. The design may be as simple as monitoring infection rates on a ward with a marker nosocomial organism, such as MRSA, for a given amount of time with the EDS in use and without it in use (e.g. compare infection rates following a year of daily EDS treatment with the infection rates in the same ward the preceding year.) However, possible problems with this include changes in infection rates due to advances in treatment or practices within the burns unit, or a single outbreak of a virulent organism having a significant impact on the infection rates for the whole year.

A more likely scenario would be for two wards or units, with a high rate of nosocomial infections, and a comparable size and treatment policy to partake in a clinical trial for a substantial amount of time. Both units would monitor the total number of infections, with one unit previously having been equipped throughout with EDS units, that were on for 14 h a day for the duration of the study. Following this, the EDS would be removed from that ward, and placed throughout the other ward for the same duration. Again infection rates would be monitored. This crossover style study, particularly if undertaken in several different case-control matched wards would provide the highest level of evidence that the EDS had an effect on infection rates.

Of note, no blinded trial would be possible given the clear visibility to all staff, patients and sample collectors of the blue light. This means that the issues raised in Chapter 5 concerning the possible effect of having a visible reminder to staff, patients and visitors, that a trial is taking place could not be overcome. It could always be argued that wards were cleaner when the blue light was on because it served to remind staff and visitors that they should maintain meticulous hygiene practises. However, the person enumerating bacteria may be blinded to the phase of the trial and sampling sites they are counting.

Outpatient clinic studies

While an increased number of repetitions of any study are always desirable, the studies carried out in the burns outpatient clinic (Chapter 6) were sufficient to successfully demonstrate the results achievable during an 8 h outpatient burns clinic using the HINS-light EDS. Future work may focus on other outpatient clinics, although the likelihood of cross-infection (and therefore the need for additional decontamination systems) must be considered. For example, it is unlikely that patients in a general medical clinic would disperse the levels of bacteria into the environment that burns patients do, or that they are as susceptible to infections. However, other areas such as an oncology unit, haematology clinic or infectious disease clinic, where patients immunocompromised

due to the effects of chemotherapy, cancers of the blood stream or HIV, may be areas to consider the impact of increased environmental cleanliness. Alternatively, environments such as emergency departments or ambulances may benefit from continuous decontamination. In these environments, the patients using the service are much less predictable and a very high turnover means that repeated cleaning may not be achieved as often as is desirable.

These communal areas represent considerably different environments to those that were previously studied in inpatient isolation rooms. Further studies would require consideration of the variables that would also have an effect on the environmental levels of bacteria. Variables such as the number of patients, pre-existing infections, patient volume, frequency of cleaning, and differences between patients in bacterial load and dispersal would all have to be considered, and where possible adjusted. However, the communal clinical environment has the potential to be one where the HINS-light EDS may have particular relevance for the reasons mentioned above, and studies following a similar protocol to that established in this work would greatly augment our understanding of its use in this setting.

The contribution of nursing 'events' to the cycle of cross contamination

While the control charts in Chapter 7 established a novel method for the measurement of increases in airborne bacteria during nursing events, these were limited to dressing and bed sheet changes. Now that a protocol has been established, it could be applied to monitor the airborne release resulting from numerous events that take place within the burns unit. These may include operations, physiotherapy, bathing and transfer of patients.

A limited number of patients with a narrow range of %TBSA were studied, and in order to determine the relative contribution to the environmental bio burden of patients with burns of different sizes, ages and levels of infection, an almost infinite amount of possible studies could be carried out. To gather the most useful data, these should be limited to pertinent studies: for example, studies of a larger variation in size of burn could be performed. Further experiments examining how the bacteria release changes with the age of a burn, as well as the presence and absence of infection may also be useful.

Further work may involve the use of an Anderson air sampler, in order to stratify the organisms collected according to their size. This would help determine how long the bacteria are likely to be suspended in the air, as the size of a particle is known to be inversely proportional to the length of time it is airborne.[388]

The creation of a statistical model to predict the amount of contamination received by a HCW carrying out dressings would certainly be useful in the creation of future infection

control guidelines for the use of burns units. It is hoped that with the publication of these results, the findings may be used for a long-overdue examination and recommendation of PPE to be worn by HCW carrying out both dressing changes and bed sheet changes, as well as anyone entering a room for a period of time during and after a dressing/bed change has taken place. In fact, these studies have already led to a policy review in the burns unit at GRI.

The significant airborne effect exerted by the HINS-light EDS may make it particularly beneficial during the events identified as creating surges in airborne bacteria. Studies into the effect on airborne levels of bacteria of the EDS during these activities were not performed due to time restraints. They may be done by repeating control charts with and without the HINS-light EDS in use. Comparing its use during the activity itself, and in the days preceding an activity would help to direct the most effective use of the HINS-light EDS: either in inactivating those airborne bacteria liberated during an event, or in inactivating surface bacteria, which would subsequently become airborne. This could be demonstrated by repeated control charts with the light on prior to the dressing change, during the dressing change, and immediately after the dressing change, and the number of 'out of control' points for each compared with studies without the light.

The two preliminary studies of the effect of the HINS-light EDS on levels of contamination received by HCW carrying out dressing/bed changes were encouraging. They indicate yet another entirely different, more targeted, application for the use of the EDS, as well as a novel way of quantifying its effect. Replication is necessary to validate the findings, either by repeating the preliminary protocol, or by carrying out an entirely new series of dressing changes, using the same method of sampling HCW gowns that was described in Section 7.3. If case-matched patients were used, a similar curve could be produced, with the use of the EDS. This would enable a direct comparison to be made of the contamination a HCW would receive carrying out a task with and without the EDS.

Similarly to the airborne studies, the decontamination effect may be exerted before or during the dressing/bed change. Studies could easily be devised along a similar protocol with the light on before the dressing/bed change or during it. With the potential to increase the output of the light, a 'booster-HINS' function may be possible if the effect took place during the dressing/bed change, where an increased intensity of light was delivered during the event.

All of the above signify the varied purposes to which the HINS-light EDS may be utilised within the hospital environment. While this work has furthered current knowledge of the clinical use of the HINS-light EDS within a burns unit, it has raised many more questions. More excitingly, it has brought to light a host of other functions and applications that had not been previously considered. The study of such applications

and the development and fine-tuning of the best use of the HINS-light EDS will warrant its deployment in burns units in the future.

8.3 The HINS-light EDS as a viable decontamination adjunct in the burns unit

The clinical experiments detailed in this work were all carried out in the context of a busy regional burns unit. Results have been consistently impressive in a plethora of hospital studies. Many patients and staff were exposed to the HINS-light EDS, and it quickly became accepted within the burns unit as a decontamination device. Its use required no extra staff time, and did not necessitate in the removal of patients or visitors, unlike other methods of environmental decontamination.[158,160,166-168,388] As it is generally used during daylight hours, it has no impact on patients' sleep or daily routine. It is quiet, unobtrusive, and aesthetically pleasing. Unlike other decontamination devices there are no known detrimental effect to the materials or surfaces it illuminates. A detailed safety analysis has demonstrated that exposure to the light system is safe to humans. Furthermore, the light uses LEDs, which present many advantages over incandescent light sources.[197] These include:

- Efficiency: Currently the best white LEDs produces around 250 lumens per Watt (lm/W), compared with 16 lm/W from incandescent and 100 lm/W from fluorescent bulbs;[315]
- Economy: It is estimated that if white LEDs replaced other light sources, about 270 million tons of CO_2 per year would be saved;[293,322]
- Longevity: estimated at between 30 000 and 50 000 hours of use, compared with 750-2000 hours for incandescent bulbs and 8000-10 000 hours for fluorescent lights;[416]
- A quick on/off time;
- The possibility of dimming by lowering the input current;
- Minimal heat release;
- Physical robustness due to solid components

Disadvantages of LED that may need to be addressed include:

- Blue hazard: concern that blue and white LED may risk damage to the eye[293]
- Temperature and current dependence of chromaticity[322]
- Droop: Increased currency decreases the efficiency of the LED[315]
- High initial price compared with incandescent and fluorescent lights

However, the first three of these are not a concern for the HINS-light EDS when used at the intensity and current used throughout this clinical work, and advocated by its developers. Although the initial cost would need to be met, encouraging studies showing

comparable effects using a single light per isolation room have indicated that this may be less than was originally supposed. Furthermore, if the EDS were to become commercially available and mass-produced, the cost would undoubtedly fall in comparison to the current hand-made prototypes for research purposes.

In conclusion, the favourable reception the HINS-light EDS has received during three years of clinical research in a burns unit, coupled with a consistently impressive record in significantly decreasing decontamination of the clinical environment provide a legitimate claim for its use in future hospital cleaning procedures.

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Appendix A: Air sampling correction table

Table A.1: Conversion tables for SAS 180 air sampler, Cherwell Laboratories, UK.

Raw count (r)	Probable count (Pr)	Raw count (r)	Probable count (Pr)	Raw count (r)	Probable count (Pr)	Raw count (r)	Probable count (Pr)	Raw count (r)	Probable count (Pr)	Raw count (r)	Probable count (Pr)
1	1	38	42	75	92	112	156	148	246	184	399
2	2	39	43	76	93	113	158	149	249	185	405
3	3	40	44	77	95	114	160	150	252	186	412
4	4	41	45	78	96	115	162	151	255	187	418
5	5	42	46	79	98	116	165	152	258	188	425
6	6	43	48	80	99	117	167	153	261	189	432
7	7	44	49	81	101	118	169	154	265	190	439
8	8	45	50	82	102	119	171	155	268	191	447
9	9	46	51	83	104	120	173	156	271	192	455
10	10	47	53	84	106	121	175	157	275	193	463
11	11	48	54	85	107	122	178	158	278	194	471
12	12	49	55	86	109	123	180	159	282	195	480
13	13	50	57	87	110	124	182	160	286	196	489
14	14	51	58	88	112	125	185	161	289	197	499
15	15	52	59	89	114	126	187	162	293	198	508
16	17	53	60	90	116	127	189	163	297	199	519
17	18	54	62	91	117	128	192	164	301	200	530
18	19	55	63	92	119	129	194	165	305	201	542
19	20	56	64	93	121	130	196	166	309	202	554
20	21	57	66	93	122	131	199	167	313	203	597
21	22	58	67	95	124	132	201	168	317	204	580
22	23	59	69	96	126	133	204	169	322	205	595
23	24	60	70	97	128	134	206	170	326	206	611
24	25	61	71	98	130	135	209	171	331	207	627
25	26	62	73	99	131	136	221	172	335	208	646
26	28	63	74	100	133	137	214	173	340	209	666
27	29	64	76	101	135	138	217	174	344	210	687
28	30	65	77	102	137	139	220	175	349	211	712
29	31	66	78	103	139	140	222	176	354	212	739
30	32	67	80	104	141	141	225	177	359	213	770
31	33	68	81	105	142	142	228	178	365	214	807
32	34	69	83	106	144	143	231	179	370	215	851
33	36	70	84	107	146	144	234	180	375	216	905
34	37	71	86	108	148	145	237	181	381	217	978
35	38	72	87	109	150	146	240	182	387	218	1088
36	39	73	88	110	152	147	243	183	393	219	1307
37	40	74	90	111	154						

Appendix B: Raw data from clinical studies

Table B.1: Section 5.3 *Raw data from two-day exposure, with samples taken at three times of day involving Patient A.*

	Site	0800h			1500h			2200h		
		Pre-HINS	During-HINS	Post-HINS	Pre-HINS	During-HINS	Post-HINS	Pre-HINS	During-HINS	Post-HINS
1	door handle	970	231	96	335	395	817	456	342	430
2	door	1	0	0	115	90	1	2	0	59
3	main light switch	55	7	141	15	16	8	1	0	27
4	pt chair L arm	970	873	1000	44	168	434	331	779	1000
5	pt chair R arm	826	318	336	6	78	766	1000	732	313
6	low drip	140	73	145	57	116	39	9	10	63
7	high drip	20	43	134	26	46	102	153	152	108
8	top L sheet	48	53	262	823	428	15	272	71	31
9	bottom L sheet	316	394	TNTC	238	0	13	22	47	29
10	top R sheet	84	76	129	252	370	11	86	713	25
11	bottom R sheet	324	133	451	706	0	151	205	30	358
12	Bottom bed rail	151	203	156	203	286	289	684	5	615
13	Bottom bed rail	60	230	167	654	123	327	161	4	182
14	Bottom bed rail	198	222	126	40	354	477	115	58	359
15	locker top	400	38	218	26	36	52	290	70	38
16	locker top	225	144	299	16	213	42	20	45	182
17	ledge behind bed	201	96	179	181	106	0	8	10	171
18	ledge behind bed	1000	330	101	113	190	165	43	53	193
19	ledge behind bed	168	151	61	58	103	80	112	13	194
20	upper ledge behind bed	547	102	212	119	120	8	29	29	187
21	upper ledge behind bed	393	202	57	194	214	237	354	71	210
22	upper ledge behind bed	257	401	43	26	230	186	308	17	252
23	table top	240	178	185	87	74	128	384	68	2
24	table top	194	47	286	467	52	58	77	208	7
25	table top	1000	56	222	6	100	0	492	2	1
26	table top	70	83	45	5	44	65	114	394	16
27	toilet light switch	662	1	259	358	4	0	22	0	2
28	toilet door handle	259	242	180	49	105	521	23	28	68
29	air con	81	37	221	59	0	57	92	7	47
30	air con	193	38	180	43	0	75	64	11	157
31	HINS box	98	13	189	28	71	0	27	6	31
32	HINS box	27	15	125	91	53	0	6	6	42
33	black bin	107	32	82	93	116	0	59	25	209
34	black bin	89	30	54	352	40	1	54	11	101
35	yellow bin	143	59	50	441	4	0	21	17	140
36	yellow bin	130	45	44	0	87	0	23	12	133
37	sink	19	42	36	29	0	0	1	22	24
38	sink	23	23	25	0	0	0	0	3	11
39	soap dispenser	0	0	4	4	1	0	0	0	0
40	towel dispenser	2	0	0	0	3	0	0	2	3
Total bacterial cfu per period		**8828**	**5261**	**8168**	**7182**	**4436**	**5126**	**6120**	**4073**	**6624**
Mean bacterial cfu/plate		**220.7**	**131.5**	**204.2**	**179.6**	**110.9**	**128.2**	**153.0**	**101.8**	**165.5**

Table B.2: Section 5.4 *Raw data from two-day exposure, with samples taken near two further patients.*

Sample	Site	Patient B (n=50)			Patient C (n=48)		
		Pre-HINS	*During-HINS*	*After-HINS*	*Pre-HINS*	*During-HINS*	*After-HINS*
1	Door	10	0	4	1	0	1
2	light switch	7	1	2	31	0	1
3	HINS power near door	8	9	12	36	46	9
4	HINS power near door	129	7	34	40	26	9
5	HINS power near door	21	11	26	13	18	24
6	lower ledge	1	5	8	10	6	1
7	lower ledge	19	3	1	14	21	16
8	lower ledge	11	1	13	3	7	5
9	lower ledge	2	1	1	7	0	13
10	upper ledge	13	12	13	13	3	9
11	upper ledge	12	3	12	29	1	11
12	upper ledge	78	2	8	65	10	14
13	upper ledge	6	2	2	48	6	4
14	left cot rail	25	1	4	78	14	4
15	left cot rail	1	0	0	224	12	11
16	left cot rail	1	2	1	26	15	5
17	left cot rail	2	0	1	4	15	34
18	right cot rail	3	4	4	5	51	4
19	right cot rail	2	1	2	97	133	53
20	right cot rail	4	1	2	79	21	528
21	right cot rail	3	0	0	12	3	8
22	foot cot rail	5	3	4	41	36	24
23	foot cot rail	5	0	17	33	38	28
24	foot cot rail	21	4	2	9	17	51
25	foot cot rail	4	0	2	28	19	12
26	table	2	1	3	97	167	24
27	table	3	1	0	110	19	6
28	table	19	1	1	9	29	23
29	table	4	3	7	15	92	9
30	locker	27	8	9	33	135	10
31	locker	39	1	6	25	6	2
32	locker	7	1	7	41	114	0
33	locker	2	2	12	39	8	1
34	sink	1	0	3	-	-	-
35	sink	6	0	0	-	-	-
36	towel holder	1	2	6	18	4	12
37	soap	13	1	9	15	10	7
38	black bin	18	0	10	14	8	3
39	black bin	18	1	11	20	8	15
40	yellow bin	6	12	7	14	2	49
41	yellow bin	7	2	7	15	3	18
42	window ledge	1	0	6	7	8	12
43	window ledge	2	2	5	16	11	8
44	window ledge	9	4	9	26	9	7
45	window ledge	10	0	12	15	3	1
46	toilet door	274	0	8	0	0	1
47	toilet light switch	53	1	20	2	0	1
48	HINS power near window	19	2	1	4	15	6
49	HINS power near window	27	2	6	10	6	5
50	HINS power near window	7	5	8	16	6	19
Total bacterial cfu		**968**	**125**	**348**	**1507**	**1181**	**1118**
Mean bacterial cfu/plate		**19.4**	**2.5**	**7.0**	**31.4**	**24.6**	**23.3**

Table B.3: Section 5.5 *Raw data from five-day exposures.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS 1 Day 2*	*During-HINS 2 Day 5*
1	door handle	24	24	53
2	door	5	5	16
3	main light switch	65	65	2
4	main light switch	6	6	0
5	pt chair L arm	171	171	84
6	pt chair R arm	649	649	101
7	HINS box 1	183	183	39
8	HINS box 1	149	149	37
9	HINS box 1	160	160	21
10	HINS box 1	46	46	23
11	low drip	30	30	16
12	low drip	71	71	13
13	high drip	17	17	92
14	high drip	241	241	7
15	L bed rail	204	204	3
16	L bed rail	838	838	8
17	L bed rail	466	466	12
18	L bed rail	191	191	17
19	L bed rail	260	260	1
20	L bed rail	1010	1010	6
21	R bed rail	1010	1010	33
22	R bed rail	214	214	65
23	R bed rail	57	57	36
24	R bed rail	195	195	75
25	R bed rail	49	49	36
26	R bed rail	170	170	85
27	Bottom bed rail	60	60	34
28	Bottom bed rail	104	104	15
29	Bottom bed rail	41	41	17
30	Bottom bed rail	111	111	15
31	pt TV	132	132	52
32	pt TV	173	173	21
33	locker top	56	56	32
34	locker top	22	22	32
35	locker top	16	16	17
36	locker top	20	20	25
37	ledge behind bed	27	27	29
38	ledge behind bed	19	19	36
39	ledge behind bed	8	8	30
40	ledge behind bed	130	130	55
41	ledge behind bed	5	5	20
42	upper ledge behind bed	113	113	21
43	upper ledge behind bed	16	16	28
44	upper ledge behind bed	15	15	19
45	upper ledge behind bed	3	3	36
46	upper ledge behind bed	13	13	54
47	table top	9	9	80
48	table top	194	194	33
49	table top	121	121	54
50	table top	36	36	54
51	table top	0	0	3
52	table top	229	229	1
53	toilet light switch	1	1	6
54	toilet door handle	0	0	0
55	toilet door	9	9	15
56	HINS box 2	19	19	185
57	HINS box 2	8	8	99
58	HINS box 2	24	24	16
59	HINS box 2	136	136	18
60	black bin	81	81	24
61	black bin	47	47	11
62	yellow bin	97	97	17
63	yellow bin	27	27	7
64	sink	63	63	0
65	sink	108	108	21
66	soap dispenser	54	54	10
67	towel dispenser	35	35	22
68	window ledge	35	35	12
69	window ledge	29	29	10
70	window ledge	16	16	23
Total bacterial cfu		**8960**	**3864**	**2191**
Mean bacterial cfu/plate		**127.8**	**55.2**	**31.3**

Table B.4: Section 5.5 *Raw data from six-day exposures.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS 1 Day 4*	*During-HINS 2 Day 6*	*Post-HINS Day 9*
1	door handle	67	87	7	286
2	door	5	0	0	1
3	main light switch	66	17	1	37
4	main light switch	14	3	2	52
5	pt chair L arm	23	21	37	47
6	pt chair R arm	29	12	12	22
7	HINS box 1	27	20	25	69
8	HINS box 1	49	22	28	290
9	HINS box 1	35	18	21	148
10	HINS box 1	59	17	23	49
11	low drip	14	7	9	117
12	low drip	188	103	4	95
13	high drip	75	11	4	544
14	high drip	31	7	6	112
15	L bed rail	114	7	6	50
16	L bed rail	7	14	4	35
17	L bed rail	27	24	11	64
18	L bed rail	3	8	9	661
19	L bed rail	11	6	3	35
20	L bed rail	10	2	2	20
21	R bed rail	31	106	2	208
22	R bed rail	24	210	1	67
23	R bed rail	20	11	0	76
24	R bed rail	48	10	5	179
25	R bed rail	55	56	6	81
26	R bed rail	15	16	5	74
27	Bottom bed rail	25	9	19	137
28	Bottom bed rail	30	8	25	21
29	Bottom bed rail	83	51	37	174
30	Bottom bed rail	40	16	20	107
31	pt TV	19	22	15	35
32	pt TV	20	23	11	50
33	locker top	18	75	7	31
34	locker top	34	33	13	68
35	locker top	68	28	18	81
36	locker top	69	9	15	49
37	ledge behind bed	37	77	16	137
38	ledge behind bed	51	38	27	136
39	ledge behind bed	32	32	27	39
40	ledge behind bed	12	38	13	69
41	ledge behind bed	384	31	16	38
42	upper ledge behind bed	44	84	66	321
43	upper ledge behind bed	185	32	28	279
44	upper ledge behind bed	47	25	35	33
45	upper ledge behind bed	30	17	8	15
46	upper ledge behind bed	10	34	8	6
47	table top	34	11	20	21
48	table top	47	19	3	45
49	table top	34	24	1	14
50	table top	30	8	10	48
51	table top	4	5	4	121
52	table top	0	8	1	0
53	toilet light switch	33	21	41	126
54	toilet door handle	1	3	0	2
55	toilet door	6	1	18	54
56	HINS box 2	13	21	6	66
57	HINS box 2	9	1	15	37
58	HINS box 2	13	2	15	19
59	HINS box 2	69	18	43	59
60	black bin	48	18	22	50
61	black bin	40	21	15	40
62	yellow bin	30	31	41	35
63	yellow bin	128	3	2	4
64	sink	81	5	5	16
65	sink	42	26	25	28
66	soap dispenser	127	12	12	24
67	towel dispenser	97	10	30	43
68	window ledge	63	14	15	43
69	window ledge	113	21	18	23
70	window ledge	49	20	18	44
Total bacterial cfu		**2905**	**1820**	**1036**	**5607**
Mean bacterial cfu/plate		**48.5**	**26.0**	**14.8**	**80.1**

Table B.5: Section 5.5 *Raw data from seven-day exposures.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS 1 Day 4*	*During-HINS 2 Day 6*	*Post-HINS Day 9*
1	light switch nr door	3	1	32	20
2	HINS box 1	20	31	17	71
3	HINS box 1	70	39	11	22
4	HINS box 1	45	10	12	42
5	HINS box 1	25	39	6	21
6	head bed rail	30	194	0	20
7	head bed rail	41	10	1	13
8	head bed rail	54	6	3	32
9	head bed rail	36	19	3	16
10	head bed rail	16	34	3	12
11	foot bed rail	23	3	6	58
12	foot bed rail	79	2	3	55
13	foot bed rail	8	2	4	53
14	foot bed rail	15	2	8	30
15	foot bed rail	57	7	3	75
16	overbed table	14	2	7	2
17	overbed table	21	5	2	94
18	overbed table	14	0	2	564
19	overbed table	28	7	1	316
20	overbed table	10	5	7	81
21	overbed table	15	3	6	66
22	pt tv	21	8	2	27
23	pt tv	60	2	0	8
24	lower ledge	132	59	2	28
25	lower ledge	138	43	13	69
26	lower ledge	71	25	7	88
27	lower ledge	13	24	4	53
28	lower ledge	17	15	5	38
29	lower ledge	38	7	6	24
30	upper ledge	126	35	10	27
31	upper ledge	116	38	9	107
32	upper ledge	161	44	4	42
33	upper ledge	214	34	2	55
34	upper ledge	145	13	14	53
35	upper ledge	36	13	4	84
36	locker top	13	5	4	6
37	locker top	23	6	4	5
38	locker top	13	4	5	37
39	locker top	6	5	11	21
40	lamp	2	2	0	7
41	obs machine	123	0	1	6
42	obs machine	87	0	6	110
43	window sil	26	30	7	203
44	window sil	25	2	6	300
45	window sil	13	7	20	122
46	toilet door	3	23	0	0
47	toilet door handle	751	0	50	344
48	toilet light switch	3	0	0	1
49	towel holder	47	11	3	26
50	towel holder	13	10	3	27
51	soap	15	4	9	12
52	sink	2	0	0	10
53	black bin	111	6	9	29
54	black bin	72	5	15	16
55	black bin	140	12	5	22
56	black bin	131	9	23	19
57	HINS box 2	24	7	2	66
58	HINS box 2	29	2	16	87
59	HINS box 2	41	4	4	86
60	HINS box 2	222	8	2	138
Total bacterial cfu		**3846**	**942**	**426**	**4068**
Mean bacterial cfu/plate		**64.1**	**15.7**	**7.1**	**67.8**

Table B.6: Section 5.6 *Raw data from seven-day exposures using single HINS-light EDS, Patient G study.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS 1 Day 2*	*During-HINS 2 Day 4*	*During-HINS 3 Day 7*	*Post-HINS Day 9*
1	door high	0	2	0	1	0
2	door low	32	0	14	0	2
3	handle	19	12	15	3	3
4	light switch	81	4	3	10	6
5	near HINS box top left	22	11	15	1	10
6	near HINS box top right	11	3	5	0	9
7	near HINS box bottom left	14	30	17	2	3
8	near HINS box bottom right	30	37	10	2	4
9	Left arm chair front	33	97	113	46	315
10	Left arm chair back	8	185	11	18	172
11	Right arm chair front	2	361	21	10	19
12	Right arm chair back	12	104	23	20	69
13	upper ledge nearside 1	52	17	10	6	15
14	upper ledge nearside 2	51	5	19	10	15
15	upper ledge nearside 3	37	29	17	10	3
16	upper ledge far side 1	32	9	5	5	26
17	upper ledge far side 2	38	10	27	4	22
18	upper ledge far side 3	56	12	12	5	15
19	lower ledge nearside 1	50	16	22	2	35
20	lower ledge nearside 2	56	33	12	6	34
21	lower ledge nearside 3	34	17	9	5	42
22	lower ledge far side 1	72	7	4	10	0
23	lower ledge far side 2	35	12	22	18	19
24	lower ledge far side 3	40	9	8	4	28
25	tv high	0	1	4	2	9
26	tv low	3	0	6	7	4
27	left bed rail head 1	20	2	7	1	208
28	left bed rail head 2	42	5	18	8	10
29	left bed rail middle	45	6	7	13	25
30	left bed rail foot 1	301	24	6	6	31
31	left bed rail foot 2	60	6	3	4	3
32	right bed rail head 1	24	2	3	5	17
33	right bed rail head 2	16	55	9	6	57
34	right bed rail middle	663	49	9	22	20
35	right bed rail foot 1	126	3	8	9	7
36	right bed rail foot 2	15	22	15	3	11
37	top bed rail left 1	18	11	10	4	8
38	top bed rail left 2	7	14	3	0	85
39	top bed rail right 1	20	2	14	8	85
40	top bed rail right 2	25	11	26	4	4
41	locker top top right	45	6	14	10	20
42	locker top top left	10	10	12	3	5
43	locker top bottom right	10	5	4	4	12
44	locker top bottom left	32	8	3	3	10
45	bedside table head far side	28	3	51	2	8
46	bedside table head middle	5	27	6	0	4
47	bedside table head nearside	37	11	34	4	16
48	bedside table foot far side	68	1	29	2	2
49	bedside table foot middle	22	8	9	2	5
50	bedside table foot nearside	168	7	12	30	8
51	drip top	3	2	2	1	1
52	drip bottom	45	4	6	0	62
53	toilet door top	1	0.0	0.0	0.0	0
54	toilet door bottom	0	0	1	0	0
55	toilet door handle	54	9	9	9	33
56	toilet light switch	2	0	0	0	0
57	far HINS box top left	29	18	8	6	11
58	far HINS box top right	21	14	10	1	9
59	far HINS box bottom left	85	2	9	2	3
60	far HINS box bottom right	37	6	9	0	15
61	black bin far	32	33	20	5	7
62	black bin near	31	9	56	14	15
63	yellow bin far	26	8	19	4	5
64	yellow bin near	32	8	31	4	26
65	towel right	23	7	9	7	17
66	towel left	11	6	16	8	16
67	sink right	6	10	8	0	1
68	sink left	10	3	9	3	2
69	tap	5	2	0	4	2
70	soap	11	4	6	7	13
Total bacterial cfu		**3164**	**1463**	**966**	**434**	**1778**
Mean bacterial cfu/plate		**45.2**	**20.9**	**13.8**	**6.2**	**25.4**

Table B.7: Section 5.6 *Raw data from seven-day exposures using single HINS-light EDS, Patient H study.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS 1 Day 4*	*During-HINS 2 Day 7*	*Post-HINS Day 9*
1	door high	3	9	0	0
2	door low	1	0	8	20
3	handle	56	37	78	117
4	light switch	179	19	4	1
5	near HINS box top left	107	392	79	156
6	near HINS box top right	223	392	113	143
7	near HINS box bottom left	413	409	125	133
8	near HINS box bottom right	319	390	107	132
9	Left arm chair front	767	251	71	146
10	Left arm chair back	339	86	124	54
11	Right arm chair front	156	82	7	11
12	Right arm chair back	56	116	8	15
13	upper ledge nearside 1	189	174	61	38
14	upper ledge nearside 2	277	70	40	59
15	upper ledge nearside 3	180	29	29	85
16	upper ledge far side 1	189	24	36	54
17	upper ledge far side 2	130	22	17	42
18	upper ledge far side 3	200	34	24	78
19	lower ledge nearside 1	383	146	36	42
20	lower ledge nearside 2	239	162	18	40
21	lower ledge nearside 3	177	90	29	53
22	lower ledge far side 1	174	60	29	50
23	lower ledge far side 2	188	16	23	34
24	lower ledge far side 3	155	18	33	45
25	tv high	132	538	16	18
26	tv low	178	451	12	45
27	left bed rail head 1	14	138	120	143
28	left bed rail head 2	621	219	210	703
29	left bed rail middle	169	283	15	108
30	left bed rail foot 1	177	12	6	1
31	left bed rail foot 2	26	117	118	50
32	right bed rail head 1	11	9	71	29
33	right bed rail head 2	37	151	162	9
34	right bed rail middle	34	103	127	174
35	right bed rail foot 1	23	72	41	72
36	right bed rail foot 2	159	166	17	163
37	top bed rail left 1	36	46	20	50
38	top bed rail left 2	177	49	40	52
39	top bed rail right 1	244	38	20	19
40	top bed rail right 2	289	59	47	47
41	locker top top right	61	120	27	39
42	locker top top left	26	35	33	24
43	locker top bottom right	82	75	18	21
44	locker top bottom left	128	45	31	33
45	bedside table head far side	22	88	0	32
46	bedside table head middle	117	107	22	8
47	bedside table head nearside	637	17	19	10
48	bedside table foot far side	69	88	5	6
49	bedside table foot middle	100	17	2	17
50	bedside table foot nearside	60	64	6	12
51	drip top	18	24	11	67
52	drip bottom	14	32	1	53
53	toilet door top	1	0.0	0.0	0
54	toilet door bottom	2	1	1	3
55	toilet door handle	57	63	36	23
56	toilet light switch	1	1	17	2
57	far HINS box top left	117	34	34	10
58	far HINS box top right	30	53	5	9
59	far HINS box bottom left	42	42	21	362
60	far HINS box bottom right	76	52	24	52
61	black bin far	64	98	20	82
62	black bin near	54	49	22	84
63	yellow bin far	92	29	27	57
64	yellow bin near	76	13	37	86
65	towel right	196	11	21	20
66	towel left	406	19	25	51
67	sink right	42	6	18	31
68	sink left	46	26	10	308
69	tap	34	8	12	4
70	soap	57	36	31	31
Total bacterial cfu **Mean bacterial cfu/plate**		**10154** **145.1**	**6732** **96.2**	**2677** **38.2**	**4768** **68.1**

Table B.8: Section 5.6 *Raw data from seven-day exposures using single HINS-light EDS, Patient I study.*

Sample	Site	*Pre-HINS Day 0*	*During-HINS Day 7*	*Post-HINS Day 9*
1	door high	1	1	26
2	door low	1	0	0
3	handle	63	16	38
4	light switch	4	4	38
5	near HINS box top left	21	24	272
6	near HINS box top right	5	33	33
7	near HINS box bottom left	23	26	196
8	near HINS box bottom right	34	28	19
9	Left arm chair front	59	21	57
10	Left arm chair back	45	72	91
11	Right arm chair front	20	22	60
12	Right arm chair back	184	123	138
13	upper ledge nearside 1	41	33	103
14	upper ledge nearside 2	19	35	134
15	upper ledge nearside 3	20	19	133
16	upper ledge far side 1	39	35	142
17	upper ledge far side 2	51	24	10
18	upper ledge far side 3	37	54	1
19	lower ledge nearside 1	27	26	67
20	lower ledge nearside 2	15	18	148
21	lower ledge nearside 3	13	21	152
22	lower ledge far side 1	34	30	120
23	lower ledge far side 2	37	40	128
24	lower ledge far side 3	27	42	145
25	tv high	11	8	16
26	tv low	23	21	45
27	left bed rail head 1	13	11	34
28	left bed rail head 2	15	15	25
29	left bed rail middle	42	30	76
30	left bed rail foot 1	22	16	26
31	left bed rail foot 2	5	11	37
32	right bed rail head 1	10	15	7
33	right bed rail head 2	4	89	3
34	right bed rail middle	1	5	154
35	right bed rail foot 1	10	18	18
36	right bed rail foot 2	22	34	26
37	top bed rail left 1	19	31	218
38	top bed rail left 2	44	31	147
39	top bed rail right 1	33	15	14
40	top bed rail right 2	59	85	17
41	locker top top right	10	26	38
42	locker top top left	22	18	20
43	locker top bottom right	7	18	17
44	locker top bottom left	34	22	8
45	bedside table head far side	31	61	61
46	bedside table head middle	16	20	64
47	bedside table head nearside	50	84	49
48	bedside table foot far side	29	66	31
49	bedside table foot middle	58	88	16
50	bedside table foot nearside	7	6	135
51	drip top	0	3	0
52	drip bottom	0	25	0
53	toilet door top	2	2.0	14
54	toilet door bottom	1	1	8
55	toilet door handle	33	7	33
56	toilet light switch	3	0	10
57	far HINS box top left	20	7	12
58	far HINS box top right	20	7	45
59	far HINS box bottom left	56	8	36
60	far HINS box bottom right	19	14	15
61	black bin far	22	20	18
62	black bin near	30	4	12
63	yellow bin far	19	17	22
64	yellow bin near	17	26	20
65	towel right	46	13	7
66	towel left	33	9	120
67	sink right	17	11	28
68	sink left	14	25	10
69	tap	21	5	18
70	soap	36	13	3
Total bacterial cfu **Mean bacterial cfu/plate**		**1826** **33.0**	**1808** **25.8**	**3984** **56.9**

Table B.9: Section 6.4 *Raw data from outpatient Clinic A studies with and without HINS-light EDS (BPA sampling).*

Sample	Site	*Before control clinic*	*After control clinic*	*Before intervention clinic*	*After intervention clinic*
1	bin	0	3	4	3
2	bin	2	10	1	0
3	bin	3	1	17	5
4	bin	3	2	5	10
5	apron dispenser	0	6	3	7
6	apron dispenser	0	7	0	5
7	apron dispenser	2	3	2	5
8	apron dispenser	4	7	0	11
9	glove dispenser	41	61	7	8
10	glove dispenser	31	52	12	10
11	soap dispenser	3	23	1	9
12	soap dispenser	1	15	0	1
13	tap	0	3	4	5
14	tap	0	4	2	1
15	towel dispenser	2	47	3	8
16	towel dispenser	2	71	1	5
17	dressing trolley	13	8	3	7
18	dressing trolley	14	11	3	5
19	dressing trolley	8	6	2	0
20	dressing trolley	9	10	0	1
21	top shelf	10	55	1	2
22	top shelf	11	38	0	2
23	top shelf	0	26	1	3
24	top shelf	18	48	1	2
25	bottom shelf	5	10	0	0
26	bottom shelf	8	47	0	0
27	bottom shelf	11	44	1	0
28	bottom shelf	13	60	0	1
29	back of worktop	8	7	6	1
30	back of worktop	12	1	1	0
31	back of worktop	7	1	2	1
32	front of worktop	6	2	2	0
33	front of worktop	4	5	0	2
34	front of worktop	3	2	6	20
35	lamp	0	29	4	21
36	lamp	9	1	7	11
37	patient couch	7	4	0	20
38	patient couch	15	5	1	7
39	patient couch	11	3	3	3
40	patient couch	7	2	0	3
41	patient couch	7	8	0	5
42	patient couch	8	3	1	12
43	HINS power supply	9	92	1	2
44	HINS power supply	18	68	0	6
45	chair	14	49	0	8
46	chair	14	40	1	10
47	chair	12	41	1	9
48	chair	10	33	0	13
49	light switch	0	1	0	56
50	door handle	3	6	0	0
Total bacterial cfu **Mean bacterial cfu/plate**		**398** **8.0**	**1081** **21.6**	**110** **2.2**	**326** **6.5**

Table B.10: Section 6.4 *Raw data from outpatient Clinic B studies with and without HINS-light EDS (BPA sampling).*

Sample	Site	*Before control clinic*	*After control clinic*	*Before intervention clinic*	*After intervention clinic*
1	bin	1	1	2	12
2	bin	0	4	2	8
3	bin	0	7	0	11
4	bin	0	19	1	77
5	apron dispenser	0	30	0	14
6	apron dispenser	0	21	0	12
7	apron dispenser	0	23	0	10
8	apron dispenser	0	25	1	5
9	glove dispenser	14	18	18	11
10	glove dispenser	7	21	19	9
11	soap dispenser	2	15	1	21
12	soap dispenser	0	1	2	0
13	tap	0	17	0	3
14	tap	0	14	21	5
15	towel dispenser	8	59	38	45
16	towel dispenser	6	186	23	42
17	dressing trolley	5	68	11	74
18	dressing trolley	3	108	23	117
19	dressing trolley	9	3	16	11
20	dressing trolley	7	1	13	9
21	top shelf	5	3	13	8
22	top shelf	10	1	6	13
23	top shelf	12	1	17	15
24	top shelf	3	0	17	17
25	bottom shelf	2	0	12	14
26	bottom shelf	4	1	10	30
27	bottom shelf	6	1	12	6
28	bottom shelf	1	4	5	8
29	back of worktop	4	0	0	1
30	back of worktop	1	2	4	10
31	back of worktop	1	2	12	13
32	front of worktop	1	0	8	1
33	front of worktop	1	7	3	1
34	front of worktop	10	33	24	19
35	lamp	16	36	43	1
36	lamp	94	20	40	11
37	patient couch	31	77	13	3
38	patient couch	12	109	7	20
39	patient couch	43	32	7	55
40	patient couch	4	23	11	16
41	patient couch	2	11	16	15
42	patient couch	5	5	5	3
43	HINS power supply	10	11	3	4
44	HINS power supply	7	9	14	11
45	chair	13	30	16	15
46	chair	19	16	4	34
47	chair	15	13	3	1
48	chair	13	40	11	23
49	light switch	8	14	1	9
50	door handle	1	0	16	0
Total bacterial cfu		**416**	**1142**	**544**	**873**
Mean bacterial cfu/plate		**8.3**	**22.8**	**10.8**	**17.5**

Table B.11: Section 6.5 *Raw data from outpatient clinic studies with and withoutHINS-light EDS (TSA sampling).*

Sample	Site	*Before control clinic*	*After control clinic*	*Before intervention clinic*	*After intervention clinic*
1	bin	8	1	2	3
2	bin	5	6	1	8
3	bin	3	20	3	2
4	bin	2	11	9	0
5	apron dispenser	5	25	5	7
6	apron dispenser	8	24	4	9
7	apron dispenser	1	37	2	10
8	apron dispenser	4	19	6	5
9	glove dispenser	130	37	19	32
10	glove dispenser	114	18	29	34
11	soap dispenser	4	15	0	25
12	soap dispenser	0	46	0	7
13	tap	1	17	0	0
14	tap	2	15	0	37
15	towel dispenser	0	3	19	6
16	towel dispenser	3	1	40	9
17	dressing trolley	5	3	8	47
18	dressing trolley	2	2	17	13
19	dressing trolley	8	9	0	8
20	dressing trolley	27	12	0	16
21	top shelf	16	17	52	27
22	top shelf	30	19	0	26
23	top shelf	7	23	3	12
24	top shelf	5	13	1	6
25	bottom shelf	16	18	2	6
26	bottom shelf	8	8	0	26
27	bottom shelf	2	3	2	3
28	bottom shelf	8	0	4	2
29	back of worktop	1	3	6	2
30	back of worktop	8	4	3	1
31	back of worktop	3	5	10	26
32	front of worktop	1	0	0	6
33	front of worktop	6	2	24	14
34	front of worktop	9	28	148	1
35	lamp	6	136	15	13
36	lamp	24	42	36	5
37	patient couch	4	37	175	4
38	patient couch	6	29	157	6
39	patient couch	4	22	7	8
40	patient couch	15	34	0	42
41	patient couch	26	25	3	17
42	patient couch	33	15	50	29
43	HINS power supply	28	22	76	37
44	HINS power supply	22	57	55	78
45	chair	11	54	2	16
46	chair	3	48	3	30
47	chair	12	33	1	9
48	chair	8	28	3	4
49	light switch	35	47	13	6
50	door handle	14	5	0	1
Total bacterial cfu **Mean bacterial cfu/plate**		**703** **14.1**	**1098** **22.0**	**1015** **20.3**	**741** **14.8**

Total number of samples taken = 2830.

Appendix C: Peer review

During the course of this research, work was submitted to peer-review for publication and presentation. In addition, funding was applied for from several bodies through a peer-review process. The following pieces were accepted, with papers, abstracts and posters included for reference.

Full papers

- Bache SE, Maclean M, Gettinby G, Anderson JA, MacGregor SJ, Taggart I. Quantifying bacterial transfer from patients to staff during burns dressing changes: implications for infection control. Burns 2013; 39: 220-8.
- Bache SE, Maclean M, MacGregor SJ, Anderson JA, Gettinby G, Coia J, Taggart I. Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings. *Burns* 2012; 38(1): 69-76.

Abstracts

- Bache SE, Maclean M, Anderson JG, Gettinby G, Coia J E, MacGregor S J, Taggart I. Laboratory inactivation of healthcare-associated isolates by a visible HINS-light source and its clinical application in the burns unit (Abstract). *Burns* 2011; 37(S1): S6.
- Bache SE, Maclean M, MacGregor SJ, Anderson J, Gettinby G, Woolsey G A, Taggart I. Application of a novel light technology for disinfection on the burns unit (Abstract). *Journal of Burn Care and Research* 2010; 31(2): S113.

Oral presentations

- Bache SE, Maclean M, Anderson JG, MacGregor SJ, Gettinby G, Taggart I. A model to predict transfer of bacteria form patients to staff during burns dressing changes. International Society for Burn Injuries conference, Edinburgh, 2012
- Bache SE, Maclean M, MacGregor SJ, Anderson JG, Gettinby G, Coia J, Taggart I. Patient-safe continuous environmental disinfection in the burns unit using a visible light source (HINS-light EDS). British Association of Plastic and Reconstructive Surgery winter scientific meeting, London, December 2011.
- Bache SE, Maclean M, Anderson JG, MacGregor SJ, Gettinby G, Taggart I. The role of airborne and staff contamination in the cycle of cross-contamination between burns patients. Scottish plastic surgery trainees meeting, Dunkeld, October 2011. **Best presentation award.**

Oral presentations (continued)

- Bache SE, Maclean M, Anderson JG, Gettinby G, MacGregor SJ, Taggart I. Laboratory inactivation of healthcare-associated isolates by a visible HINS-liht source and its clinical application in the burns unit. European Burn Association Congress, The Hague, September 2011.
- Bache SE, Maclean M, MacGregor SJ, Anderson JG, Gettinby G, Watson SB, Taggart I. Reducing bacterial contamination in the burns dressing clinic using a novel light-based method of continuous disinfection. International Society for Burn Injuries Conference, Istanbul, June 2010.
- Bache SE, Maclean M, MacGregor SJ, Anderson JG, Gettinby G, Woolsey GJ, Taggart I. Application of a novel light technology for disinfection on the burns unit. American Burn Association annual meeting, Boston, March 2010.

Poster presentation

- Bache SE, Maclean M, MacGregor SJ, Anderson JG, Watson SB, Taggart I. The HINS-light in practice: a novel method of continuous disinfection on the burn unit. Birmingham international burn congress, Birmingham September 2010.
- Bache SE, Morhij R, Yousif S, Taggart I. A survey of protective equipment worn by healthcare workers during burns dressing changes in the UK. International Society for Burn Injuries conference, Edinburgh September 2012.

Peer-reviewed research grants awarded

- Research award, Tenovus Scotland. National research grant towards cost of consumables.
- Small research support grant, The Royal College of Surgeons of Edinburgh. National research grant issued towards cost of consumables.
- Stephen Plumpton award, Canniesburn Plastic Surgery Unit. Local award towards research costs.

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Quantifying bacterial transfer from patients to staff during burns dressing and bed changes: Implications for infection control

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ABSTRACT

Routine nursing activities such as dressing/bed changes increase bacterial dispersal from burns patients, potentially contaminating healthcare workers (HCW) carrying out these tasks. HCW thus become vectors for transmission of nosocomial infection between patients. The suspected relationship between %total body surface area (%TBSA) of burn and levels of bacterial release has never been fully established.

Bacterial contamination of HCW was assessed by contact plate samples ($n = 20$) from initially sterile gowns worn by the HCW during burns patient dressing/bed changes. Analysis of 24 gowns was undertaken and examined for relationships between %TBSA, time taken for activity, and contamination received by the HCW.

Relationships between size of burn and levels of HCW contamination, and time taken for the dressing/bed change and levels of HCW contamination were best described by exponential models. Burn size correlated more strongly ($R^2 = 0.82$, $p < 0.001$) than time taken ($R^2 = 0.52$, $p < 0.001$), with levels of contamination received by the HCW. Contamination doubled with every 6–9% TBSA increase in burn size.

Burn size was used to create a model to predict bacterial contamination received by a HCW carrying out bed/dressing changes. This may help with the creation of burn-specific guidelines on protective clothing worn by HCW caring for burns patients.

1. Introduction

Advances in fluid resuscitation, organ support, and early excision and grafting have all improved survival rates following a severe burn [1]. However, this has also had the effect of shifting the cause of morbidity and mortality away from hypovolemia and towards sepsis. Sepsis is a primary risk factor of mortality following a burn [2,3]. It is now estimated that in patients with burns over 40% total body surface area (TBSA), 75% of all deaths are related to infection and/or inhalation injury [1]. Following a severe burn, physical, non-specific and specific immune defences are all affected, leading to a state of immunosuppression. Coupled with large bacteria-

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harbouring wounds, this renders burns patients both susceptible to infection and potent dispersers of bacteria [4]. The consequences of nosocomial propagation can be felt throughout the entire hospital, increasing costs and the risk of outbreaks of multidrug-resistant bacteria on the burns unit and beyond [5].

Transmission of infection between burns patients mainly occurs through airborne transmission or direct and indirect contact [1,6]. Routine nursing activity may create periods of increased bacterial dispersal into the air and onto surfaces and other individuals present in the vicinity. The present study examines the contamination of healthcare workers (HCW) resulting from burn wound dressing changes, which are often coupled with bed sheet changes.

Dressing changes on even small non-burn wounds create airborne dispersal of bacteria [7]. Bed sheet changes have also been shown to liberate bacteria into the air [8]. In the 1970s, attempts were made to link the size of a burn and the airborne dispersal of *Staphylococcus aureus* during a dressing change, which implied that the size of the burn was related to levels of bacteria found on settle plates over a period of days [9]. More recently, it was shown that 31% of dressing changes on methicillin resistant *S. aureus* (MRSA) positive burns patients liberated the organism into the air [10].

HCW uniforms are a potential reservoir of infection [11–13], and their contamination can be directly attributed to patients [14,15]. Not only can bacteria be transferred from burns patients to uniforms during dressing changes, but also laboratory simulations have demonstrated that these bacteria can be transferred from the uniform to patients [17,18]. Despite this, there is little consensus for the appropriate protective attire to be worn by HCW carrying out dressing changes on burns patients. In a survey of US burns units, only 24% of units required full protective coverage on entering a patient's room and changing a dressing [19]. UK guidelines are similarly vague and not burns-specific [20–22]. Quantitative data on key issues may help in their development. In this context, the current study was set up to address the hypothesis that the level of contamination received by a HCW would be related to the size of the burn and the time taken for the dressing change.

2. Materials and methods

2.1. Setting

Quantification of HCW contamination was carried out during burn dressing changes. For patients with larger burns, the dressing change would usually also incorporate a bed sheet change while rolling the patient to apply bandages (hereafter termed 'dressing/bed change'). Data including age of burn, recent routine wound swab results, time taken for the dressing/bed change to take place and the %TBSA burn were recorded for each patient. Patients were treated according to standard practice on our burns unit. We aim for early excision and split thickness skin autograft or coverage with a dermal substitute in all deep dermal and full thickness burns. Patients with superficial burns, or those deemed too sick for surgical intervention are managed conservatively with dressings and topical agents. Patients with burn wounds over 10 days old were excluded from the study.

2.2. Sample standardisation

To ensure that samples were taken from a standardised baseline, HCW were asked to don sterile, impermeable, disposable full-body gowns over their uniforms prior to performing dressing/bed changes. This was done to eliminate natural variations in bacterial contamination between different HCWs before the beginning of the dressing/bed change. It also provided a consistent sampling material, which was preferable to sampling from a variety of textures and surfaces including cotton and skin. Gowns were thus worn by the HCW only to facilitate the study design and sampling objectives. Usually, disposable plastic aprons would be worn over uniforms as routine bed/dressing changes are carried out. All HCW maintained standard hand hygiene by decontaminating hands and putting on fresh disposable gloves before entering the patient's room to carry out the nursing activity. Thereafter, with the exception of wearing disposable gowns rather than disposable plastic aprons over uniforms, the HCW carried out the dressing/bed change in the usual manner. Gloves were removed and hands washed following the dressing change and gown sampling, before leaving the room.

Samples were taken from the two most 'involved' HCW carrying out the dressing change, each of whom would usually stand either side of the bed and carry out undressing and redressing of wounds alongside one another. For smaller burns, one HCW often carried out the dressing change alone, and only one set of samples was obtained. Sampling during dressing/bed changes on any one patient was only carried out once.

2.3. Sampling sites

Following the dressing/bed change, and while the HCW was still wearing the disposable gown, and remained in the patient's room, the gown was sampled. To estimate the contamination that would be received during a dressing/bed change by a HCW who had not been wearing an apron, samples were taken from 20 sites across the front of the gown. The 20 'no apron' sites are illustrated in Fig. 1. Of note, the sites are all across the front of the gown, as it was the aim of the study to collect samples from areas that were likely to become most contaminated during dressing/bed changes. In order to estimate the protection afforded had a disposable plastic apron been worn, a subset of 15 'with apron' sites were analysed separately. These excluded five sampling sites on the chest and abdomen that would normally be covered by a disposable apron. These are also demonstrated in Fig. 1.

2.4. Bacteriological methods

Samples were taken from the 20 sites using 25 cm^2 Baird Parker Agar (BPA) contact plates that were pressed firmly against the sampling site for approximately 2 s, by the same investigator (SEB). BPA allows for selective isolation of staphylococcal-type organisms, which are an accepted marker of bacteria originating from a human source [23]. A selective

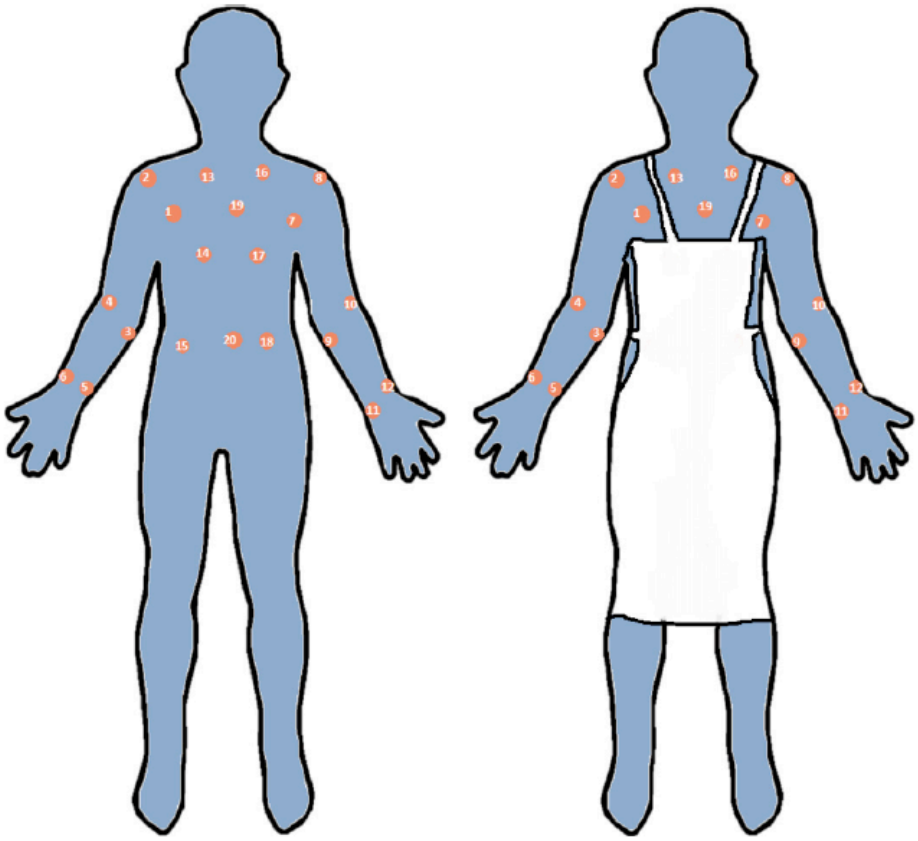

Fig. 1 – Diagram to demonstrate sampling sites on the front of HCW gowns. The image on the left shows the positions of all 20 sampling sites (termed 'no apron' sites). The image on the right highlights the 15 sampling sites left exposed if the HCW had been wearing an apron (termed 'with apron' sites). The two sets of samples were analysed separately.

agar was chosen over a non-selective agar as preliminary studies indicated that non-selective agar yielded too many bacterial colony-forming units (cfu) per agar plate to accurately enumerate. Contact agar plates allow direct sample collection from the contaminated gowns, and enable accurate reproduction of sampling due to the defined surface area of the agar plates. Sample plates were incubated at 37 °C for 48 h before enumeration.

The time taken for the dressing/bed change to take place was measured from when the HCW entered the patient's room to commence the dressing/bed change (the point at which they would usually don a plastic apron). It finished at the point when the dressing and bed change (if that was also being carried out) was completed, when they would usually remove their apron and gloves prior to leaving the room. At this point the gown was sampled. Any further activities, including tidying the room, assisting with feeding, or brushing the patient's hair or teeth were not included in the time taken for dressing/bed change. The gown was sampled before these extra activities took place. This meant that the contamination measured was that received only during the dressing/bed change. It was not possible to separate the dressing and bed change components of the activity, as the bed sheet change was often integrated into the dressing change when the patient was rolled for application of bandages. We intended to mimic real-life situations as much as possible and did not want to inconvenience the patient or HCW, or prolong the activity by carrying out separate dressing changes and bed changes, during what can be a distressing and uncomfortable time.

2.5. *Statistical analysis*

In undertaking the study consideration was given to power and sample size required for the purposes of the regression and correlation analysis. It was estimated that measurements would be required on bacterial cfu and associated %TBSA for a minimum of 10 patients in order to have in excess of 90% statistical power to detect a correlation of 0.9 with 95% confidence. A random sample size of between 10 and 15 patients was planned with replicate cfu measurements being observed on up to two HCW carrying out dressing/bed changes per patient.

HCW bacterial contamination was expressed as mean number of bacterial cfu per 25 cm^2 agar plate, or mean cfu/plate. For each sampling session this was calculated for all 20

'no apron' sites, and also for the 15 'with apron sites', excluding those 5 sites that would have been covered by a disposable plastic apron, had one been worn. Statistical analysis was carried out using NCSS Windows Version 7 software. Relationships were examined for between three variables: %TBSA and HCW contamination; time taken for the dressing/bed change and HCW contamination; %TBSA and time taken for the dressing/bed change. Separate analysis was carried out on all 20 'no apron' sites, and on the 15 'with apron' sampling sites. Mathematical modelling was used to identify equations which best described the three relationships. These were used to predict the contamination a HCW would receive during dressing/bed change of a burn patient by % TSBA. The coefficient of determination, R^2 was used to measure how well the model fitted to the observed data and $p < 0.05$ was considered significant.

3. Results

3.1. Patient demographics and wound information

Samples were collected from the gowns of 24 HCW carrying out dressing changes on 15 different patients, with a mean burn size of 19%TBSA (range 1–51%TBSA). Mean age of patient was 39 years (range 19–85 years). Samples were taken a mean of 6.4 days after the burn (range 2–10 days). Mean time taken for the dressing change was 45 min (range 10–90 min). The most common organism identified on routine wound swabs was *S. aureus*. *Bacillus* sp., coliforms, and *Streptococcus* sp. were also commonly isolated. Results are summarised in Table 1.

3.2. Relationship between time taken for dressing/bed change and %TBSA

A significant relationship was demonstrated between the time taken for the dressing/bed change to take place and the size of the burn (%TBSA). This was explained by a linear correlation (coefficient of determination, $R^2 = 0.76$; $p < 0.001$). This is demonstrated in Fig. 2.

3.3. Analysis of 20 'no apron' sites

The variation in contamination received by a HCW during a dressing/bed change when 20 'no apron' sampling sites were analysed was examined in relation to %TBSA of the burn and time taken for the dressing/bed change. Both relationships were explained by exponential models. These were as follows:

Relationship between HCW contamination and %TBSA (coefficient of determination, $R^2 = 0.82$; $p < 0.001$):

Mean cfu/plate = $8.59\ \mathrm{Exp}^{0.080 \times \%\mathrm{TBSA}}$

Relationship between time taken in min for dressing/bed change and HCW contamination (coefficient of determination, $R^2 = 0.52$; $p < 0.002$):

Mean cfu/plate = $17.44\ \mathrm{Exp}^{0.034 \times \text{time taken in min}}$

These curves are illustrated in Fig. 3. Both charts demonstrate an exponential relationship between the variable (%TBSA or time taken for the dressing/bed change to take place) and the contamination received by the HCW. However, although they are both significant relationships, time taken correlates less strongly than %TBSA as shown by the lower R^2. %TBSA is a more accurate predictor of HCW contamination than time taken for the dressing/bed change to take place.

3.4. Analysis of 15 'with apron' sites

The variation in contamination received by a HCW during a dressing/bed change when 15 'with apron' sampling sites was examined in relation to %TBSA of the burn and time taken for the dressing/bed change. Both relationships were explained by exponential models. These were as follows:

Relationship between HCW contamination and %TBSA (coefficient of determination, $R^2 = 0.86$; $p < 0.001$):

Mean cfu/plate = $2.05\ \mathrm{Exp}^{0.110 \times \%\mathrm{TBSA}}$

Relationship between HCW contamination and time taken in min for dressing/bed change (coefficient of determination, $R^2 = 0.44$; $p = 0.007$):

Mean cfu/plate = $15.98\ \mathrm{Exp}^{0.034 \times \text{time taken in min}}$

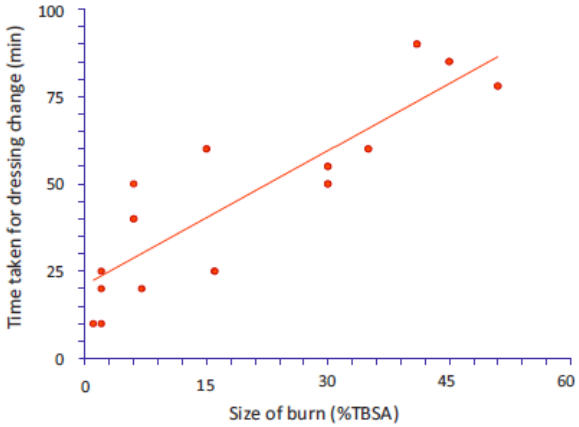

Fig. 2 – Chart demonstrating linear relationship between %TBSA of the burn, and time taken in min to complete the dressing/bed change.

Table 1 – Summary of all 24 studies of HCW carrying out dressing/bed changes on 15 patients. Details were taken of: size of burn as % TBSA; site of burn (UL, upper limb; LL, lower limb; AT, anterior trunk; PT, posterior trunk; and HN, head and neck); depth of burn (SPT, superficial partial thickness; DPT, deep partial thickness; and FT, full thickness); age of burn in days; the %TBSA that has been harvested as a split thickness skin graft; the %TBSA that has been covered by autograft or dermal substitute; recent wound swabs; whether a dressing change and bed change took place; time taken for the dressing/bed change; and the mean cfu per plate for all 20 'no apron' sites, and the 15 'with apron' sites.

Study no.	Patient	Pt age (years)	%TBSA burn	Site of burn	Depth of burn	Age of burn (days)	%TBSA donor site harvested	%TBSA covered in skin or substitute	Wound swab results	Dressing change	Bed sheet change	Time taken (min)	Mean cfu/plate 20 sites	Mean cfu/plate 15 sites
1	A	19	1	UL	DPT	6	0	0	Not taken	Yes	No	10	23	18
2	B	24	2	AT	SPT	6	0	0	Not taken	Yes	No	25	12	9
3	C	26	2	AT	SPT	6	0	0	Not taken	Yes	No	10	14	5
4	D	44	2	UL	SPT	10	0	0	Not taken	Yes	No	20	13	4
5	E	34	6	AT	DPT/FT	8	6	6	*Staphylococcus aureus*, *Bacillus* sp.	Yes	No	40	40	27
6	E	34	6	AT	DPT/FT	8	6	6	*S. aureus*, *Bacillus* sp.	Yes	No	40	13	5
7	F	33	6	LL	DPT	9	6	6	coliforms, *S. aureus*, Gp G *Streptococcus*, *Bacillus* sp.	Yes	No	50	1	1
8	G	22	7	UL	SPT	8	0	0	coliforms, *S. aureus*, Gp A *Streptococcus*, *Bacillus* sp.	Yes	No	20	50	22
9	H	45	15	UL, AT, HN	FT	6	9	15	*S. aureus*,, *Bacillus* sp., *Clostridium perfringens*	Yes	Yes	55	54	41
10	H	45	15	UL, AT, HN	FT	6	9	15	*S. aureus*,, *Bacillus* sp., *C. perfringens*	Yes	Yes	55	50	21
11	I	85	16	AT	DPT/FT	120	0	0	*S. aureus*, *Bacillus* sp.	Yes	Yes	25	101	90
12	I	85	16	AT	DPT/FT	120	0	0	*S. aureus*,, *Bacillus* sp.	Yes	Yes	25	20	20
13	J	39	30	UL, LL, AT, PT	DPT/FT	7	9	15	*S. aureus*, *Streptococcus pneumonia*	Yes	Yes	50	108	118
14	J	39	30	UL, LL, AT, PT	DPT/FT	7	9	15	*S. aureus*, *S. pneumoniae*	Yes	Yes	50	97	52
15	K	46	30	UL, LL, PT	DPT/FT	6	0	0	*S. aureus*, *Streptococcus* sp., *Bacillus* sp.	Yes	Yes	55	28	7
16	K	46	30	UL, LL, PT	DPT/FT	6	0	0	*S. aureus*, *Streptococcus* sp., *Bacillus* sp.	Yes	Yes	55	25	26
17	L	55	35	UL, LL, AT,	DPT/FT	4	0	0	Methicillin resistant *S. aureus* (MRSA)	Yes	Yes	60	177	126
18	L	55	35	UL, LL, AT,	DPT/FT	4	0	0	MRSA	Yes	Yes	60	66	71
19	M	29	41	UL, PT, HN	FT	8	18	18	coliforms, *S. aureus*, *S. pneumonii*, *bacillus* sp.	Yes	Yes	90	142	96
20	M	29	41	UL, PT, HN	FT	8	18	18	coliforms, *S. aureus*, *S. pneumonii*, *bacillus* sp.	Yes	Yes	90	294	233
21	N	45	43	UL, LL, AT, HN	FT	2	1	18	No growth	Yes	Yes	85	287	259
22	N	45	43	UL, LL, AT, HN	FT	2	1	18	No growth	Yes	Yes	85	420	341
23	O	40	51	UL, AT, PT, HN	FT	6	4	32	*Enterococcus doacae*	Yes	Yes	78	662	569
24	O	40	51	UL, AT, PT, HN	FT	6	4	32	*E. cloacae*	Yes	Yes	78	333	569

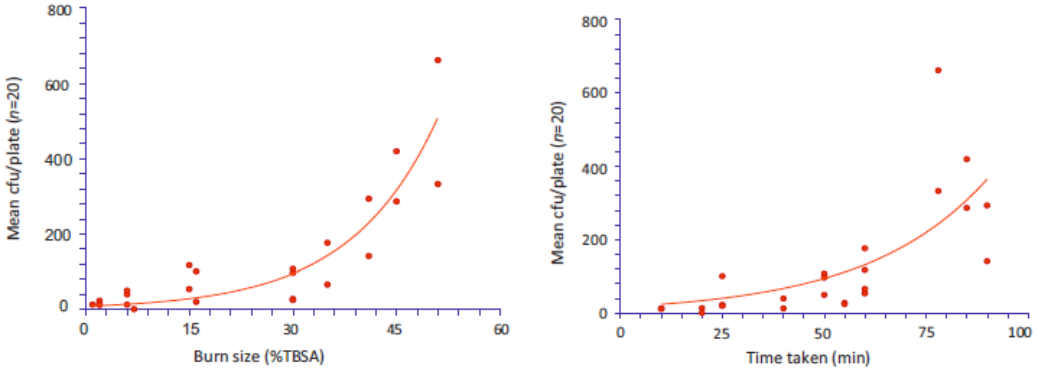

Fig. 3 – Charts demonstrating exponential relationships between %TBSA and mean cfu per plate (left) and time taken in minutes for dressing change and mean cfu per plate (right) when all 20 'no apron' sampling sites on a HCW gown are analysed.

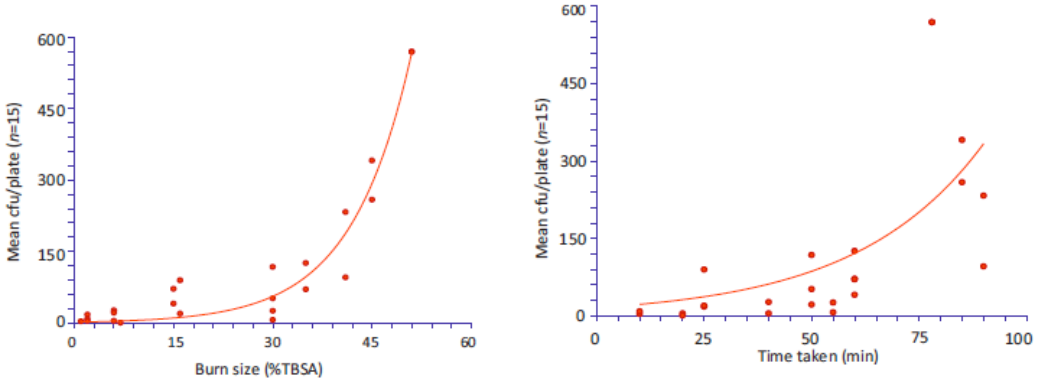

Fig. 4 – Chart demonstrating exponential relationships between %TBSA and mean cfu per plate (left) and time taken in minutes for dressing change and mean cfu per plate (right) when 15 'with apron' sampling sites on a HCW gown are analysed.

These curves are illustrated in Fig. 4. Again, both charts demonstrate an exponential relationship between the variable (%TBSA or time taken for the dressing/bed change to take place) and the contamination received by the HCW. However, although they are both significant relationships, time taken correlates less strongly than %TBSA as shown by the lower R^2. %TBSA is a more accurate predictor of HCW contamination than time taken for the dressing/bed change to take place.

3.5. *Predicted contamination of HCW*

Using the above statistical models, the expected mean number of bacterial cfu per 25 cm^2 plate from a HCW performing a burns dressing/bed change can be predicted. This was produced from data sets for all 20 'no apron' sites and the 15 'with apron' sites. These values are summarised in Table 2. It was found that for every 9%TBSA increase in burn size, the mean number of cfu/plate doubled when all 20 sites were analysed. This was true for every 6%TBSA increase in burn size when 15 'with apron' sites were analysed.

Table 2 – Predicted mean contamination received by HCW performing a burn dressing/bed change. All 20 'no apron' sites, and the 15 'with apron' sites that would be left exposed if the HCW donned a plastic apron are analysed separately for comparison. Results are expressed as mean bacterial cfu per 25 cm^2 agar plate.

%TBSA	Predicted mean cfu per 25 cm^2 plate 20 'no apron' sites	Predicted mean cfu per 25 cm^2 plate 15 ' with apron' sites
5	13	4
10	19	6
15	29	11
20	43	18
25	64	32
30	95	56
35	141	97
40	211	168
45	314	292
50	469	507

4. Discussion

The consequences of nosocomial infections from a burns patient cross-contaminating other patients are potentially devastating [1,24]. Prevention of cross-contamination is thus becoming an increasingly important area of burn care research. The potential for HCW to act as vectors of transmission between patients, and the increased bacterial dispersal during dressing and bed sheet changes on burns patients has long been known [6–9,11–18]. The current study highlights high levels of HCW contamination following a dressing/bed change and quantifies levels of bacterial contamination for the first time.

During a dressing/bed change the HCW can be expected to come into contact with the patient, their dressings and the surrounding environment, all of which are likely to be heavily contaminated on the burns unit. A HCW who has become contaminated by carrying out a dressing change will proceed to make contact with other patients or environmental surfaces, dispersing organisms, where they can survive for several weeks and form an environmental reservoir [25–27]. The environment may then contaminate another patient directly or indirectly via the hands or uniform of a HCW acting as a carrier for nosocomial infection [28,4,29].

Guidelines on the use of protective clothing for HCW during burns dressing/bed changes are not burns-specific. Based on the results of this study, they may require to be revised with consideration of the amount of contamination received by HCW during performance of these routine nursing activities. The use of gloves and meticulous hand hygiene for all dressing changes is accepted practise and was not examined here [15,30]. Of note, WHO recommend a '5 moments for hand hygiene' approach whereby hands should be cleaned before and after all procedures and contact with patient surroundings [31]. It may be argued that the HCW in this study should have been encouraged to wash their hands several times during the activity, rather than just at the beginning and end. However as they were in constant contact with the environment, patient, and open wounds throughout the duration of the activity, dividing the dressing/bed change into distinct 'moments for hand hygiene' was difficult. One compromise that may be employed in the future is to encourage a pause for hand hygiene and change of gloves only, between removing dressings and applying fresh dressings. The compliance with these recommendations is however unlikely to affect the levels of bacteria found on the gowns, as they concern only hand hygiene.

Disposable full-body gowns were only worn for this study to enable sampling from a surface that was known to be sterile prior to the nursing activities. Standard practice on our unit is for plastic aprons to be worn for most dressing and bed changes, excluding those taking place in ICU or on known heavily contaminated patients. The results of this study have led to a review of our clinical practice, and revised guidelines on protective attire worn by HCW.

The mathematical models produced indicate that a HCW performing a dressing change on a patient with a 15%TBSA burn could be expected to become contaminated with a mean of 29 bacterial cfu/25 cm^2 if they wore no protective clothing and 11 bacterial cfu/25 cm^2 if a plastic apron was worn, supposing absolute protection is afforded by the apron. For large burns, prediction of levels of contamination when a HCW wears or does not wear an apron highlights the limitation of relying only on the apron as a means of prevention of HCW contamination. For example, 50% TBSA burn is estimated to produce 469 cfu/plate when wearing 'no apron', compared to 507 cfu/plate 'with apron'. The majority of samples were collected from the forearms, arms, shoulders and chest: areas that of skin and uniform which would not be protected or cleaned during hand washing and may come into contact with other patients or equipment. Before the study was initiated, HCW were encouraged to act exactly as they would were they wearing an apron. Whilst this was the agreed intention, it is nevertheless possible that they may have been less careful than usual knowing they were covered by a gown, or more careful as they were conscious they were part of a study. Regardless of this possible effect, the results highlight the need for a review of protective guidelines for HCW.

Burns between 2 and 10 days old were examined, although numerous factors such as the site of the burn, whether debridement had taken place, donor site size, comorbidities and bacteria isolated from the wound were unable to be controlled. Despite the inclusion criteria being fairly broad, %TBSA was still shown to be an important predictor of HCW contamination. Future studies would be useful to monitor the change in HCW contamination as a burn progresses towards healing, or as the patient becomes colonised with increasingly resistant organisms. Furthermore, BPA was used throughout to monitor staphylococcal-type bacteria, but other selective media may be used in the future to identify other organisms that colonise burns wounds, such as Gram-negatives, which may show different transfer characteristics between patients and HCW. Were the studies to be repeated on a larger sample size, quantitative analysis of wound contamination may be attempted, although this would only be an estimate. However this would not be helpful in predicting contamination and thus guiding HCW on which protective attire to wear; results not being known until after the dressing/bed change had taken place.

Despite the relatively small sample size an excellent correlation of 82% was demonstrated, enabling the production of mathematical models. The largest burn studied was 51% TBSA so extrapolation to predict contamination from larger burns was not attempted. Although further studies may help to show the contamination produced by much bigger burns, at the upper limits of %TBSA tested, many agar plates were very heavily contaminated, and much more contamination would probably render the number of bacterial cfu uncountable. Suffice to say contamination to at least the same extent would be expected for burns over 51% TBSA. It is important to note that all results are reported as cfu per 25 cm^2 plate, and the total contamination across a whole gown would be many times this figure. What is not known is what constitutes a 'significant number' of bacteria. Further work would need to be carried out to determine the

transfer rate from the HCW to another surface or patient. In the absence of this, an arbitrary figure may be assigned as a pre-determined cut off point above which full-body protection should be worn. The cost of full body protection must also be considered and weighed up against the perceived risk of transfer from a HCW.

It is logical to assume that in general a larger burn will take longer to dress, and indeed this was shown by a linear relationship between %TBSA and total time taken (Fig. 2). Although time taken was related to the level of HCW contamination, it explained less of the variation than burn size, with a lower coefficient of determination, R^2. Furthermore, as the time taken for the dressing change will not be known until after the event, and may depend on HCW experience, %TBSA was preferentially considered to predict HCW contamination. A rough guide is that for every 6–9%TBSA increase in burn size, bacterial contamination doubles.

This study increases knowledge of the transfer of bacteria from burns patients to HCW. It highlights the need for guidelines on protective clothing worn by HCW to be developed, as burns patients have been shown to disperse high levels of bacteria onto HCW. For the first time, a quantitative analysis of bacterial contamination received by HCW performing burns dressing and bed changes have been performed. The risks of HCW contamination must be balanced against the cost of protective measures and resources available to burns units worldwide.

Conflict of interest

All authors declare no financial or personal associations that could inappropriately influence this work.

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Ethical approval

Ethical approval was granted by the local REC for the study of levels of environmental bacterial contamination around burns patients, as part of a larger body of work. All samples were taken from disused gowns and no direct patient involvement was required to carry out this research. Verbal consent was obtained from the patient and HCW prior to the study.

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Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings☆

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ABSTRACT

Infections are the leading cause of morbidity and mortality in burn patients and prevention of contamination from exogenous sources including the hospital environment is becoming increasingly emphasised. The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is bactericidal yet safe for humans, allowing continuous disinfection of the environment surrounding burn patients. Environmental samples were collected from inpatient isolation rooms and the outpatient clinic in the burn unit, and comparisons were then made between the bacterial contamination levels observed with and without use of the HINS-light EDS. Over 1000 samples were taken. Inpatient studies, with sampling carried out at 0800 h, demonstrated a significant reduction in the average number of bacterial colonies following HINS-light EDS use of between 27% and 75%, ($p < 0.05$). There was more variation when samples were taken at times of increased activity in the room. Outpatient studies during clinics demonstrated a 61% efficacy in the reduction of bacterial contamination on surfaces throughout the room during the course of a clinic ($p = 0.02$). The results demonstrate that use of the HINS-light EDS allows efficacious bacterial reductions over and above that achieved by standard cleaning and infection control measures in both inpatient and outpatient settings in the burn unit.

1. Introduction

The sequelae of burn wound infections can be devastating to the burn patient, causing progression of burn depth, graft loss, increased scarring, and subsequent sepsis, leading to multi-organ failure, and death or a significantly prolonged hospital stay. Due to advances in resuscitation and early excision regimes, it is now estimated that 75% of deaths in patients

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with burns over 40% of the total body surface area (TBSA) are related to sepsis from burn wound infection or other infectious complications and/or inhalation injury [1,2]. Destruction of the skin barrier, a state of immunosuppression, and large wound areas of nutrient rich, bacteria harbouring eschar render burn patients unique in their tendency to disperse bacteria into the surrounding environment and their susceptibility to developing infections [3]. The spread of healthcare-associated infections (HAI) is an increasing worry as new strains of multi-drug resistant bacteria emerge, with a diminishing number of effective antimicrobials, leading to severe sepsis and outbreaks in burn units. Efforts to improve hand hygiene and limit the use of broad-spectrum antibiotics are important in reducing nosocomial infection rates on the burn unit, but the impact of environmental cleanliness is also becoming increasingly acknowledged [1]. The environment surrounding burn patients has been shown to be a reservoir for pathogens, and a potential source of cross-contamination between patients [4,5]. Bacteria surviving on inanimate surfaces for weeks or months can contaminate patients or healthcare workers, who become colonised, spreading HAI amongst patients [4–8].

Novel methods of cleaning and decontamination within hospitals have been developed, including hydrogen peroxide vapour (HPV), ultraviolet light (UV-light), and super-oxidised water [9–11]. These enable efficient temporary disinfection of the environment, but the effect is only transient and within a matter of hours the number of microorganisms begins to return to pre-decontamination levels [12]. Furthermore, they are time-consuming, requiring the removal of patients from the room, which limits their usefulness in a busy burn unit, and particularly in a burns outpatient clinic. The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is a ceiling-mounted lighting unit, which allows continuous decontamination of the clinical environment, killing bacteria through photodynamic inactivation while being safe to humans [13]. The decontamination technology uses a narrow bandwidth of visible blue-violet light, with a peak output at 405 nm. This has previously been demonstrated *in vitro* to kill a wide spectrum of pathogenic bacteria, including meticillin-resistant *Staphylococcus aureus* (MRSA), meticillin-sensitive *S. aureus* (MSSA), *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Acinetobacter sp.* in a dose-dependent and species-dependent fashion [14,15].

The present study focused on assessing the use of the HINS-light EDS in two different burn unit environments: an isolation room housing a burn inpatient, and the burn outpatient clinic, through which several patients pass each day, so total decontamination of the room between patients is almost impossible to achieve. The propensity of burn patients to disperse pathogens into the environment means that environmental bacterial contamination is higher on the burn unit than most other hospital wards, which increases the risk of healthcare workers contaminating their hands and uniforms, and transmitting HAI to other patients in their care. This study assessed whether use of the HINS-light EDS had a significant effect on reducing the levels of environmental bacterial contamination in both the inpatient and outpatient settings, therefore potentially aiding in reducing the risk of cross-contamination of infectious pathogens from the environment to patients.

2. Materials and methods

2.1. Setting

Glasgow Royal Infirmary (GRI) has a dedicated 13-bed burn unit, arranged as six single isolation rooms, one three-bed high dependency bay, one four-bed open bay and an outpatient clinic area. Intubated patients are treated in a separate general intensive care unit. Throughout all studies, GRI burn unit infection control and isolation policies were adhered to [16]. These state that disposable gloves and aprons are donned by staff on entering isolation rooms and hands are decontaminated before and after entering the room with alcohol gel or soap and water. Appropriate ethical approval was obtained.

All air-conditioning units in the ward contain High Efficiency Particulate Air (HEPA) filters and isolation rooms are maintained at a negative pressure. Domestic staff clean inpatient isolation rooms daily, usually around 1100 h, using chlorine-based detergents. Table tops and locker tops are wiped down periodically by nursing staff using hard surface disinfectant wipes. Following vacation of the room, a "terminal clean" is carried out. The outpatient clinic room is cleaned before the start of a clinic, around 0800 h by domestic staff, using chlorine-based detergents. The clinic nurse cleans the worktop, examination couch and any equipment used, using hard surface disinfection wipes between each patient.

2.2. HINS-light EDS

HINS-light EDS prototype units were installed in the burn unit. Two units were installed in the ceiling of two test inpatient isolation rooms and one unit in the ceiling of the smaller outpatient clinic room. Light was generated from a matrix of light-emitting diodes (LEDs), emitting a narrow bandwidth of blue-violet light centred on 405 nm wavelength. White LEDs are also incorporated into the HINS-light EDS such that the illumination effect is predominantly white. The HINS-light EDS units were connected to mains electricity and simply switched on and off at the wall. Minimal staff training was required and there was no disruption of the normal hospital routine. The HINS-light EDS is designed to treat an area of approximately 10 m^2, with sufficient intensity to cause inactivation of exposed bacteria. Rigorous safety analysis has been carried out to standards set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and the American Conference of Governmental Industrial Hygienists (ACGIH). It has shown that the intensity levels used in the hospital are well below the threshold limit for any adverse effects occurring, as established by ACGIH [17–19].

2.3. Bacteriological methods

Methods were based on previous work evaluating the efficacy of the HINS-light EDS in clinical environments [13]. Environmental bacterial samples were collected from surfaces in each

room using 25 cm^2 Baird Parker with egg yolk telurite contact agar plates (BPA plates; Cherwell Laboratories Ltd, Bicester, UK), by the same researcher (SEB). Contact plate sampling, which enables microorganisms to be directly collected on an agar surface, was selected as the most appropriate method of assessing bacterial counts on environmental surfaces. Sample collection using broad spectrum contact agar plates, such as tryptone soya or blood agar yielded plates with too many bacterial colony forming units (cfu) to accurately enumerate in preliminary studies. Therefore, Baird Parker agar, a selective agar for staphylococcal bacteria, and an accepted marker of hospital environmental contamination in studies of hospital cleanliness, was used in the present study [20]. Staphylococci are known to survive on environmental surfaces for significant periods of time and can be transmitted between patients, staff and the environment [20,23–25]. Studies have shown the association between levels of environmental contamination with *S. aureus* and the size of the burn wound [21]. Furthermore, analysis of GRI burn wound swabs from the previous two years showed that MSSA and MRSA accounted for approximately 50% of all positive routine admission and twice-weekly surveillance wound cultures. It was therefore felt that an agar that selected for the commonest pathogens was justified, using the most accurate environmental sampling technique available.

Between forty and fifty sites on frequently-touched surfaces were identified around each room being studied, and bacterial samples were collected by directly pressing the contact agar plates onto the sampling site, with samples being taken from the same sites each time. After collection, contact plates were incubated at 37 °C (98.6 °F) for 48 h and the number of bacterial cfu on each contact agar plate was enumerated. Raw counts were statistically analysed by a chartered statistician.

2.4. *Inpatient studies*

The first part of the study was carried out in an inpatient isolation room containing a 49-year-old male, Patient A, with 45% TBSA full thickness (third degree) flame burns, one month after admission. Routine wound surveillance swabs had isolated MRSA and *P. aeruginosa*, and mixed coliforms immediately before and during the study. Forty sampling sites ($n = 40$) were identified around the room (Table 1). For each study, contact plate samples were collected during three phases: before the HINS-light EDS was in use (*pre-HINS*); after the HINS-light EDS had been on for two days (*during-HINS*); and after the HINS-light EDS had been switched off for a further two days (*post-HINS*).

Pre-HINS sampling was first carried out at 0800 h. Immediately after this, the HINS-light EDS was switched on and remained on for 14 h during daylight hours, for two consecutive days. *During-HINS* samples were collected at 0800 h from the same 40 sites following this two-day use of HINS-light EDS. The HINS-light EDS was then switched off for two consecutive days, after which time *post-HINS* samples were collected at 0800 h, again from the same 40 sampling sites. This study was repeated over three consecutive weeks using identical methods with the same patient in the same room but with sample collection at 1500 h, and then 2200 h in order to assess the efficacy of the HINS-light EDS when samples were collected at differing times of day.

To address reproducibility, the 0800 h sampling protocol was repeated in rooms occupied with two further patients. Patient B was a 35-year-old female with 25% TBSA mixed deep dermal and full thickness (second and third degree) flame burn, housed in the same isolation room that Patient A had previously occupied. Her routine wound surveillance swabs had isolated MRSA and mixed coliforms. Patient C was a 55-year-old female with 40% TBSA full thickness (third degree) burn in a different room of the unit, with a mirror-image layout. Her routine wound surveillance swabs had isolated MRSA and *P. aeruginosa*. Ten extra sampling sites were included in the studies on Patients B and C, along both bed rails, as these two patients were bed-bound, and the bed rails were constantly upright, and an important potential site of contamination (Table 1) ($n = 50$).

Table 1 – Environmental sampling sites used in inpatient and outpatient rooms on the burn unit, with the number of samples taken from each site stated.

Inpatient isolation rooms		Outpatient clinic room	
Sampling site	No. samples	Sampling site	No. samples
Bed sheet	4	Waste bin	4
Locker top	2	Apron dispenser	4
Ledge	6	Glove dispenser	2
Table	4	Sink area	6
Foot of bed rail	3	Dressings trolley	4
Drip stand	2	Dressings shelves	8
Patient chair	2	Worktop	6
Light switches	2	Lamp	2
Door handles	3	Examination couch	6
Air con supply	2	Patient chair	4
Waste bins	4	Power supply	2
Sink area	4	Light switch	1
Bed cot sides	10 (Studies B and C)	Door handle	1
Total	40 (50)	Total	50

2.5. Outpatient studies

Fifty sampling sites were identified on frequently touched surfaces around the outpatient clinic room ($n = 50$) (Table 1). *Before clinic* samples were collected at 0830 h, shortly after the room had been cleaned. Clinics ran between 0900 h and 1600 h, and between seven and 12 burns patients were seen per clinic. *After clinic* samples were collected at 1630 h from the surfaces, immediately adjacent to where the 50 sites had been sampled before clinic. Samples were collected 30 min before and 30 min after two clinics when the HINS-light EDS was switched off (*HINS off*) and two clinics when the HINS-light EDS was switched on continually for 8 h during the clinic (*HINS on*).

2.6. Statistical analysis

The *pre-HINS* and *post-HINS* sampling periods in the inpatient room studies acted as controls for each *during-HINS* sampling period. A rise in the average number of bacterial cfu in the *post-HINS* samples indicated that reductions seen in *during-HINS* samples were not due to a general decrease in bacterial shedding by the patient over the two days, but the effect of the HINS-light EDS. For the outpatient clinic investigation, the study was repeated during two clinics in the absence of the HINS-light EDS. This acted as a control to show the expected increase in contamination levels usually seen throughout the course of a typical burns outpatient clinic. Statistical software (Minitab version 15) was used and a log-transformation was found to normalise data and equalise variances when analysing cfu data. For the inpatient studies, analysis of variance (ANOVA) and Tukey pair-wise comparisons were undertaken. The cfu counts per plate were compared between the three periods, *pre-HINS*, *HINS* and *post-HINS*. A 95% confidence interval (CI) was calculated for the differences obtained between the means of the three sampling periods. For the outpatient studies, the differences in cfu count *before clinic* and *after clinic* was compared with and without the use of the HINS-light EDS. Results were displayed using mean values and statistical testing was carried out at the 5% significance level ($p \leq 0.05$).

3. Results

3.1. Inpatient studies

Results from the five inpatient studies are summarised in Table 2. Samples collected in Patient A's room at 0800 h demonstrated a statistically significant reduction of 43% in the average number of Baird Parker agar isolated bacterial cfu following two days of HINS-light EDS use ($p = 0.043$). After the light had been switched off for two days, bacterial numbers recovered to pre-decontamination levels, a 48% rise, ($p = 0.040$). Sample collection at 1500 h demonstrated a 45% reduction in bacterial contamination following two days of HINS-light EDS use, which was not statistically significant ($p = 0.252$). The study with samples collected at 2200 h, produced a 39% reduction in the number of cfu

Table 2 – Results and statistical analysis of the data on the effects of the HINS-light EDS on Baird Parker isolated bacterial contamination levels in a burns unit isolation room (i) occupied with three different patients (A–C) and (ii) when environmental samples are taken at three different times of day from the room occupied by Patient A.

Time of sample collection	Patient	Sample number (n)	Mean plate counts [cfu/plate] (standard error, SE)			Mean cfu reduction by EDS use (95% CI)	% reduction by EDS use	Sig. reduction by EDS use ($p < 0.05$)	Mean cfu increase after EDS switched off (95% CI)	% increase after EDS switched off	Sig. increase after EDS switched off ($p < 0.05$)
			Pre-HINS	During-HINS	Post-HINS						
0800 h	A	40	206.7 (29.5)	117.8 (29.5)	173.8 (29.5)	88.9 (5.7, 183.5)	43	Yes ($p = 0.043$)	56.0 (−38.6, 150.6)	48	Yes ($p = 0.040$)
1500 h	A	40	165.4 (28.2)	90.6 (28.2)	107.8 (28.2)	74.8 (15.6, 165.2)	45	No ($p = 0.252$)	17.2 (−73.1, 107.6)	19	No ($p = 0.149$)
2200 h	A	40	132.1 (25.4)	80.9 (25.4)	129.6 (25.4)	51.2 (−30.1, 132.5)	39	No ($p = 0.054$)	48.7 (−32.6, 130.0)	60	Yes ($p = 0.005$)
0800 h	B	50[a]	22.5 (3.4)	5.6 (3.4)	10.1 (3.4)	16.9 (6.4, 27.4)	75	Yes ($p < 0.0001$)	4.5 (−6.1, 15.0)	80	Yes ($p < 0.0001$)
0800 h	C	48[b]	25.3 (8.1)	18.5 (8.1)	17.2 (8.1)	6.8 (−18.6, 32.1)	27	Yes ($p = 0.022$)	−1.3 (−24.0, 26.6)	−7	No ($p = 0.692$)

[a] Ten extra sampling sites on cot sides included in study with Patient B ($n = 50$).
[b] Ten extra sampling sites on cot sides were included in study with Patient C, but two sites excluded on statistical grounds ($n = 48$), see Section 3.

following two days of HINS-light EDS use, again not statistically significant ($p = 0.054$). After the light had been switched off again for two days there was a statistically significant 60% rise in bacterial contamination ($p = 0.005$).

The results from 0800 h sampling carried out in the room occupied by Patient B confirmed these findings. A significant 75% reduction in the average number of cfu was achieved following two days of HINS-light EDS use ($p < 0.0001$). When the light was switched off again, the average number of cfu rose by 80% ($p < 0.0001$). In the study involving Patient C, the average number of bacterial cfu increased slightly from 25.2 to 25.5 cfu following the use of the HINS-light EDS. However, the statistical analysis indicated an exceptionally unusual observation associated with the two samples from the sink site in the *during-HINS* sampling period. From the least squares fitted model, the standardised residual was estimated to be 8.1 and the pattern associated with the sink site was inconsistent with all other sites. A further analysis was undertaken excluding samples from the sink site ($n = 48$) and this demonstrated a significant 27% reduction from 25.3 to 18.5 cfu ($p = 0.022$). There was a small (7%) decrease in the average number of cfu when the light was switched off again for two days, but this was not statistically significant ($p = 0.692$).

3.2. Outpatient studies

Results of the outpatient studies are summarised in Table 3. For studies both with and without HINS-light EDS intervention, 50 samples were collected at the start and end of two clinics. The combined results were analysed using a block design to take account of the findings from the two clinics. The difference between clinics with and without HINS-light EDS was then compared. The mean number of Baird Parker agar isolated bacterial cfu per plate before *HINS off* clinics was 8.1 cfu, and rose to 22.2 cfu during the course of the clinics. This increase in contamination levels was expected, due to the dispersal of bacteria into the air and onto environmental surfaces during dressing changes and wound care of between seven and 12 patients a day. During *HINS on* clinics, the mean number of bacterial colonies at the start of the clinic was 6.5 cfu, and only rose to 12.0 cfu by the end of the clinic. This indicated that the amount of additional contamination of the room, released throughout the course of a burn outpatient clinic, was reduced by an average of 8.6 cfu per plate by the HINS-light EDS. This was the equivalent of a significant 61% efficacy ($p = 0.02$).

4. Discussion

The consequences of HAI for burn patients and the burn unit as a whole are serious and multiple. Prevention, identification and eradication of nosocomial infections is thus becoming an increasingly important area of burn care research [1]. The vital importance of infection control and isolating burn patients has been recognised for many decades [22]. More recently, the role of the burn unit environment in harbouring pathogens including MRSA that can survive on dry surfaces for weeks or months has been acknowledged [23–25]. A 42% transmission rate of MRSA to the hands of healthcare workers who had no direct patient contact, as a result of touching contaminated surfaces has previously been demonstrated [26,27]. The reduction of the environmental reservoir of nosocomial infection is imperative and the current study adds further evidence of the role that the HINS-light EDS may have in achieving this.

Previously published data on the use of the HINS-light EDS as a method of decontamination for hospital inpatient environments, demonstrated reductions in the total number of environmental staphylococcal-type bacteria of between 56% and 86%, when samples were collected at 0800 h [13]. The current study logically develops that work by investigating the reduction achieved at three different times of day, in rooms housing different burn patients, and examining specifically its use in both the inpatient and outpatient setting in one of the most important areas for infection control in the hospital: the burn unit. In the burn inpatient isolation room, the HINS-light EDS has proved to have a significant benefit in reducing environmental contamination levels by between 27% and 75% on samples taken at 0800 h, over and above the hospital's current stringent infection control and hygiene measures. This effect was achieved with an exposure of 14 h a day for two consecutive days, with the light being switched off overnight, in order that it did not affect the patient's sleep.

Differences in the levels of bacterial contamination during daylight hours – likely due to direct contamination by patients or staff, or cleaning by domestic staff – is reflected on sampling at 1500 h and 2200 h when there was much more variability of activity within the room. There is no logical reason to suspect that the HINS-light EDS would be any less effective at these times of day than at 0800 h: indeed it might be expected that 0800 h sampling would produce the least dramatic reduction in contamination levels as the HINS-light EDS had been switched off overnight immediately before samples were

Table 3 – Results and statistical analysis of data on the effect of use of the HINS-light EDS on Baird Parker isolated bacterial contamination levels during burns outpatient clinics.

HINS-light EDS on/off during clinics	Sample number (*n*)	Mean plate counts (cfu/plate)		Mean increase in cfu/plate during clinic	Reduction in increase of cfu with EDS on (95% CI)	Efficacy of reduction in increase of cfu with EDS on (95% CI)	Sig. reduction in increase of cfu with EDS on
		Before clinic	After clinic				
HINS off clinics	100	8.1	22.2	14.1	8.6 (1.4, 15.8)	61.3% (10%, 113%)	Yes ($p = 0.02$)
HINS on clinics	100	6.5	12.0	5.5			

taken. The main advantage to sampling at 0800 h is that the activity levels in the room had been relatively constant overnight before the samples were taken, as the patient was asleep in bed and staff had minimal input, preventing large surges or reductions in numbers of bacteria. This allowed a steady level of bacteria and a reliable estimate of contamination levels to be achieved when samples were taken. Although a similar pattern of reduction was demonstrated at the other times of day, there seemed to be considerable variability in staff and patient activity. This was thought to affect contamination levels and produce results that were not significant. For future studies involving environmental contamination, 0800 h sampling is recommended as a model to achieve the most reproducible conditions possible so that the effect of an intervention can be seen.

An incidental observation was the variability in bacterial deposition demonstrated between the three inpatients. Patient A produced higher environmental contamination, with *pre-HINS* levels of 206.7 cfu per plate. Patients B and C had starting populations of 22.5 and 25.3 cfu per plate respectively. There are several possible explanations for this: Patient A was ambulant around the room during the studies, although he was confined to his room. Furthermore he had loose motions on several occasions during the study, and although no infective cause for this was found, and it was assumed to be secondary to antibiotic treatment, it meant he had to go to the en-suite bathroom several times during the day and night. He had the highest % TBSA burns, although comparable with Patient C, and all three patients had MRSA isolated from their wounds. He was also noted to have very dry flaky skin and hair, and was consequently likely to be a relatively heavy shedder of squames when compared to other patients. The exceptional counts observed for one patient at the sink location was thought to arise from gross direct contamination immediately prior to sampling. The contamination must have taken place within the room as agar plates were sealed before being removed from the room for incubation. The level of contamination may have arisen from a number of activities but none could be identified with any confidence.

The outpatient clinic was used as an example of a communal patient room in the burn unit, where it was recognised that organisms may be passed from one patient, onto a surface and thence directly to the next patient in the room. As expected, the starting numbers of bacteria were lower than in isolation rooms housing a patient constantly over long periods of time, however a significant rise in the numbers of bacteria on surfaces at the end of the clinic was seen, despite these being patients with relatively small or partly healed burns. Even though the HINS-light EDS was only on for a total of 8 h, and the room was relatively much cleaner than the inpatient rooms to begin with, significant reductions in the increase of environmental bio burden released during a clinic were still demonstrated, with a 61% efficacy. This may lead to the use of the HINS-light EDS in other communal patient rooms, such as the physiotherapy room or bathroom, where decontamination of all surfaces is unachievable between each patient due to time limitations.

Previous studies into the bactericidal nature of 405 nm HINS-light have demonstrated the effect on a wide range of Gram-positive and Gram-negative organisms [15], and although levels of staphylococcal organisms were used as the marker for the current study it is important to bear in mind that levels of Gram-negative organisms will also have been reduced through use of the HINS-light EDS. The HINS-light EDS has a unique advantage in its ability to be used continuously throughout daylight hours in inpatient isolation rooms, and constantly through the day and night in other areas of the burn unit. It is efficient, simple to run, unobtrusive, and is neither dependent on staff compliance nor requires any additional staff time to implement. It must be stressed that the HINS-light EDS is not designed to replace standard cleaning routines, and the importance of wiping down surfaces, washing hands and using gloves and gowns remains. Rather, it augments current infection control methods. The HINS-light EDS is thought to have its main effect against the ubiquitous bacterial reservoirs dispersed into the air during periods of activity in the room, such as bed changes or burn dressing changes, settling on hard surfaces around the source. When surfaces are touched directly by a patient or healthcare worker, the density of organisms is more likely to be greater, so a longer exposure to the HINS-light EDS is required to decontaminate. It is probable that routine physical cleaning would take place before this, so the HINS-light EDS is not a replacement for excellent physical cleanliness in burn units, but has still been shown to maintain consistently lower levels of environmental bacteria than that achieved by physical cleaning alone.

The study of the inpatient rooms was limited in that it only examined the effect of the HINS-light EDS for a relatively short period of between 8 h and 14 h a day on two consecutive days. It is not yet known if leaving the system on for longer periods of time (for example overnight in the outpatient clinic, or at lower levels during the night in the inpatient rooms, or for more consecutive days) would continue to reduce overall levels of bacteria, or if the contamination levels would plateau after a time: this is an area of interest for future studies. Although HINS-light has wide bactericidal activity, as demonstrated *in vitro* [14,15], this study focused on the reduction of staphylococcal type organisms, which account for over 50% of wound contaminations and infections in the GRI burn unit and give an indication of organisms which have originated from a human source, and are thus potential pathogens. While the experiment could be repeated using an agar that would allow estimation of total viable counts of all bacteria, the large number of cfu arising from some surfaces would also make accurate enumeration very difficult. Future work may address the impact of the HINS-light EDS on Gram-negative organisms, by sampling using an agar that selects for Gram-negatives alone. Further laboratory studies on the effect of the HINS-light EDS on bacteria subject to various stressing factors, or the formation of biofilms would also be of interest.

These studies provide convincing evidence that this novel technology achieves a reduction in environmental contamination levels. To demonstrate that this translates into a reduction in colonisation and infection in burn patients, in the context of the huge numbers of variables in the patients, burns and treatment administered, would be the ideal next stage, but would probably require a multi-centre trial over months or years. Such difficulties account for the paucity of evidence that many other established infection control methods and

disinfection technologies have achieved reductions in infection rates. Rather, a logical and pragmatic approach has been adopted that a cleaner environment and cleaner hands are likely to result in the transfer of fewer numbers of bacteria to patients, and thus generate fewer infections. The impact of surface disinfection in hospitals cannot be dismissed due to the lack of outcome trials, as HAI as an outcome has reasonably low frequency, so any potential trial would suffer from low statistical power [28,29].

The findings of this work provide evidence that the HINS-light EDS is an effective treatment for the reduction of environmental bacterial contaminants in different clinical situations on the burn unit. The percentage reduction observed for counts taken at different times during the day were broadly comparable for the room containing the same patient. In contrast, the percentage reduction at the same time of day for rooms housing different patients varied considerably. This is not unexpected, as contamination levels are known to differ depending on the patient, the size of burn and the patient environment [30]. A total of 34 different burn patients were treated in the outpatient clinic room, yet the presence of the HINS-light EDS in the room while they were being treated significantly reduced the environmental bacterial contamination they produced. These results suggest that for burn patients, the HINS-light EDS can potentially make an important additional contribution to the reduction of nosocomial infections which originate from transmission of pathogens from the environment, by significantly reducing the contamination of the surrounding environment.

Conflict of interest statement

The intellectual property rights of the HINS-light EDS belong to the University of Strathclyde. As co-inventors, MM, SJM and JGA have a share of the intellectual property rights. SEB, GG JEC and IT have no claim to intellectual property. The University has made all HINS-light EDS for research purposes only and no commercial company manufactures or sells this technology.

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Bache SE, Maclean M, Anderson JG, MacGregor SJ, Gettinby G, Taggart I. A model to predict transfer of bacteria form patients to staff during burns dressing changes. Presented at the International Society for Burn Injuries meeting, Edinburgh, Spetember 2012.

Introduction The prevention of cross-contamination of nosocomial infections between burns patients is imperative. Transmission of infection between patients mainly occurs through direct and indirect contact, and healthcare workers (HCW) may act as vectors of transmission. There is limited evidence that bacteria are transferred from burns patients to the uniforms of HCW caring for them. Studies were designed to examine the contamination of HCW resulting from dressing changes on patients with different % total body surface area (TBSA) of burn.

Methods HCW were asked to wear sterile, disposable, impermeable theatre gowns to carry out a dressing change. Samples were taken from 20 set sites across the chest, abdomen and arms of the gowns using 25cm^2 Baird Parker contact agar plates. These were incubated and enumerated. The size of the burn and time taken to complete the dressing change was noted. Samples were taken from the gowns of 25 HCW carrying out dressing changes on 16 different patients with burns between 1% and 51% TBSA. Statistical analysis determined the mean number of bacterial colony forming units per plate (cfu/plate) on the gown of each HCW carrying out a dressing change. From these results a mathematical model was used to predict the mean levels of contamination a HCW would receive during dressing changes on different %TBSA burns.

ResultsThe contamination received by a HCW carrying out a dressing change correlated strongly with the % TBSA of the patient, and was explained by an exponential model. This model allowed the contamination a HCW may be expected to receive when carrying out a dressing change to be predicted by the % TBSA of the burn. For example, a 10%TBSA burn would be expected to contaminate the HCW with a mean of 20 cfu/plate; a 20%TBSA burn would produce a mean of 43 cfu/plate, and a 40% burn would produce a mean of 212 cfu/plate.

Conclusions This study shows that even relatively small sized burns liberate considerable levels of bacteria onto HCW, with the potential for cross-contamination to occur. There is little consensus amongst US or UK burns units for the appropriate protective attire to be worn by HCW carrying out dressing changes on burns patients. This information may be useful when designing future guidelines for the use of protective attire during dressing changes on the burns unit.

Bache SE, Maclean M, MacGregor SJ, Anderson JG, Gettinby G, Coia J, Taggart I. Patient-safe continuous environmental disinfection in the burns unit using a visible light source (HINS-light EDS). Presented at the British Association of Plastic and Reconstructive Surgery winter scientific meeting, London, December 2011.

Introduction and aims Effective environmental decontamination of the burns unit is important as burns patients are not only highly susceptible to infection but readily disperse pathogens into the surrounding environment. The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is a ceiling-mounted light source designed to provide continuous disinfection of the burns unit environment.

Materials and methods Burns unit inpatient and outpatient settings were studied, comparing environmental contamination with and without the addition of a HINS-light EDS, continuing standard hospital cleaning throughout. Five clinical isolates including MRSA and *Acinetobacter baumannii* were exposed to the light to determine inactivation times.

Results Inpatient studies demonstrated a reduction in environmental bio-burden of between 27% and 89% ($p<0.02$), when the light was on for between two and seven days. Outpatient studies showed a 61% efficacy in reducing bacterial contamination produced during a clinic ($p=0.02$). All bacterial species tested were killed on exposure to the HINS-light EDS, with significant reductions by two hours ($p<0.02$).

Conclusions Infection is the leading cause of morbidity and mortality in burns patients. The effectiveness of the HINS-light EDS in a variety of settings offers great scope for its future use in reducing healthcare-associated infections (HAI) in burns patients.

Bache SE, Maclean M, Anderson JG, MacGregor SJ, Gettinby G, Taggart I. The role of airborne and staff contamination in the cycle of cross-contamination between burns patients. Presented at Scottish plastic surgery trainees meeting, Dunkeld, October 2011.

Introduction Up to 75% of deaths in patients with burns over 40% total body surface area (TBSA) are related to sepsis from burn wounds or other infections and/or inhalation injury. The prevention of cross-contamination of nosocomial infections between burns patients is imperative. Bacteria can be passed between patients by direct contact; via airborne particles; or via a vector such as a staff member or the inanimate surrounding environment. Studies were designed to show the impact of a dressing and sheet change on both airborne bacteria levels and staff contamination.

Methods **1.** A SAS Super 180 sieve impaction sampler was used to take air samples at regular intervals throughout a dressing and sheet change on a patient with significant burns. Each sample was enumerated and the total number of bacterial colony forming units per m^3 of air (cfu/m^3) plotted to demonstrate trends in the number of airborne bacteria released throughout the course of the event. Statistical analysis was performed using Minitab to establish a control chart. **2.** Staff nurses were asked to wear sterile theatre gowns to carry out a wound dressing change. Twenty samples were taken from each gown using contact agar plates, and the total number of cfu per $25cm^2$ plate enumerated and an average taken. This was repeated 23 times on nurses carrying out dressing changes on burns of different sizes.

Results High levels of airborne bacteria (up to 350 cfu/m^3) were demonstrated during a burn dressing and sheet change, which persisted after the event had finished. Staff nurses were found to be contaminated with levels between 13 cfu /25 cm^2 for a 1% TBSA burn and 662 cfu/25 cm^2 for a 51% TBSA burn.

Conclusions The study provides evidence for two conclusions: firstly, there are high levels of airborne bacteria in the room of a patient with significant burns throughout a burn wound dressing and sheet change. Secondly, even relatively small burns liberate bacteria onto the nurse carrying out a dressing change. Plastic aprons alone would not prevent this from contaminating the nurse's uniform. Of note there are currently no national burn-specific guidelines for the use of personal protective equipment (PPE) during dressing changes.

Bache SE, Maclean M, Anderson JG, Gettinby G, MacGregor SJ, Taggart I. Laboratory inactivation of healthcare-associated isolates by a visible HINS-liht source and its clinical application in the burns unit. Presented at the European Burn Association Congress, The Hague, September 2011.

Rationale Large areas of bacteria-harbouring eschar predispose burns patients to disperse many pathogens into their surrounding environment. This becomes a bacterial reservoir, and a potential source of cross-contamination. The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) has previously been shown to reduce environmental bio-burden in the burns unit for up to two days. The maintenance of this decontamination effect over longer periods in the clinical environment, alongside controlled laboratory exposures of five nosocomial isolates from burns patients was examined.

Methods 1) Laboratory experiments: clinical isolates of *Staphylococcus aureus*, MRSA, *Streptococcus pyogenes,* multi-drug resistant *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were spread onto agar surfaces and exposed to the HINS-light EDS to establish the rate of inactivation. 2) Clinical experiments: three burns patients were nursed in isolation rooms containing ceiling-mounted HINS-light EDS. Environmental samples were taken from frequently touched sites, and results compared before and after use of the light. The rooms were treated with HINS-light EDS for five, six and seven days, respectively.

Results 1) All bacteria were inactivated *in vitro*, with significant reductions demonstrated at between two and four hours. 2) Levels of environmental contamination surrounding the burns patient were reduced by 75%, 70%, and 89% and perpetuated following five, six, and seven days' exposure to the HINS-light EDS.

Conclusion Five significant nosocomial isolates from burns patients were inactivated within a matter of hours in laboratory conditions. The safe wavelength of the HINS-light EDS means it can be used continuously in the presence of patients and staff. This unique benefit of continuous disinfection allows maintenance of environmental cleanliness, augmenting that achieved by current infection control practice, and despite the ongoing dispersal of bacteria into the environment in the dynamic clinical situation. The decontamination effect is sustained during periods of at least one week.

Bache SE, Maclean M, MacGregor SJ, Anderson JG, Gettinby G, Watson SB, Taggart I. Reducing bacterial contamination in the burns dressing clinic using a novel light-based method of continuous disinfection. Presented at the International Society for Burn Injuries Conference, Istanbul, June 2010.

Background Infection is the leading cause of morbidity in burns patients. During burns dressing clinics, many patients may be seen in quick succession. The burns patient has a propensity to disperse pathogens into the environment during dressing changes. Pathogens dispersed into the air and surviving on environmental surfaces pose a significant risk of cross-infection between patients.

A novel, safe, light-based technology, termed the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) permits continuous disinfection in addition to standard hospital cleaning procedures. We have already shown this to be advantageous in the inpatient setting. We set out to determine whether the presence of a HINS-light EDS in a burns outpatient clinic could usefully reduce the environmental contamination of a room used for burns dressings compared to standard procedures alone.

Methods Following the morning clean of a clinic room, samples were taken from 50 sites around the room using contact agar plates. A nurse-led burns clinic was then held, using standard cleaning procedures ("HINS-off clinic"). Samples were taken at the end of the day from the same 50 sites and the average number of colony forming units (CFUs) from each plate was calculated. The identical sampling procedure was undertaken on a different day with the addition of a ceiling mounted HINS-light ("HINS-on clinic") running continuously throughout the clinic. The study was replicated a second time, producing a total of 100 plates from sites before and after each clinic and statistical analyses were carried out on the results using parametric analysis of variance and confidence interval methods.

Results The average number of CFUs per agar plate before the "HINS-off clinic" was 8.2. This was comparable with an average number of 6.5 CFUs before the "HINS-on clinic". Following the "HINS-off clinic", an average of 22.2 CFUs were counted per plate, compared with 12.0 CFUs following the "HINS-on clinic". Overall, the presence of the HINS-light EDS reduced the increase in bacterial contamination due to clinic activity on average by 8.6 CFUs per plate (95% CI 1.4 to 15.8) which was a percentage reduction of 61.3% (95% CI 10% to 112.6%). This reduction was statistically significant (p=0.02)

Conclusions Prevention of cross-infection is crucial for burns patients. Inanimate surfaces within clinical rooms have been shown to act as reservoirs for pathogens. The addition of a HINS-light EDS, in conjunction with current cleaning practices provides an efficient, continuous method of disinfection that significantly reduces environmental contamination in the dressings clinic room compared with standard cleaning alone. We believe that this may help to decrease cross-infection between successive patients.

Bache SE, Maclean M, MacGregor SJ, Anderson J, Gettinby G, Woolsey G A, Taggart I. Application of a novel light technology for disinfection on the burns unit (Abstract). *Journal of Burn Care and Research* 2010; 31(2): S113. Presented at American Burn Association annual meeting, Boston, March 2010.

Introduction: Environmental contamination from patients is an important source of cross-infection and outbreaks within burns units. The use of a High Intensity Narrow Spectrum (HINS) light source that is bactericidal yet safe for patients and staff, has facilitated continuous disinfection of surfaces within the burns unit. We present results of the utilization of this novel technology.

Methods: Two ceiling-mounted HINS-light environmental decontamination systems were installed into a standard isolation room on the burns unit containing a patient with large burns. To assess the efficacy of HINS-light for environmental decontamination, bacteria levels on various environmental surfaces within the room were monitored using contact agar plates. Fifty sampling sites were identified and pre-HINS bacterial levels on environmental surfaces were assessed. The HINS-light was then switched on constantly during daylight hours and further samples were taken after two days to assess bacteria levels during HINS-light exposure. The HINS-light was then switched off and sampling was repeated after a further two days to measure post-HINS levels. All samples were collected at 1500 hours. Two further investigations were carried out, with samples being taken at 0800 hours and 2200 hours using the same methodology to assess the pre-HINS, during-HINS and post-HINS levels of environmental contamination at different times of the day. Standard hospital isolation precautions were followed throughout.

Results: Pre-HINS sampling at 1500 hours showed an average of 192 colony forming units (CFU) per plate which was reduced to 112 CFU after two days exposure to the HINS-light (42% reduction). Further samples taken after the light had been switched off for two days showed that levels recovered to an average of 148 CFU (32% increase). Overall, the presence of the HINS-light reduced environmental contamination. This was more clearly demonstrated with studies carried out at 0800 hours and 2200 hours, when the level of activity in the room was more constant, correlating well with previous studies that showed approximately a 60% reduction in environmental bioburden.

Conclusions: Large wound areas and a state of immunosuppression render burns patients susceptible to infection therefore reduction of environmental bacterial contamination is an important factor in the control of hospital acquired infections within burns units. HINS-light provides a method of environmental disinfection in addition to standard isolation precautions.

The HINS-light EDS in practice: a novel method of continuous environmental disinfection on a burns unit

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Introduction

The High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) is a new decontamination technology which uses visible blue light centred around 405nm wavelength, that has been shown in laboratory conditions to kill important bacteria commonly found in the burns unit. It is safe to humans enabling it to be used continuously throughout the day, reducing contamination and decreasing the environmental reservoir of bacteria that contribute to cross-contamination and nosocomial infections. We present results of the clinical work with this novel technology.

Laboratory experiments

A variety of pathological bacteria have been exposed to HINS-light wavelengths in the laboratory setting. To date over 20 different bacterial species have been tested and all were inactivated in a dose-dependent and species-dependent fashion. Gram positive organisms, including MRSA were particularly susceptible to photodynamic inactivation by HINS-light exposure.

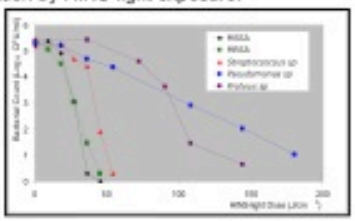

Fig 1: HINS-light inactivation curves for some important bacteria on the burns unit

Clinical studies

HINS-light EDS units were installed in two burns unit inpatient isolation rooms containing a patient with significant burns, and the burns outpatient clinic where typically around ten patients a day are seen. Fifty sampling sites were identified around each room, and environmental surface samples taken using Baird Parker contact agar plates. Plate counts were then enumerated and the mean values calculated.

This work is supported by the Stephen Forrest Trust & Burns Fund

Fig 2: HINS-light EDS installed in burns outpatient clinic and inpatient isolation room (top left corner of each photograph)

Inpatient studies

Environmental samples taken before, during and after the HINS-light EDS was switched on for two days, demonstrated a reduction in the average number of bacterial colony forming units (cfu) of between 46% and 55%. This was replicated when the sampling was done at different times of day, in two different isolation rooms, and on different patients. Samples taken at 0800h were used as a model for future studies as they were most reproducible and statistically significant (p=0.02)

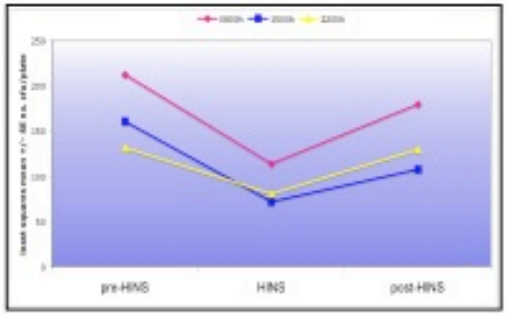

Fig 3: Mean cfu/plate before, during and after HINS light EDS treatment, with samples collected at three different times of day (n=50)

Outpatient studies

Samples were taken before and after nurse-led dressing clinics that took place with and without the HINS-light EDS. Without the HINS-light EDS, environmental contamination rose from 8.1 to 22.2 cfu. When the HINS-light EDS was on, levels rose from 6.5 to just 11.9 cfu. ie the amount of environmental contamination was reduced by an average of 8.6 cfu, giving a 61% efficacy (p=0.02).

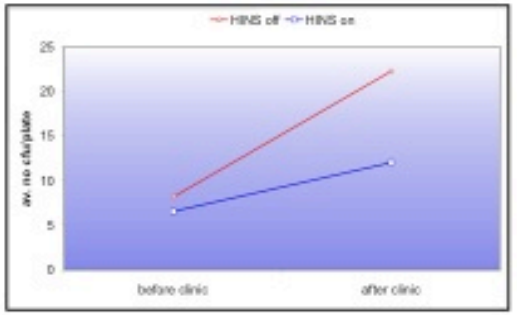

Fig 4: Mean cfu/plate before and after two burns clinics, with HINS-light EDS switched off (blue) and on (red) (n=100)

Conclusion

Hospital environmental surfaces contribute to cross-transmission of pathogenic microbes which can survive for days or weeks on dry surfaces. HINS-light EDS can achieve statistically significant reductions of levels of environmental bacterial contamination in the burns unit over and above current infection control and cleaning policies. Its efficacy in a variety of clinical situations in the burns unit give great scope for its future use in reducing cross-contamination and nosocomial infection in burns patients.

References: Maclean M, MacGregor S J, Anderson J G et al. Environmental decontamination of a Hospital Isolation Room using High-Intensity Narrow-Spectrum Light. J Hosp Infect 2010; in press, doi:10.1016/j.jhin.2010.07.010
Maclean M, MacGregor S J, Anderson J G et al. Inactivation of Bacterial Pathogens Following Exposure to Light from 405 nanometer Light Emitting Diode Array. Appl Environ Microbiol 2009; 75(7): 1932-1937. doi:10.1128/AEM.01892-08

A survey of protective equipment worn by healthcare workers during burns dressing changes in the UK

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Background

The primary mode of cross-contamination between burns patients is direct or indirect contact, either with contaminated equipment or the hands and uniforms of healthcare workers (HCW) caring for patients.

Dressing changes liberate bacteria from burns wounds and have been identified as a time when bacteria can be passed from patient to HCW. This is relative to the size of the burn.[1] Despite this, there are no burns-specific guidelines on the use of personal protective equipment (PPE) by HCW during dressing changes in the US or UK. UK guidelines by NICE and SIGN are vague and not specific to the unique infection control requirements of burns patients.[2] A questionnaire of all US burn centres, a wide variety of infection control practices were reported[3].

Aim : To determine whether or not there is consensus between UK burns units about the use of PPE during dressing changes

Methods

- All 16 adult burns units and facilities registered with the British Burns Association (BBA) were approached to take part in a telephone survey in 2011.
- The nurse in charge was asked to answer questions on the demographics of the unit, infection control policies, and the use of PPE by staff carrying out dressing changes.

Figure 1: Telephone questionnaire

Results

Fifteen Units took part in the survey: twelve were adult only and three were mixed adult and children wards.

Several units shared with other specialties including plastic surgery, maxillofacial surgery and trauma and orthopaedics, although seven were exclusively burns units.

The frequency with which wound swabs were carried out, and location for dressing changes are illustrated in Figures 2&3.

Figure 2: Frequency of wound swabs on UK burns units

Figure 3: Main location for dressing changes on UK burns units

Only one unit reported following a burns-specific policy for the use of PPE during dressing changes. The others adhered to generic hospital or national guidelines. All units reported the use of routine hand hygiene measures and the use of gloves for all patient contact.

Only three units stated they would wear different PPE if a burn was over 16% TBSA: In this situation one unit reported a policy of HCW changing into clean scrubs for dressing changes on burns over 16% TBSA, and two reported a policy of wearing full-length gowns over uniforms (Figure 4).

Most units said they paid more attention to the presence of known infection, with five units having a policy to change into scrubs, and nine of wearing full-length gowns regardless of %TBSA.

Results cont.

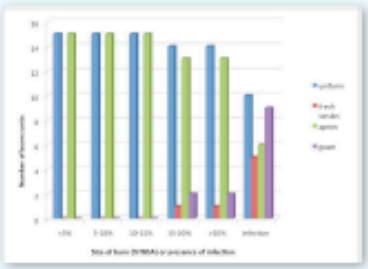

Figure 4: PPE worn by HCW carrying out burns dressing changes

One unit also reported wearing hats, and three wearing visors when carrying out dressing changes on patients with known infection (Figure 5.)

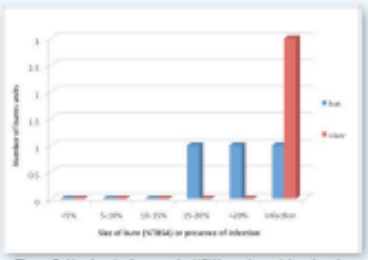

Figure 5: Head protection worn by HCW carrying out dressing changes

Conclusions

- Most burns units report the wearing of increased PPE (fresh scrubs or full-length gowns) if a wound is known to be infected. However, this can be difficult to diagnose, with positive swabs reported days after the wound has become infected.
- There is lack of agreement between the UK burns units about which PPE should be worn for dressing changes on burns of different sizes, although size of burn is related to levels of bacteria dispersal during a dressing change.
- Burns-specific PPE guidelines may be of value to reflect the specific infection control risks of burns patients.

References
1. Bache SE, Maclean M, Anderson JG, MacGregor SJ, Gettinby G, Taggart I. A model to predict transfer of bacteria from patients to staff during burns dressing changes. ISBI 2012 oral presentation.
2. NICE Infection control guidelines: Prevention of healthcare associated infection in primary and community care June 2003. (Accessed November 3 2011, at www.nice.org.uk)
3. Hodle AE, Richter KP, Thompson RM. Infection control practices in US burns units. J Burn Care Res 2006; 27: 142-61.

Glossary and abbreviations

ACGHI	American conference of government hygienists
ACH	Air changes per hour
AK	Actinic keratosis
ALA	Aminolevulinic acid
Allograft	Skin graft harvested from a human who is not the intended recipient
AMD	Age related macular degeneration
ANOVA	Analysis of variance
Autograft	Skin graft harvested from the intended recipient patient
ASHRAE	American Society of Heating, Refrigerating and Air Conditioning Engineers
BCC	Basal cell carcinoma
Biobrane	A biological dressing
BPA	Baird Parker agar
cfu	Colony forming unit (of bacteria)
CNS	Coagulase negative *Staphylococci*
CP	Coproporphyrin
Dermis	The layer of skin below the epidermis and above underlying connective tissues
EDS	Environmental decontamination system
Epidermis	The most superficial layer of cells of the skin
Eschar	Non-viable or dead tissue, in this case as a result of a burn
Escharotomies	Pressure-relieving incisions through areas of deep burn eschar
GE	Germicidal efficiency
Glottis	The combination of the vocal cords and the space between them, marking the distinction between the upper and lower airways
GRI	Glasgow Royal Infirmary
HAI	Hospital acquired infections (also known as nosocomial infections)
HCW	Healthcare workers
HINS-light EDS	High Intensity Narrow Spectrum light Environmental Decontamination System
HVAC	Heating, ventilation and air conditioning
Integra	A biological skin substitute
LED	Light emitting diode
MAL	Methyl aminolevulinate
Matriderm	A biological skin substitute
MDR-*A. baumannii*	Multi drug resistant *Acinetobacter baumannii*
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
NA	Nutrient agar
NB	Nutrient broth
Pa	Pascals
PDI	Photodynamic inactivation (also known as photodynamic therapy, PDT)
PpIX	Protoporphyrin IX
ROLEST	Robertson Trust Laboratory for Electronic Sterilisation Technologies
ROS	Reactive oxygen species
RPE	Retinal pigment epithelium
SCC	Squamous cell carcinoma
TBSA	Total Body Surface Area: measure of burn size
TLV	Threshold limit values
TSA	Tryptone soya agar
TSB	Tryptone soya broth
UVGI	ultraviolet germicidal irradiation
Xenograft	Skin graft harvested from another species (usually porcine)