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Evaluation of amoxicillin, metronidazole and gentamicin dosage regimens for use in antibiotic prophylaxis in colorectal surgery

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DECLARATION

‘This thesis is the result of the author’s original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.’

Signed: 

Date: 15th February 2020

Dedicated to my parents, wife and son

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ABSTRACT

Surgical site infection (SSI) is one of the most frequent healthcare associated infections in Scotland and has a significant clinical and financial burden to hospitals and society. Colorectal surgery is associated with the highest SSI rate among elective operations and from the various measures to prevent SSIs, antimicrobial prophylaxis is one of the most effective. It is important to maintain free antibiotic concentrations in serum and tissue above the minimum inhibitory concentration (MIC) breakpoints of microorganisms commonly associated with SSIs until skin closure.

This thesis demonstrates the value of using population pharmacokinetic (PopPK) modelling to assess an antibiotic prophylaxis regimen in colorectal surgery with the aim of identifying optimal dosing regimens.

PopPK models were developed for amoxicillin, metronidazole, and gentamicin using NONMEM® in order to determine the probability of maintaining free drug concentrations above the MIC breakpoints of the following microorganisms: methicillin-sensitive *Staphylococcus aureus*, *Escherichia coli*, *Bacteroides fragilis* group, enterococci, and *Streptococcus anginosus* group.

Pharmacokinetics for all three antibiotics were best described by a one-compartment model. Elimination and distribution of amoxicillin and metronidazole were affected by body weight. Elimination of gentamicin was influenced by creatinine clearance and height. Distribution was affected by height.

The findings of this study support 1000 mg of amoxicillin being re-dosed intra-operatively every 4 hours, however, in patients at high risk of infective endocarditis, additional doses are required every 2 hours. Following a dose of 500 mg metronidazole, a re-dosing interval of 8 hours would be acceptable for patients with normal weight (BMI <25 kg/m^2), whereas for patients with a BMI ≥25 kg/m^2 the results suggest that an additional dose of 500 mg should be given 4 hours after the initial dose. Finally, gentamicin doses that are based on 5 mg/kg ideal body weight and banded according to height are recommended to be re-dosed at 5 hours.

LIST OF PUBLICATIONS

Kathriarachchi, U.L., Vidhate, S.S., Al-Tannak, N., Thomson, A.H., da Silva Neto, M.J.J. and Watson, D.G. (2018) 'Development of a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography (HILIC)'. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences*, 1089, pp. 78-83.

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LIST OF ABBREVIATIONS AND ACRONYMS

-2LL	Minus twice the log of the likelihood
AJBW	Adjusted body weight
*f*AUC/MIC	Area under the free concentration time curve to minimum inhibitory concentration ratio
BMI	Body mass index
BSV	Between subject variability
CI	Confidence interval
CL	Clearance
CRCA	Creatinine clearance based on adjusted body weight
CRCL	Creatinine Clearance
CTA	Cumulative target attainment
CV	Coefficient of variation
CWRES	Conditional weighted residuals
EBL	Estimated blood loss
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HAI	Healthcare Associated Infection
HT	Height
IBW	Ideal body weight
IE	Infective endocarditis
IV	Intravenous
k	Elimination rate constant
LBW	Lean body weight
LC-MS	Liquid chromatography coupled with mass spectrometry

LOQ	Limit of quantification
MBW	Maximum body weight
MCS	Monte Carlo Simulation
MIC	Minimum inhibitory concentration
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NHSGGC	NHS Greater Glasgow and Clyde Health Board
NONMEM®	Nonlinear mixed effects modelling software package
OFV	Objective function value
PD	Pharmacodynamic
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/pharmacodynamic
PopPK	Population pharmacokinetics
PsN	Perl-speaks-NONMEM®
PTA	Probability of target attainment
RE	Residual error
RSE	Relative standard error
SIGN	Scottish Intercollegiate Guidelines Network
SSI	Surgical site infection
$t_{1/2}$	Half-life
TDM	Therapeutic drug monitoring
%*f*T>MIC	Percentage probability of achieving free antibiotic concentrations above the minimum inhibitory concentration
TVCL	Typical value of clearance
TVV	Typical value of volume

V	Volume of distribution
VPC	Visual predictive check
WT	Total body weight

1 Introduction

1.1 General background

Guidelines for surgical antimicrobial prophylaxis recommend maintaining adequate serum and tissue antibiotic concentrations for the entire duration of surgical procedures. This is important because it reduces the risk of surgical site infections (SSIs) (Zelenitsky et al. 2002, Zanetti et al. 2001). Amongst all types of surgery, colorectal surgery has the highest rates of SSIs, which presents a substantial clinical and financial burden to hospitals and society (Coello et al. 2005, Kirkland et al. 1999, Broex et al. 2009).

NHS Greater Glasgow and Clyde (NHSGGC) guidelines for antibiotic prophylaxis for patients receiving colorectal surgery recommend administering intravenous (IV) amoxicillin, metronidazole and gentamicin prior to skin incision. Additional doses are administered during prolonged procedures due to the short elimination half-lives of these antibiotics. However, as highlighted in the Scottish Intercollegiate Guidelines Network (SIGN) "Antibiotic Prophylaxis in Surgery" guideline, the optimal frequency of administration of these antibiotics in patients receiving prolonged surgery is not clear (Scottish Intercollegiate Guidelines Network 2014). For example, the Scottish Antimicrobial Prescribing Group recommends re-dosing amoxicillin every 4 hours and both metronidazole and gentamicin every 8 hours (Scottish Antimicrobial Prescribing Group 2016), whereas other national and international recommendations suggest re-dosing antibiotics if the duration of the procedure exceeds the elimination half-life ($t_{1/2}$) of the antibiotic (Scottish Intercollegiate Guidelines Network 2014, Bratzler et al. 2013, National Institute for Health and Care Excellence 2008). Since the elimination $t_{1/2}$ of amoxicillin is approximately 1 hour, a rapid decline in antibiotic concentrations could lead to amoxicillin being re-dosed every hour to ensure that levels are maintained above the typical minimum inhibitory concentrations (MICs) against sensitive organisms. In addition, there is no consensus on the optimal doses of gentamicin and metronidazole when used for prophylaxis (Zelenitsky et al. 2016, Hobbiss et al. 1988). Within Scotland, gentamicin

prophylactic dosing regimens used in colorectal surgery range from 2 mg/kg to 4 mg/kg and there are concerns about the potential for toxicity with higher doses (4.5 – 5 mg/kg) of gentamicin (Dubrovskaya et al. 2015, Hayward et al. 2018). It is known that other national and international recommendations suggest doses of these antibiotics that are higher than the doses currently recommended by NHSGGC (Asin-Prieto et al. 2015b, Bratzler et al. 2013). No studies have investigated concentrations achieved by the combination of antibiotics and dosage regimens used within NHSGGC for colorectal surgery.

The present study aimed to address these questions by analysing serum concentrations of amoxicillin, gentamicin, and metronidazole in patients undergoing colorectal surgery to determine whether they were adequate for the whole duration of the surgical procedures.

1.2 Surgical site infections

The term surgical site infection (SSI) was created in 1992 by the Centers for Disease Control and Prevention and refers to an infection resulting from a surgical incision. The term was devised to avoid ambiguity with infections arising from traumatic wounds. An SSI is defined as an infection occurring at the surgical site within 30 days after the procedure or within one year of implanted material. SSIs are divided into three categories: superficial incisional, deep incisional, and organ/space. These categories have been adopted internationally and are summarised in Table 1.

The 2017 Health Protection Scotland annual report on Healthcare Associated Infection (HAI) highlighted that an SSI was one of the most frequent causes of HAI, estimated as 16.5% of inpatient HAIs in Scotland (Health Protection Scotland 2018). Furthermore, the Surveillance of Surgical Site Infection Annual Report for procedures carried out from January 2008 - December 2012 stated that from twelve procedure categories, which include cardiac, orthopaedic and gynaecological procedures, colorectal surgery had the highest rates of SSI; in 2012 the inpatient SSI

Table 1 Centers for Disease Control and Prevention surgical site infection categories (Mangram et al. 1999).

Superficial incisional SSI
Infection occurs within 30 days after the operation and infection involves only skin and subcutaneous tissue of the incision and at least one of the following: 1. Purulent drainage, with or without laboratory confirmation, from the superficial incision. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision. 3. At least one of the following signs or symptoms of infection: pain or tenderness, localised swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative. 4. Diagnosis of superficial incisional SSI made by a surgeon or attending physician.
Deep incisional SSI
Infection occurs within 30 days after the operation if no implant is left in place or within one year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissue (e.g. fascia, muscle) of the incision and at least one of the following: 1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site. 2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localised pain or tenderness, unless incision is culture-negative. 3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of deep incisional SSI made by a surgeon or attending physician.
Organ/space surgical SSI
Infection occurs within 30 days after the operation if no implant is left in place or within 90 days if implant is in place and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs and spaces) other than the incision which was opened or manipulated during an operation and at least one of the following: 1. Purulent drainage from a drain that is placed through a stab wound into the organ/space. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space. 3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of organ/space SSI made by a surgeon or attending physician.

Key: SSI, surgical site infection.

rate was 14.8% for large bowel surgical procedures (Health Protection Scotland 2013). European data were consistent with Scottish data with colon surgery also presenting the highest rate of SSI (9.9%) (Health Protection Scotland 2013). Data from Public Health England, between April 2012 to March 2017, were also similar showing a cumulative SSI incidence in large bowel surgery of 9.2%, the highest across seventeen types of operation (Public Health England 2017).

SSIs increase post-operative morbidity, mortality and cost of treatment, and are a substantial clinical and financial burden to hospitals and society. In Scotland, the annual cost of HAI is estimated at £137 million and an additional 318 172 bed days in order to treat these patients; this equates to a large teaching hospital occupied for a year (Health Protection Scotland 2014). Broex et al. (2009) estimated that on average the costs of treating a patient were doubled if they developed an SSI and this was mainly due to a longer length of hospital stay. SSIs have a serious adverse impact on the health of patients causing pain, suffering, and, in extreme cases, death. Coello et al. (2005) looked at SSI surveillance data from 140 English hospitals between October 1997 and June 2001 in nine categories of surgery and found a significant association between SSIs and mortality in patients with hip prosthesis, vascular surgery and large bowel surgery, who develop deep incisional and organ/space SSI. Furthermore, SSIs result in an increased use of antimicrobials, leading to emergence of bacterial resistance.

1.2.1 Pathogenesis and microbiology of surgical site infection in bowel surgery

The risk of developing an SSI following colorectal surgery originates mostly from the endogenous bacteria present in the colon at the time of surgery. The human gastrointestinal tract contains numerous and diverse bacteria; it is reported that one gram of faeces contains about 10^8 to 10^{11} bacteria (Sender et al. 2016).

Operations can be split into four classes (Table 2) with the risk of SSI depending on the degree of bacterial contamination from the surgical site (Culver et al. 1991). When the gastrointestinal tract is entered with adequate technique and there are no existing intra-abdominal infections, colorectal surgery is classified as clean-contaminated.

Table 2 Classification of surgical operations (Culver et al. 1991).

Class	**Definition**
Clean	Operations in which no inflammation is encountered and the respiratory, alimentary or genitourinary tracts are not entered. There is no break in aseptic operating theatre technique.
Clean-contaminated	Operations in which the respiratory, alimentary or genitourinary tracts are entered but without significant spillage.
Contaminated	Operations where acute inflammation (without pus) is encountered, or where there is visible contamination of the wound. Examples include gross spillage from a hollow viscus during the operation or compound/open injuries operated on within four hours.
Dirty	Operations in the presence of pus, where there is a previously perforated hollow viscus, or compound/open injuries more than four hours old.

Colorectal surgery involves the opening of the colon and/or rectum (hollow viscus) and the high numbers of bacteria present are considered the major cause of contamination of the surgical site. The most frequent organisms associated with SSIs include aerobic Gram-negative bacteria (e.g., *Escherichia coli*) and anaerobes (e.g., *Bacteroides fragilis*). Other sources of contamination of the surgical site include skin flora and bacteria from the operating-room environment, healthcare professionals and instruments. Gram-positive aerobes (e.g., methicillin-sensitive

Staphylococcus aureus, or MSSA) are the usual cause of SSIs from this source of contamination (Fry 2013, Poggio 2013).

In addition to the *inoculum* of bacteria that contaminates the surgical site, there are numerous clinical variables associated with SSIs following colorectal surgery. Patient-related factors include advanced age, obesity, diabetes, smoking, malignancy, cardiac disease, and lung/liver/renal chronic disease. Procedure-related factors include the length of the operation, traffic in and out of theatre, contaminated instruments, hair removal strategy, antimicrobial prophylaxis, excessive electrocautery, drains, intra-operative hypothermia, and theatre ventilation (Fry 2013, Poggio 2013).

1.2.2 Diagnosis and surveillance of surgical site infection following bowel surgery

The discharge of pus from the surgical site is the most frequent sign of an SSI. Redness and induration is also used as a diagnostic sign of SSI, however, redness is also seen in the normal inflammatory response and induration may not be detected in overweight patients. The discharge of serous fluid may also be normal, particularly if the culture shows a light growth of a skin contaminant such as *Staphylococcus epidermidis* (Fry 2013). In more serious cases patients present with clinical signs of sepsis. An SSI may also prevent healing by causing the wound edges to separate or cause an abscess to develop in the deeper tissues (National Institute for Health and Care Excellence 2008). To allow national and international comparison of rates of SSIs and to quantify care improvement, it is important to use consistent definitions and a standardised methodology to assess SSIs. In Scotland, Health Protection Scotland provides guidance for SSI surveillance and in April 2017 included elective large bowel procedures within the mandatory requirements of SSI surveillance. Surveillance is a multidisciplinary activity that includes infection control teams, nurses, and clinicians working together to monitor and lower the incidence of SSI at hospital level. At both Scottish and international levels, the objectives of SSI surveillance include monitoring trends, assessing the impact of

interventions, and prioritising the allocation of resources (Health Protection Scotland 2017, World Health Organization 2018). In Scotland, SSI surveillance is mandatory on all patients within the following four operation categories: caesarean section, hip arthroplasty, elective large bowel and elective vascular procedures. The large bowel SSI surveillance includes inpatient and readmission for up to 30 days (Health Protection Scotland 2017). An NHSGGC large bowel surgery SSI surveillance report (unpublished) from July 2016 to October 2018 shows a cumulative SSI incidence rate of 3.6% (70 SSIs: 1969 procedures). NHS England surveillance data from April 2018 to March 2019 show an SSI incidence of 9% for large bowel surgery with high inter-hospital variation (0.3 – 24.9%) (Public Health England 2019). European data for 2017 reported an SSI incidence of 6.4% and 10.1% for laparoscopic and open large bowel surgery respectively (European Centre for Disease Prevention and Control 2019).

1.2.3 Prevention of surgical site infections in elective bowel surgery

The principles of SSI prevention are divided into pre-operative, intra-operative, and post-operative measures. The pre-operative measures include decontaminating the skin, sterilising instruments and equipment, and minimising airborne particles in order to avoid the introduction of microorganisms into the operative site. Antibiotics are also administered before incision to prevent multiplication of microorganisms at the surgical site. The intra-operative measures include minimising tissue damage by surgical technique and peri-operative warming to enhance patients' defences against infection. It is also important to prevent micro-organisms from accessing the wound post-operatively by using appropriate wound dressing techniques. Recommendations for these three phases are discussed in the National Institute for Health and Care Excellence SSIs prevention and treatment guideline (National Institute for Health and Care Excellence 2008). The implementation of surveillance programmes also reduces the rates of SSI through increasing hospital staff awareness of the problem by providing quantitative measures from surveillance data (Haley et al. 1985).

Though all these measures are important to prevent SSIs, this research will focus on antibiotic prophylaxis in colorectal surgery.

1.3 Antibiotic prophylaxis in surgery

Experimental studies in animals by Burke (1961) provided the scientific rationale for administering pre-operative prophylactic antibiotics to prevent SSIs, by demonstrating suppression of infection when antibiotics were administered before bacteria contaminated the surgical site. This important finding changed the previous practice of administering antibiotics in the recovery room at the end of the procedure. The reduction in SSIs when administering pre-operative antibiotics in colorectal surgery was demonstrated in a prospective clinical trial in the mid-sixties (Bernard and Cole 1964).

The SSI risk from colorectal surgery is highly associated with the amount of endogenous contamination from the lumen of the colon and the role of antibiotic prophylaxis is to reduce the *inoculum* of bacteria at the surgical site. As the high numbers of bacteria that are released when the large bowel is opened are considered the major cause of contamination of the surgical site, it is generally accepted that antibiotic prophylaxis is the most significant measure to prevent SSIs. The benefit of using antibiotic prophylaxis in colorectal surgery is well accepted; the rate of SSIs without antibiotic prophylaxis is reported to be as high as 40% (Ludwig et al. 1993) compared to rates of 11% with antibiotic prophylaxis (Song and Glenny 1998).

1.3.1 Antibiotic choice

The choice of antibiotics is based on their capacity to eradicate the most frequent microorganisms that cause SSI by type of surgery. In colorectal surgery, the targeted pathogens are aerobic and anaerobic bacteria including *Escherichia coli*, MSSA, and *Bacteroides fragilis*. Broad-spectrum antibiotic cover is generally recommended for

antibiotic prophylaxis in colorectal surgery and NHSGGC uses a combination of IV amoxicillin, metronidazole and gentamicin prior to incision; if beta-lactam allergy is present the amoxicillin is replaced with teicoplanin (Appendix 1, NHSGGC Clinical Guideline: Antibiotic Prophylaxis in Gastrointestinal and Vascular Surgery). Other institutions only recommend IV metronidazole and gentamicin as enterococci are not considered to be pathogenic and are therefore unlikely to cause infection. Furthermore, regimens (including amoxicillin) that are active against enterococci do not show lower infection rates. However, a few cases of *Streptococcus anginosus* group (also known as the *Streptococcus milleri* group) infections after colorectal surgery led to a local decision to include amoxicillin in the antibiotic prophylaxis regimen to provide cover against this group of bacteria. Similar cases have been reported elsewhere (Tresadern et al. 1983).

In addition to providing adequate bacterial cover, the antibiotic prophylaxis regimen should avoid antibiotics that lead to a high-risk of *Clostridium difficile* infection (such as cephalosporins, fluoroquinolones, clindamycin, and carbapenems) (Scottish Intercollegiate Guidelines Network 2014).

1.3.1.1 Amoxicillin

Amoxicillin is a penicillin antimicrobial that was licensed in 1972. It is a weak acid with a sulphur-containing, five membered, thiazolidine ring adjacent to the beta-lactam ring (Figure 1) and it has a low molecular weight (365.40 g/mol).

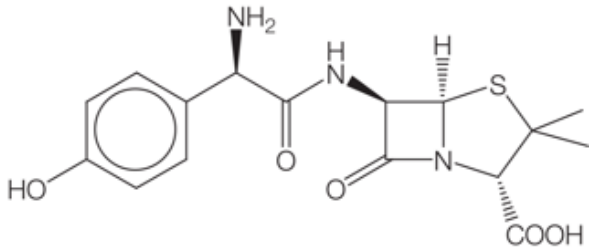


Figure 1 Chemical structure of amoxicillin.

Amoxicillin is bactericidal by inhibiting bacterial cell wall synthesis (Bryskier 2005). It is available in oral and parenteral formulations, has a good oral bioavailability, distributes well into most extracellular fluids but does not significantly enter cells (White and Andrews 1999). Protein binding is low (17%) (Sutherland et al. 1972).

Amoxicillin is mostly excreted in the urine by glomerular filtration and tubular secretion. It has a short elimination $t_{1/2}$ of approximately 1 h and therefore requires frequent administration unless the patient has renal impairment, in which case accumulation occurs if the dosing regimen is not adjusted (Humbert et al. 1979, Spyker et al. 1977). It is converted to penicilloic acid, a microbiologically inactive derivative, by cleavage of the beta-lactam ring by liver enzymes and/or thermal degradation. Amoxicillin is effective against Gram-positive (most streptococci and some enterococci) and Gram-negative bacteria (the majority of the Haemophilus and Moraxella strains and the occasional coliform). Therapeutic concentrations are achieved in different tissues or fluids, such as lung tissue, maxillary sinuses, interstitial fluid and cerebrospinal fluid of patients with meningitis (Bryskier 2005). Research by Martin et al. (1995) in 17 patients undergoing colorectal surgery determined the amoxicillin and clavulanic acid concentrations in serum, abdominal wall fat, epiploic fat, and colonic wall tissue of two clavulanic acid dosing regimens (200 and 400 mg) administered with 2000 mg of amoxicillin at induction of anaesthesia and re-dosed 2 hours after the first dose. The effective antibacterial concentrations used in the methodology of the study were defined as clavulanic acid concentrations above 2 mg/L. For beta-lactamase producing bacteria this concentration decreases the amoxicillin MIC from the resistant to the susceptible category. The results showed that the clavulanic acid and amoxicillin concentrations exceeded the concentrations found to be effective in vitro in all of the serum samples. Effective concentrations of amoxicillin (for susceptible bacteria) were also found in all tissue samples, however, effective clavulanic acid concentrations in tissues were only found in 11% of the samples with the 200 mg doses and 72% with the 400 mg doses.

Beta-lactams are time-dependent antibiotics (DeRyke et al. 2006) which means that their bactericidal effect is associated with the amount of time that concentrations are above the MIC of the infective organisms. To achieve the NHSGGC aim of covering the *Streptococcus anginosus* group with amoxicillin, a serum concentration above the MIC breakpoint of 0.5 mg/L is required (European Committee on Antimicrobial Susceptibility Testing 2018). There are no pharmacokinetic /pharmacodynamic (PK/PD) studies of amoxicillin in colorectal surgery; previous Monte Carlo simulation (MCS) studies of other beta-lactams in abdominal surgery used unbound antibiotic concentrations above the MIC for the whole duration (100%) of the surgical procedure as the PK/PD target (Moine and Fish 2013, Zelenitsky et al. 2016).

Amoxicillin is generally very well tolerated; the most important side-effect of amoxicillin is hypersensitivity, including rashes and cases of fatal anaphylaxis. Between 1 – 10% of patients exposed to beta-lactams will report an allergic reaction but much smaller numbers will have anaphylaxis (Idsoe et al. 1968).

1.3.1.2 Metronidazole

Metronidazole is a 5-nitroimidazole (Figure 2) and was licensed in 1963. It has a low molecular weight (171.16 g/mol) and is bactericidal by inhibiting nucleic acid synthesis resulting in bacterial cell death (Dubreuil 2005).

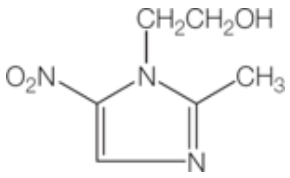


Figure 2 Chemical structure of metronidazole.

Metronidazole is active against anaerobic bacteria, the oral bioavailability of metronidazole is close to 100% and protein binding is low (15%) (Dubreuil 2005, European Committee on Antimicrobial Susceptibility Testing 2010). It penetrates

well into all body tissues and fluids (Dubreuil 2005). Martin et al. (1991) determined concentrations of metronidazole in serum and different abdominal tissues after a single dose of 1000 mg was given to 11 patients undergoing colorectal surgery. Adequate concentrations (above the MIC of susceptible bacteria) in the colonic wall at surgical anastomosis were found in 91% of patients; the percentage dropped in the abdominal wall fat and epiploic fat to 40 and 60% respectively at surgical closure.

The elimination $t_{1/2}$ of IV metronidazole is 7.3 – 7.9 h (Houghton et al. 1979, Mattila et al. 1983). It is mostly metabolised in the liver to five metabolites and the main (hydroxyl) metabolite has 30 to 65% the antimicrobial activity of metronidazole. Excretion is mostly urinary of which approximately 20% of the total dose is the unchanged parent drug and 24 – 28% is the hydroxyl metabolite (Dubreuil 2005, Lau et al. 1992). The area under the unbound concentration-time curve over MIC ratio (*f*AUC/MIC) is the PK/PD parameter best correlated with efficacy (Sprandel et al. 2006). The metronidazole dosage regimen used by NHSGGC aims to cover the *Bacteroides fragilis* group (MIC breakpoint of 4 mg/L) (European Committee on Antimicrobial Susceptibility Testing 2018). Metronidazole PK/PD parameters reported in patients undergoing colorectal surgery are summarised in Table 3.

Metronidazole is well tolerated and gastrointestinal side-effects are usually mild but neurotoxicity has been reported in patients receiving prolonged or high dose therapy (Lau et al. 1992).

1.3.1.3 Gentamicin

Gentamicin was licensed in 1964 and is an antimicrobial composed of three aminoglycosides, gentamicin C_1, gentamicin C_2, and gentamicin C_{1a} (Weinstein et al. 1963). It is rapidly bactericidal by inhibiting protein synthesis and has a low molecular weight (449.56 – 477.61 g/mol) (Veyssier and Bryskier 2005). Aminoglycosides are strong bases with an amino sugar and an aminocyclitol joined by a glycosidic link (Figure 3) (Lovering 1999).

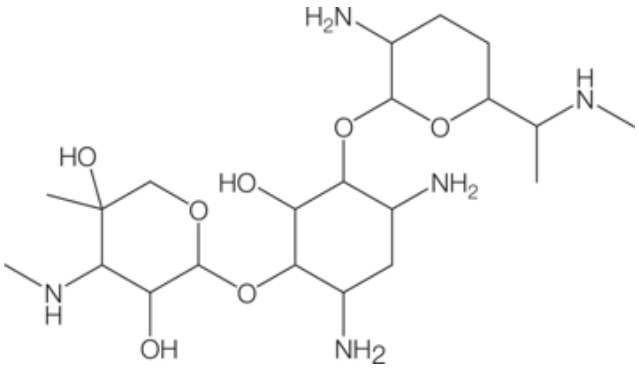


Figure 3 Chemical structure of gentamicin.

The binding of gentamicin to serum proteins is very low or non-existent under normal conditions (Gordon et al. 1972) although it could be significantly higher in certain diseases associated with low concentrations of the divalent cations, calcium and magnesium (Ramirez-Ronda et al. 1975). Gentamicin is not absorbed after oral administration; the serum elimination $t_{1/2}$ is 2 – 2.5 h in patients with normal renal function and excretion is almost exclusively via glomerular filtration (Veyssier and Bryskier 2005). Aminoglycosides distribute well into most body fluids with a volume of distribution equivalent to that of extracellular fluids (Veyssier and Bryskier 2005, Lovering 1999). Research by Martin et al. (1993) in 13 patients undergoing colorectal surgery determined concentrations in serum and different abdominal tissues after a single dose of an aminoglycoside (netilmicin 6 mg/kg). The netilmicin concentrations in all of the tested tissues (except in the abdominal wall and epiploic fat tissues of one patient at the time of skin closure) were above the MIC of susceptible bacteria.

Gentamicin is active against Gram-negative and Gram-positive bacteria and requires to be actively transported across the bacterial cell membrane. It is not active against anaerobes and streptococci as these bacteria do not have the required carrier proteins (Veyssier and Bryskier 2005). Aminoglycosides were historically thought to be concentration-dependent killers meaning their bactericidal effect depended on achieving a high ratio of the peak serum concentration to the MIC. However, recent evidence shows that the *f*AUC/MIC better relates to clinical efficacy (Muller et al.

2018). The gentamicin dosage regimen used by NHSGGC aims to cover MSSA (MIC breakpoint of 1 mg/L) and Gram-negative bacteria, including *Escherichia coli* (MIC breakpoint of 2 mg/L) (European Committee on Antimicrobial Susceptibility Testing 2018). Gentamicin PK/PD parameters reported in patients undergoing colorectal surgery are summarised in Table 3.

Gentamicin has a narrow therapeutic index and therapeutic drug monitoring (TDM) may prevent dose related toxicity when treating infections. Nephrotoxicity and ototoxicity are important side-effects of gentamicin (Hayward et al. 2018). A recent systematic review on adverse effects of a single dose of gentamicin in adults (Hayward et al. 2018) included 24107 patients receiving a dose ranging from 1 mg/kg to 480 mg per dose. Acute kidney injury was described in approximately 10% of patients, however, persistent renal impairment and other adverse effects were rare. No cases of ototoxicity were identified. This systematic review supports the safe use of a single-dose of gentamicin in surgical antibiotic prophylaxis.

Table 3 Metronidazole and gentamicin pharmacokinetic/pharmacodynamic parameters in studies of patients undergoing elective colorectal surgery.

Antibiotic	**Patient characteristics**	**CL**	**V**	**$t_{1/2}$**	**PK/PD target**
Metronidazole (Ventura et al. 2008)	Sex, 19 M/14 F; age 59 (19)* years; weight, 73 (13)* kg; CRCL, 78 (29)* ml/min	3.15 (1.20)* L/h	0.68 (0.20)* L/kg	11.8 (5.1)* h	Concentrations in plasma >8 mg/L
Metronidazole (Asin-Prieto et al. 2015b)	Sex, 37 M/26 F; age 69.1 (12.3)* years; weight, 68.6 (10.4)* kg; CRCL, 76.5 (27.2)* ml/min	3.53 L/h BSV 44.56%	V_1 27.67 L BSV 57.60% V_2 16.86 L	8.77 (N/R) h	*f*T>MIC of 100% of the surgery length
Gentamicin (Ventura et al. 2008)	Sex, 19 M/14 F; age 59 (19)* years; weight, 73 (13)* kg; CRCL, 78 (29)* ml/min	4.71 (1.95)* L/h	0.23 (0.06)* L/kg	2.3 (1.4)* h	C_{max} >9 mg/L and C_{max}/MIC ≥8
Gentamicin (Markantonis et al. 2004)	Sex, 11 M/5 F; age 61 (3)* years; weight, 75 (4)* kg; CRCL, 88.5 (6.6)** ml/min	5.31 (0.40)** L/h	0.22 (0.02)** L/kg	2.19 (0.24)** h	N/R
Gentamicin (Zelenitsky et al. 2000)	34 patients, Sex N/R; age N/R; weight N/R; CRCL N/R	0.091 (N/R) L/h/kg	0.26 (N/R) L/kg	1.98 (N/R) h	N/R

*Key: *data are presented as mean (SD), **mean (SEM). CL, clearance; V, volume of distribution; $t_{1/2}$, elimination half-life; PK/PD, pharmacokinetic/pharmacodynamic; sex (number of M males, F females); CRCL, creatinine clearance estimated by the Cockcroft-Gault formula (Cockcroft and Gault 1976); BSV, between-subject variability; V_1, volume of distribution of the central compartment; V_2, volume of distribution of the peripheral compartment; fT>MIC, probability of achieving free antibiotic concentrations above the minimum inhibitory concentration; C_{max}, maximum antibiotic concentration; MIC, minimum inhibitory concentration; SD, standard deviation; SEM, standard error of the mean; N/R, not reported.*

1.3.2 Antibiotic timing and dosing

Early or delayed administration of antibiotics reduces the efficacy of the antibiotic at the time of surgery and is associated with an increased risk of SSI (Classen et al. 1992, Scottish Intercollegiate Guidelines Network 2014), therefore it is important to administer IV antibiotics just before the start of the operation. The SIGN 104 guideline for antibiotic prophylaxis in surgery recommends administering antibiotics as close to the time of incision as possible and within 60 minutes of surgical incision (Scottish Intercollegiate Guidelines Network 2014). The guideline also states that it is good practice for the prophylactic dose to be the same as the dose used to treat infections.

In order to achieve adequate serum and tissue concentrations, it is important to consider the antibiotic pharmacokinetic (PK) and pharmacodynamic (PD) properties as well as patient factors when choosing the dose. For most procedures IV administration is the preferred route, as it will provide rapid and predictable concentrations. NHSGGC guidelines (Appendix 1, NHSGGC Clinical Guideline: Antibiotic Prophylaxis in Gastrointestinal and Vascular Surgery) recommend (for patients undergoing colorectal surgery who are not allergic to beta-lactams) the administration of IV amoxicillin 1000 mg, metronidazole 500 mg and gentamicin, with gentamicin doses banded according to height (HT) based on 3 mg/kg ideal body weight (IBW), capped at 300 mg. There is a considerable variation in the recommended doses used in surgical antibiotic prophylaxis; in Scotland the gentamicin dose in colorectal surgery ranges from 2 – 4 mg/kg. The joint guidelines for antimicrobial prophylaxis in surgery from the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Surgical Infection Society, and the Society for Healthcare Epidemiology of America recommend 5 mg/kg of total body weight (WT). In obese patients who weigh at least 20% above their IBW, the dose is calculated using the adjusted body weight (AJBW) equation: dosing weight = IBW + 0.4 x (WT – IBW). The correction factor value (0.4) was determined by Bauer et al. (1983) for gentamicin dosing in obese patients. The

guidelines also recommend doses of 2000 mg of ampicillin (a beta-lactam with similar properties to amoxicillin), and 500 mg of metronidazole (Bratzler et al. 2013). Licensed indications for metronidazole include antibiotic prophylaxis with a single pre-operative dose of 1000 – 1500 mg (30 – 60 minutes before the operation) or alternatively 500 mg immediately before, during, or after the operation, then 500 mg 8 hourly (Electronic Medicines Compendium 2018a). Indications for amoxicillin include prophylaxis of infective endocarditis with a single dose of 2000 mg (Electronic Medicines Compendium 2018c). Gentamicin is only licensed for the treatment of infections (Electronic Medicines Compendium 2018b).

1.3.3 Intra-operative re-dosing

The aim of antibiotic prophylaxis is to achieve serum and tissue concentrations above the MIC of common organisms involved in SSI. The importance of maintaining adequate concentrations for the whole duration of the procedure from incision to skin closure has been clinically demonstrated by lower SSI rates in colorectal and cardiac surgery (Zelenitsky et al. 2002, Zanetti et al. 2001). In colorectal surgery, the extraction of the colonic specimen is the presumed inoculation point of surgical wounds and is the time at which antibiotic cover is particularly important. This time is usually near the end of the procedure, which reiterates the importance of maintaining adequate concentrations for the whole duration of the procedure. In order to achieve adequate concentrations at the surgical site during prolonged procedures, it is generally accepted that antibiotics with a short elimination $t_{1/2}$ should be re-dosed intra-operatively (Bratzler et al. 2013, Scottish Intercollegiate Guidelines Network 2014, Scottish Antimicrobial Prescribing Group 2016).

For prolonged surgery, NHSGGC follow the 2016 Scottish Antimicrobial Prescribing Group re-dosing recommendations. An additional amoxicillin dose 4 hours after the first dose and additional amoxicillin, metronidazole and gentamicin doses 8 hours after the first dose should be administered intra-operatively (Appendix 1, NHSGGC Clinical Guideline: Antibiotic Prophylaxis in Gastrointestinal and Vascular Surgery)

(Scottish Antimicrobial Prescribing Group 2016). Blood loss and fluid replacement are also known to reduce serum antibiotic concentrations (Scottish Intercollegiate Guidelines Network 2014); this is also covered in the NHSGGC guidelines, which recommend re-dosing amoxicillin, metronidazole and gentamicin (at half the prophylaxis dose) if the estimated blood loss (EBL) is greater than 1.5 L. Teicoplanin is not re-dosed in prolonged surgery or major blood loss. The joint guidelines for antimicrobial prophylaxis in surgery from the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Surgical Infection Society, and the Society for Healthcare Epidemiology of America recommend re-dosing antibiotics if the duration of surgery exceeds twice the elimination $t_{1/2}$ of the relevant antibiotic. The guidelines suggest re-dosing ampicillin 2 hours from the first pre-operative dose and state that metronidazole and gentamicin may need to be re-dosed in unusually long procedures (typical case length of surgery not specified) (Bratzler et al. 2013).

Large bowel surgery SSI surveillance data collected in NHSGGC from October 2017 to April 2018 (unpublished) showed that 52.1% of prescription charts or anaesthetic sheets had no documented evidence of re-dosing of antibiotic prophylaxis (data from 139 procedures lasting more than 4 hours).

1.3.4 Duration of prophylaxis

Surgical antibiotic prophylaxis should be administered as a single dose. Exceptions include major blood loss and/or prolonged surgery where antibiotics are re-dosed intra-operatively, or in specific operations such as open heart surgery and certain types of orthopaedic, ear nose and throat, and head and neck surgery where antibiotics may continue post-operatively up to 24 – 48 hours (Scottish Intercollegiate Guidelines Network 2014). A systematic review of randomised controlled trials on antimicrobial prophylaxis in colorectal surgery showed no difference in the rates of SSI comparing a single-dose regimen with a multiple-dose regimen (Song and Glenny 1998). The risks of prolonging surgical antibiotic

prophylaxis after skin closure are an increase in *Clostridium difficile* infection and antibiotic resistance (Scottish Intercollegiate Guidelines Network 2014).

1.3.5 Prophylaxis against infective endocarditis

Infective endocarditis (IE) is associated with high mortality and serious complications. The rationale for IE antibiotic prophylaxis is to prevent the attachment of bacteria onto the endocardium after transient bacteraemia following invasive procedures. This led the European Society of Cardiology to recommend antibiotic prophylaxis in patients at high risk of IE who are undergoing high-risk procedures (Habib et al. 2015). The three high-risk IE categories identified are: patients with a prosthetic valve or with prosthetic material used for cardiac valve repair; previous history of IE; patients with untreated cyanotic congenital heart disease and those with congenital heart disease who have post-operative palliative shunts, conduits or other prostheses. If high-risk patients undergo high-risk invasive procedures, including gastrointestinal procedures that require antibiotic prophylaxis to prevent SSI, the European Society of Cardiology suggests that the antibiotic regimen includes enterococci cover. The antibiotics suggested are ampicillin, amoxicillin or vancomycin (if beta-lactam allergy is present). The guidelines do not specify antibiotic prophylaxis dosing/re-dosing information but recommend a single-dose of 2000 mg, orally or IV, of amoxicillin or ampicillin in adults with no beta-lactam allergy 30 – 60 minutes before high-risk dental procedures. The British Society for Antimicrobial Chemotherapy guidelines for the prevention of IE state that the standard antibiotic prophylaxis regime for surgical operations involving the intestinal mucosa may need modified. Also, they mention that colonic surgery has been anecdotally associated with cases of IE and patients should receive prophylaxis (Gould et al. 2006). The guidelines recommend a single dose of IV amoxicillin (1000 mg) and gentamicin (1.5 mg/kg) given just before the procedure or at induction of anaesthesia to provide cover for enterococci, streptococci and staphylococci. The British Society for Antimicrobial Chemotherapy guidelines do not mention re-dosing if surgery is prolonged or there is major blood loss. For

enterococci, an amoxicillin serum concentration above the MIC breakpoint of 4 mg/L is required (European Committee on Antimicrobial Susceptibility Testing 2018).

1.4 Antimicrobial assays

Antibiotic assays are used in clinical practice and research to measure antibiotic concentrations in blood with the aim of optimising therapy. TDM has traditionally been used for antimicrobials with a narrow therapeutic index such as gentamicin, whereas TDM of beta-lactam and nitroimidazole antibiotics is not common in clinical practice and is mostly academic. Immunoassay techniques are commercially available for frequently assayed drugs such as gentamicin; they have the advantage of having a very short turnaround time and are very sensitive and reproducible (White and Lovering 1999). Liquid chromatography coupled with mass spectrometry (LC-MS) is an increasingly popular technique due to its rapid and sensitive nature. The role of LC-MS in drug discovery, development, and TDM is well established (Unger et al. 2013) and several LC-MS methods have been reported for analysis of amoxicillin and metronidazole in plasma (Silva et al. 2009, Khuroo et al. 2008, Yoon et al. 2004, Dong et al. 2013, Wen et al. 2008, Gaikwad et al. 2013). Kathriarachchi et al. (2018) developed a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography. Compared to other published chromatography techniques, this method retains more polar ionisable compounds, such as amoxicillin, which is otherwise poorly retained by reversed phase methods. It also provides higher sensitivity when coupled with an electrospray mass spectrometry detection system.

1.5 Population pharmacokinetics

Population pharmacokinetics (PopPK) is the study of the variability in drug concentrations between individuals when a specific dose is administered to the target patient population (Aarons 1991). The aim of PopPK is to estimate the typical values of PK parameters such as clearance (CL) and volume of distribution (V) and to

determine the influence of clinical factors, such as age, sex, weight, or renal function on PK variability. It is from a knowledge of these factors that drug doses are usually determined, and PopPK is therefore an essential tool to personalise dosing regimens in specific patient groups (Anon. 2003, Mould and Upton 2012).

Specialised data analysis techniques and software are required in order to analyse sparse concentration data from a large patient population, which comprised early PopPK studies. These packages use nonlinear mixed effects modelling techniques to analyse all the data simultaneously and estimate between subject variability (BSV) and residual error (RE) in addition to population parameter values. The nonlinear mixed effects modelling program NONMEM®, originally developed by Beal and Sheiner in 1980, was the first software package for PopPK modelling and is still regarded as the gold standard (Owen and Fiedler-Kelly 2014, Mould and Upton 2012).

PopPK analysis has been used to personalise surgical antibiotic prophylaxis regimens in colorectal surgery. Research by Asin-Prieto et al. (2015b) assessed the adequacy of a prophylactic regimen of pre-operative single doses of metronidazole 1500 mg and cefuroxime 1500 mg in 63 adult patients undergoing elective colorectal surgery. This study developed PK models for each drug and performed simulations to determine how clinical characteristics influenced the probability of target attainment (PTA) against organisms frequently associated with SSIs. The results of this study showed that cefuroxime would need to be re-dosed 2 hourly in patients with normal renal function and 4 hourly in patients with moderate renal impairment. However, in patients with severe renal impairment, cefuroxime plasma concentrations were adequate for up to 8 hours after the first dose. Regarding metronidazole, an additional dose at 4 hours would be required in patients weighing 90 kg. Isla et al. (2012) developed a PopPK model for cefoxitin to evaluate the adequacy of a pre-operative dose of 2000 mg re-dosed every 2 hours (if surgery is prolonged), in 56 patients undergoing colorectal surgery. Based on PTA values, the authors suggested re-dosing cefoxitin every hour if the creatinine clearance

(CRCL) was above 100 ml/min and every 1.5 hours if CRCL was 60 – 80 ml/min; considering a continuous infusion preceded by a bolus injection was also suggested. Moine and Fish (2013) used PK/PD modelling to assess the adequacy of seven antibiotics in elective colorectal surgery. The following regimens were assessed: cefoxitin 1000 mg and 2000 mg; cefotetan 1000 mg and 2000 mg; ceftriaxone 1000 mg and 2000 mg; cefazolin 1000 mg and 2000 mg; ampicillin/sulbactam 1500 mg and 3000 mg; cefuroxime 1500 mg; and ertapenem 1000 mg. This study used MCS methods to assess the influence of dose and dosing frequency on the PTA against organisms frequently associated with SSIs. Cumulative target attainment (CTA) was also determined for each antimicrobial using the MIC distributions of each organism. The authors concluded that ertapenem 1000 mg, cefuroxime 1500 mg, and cefazolin 2000 mg were the only antibiotics to achieve a CTA greater than 90% against the targeted organisms up to 4 hours after the pre-operative dose. Zelenitsky et al. (2016) used PK/PD modelling to inform dosing recommendations of antibiotic prophylaxis in abdominal surgery. Eight antibiotic regimens were assessed: cefazolin 2000 mg plus metronidazole 500 mg; cefoxitin 2000 mg; ceftriaxone 2000 mg plus metronidazole 500 mg; ertapenem 1000 mg; gentamicin 5 mg/kg plus metronidazole 500 mg; gentamicin 5 mg/kg plus clindamycin 900 mg; levofloxacin 500 mg plus metronidazole 500 mg; and levofloxacin 500 mg plus clindamycin 900 mg. The PTA against organisms frequently associated with SSIs was determined for each antibiotic at 1, 2, 3, 4, 5 and 6 h after the pre-operative dose. CTA was also determined for each antimicrobial using the MIC distributions of each organism. For gentamicin, the 5 mg/kg dose was compared with lower doses down to 1.5 mg/kg. The authors concluded that cefoxitin and clindamycin should be avoided, 2000 mg doses of cefazolin should be used in patients from 60 to 120 kg, and recommended a 3 mg/kg dose of gentamicin.

1.6 Aims and objectives

PopPK studies to optimise antibiotic exposure in surgical antibiotic prophylaxis are scarce. The antibiotic dosage regimens that are routinely used for the prophylaxis of SSIs are largely empirical and it is not known if they maintain adequate antibiotic cover during prolonged colorectal surgical procedures. The SIGN 104 guideline for antibiotic prophylaxis in surgery highlights the requirement for further research in areas such as the PD, PK, and duration of antibiotic prophylaxis and the requirement for additional intra-operative dosage by surgery type and antibiotic (Scottish Intercollegiate Guidelines Network 2014).

The aim of this research was to evaluate concentration-time profiles of metronidazole, amoxicillin and gentamicin when used for antibiotic prophylaxis in colorectal surgery with the dosing regimens recommended by NHSGGC antibiotic guidelines. The study objectives were as follows:

1. To determine whether current prophylactic dosage regimens of amoxicillin, metronidazole and gentamicin provide adequate serum concentrations to reduce the risk of infection for the duration of prolonged colorectal surgery.
2. To estimate the PK parameters of amoxicillin, gentamicin and metronidazole following administration of prophylactic doses to patients undergoing colorectal surgery.
3. To use the final PK models for simulations to explore the role of the dosage regimen on the probability of target attainment (PTA).
4. To assess the incidence of SSIs within 30 days of colorectal surgery in patients who were administered the current NHSGGC antibiotic prophylaxis regimen.

2 MATERIALS AND METHODS

2.1 Study design and setting

This was a pilot, prospective, open-label study involving patients undergoing elective, colorectal surgery. The antimicrobial pharmacist (author of this thesis) completed the Research Ethics Committee and NHSGGC Research and Development forms under supervision of the Chief Investigator (consultant colorectal surgeon) and the academic supervisors. The study was approved by the NHS East Midlands – Nottingham 1 Research Ethics Committee (REC reference: 16/EM/0209) and the NHSGGC Clinical Research and Development group (R&D reference GN16OR139). The University of Strathclyde Ethics Committee endorsed the favourable opinion of the NHS committee and also approved the study (UEC 16/28). Written consent (Appendix 2, Consent Form) was obtained from all patients following verbal and written explanations of the study (Appendix 3, Participant Information Sheet). The antimicrobial pharmacist provided the study information and took the informed consent. Recruitment occurred between June 2016 and December 2016. Twenty adult patients who were scheduled to undergo elective colorectal surgery were included in the study. The Chief Investigator had clinical responsibility for the patients.

2.2 Selection criteria

Patients were eligible if they met all of the following inclusion criteria: male and non-pregnant females, 18 years of age or older, who were undergoing elective colorectal surgery at the Glasgow Royal Infirmary and who gave written, informed consent. Patients were excluded if they were less than 18 years of age, had an allergy to beta-lactams, nitroimidazoles or aminoglycosides, had received any antibiotic within 72 hours of the start of surgery, had a renal transplant, had an estimated glomerular filtration rate of less than 20 mL/min/1.73m^2, had emergency surgery, did not have an arterial line, were pregnant or breast feeding, and patients who might not adequately understand verbal explanations or written information given in English, or who had special communication needs.

Potential participants who fitted these criteria were identified by the Chief Investigator and the antimicrobial pharmacist from the multidisciplinary team meeting outcome list. On the day of the patient's routine pre-operative assessment, which was typically 7 – 10 days before the planned surgery date, the nurse undertaking the assessment asked potential participants if they were willing to be provided with verbal and written information about the study from the antimicrobial pharmacist. If the patients agreed to this, the antimicrobial pharmacist explained the rationale for the study and provided the information leaflet (Appendix 3, Participant Information Sheet). Potential participants had a minimum of 24 hours to decide whether or not to take part in the study.

2.3 Drug administration, sampling procedure and analytical methods

2.3.1 Drug administration and blood sampling

Prior to incision at the start of surgery, the following antibiotics were administered by the anaesthetist according to routine clinical practice: an infusion of 500 mg of metronidazole given by gravity; a bolus injection of 1000 mg amoxicillin; and a bolus injection of gentamicin. The gentamicin dose was banded according to HT and based on 3 mg/kg IBW (Appendix 1, NHSGGC Clinical Guideline: Antibiotic Prophylaxis in Gastrointestinal and Vascular Surgery). Re-dosing of antibiotics was performed at 4 hours for amoxicillin and at 8 hours for amoxicillin, metronidazole and gentamicin. If the EBL was greater than 1.5 L, amoxicillin, metronidazole and gentamicin were also re-dosed. If a second antibiotic dose was required intra-operatively, the same prophylactic doses were given with the exception of gentamicin (half the prophylactic dose).

Blood samples (3 ml) for assay were withdrawn by the anaesthetist from the arterial line at the following times: pre-dose; 1 hour and 2 hours after the start of the antibiotic administration; and at skin closure. If additional antibiotic doses were required (due to prolonged surgery and/or blood loss), samples were also

withdrawn before and 1 hour after these doses. The documentation of the times of antibiotic administration and withdrawal of blood samples was undertaken by the antimicrobial pharmacist in the operating theatre. The times were recorded on the Case Report Form (Appendix 4) in real-time (at the start of administration and sampling). Blood samples were processed and stored by the antimicrobial pharmacist within the Glasgow Clinical Research Facility laboratory according to the protocol described in Appendix 5. The blood samples were collected in Vacuette® tubes containing clotting accelerator and separation gel and then were centrifuged at 3000 x g for 10 minutes at 4°C. The serum samples were stored at -80°C until batched analysis within the Strathclyde Institute of Pharmacy and Biomedical Sciences (amoxicillin and metronidazole) and within the biochemistry department at the Glasgow Royal Infirmary (gentamicin).

2.3.2 Liquid chromatography – mass spectrometry analysis

A highly sensitive method for simultaneous determination of amoxicillin and metronidazole concentrations by LC-MS was applied for the serum analysis (Kathriarachchi et al. 2018). The instrument consisted of an UltiMate 3000 high performance liquid chromatography system coupled with an Exactive benchtop Orbitrap mass spectroscopy system (Thermo Scientific, USA). Chromatographic separations were performed on a ZIC-HILIC column (150 x 4.60 mm, 3.5 µm; HiChrom, Reading UK) with a metal free guard column ZIC-HILIC (20 x 2.1 mm). The mobile phase consisted of a mixture of 0.1% volume/volume formic acid in water and 0.1% volume/volume formic acid in acetonitrile.

Serum samples were preserved at -80°C for a median of 9 months (range 6 – 11 months) and defrosted on the day of the assay. The samples were prepared by combining 100 µl of serum and 20 µl of each internal standard (ampicillin and metronidazole-D4, 50 mg/L) with 860 µl of acetonitrile. The sample injection volume was 10 µl. The assay was validated for extraction of amoxicillin and metronidazole from human serum with regard to selectivity, accuracy, precision, calibration, lower limit of quantification (LOQ), extraction recovery and matrix

effect. The method was linear in the range 0.1 to 6.4 mg/L for both drugs, with an LOQ of 0.016 and 0.008 mg/L for amoxicillin and metronidazole respectively. The intra-run coefficients of variation (CV) were <15 % for amoxicillin and metronidazole at all quality control levels (Table 4).

Table 4 Intra-run precision data for amoxicillin and metronidazole (Kathriarachchi et al. 2018).

QC level mg/L	Amoxicillin CV%	Metronidazole CV%
0.1	5.81	1.94
0.8	11.6	4.67
6.4	3.80	3.14

Key: QC, quality control; CV, coefficient of variation.

The effect of freeze thaw was assessed by re-analysing four patient samples in triplicate 9 months after the original study. The samples were stored at -80°C and were subject to two more freeze thaw cycles. The results were all within 20% of the original measurements and demonstrated that there were no issues with long-term storage and repeated freeze thawing (Kathriarachchi et al. 2018). Metronidazole stability has also been established in human plasma at room temperature up to 4 hours (Sagan et al. 2005) and serum samples containing amoxicillin may be stored refrigerated at 4°C for up to 2 days (Latte et al. 2015).

2.3.3 Immunoassay

The serum concentrations of gentamicin were determined in all samples with the MULTIGENT Gentamicin assay (Architect c16000, Abbott, Abbott Park, IL, USA), a homogeneous particle-enhanced turbidimetric inhibition immunoassay which is used for clinical TDM. The reagents of this competitive binding type assay are anti-gentamicin monoclonal antibody and gentamicin-coated microparticles. The rate of absorbance change is measured photometrically and is directly proportional to the

rate of agglutination of the particles. The higher the concentration of gentamicin in the sample, the smaller the amount of gentamicin-coated microparticles which can bind to the antibody, slowing down the rate of absorbance change. For quantification, a concentration-dependent agglutination inhibition curve is then obtained.

Serum samples were preserved at -80°C for a median of 10 months (range 7 – 13 months) and defrosted on the day of the assay. An aliquot of serum (2 µl) was run on the analyser (Architect c16000, Abbott, Abbott Park, IL, USA) by Glasgow Royal Infirmary biochemistry staff using the department standard gentamicin assay protocol. The assay used six calibrators (0.00, 0.56, 1.52, 3.00, 5.93, and 10.00 mg/L) and was linear up to a serum gentamicin concentration of 10 mg/L; the LOQ was 0.5 mg/L. Samples containing gentamicin concentrations above 10 mg/L were diluted using the automated dilution protocol, in which the system performed a 1:2 dilution of the sample and automatically corrected the concentration by multiplying the result by the appropriate dilution factor. A tri-level human serum based commercial control containing gentamicin was used for determination of precision (Liquicheck, Bio-Rad Laboratories, Irvine, CA, USA). The inter-assay CVs were 2.2% for both the medium and high controls and 4.5% for the low control.

Aqueous aminoglycoside solutions are extremely stable and may be stored frozen for several years without loss of activity (Lovering 1999). Stability has also been established in blood at room temperature up to 4 hours and in serum, at 4 – 8°C, for up to 4 weeks (World Health Organization 2002).

2.4 Data collection

Patient demographics (sex, age, weight and height), routine clinical data (serum creatinine, serum albumin) were extracted from the patient's notes/routine blood tests and recorded on the Case Report Form (Appendix 4). In addition, the following data were also recorded: antibiotic-prescribing/administration and blood sampling information, duration of surgery, EBL and volume of IV fluids administered during

surgery. CRCL and other size descriptors were also determined from the patient's clinical characteristics.

2.5 Data handling

During the development of the data set, patients were allocated an identification number. An Excel® file was created that contained this identification number, patient name, and Community Health Index number. This coding sheet was stored by the antimicrobial pharmacist on password protected files within his personal drive on a folder within the NHSGGC internal computer network. Only the antimicrobial pharmacist was able to access this folder by logging on with his NHS username and password. The file was deleted from the NHS database once data collection was complete and the data set for analysis had been finalised. The final data set contained only the patient identification (ID) numbers and no patient identifiable data.

The anonymised data set was then transferred from the NHSGGC internal computer network to the Strathclyde University computer network by email. All blood samples and paper Case Report Forms completed in the operating theatres contained only the patient ID numbers and no patient identifiable data. Hard copies of the coding sheet, Case Report Forms, and consent forms were stored within a locked filing cabinet in the Chief Investigator's office (locked) within the hospital. Personal information held within the coding sheet were only viewed by the antimicrobial pharmacist and the Chief Investigator, who are both NHS members of staff.

Concentration measurements and all other data collected in the course of the study were stored in an anonymised form on the University of Strathclyde server accessible only by the antimicrobial pharmacist and the two university supervisors. Following completion of the study, data were stored within the University of Strathclyde server (Strathcloud) and on the PURE database of the academic supervisor.

2.6 Population pharmacokinetic model estimation and pharmacodynamic analysis

Initially, antibiotic concentration-time profiles were plotted using Microsoft Excel® for Mac (2016) to identify a possible structural model. After establishing the appropriate structural model, population models were compared in order to identify the contribution of predictable components of BSV (depending on covariates such as age, WT, and renal function). The data were first described by a model where all variability between individuals was assumed to be random (base population model). Then scatter plots of individual estimates of CL and V versus clinical characteristics were examined to identify which covariates might influence the PK parameters. These factors were then tested in the population model and changes in BSV were examined.

2.6.1 Structural population model

All concentration-time data for each drug were analysed simultaneously using nonlinear mixed effects modelling. Population pharmacokinetic parameter estimates were obtained with NONMEM® 7.3.0 (ICON Development Solutions, Ellicott City, MD). Post-processing and graphical analysis of the NONMEM® results were performed with Xpose (version 4.3.5) (Jonsson and Karlsson 1999) implemented in R (version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria) (R Core Team 2014) and Microsoft Excel® for Mac (2016). PopPK models for amoxicillin, metronidazole and gentamicin were developed separately using NONMEM®. First-order conditional estimation with interaction (FOCEI) was used throughout the model building. As preliminary evaluation of the data indicated that a one-compartment model was adequate to describe the concentration-time profiles of all antibiotics, this was the only structural model that was tested. This model was parameterised as follows:

IV bolus (amoxicillin and gentamicin)

$C_{(t)} = D/V \times \exp^{-CL/V \times t}$

where $C_{(t)}$ is the concentration at time t (the dependent variable), D is the dose, V is the volume of distribution, CL is the clearance and t is the time after dose (the independent variable).

IV infusion (metronidazole)

$C_{(t)} = IR/CL \times (1 - \exp^{-CL/V \times Tinf}) \times \exp^{-CL/V \times (t - Tinf)}$

where IR is the infusion rate, t is the time after the start of the infusion, and Tinf is the duration of the infusion.

The one-compartment model was parameterised in NONMEM® to give estimates of CL and V using the ADVAN1 and TRANS2 subroutines as follows:

$TVCL = \theta_1$

$TVV = \theta_2$

where θ_1 and θ_2 are fixed-effect parameters defining the typical values of CL (TVCL) and V (TVV). Individual estimates of CL and V were assumed to be log-normally distributed. The first level of random effect, unexplained BSV in these parameters, was therefore modelled as follows:

$CLi = TVCL \times \exp^{\eta i}$

$Vi = TVV \times \exp^{\eta i}$

where CLi and Vi are the individual estimates of CL and V and individual eta (η_i) is a random effect accounting for the individual differences from the typical values of these parameters. NONMEM® estimates the variance of η and provides individual η values for each patient. The distribution of individual η values was assumed to have a mean of 0 and variance of omega squared (ω^2).

The second level of random effect modelled residual error (RE), i.e. differences between the measured concentrations in each individual and the concentrations

that were predicted using individual PK parameters. RE may arise from assay errors, errors in dose or sampling time, intraindividual variability or due to the use of an inappropriate structural model. RE is defined by a quantity (ε) reflecting the differences between the model predicted concentrations and the individual's observed concentrations. RE was initially described using a combined error model that included both additive and proportional components, as shown below:

$C_{obs,ij} = C_{pred,ij} (1 + \varepsilon_{1ij}) + \varepsilon_{2ij}$

Where $C_{obs,ij}$ is the j^{th} observed concentration in the i^{th} individual, $C_{pred,ij}$ is the j^{th} predicted concentration in the i^{th} individual, and ε_i represent proportional (ε_{1i}) and additive (ε_{2i}) components of the difference between the predicted and observed j^{th} concentrations at time t. The individual epsilons (ε) were assumed to be normally distributed with a mean of 0 and variance of sigma squared (σ^2). Simpler models with only an additive or a proportional component were also considered.

2.6.2 Covariate model

Once the base population model had been selected, the following clinical characteristics were examined for a possible influence on the PK of each antibiotic: age; WT; HT; sex; albumin; serum creatinine; and CRCL estimated using the Cockcroft-Gault formula (Cockcroft and Gault 1976). Identification of covariate candidates was first carried out by visual inspection of scatterplots of parameters versus covariates. Potentially influential covariates were then added to the model, for example, a direct linear relationship between CL and WT would be described using the following format:

$TVCL = \theta_1 \times WT$

Where θ_1 is a fixed effect parameter describing the relationship between the TVCL and WT. A more complex hierarchical model that includes an intercept would be:

$TVCL = \theta_1 \times WT + \theta_2$

Allometric models with the following structure were also investigated:

$TVCL = \theta_1 \times (WT/70)^{0.75}$

As the study population had a wide range of body mass index (BMI) values, in addition to WT the following size descriptors were calculated and included in the covariate analyses:

- Ideal body weight (IBW) (Devine 1974)

  Male (kg) = 50 kg + 0.89 x (HT (cm) – 152.4)

  Female (kg) = 45.5 kg + 0.89 x (HT (cm) – 152.4)

- Adjusted body weight (AJBW) (Erstad 2004)

  AJBW = IBW + 0.4 x (WT - IBW)

- Maximum body weight (MBW)

  IBW + 20%

- Lean body weight (LBW) (Janmahasatian et al. 2005)

  Male (kg) = 9270 x WT / (6680 + 216 x BMI)

  Female (kg) = 9270 x WT / (8780 + 244 x BMI)

  BMI = WT / HT (m) squared

These corrections were only applied if WT was higher than IBW. CRCL estimates using the Cockcroft-Gault formula (Cockcroft and Gault 1976) based on the different size descriptors were also examined.

2.6.3 Model evaluation

Models were evaluated and compared using the following criteria:

(a) Goodness-of-fit plots, which were used as an indicator of model suitability and included the observed (measured) concentrations versus individual predictions and population predictions. Shrinkage in η and ε was also

calculated; estimates below 25% suggested that covariate and structural models were reliable (Karlsson and Savic 2007).

(b) The objective function value (OFV) given by NONMEM®, which is approximately equal to -2 x Log-Likelihood (-2LL). The difference in -2LL between two hierarchical models is approximately χ^2 distributed and reductions of 3.84 and 6.63 were considered to be statistically significant at $\alpha = 0.05$, and 0.01, respectively, for one extra parameter in the model.

(c) The precision of parameter estimates, which was expressed as the relative standard error [RSE(%)] and calculated as the ratio between the standard error provided by NONMEM® and the final parameter estimate, multiplied by 100.

(d) A reduction in BSV when adding a covariate that significantly reduces the OFV.

(e) The stability of parameter estimates and precision corresponding to the selected model, which was further evaluated by computing the 5^{th} and 95^{th} percentiles from the analysis of 1000 bootstrap data sets. The bootstrap simulations were executed using Perl-speaks-NONMEM® (PsN) version 4.6.0 (Lindbom et al. 2004).

(f) Conditional weighted residuals (CWRES) were plotted versus time after dose and population predicted concentrations to evaluate the structural and residual error models respectively. Plots were obtained using Microsoft Excel® for Mac (2016).

(g) A visual predictive check (VPC), constructed with the 5^{th}, 50^{th}, and 95^{th} percentiles of the observed data and the 95% confidence intervals (CI) of each of these percentiles of the simulated data (1000 data sets). The VPC was performed using PsN 4.6.0, the plots were performed with Xpose (version 4.3.5) (Jonsson and Karlsson 1999) implemented in R (version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria) (R Core Team 2014).

2.6.4 Derived pharmacokinetic parameters and predictions

Elimination rate constants (k) and elimination half-life ($t_{1/2}$) were calculated as follows:

$k = CL/V$

$t_{1/2} = 0.693/k$

Individual predicted concentrations at time t_2 were calculated using the following equation:

$C_2 = C_1 \times e^{-k \times (t2 - t1)}$

Where C_2 is the estimated concentration at time t_2, C_1 is the observed concentration at t_1, and k is the elimination rate constant.

2.6.5 Pharmacodynamic analysis

2.6.5.1 Concentration-time profile predictions

The final parameters of each PopPK model were used to predict total antibiotic concentrations at set times using NONMEM® with a dataset containing 100 simulations based on the original dataset of 20 patients. Single dose simulations were performed at set times after the pre-operative dose of each antibiotic as per current NHSGGC colorectal surgery antibiotic prophylaxis dosing guidelines. The simulated times are shown in Table 5.

Table 5 Simulated set times for each antibiotic.

Antibiotic and dose	**Simulated times (h)**
Amoxicillin 1000 mg	1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8
Metronidazole 500 mg and gentamicin (dose banded on height based on 3 mg/kg ideal body weight and capped at 300 mg)	1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8

Additional simulations at the same set times were performed with the following dosage regimens: pre-operative dose of amoxicillin 1000 mg re-dosed at 2 hours with either an additional dose of either 500 mg or 1000 mg, pre-operative dose of gentamicin 5 mg/kg WT as per the joint guidelines for antimicrobial prophylaxis in surgery from the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Surgical Infection Society, and the Society for Healthcare Epidemiology of America (Bratzler et al. 2013), pre-operative dose of gentamicin 5 mg/kg MBW (if WT higher than MBW), and a version of the current dosing guidelines but with the dose banded on HT based on a dose of 5 mg/kg IBW (Table 6). Post-processing and graphical analysis of the NONMEM® results were performed with Microsoft Excel® for Mac (2016) and Minitab 18 Statistical Software (Minitab Inc.).

Table 6 Gentamicin dose banded on height for use in surgical prophylaxis.

Doses based on 3 mg/kg ideal body weight (Devine 1974) (current NHSGGC dosing regimen) and 5 mg/kg ideal body weight (Devine 1974). The higher dosing regimen was adapted from the dosing regimen recommended by Bratzler et al. (2013).

	Male		**Female**	
Height ranges (cm)	**3 mg/kg dose (mg)**	**5 mg/kg dose (mg)**	**3 mg/kg dose (mg)**	**5 mg/kg dose (mg)**
142 -147	160	240	140	220
148 - 160	180	300	160	260
161 - 178	240	400	200	340
179 - 188	300	500	260	440
≥ 189	300	500	300	500

2.6.5.2 Calculation of probability of target attainment

The probability of target attainment (PTA) was defined as the percentage probability of achieving free antibiotic concentrations above the MIC (%*f*T>MIC). The clinical MIC breakpoints used in this study were those currently recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). For amoxicillin, the chosen breakpoints were 0.5 mg/L for the *Streptococcus anginosus* group and 4 mg/L for enterococci, for metronidazole, the breakpoint was 4 mg/L for the *Bacteroides fragilis* group, and for gentamicin the breakpoints were 1 mg/L for MSSA and 2 mg/L for *Escherichia coli* (European Committee on Antimicrobial Susceptibility Testing 2018). The free (unbound) drug concentrations were estimated using published protein binding values for each antibiotic. The protein binding values used in this this study were 17% for amoxicillin (Sutherland et al. 1972), 15% for metronidazole (Dubreuil 2005, European Committee on Antimicrobial Susceptibility Testing 2010), and 0% for gentamicin (Gordon et al. 1972).

2.7 Surgical site infection follow-up

The SSI follow-up was undertaken for each patient for 30 days after the surgical procedure. The medical notes and microbiology culture results were used as sources for identification of potential SSIs. The type of SSI was divided into incisional and organ/space SSI. Incisional SSIs were further divided into superficial incisional and deep incisional SSI (Table 1). The diagnostic criteria used to determine an SSI (Table 7) were the criteria specified on the Health Protection Scotland SSI surveillance form (Appendix 6, Surgical site infection surveillance, large bowel surgery, data collection form). The SSI rate was calculated as:

SSI rate (%) = number of SSIs / number of patients included x 100.

Table 7 Criteria used to determine a surgical site infection (Appendix 6, Surgical site infection surveillance, large bowel surgery, data collection form).

Criteria used to determine SSI	
• Purulent drainage • Localised swelling • Redness • Heat	• Localised pain or tenderness • Incision spontaneously dehisces • Incision is deliberately opened by surgeon • Fever (temperature 38 degrees or more)
• Abscess/other evidence found during direct examination, a re-operation or radiology/histopathology • Organisms isolated from an aseptically obtained culture of fluid, tissue, blood, bone or biopsy • Diagnosis by surgeon or trained healthcare worker	
Extra criteria organ/space only	
• Nausea • Vomiting • Jaundice	• Organisms seen on Gram stain • Radiographic evidence of infection

Key: SSI, surgical site infection.

3 RESULTS

3.1 Patient characteristics

Twenty adult patients (11 women and nine men) undergoing elective colorectal surgery participated in this study. Details of demographic and laboratory characteristics for each patient are provided in Table 8. The ages of the patients ranged from 18 to 81 years (mean 57 years) and WT from 48 to 102 kg (mean 74 kg); eight patients had a normal weight and 12 were overweight or obese (BMI $\geq$25 kg/m^2). Estimated CRCL ranged from 50 to 166 ml/min (mean 105 ml/min).

The surgery related characteristics of the patient group are summarised in Table 9. Laparoscopic procedures were conducted in 11 patients (55%), and nine patients (45%) underwent open resections. Eighteen patients (90%) underwent surgery for malignant indications and the remaining two patients (10%) for benign indications. Left colonic/rectal resection was the most common procedure (performed in 10 patients). A stoma was formed in five patients (25%). A blood transfusion was required in two patients (10%). The IV Hartmann's solution volume administered during surgery ranged from 1200 to 6000 ml (mean 3305 ml). The length of the surgical procedure ranged from 1.5 to 8 hours; the mean surgical time from incision to closure was 4.5 hours.

Overall, 13 patients (65%) required an additional dose of amoxicillin and two of those patients also had re-dosing of gentamicin and metronidazole due to blood loss. The remaining seven patients (35%) had no re-dosing of prophylactic antibiotics intra-operatively.

Table 8 Demographic and laboratory characteristics of the patient group.

Patient no	Sex	Age (years)	HT (cm)	WT (kg)	BMI (kg/m²)	Cr (µmol/L)	CRCL (mL/min)	Alb (g/L)
1	F	32	166	68	24.7	59	129	37
2	F	80	158	76	30.4	60	79	37
3	M	68	167	102	36.5	85	106	38
4	M	18	167	75	26.9	68	166	42
5	M	33	179	71	22.2	67	139	36
6	F	56	154	56	23.6	53	92	34
7	M	63	169	72	25.2	69	99	40
8	M	81	175	67	21.9	97	50	33
9	F	63	153	54	23.2	68	64	36
10	F	73	148	71	32.6	69	72	40
11	M	41	176	82	26.4	65	153	40
12	M	63	176	94	30.3	63	141	39
13	F	54	156	99	40.7	56	158	40
14	F	57	159	71	28.1	68	90	40
15	F	75	144	48	23.0	64	50	36
16	M	45	173	62	20.7	65	111	32
17	F	56	168	86	30.5	77	98	36
18	F	69	157	69	28.0	61	83	40
19	M	59	170	94	32.4	73	128	37
20	F	44	168	55	19.4	62	88	44
Mean		**57**	**164**	**74**	**27.3**	**67**	**105**	**38**
SD		**17**	**10**	**15**	**5.5**	**10**	**35**	**3**

Key: no, number; HT, height; WT, total body weight; BMI, body mass index; Cr, creatinine concentration; CRCL, creatinine clearance estimated by the Cockcroft-Gault formula (Cockcroft and Gault 1976); Alb, albumin concentration; SD, standard deviation.

Table 9 Surgery related characteristics of the patient group.

Patient no	Resection/anastomosis type	Stoma formation Yes/No	Laparoscopic/ open	Benign/ malignant indication	EBL (ml)	Blood products (ml)	IVs (ml)	Dur (h)
1	Left colonic/rectal resection	No	Laparoscopic	Malignant	<150	-	2500	4.5
2	Left colonic/rectal resection	No	Laparoscopic	Malignant	150	-	2500	3.7
3	Small bowel resection	No	Open	Malignant	<150	-	1500	2.1
4	Sub-total colectomy	No	Laparoscopic	Malignant	605	-	2500	6.5
5	Pelvic exenteration	Yes	Open	Malignant	1825	245	6000	6.8
6	Pelvic exenteration	No	Open	Benign	1650	588	3100	5.3
7	Left colonic/rectal resection	No	Laparoscopic	Malignant	200	-	2800	4.5
8	Right colonic resection	No	Laparoscopic	Malignant	<150	-	3500	2.8
9	Right colonic resection	No	Open	Malignant	<150	-	1200	1.5
10	Left colonic/rectal resection	Yes	Laparoscopic	Malignant	<150	-	3000	5.4
11	Abdomino-perineal resection	Yes	Open	Malignant	1600	-	4500	8.0
12	Left colonic/rectal resection	Yes	Laparoscopic	Malignant	710	-	4000	4.8
13	Left colonic/rectal resection	No	Open	Malignant	1300	-	4500	3.5
14	Abdomino-perineal resection	No	Open	Malignant	1500	-	3900	6.9
15	Left colonic/rectal resection	No	Laparoscopic	Malignant	<150	-	2150	3.8
16	Abdomino-perineal resection	No	Open	Benign	1100	-	5500	5.2
17	Left colonic/rectal resection	Yes	Open	Malignant	200	-	2500	1.9
18	Left colonic/rectal resection	No	Laparoscopic	Malignant	350	-	4000	5.3
19	Right colonic resection	No	Laparoscopic	Malignant	230	-	3750	3.1
20	Left colonic/rectal resection	No	Laparoscopic	Malignant	<150	-	2700	4.2
Mean							**3305**	**4.5**
SD							**1233**	**1.8**

Key: no; number, EBL, estimated blood loss; IVs, intravenous Hartmann's solution volume administered during surgery; Dur, duration of surgery from incision to skin closure; SD, standard deviation.

3.2 Drug administration and sampling

Amoxicillin and gentamicin were administered by bolus injection. Metronidazole was administered as an infusion over 3 to 20 minutes (median 6 minutes). Blood samples were processed and serum was stored within 0.3 to 3.2 hours (median 0.6 hours) after collection.

3.3 Amoxicillin data and analysis

3.3.1 Concentration-time profiles

A total of 99 serum amoxicillin concentration measurements were available, with a median of 5 (range 3 – 6) measurements per patient. The pre-dose sample from each patient was used as a blank during drug quantification. Four samples were excluded from the final data set; two due to uncertainty regarding the sample time and two that were taken during the distribution phase (within 17 minutes) after the second dose of amoxicillin (time of skin closure). Individual concentration-time profiles are presented on linear and log-linear scales in figures 4a and b, respectively. The shape of the profiles in figure 4b suggests that the data would be adequately described using a one-compartment model.

After the first dose of amoxicillin, the 1 hour concentrations ranged from 14.6 to 35.8 mg/L with a median of 26.9 mg/L. Concentrations at 4 hours after the first dose were available from 12 patients and ranged from 1.6 to 5.9 mg/L with a median of 3.9 mg/L.

3.3.2 Population analysis and covariate model

A one-compartment structural model was satisfactory to describe the individual concentration-time profiles. RE was initially described using a combined error model but the additive component was negligible and removal of this parameter had no influence on the OFV. A proportional error model was therefore used for the covariate analysis. Allowing covariance in BSV between CL and V led to a reduction in OFV of 18.37 and was retained for subsequent analyses.

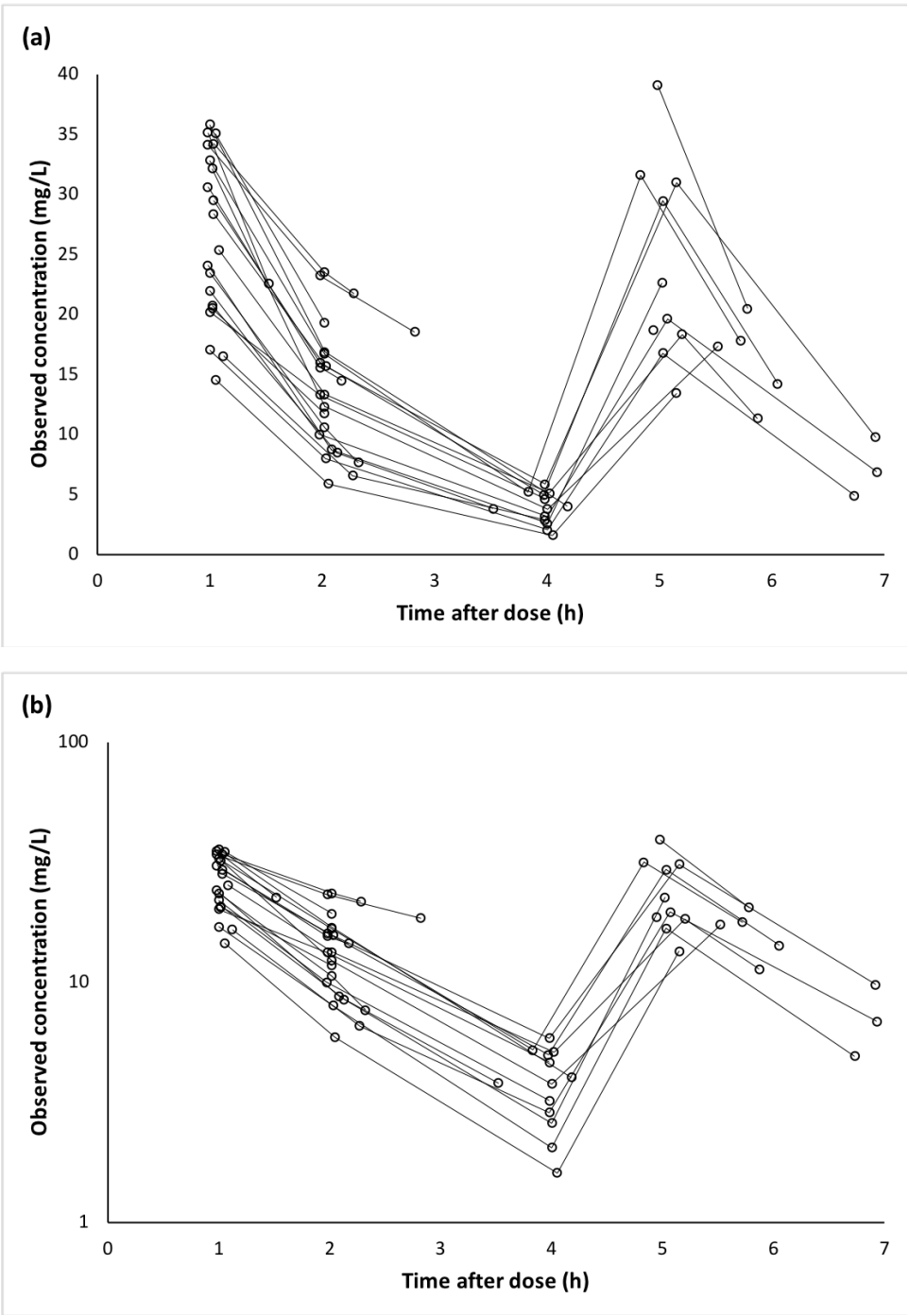


Figure 4 Amoxicillin concentration measurements in 20 colorectal surgery patients following intravenous doses of 1000 mg.

An additional dose of 1000 mg was administered to 13 patients at 4 hours. (a) Linear concentration scale, (b) log-linear concentration scale.

Figure 5 shows scatter plots of WT and AJBW versus age, HT and sex, and CRCL versus WT and AJBW. Clear relationships were identified between WT/AJBW and HT and sex, and between CRCL and WT/AJBW.

Figure 6 shows scatter plots and box plots of individual estimates of amoxicillin CL and V versus clinical characteristics. Box plots suggested that both CL and V were higher in males while the scatter plots suggested that CL declined with age and that both CL and V increased with WT, HT, and CRCL. There were no apparent relationships between CL or V and albumin (data not shown) or creatinine concentrations.

The following characteristics were tested in the covariate model: age, WT, HT and CRCL. As the data set covered a wide range of BMI values, CRCL estimates based on WT, IBW, AJBW, MBW, and LBW were also tested.

Table 10 summarises the key covariate models that were tested during model development. Including WT and LBW in the model had a negligible impact but there was a small improvement in fit when HT, IBW, MBW or AJBW was used rather than WT; allometric scaling offered no advantage. Adding CRCL achieved a further improvement in fit. Model 11 with AJBW combined with CRCL based on AJBW (CRCA) was initially considered as a final model. However, the parameters of this model had high CV, the bootstrap procedure only had a 61.7% convergence rate, there was a wide range in the 90% CI of the CL parameter estimates and the median bootstrap estimates differed from those identified in the original analysis. Therefore, model 7, which only included AJBW, was chosen as the final model.

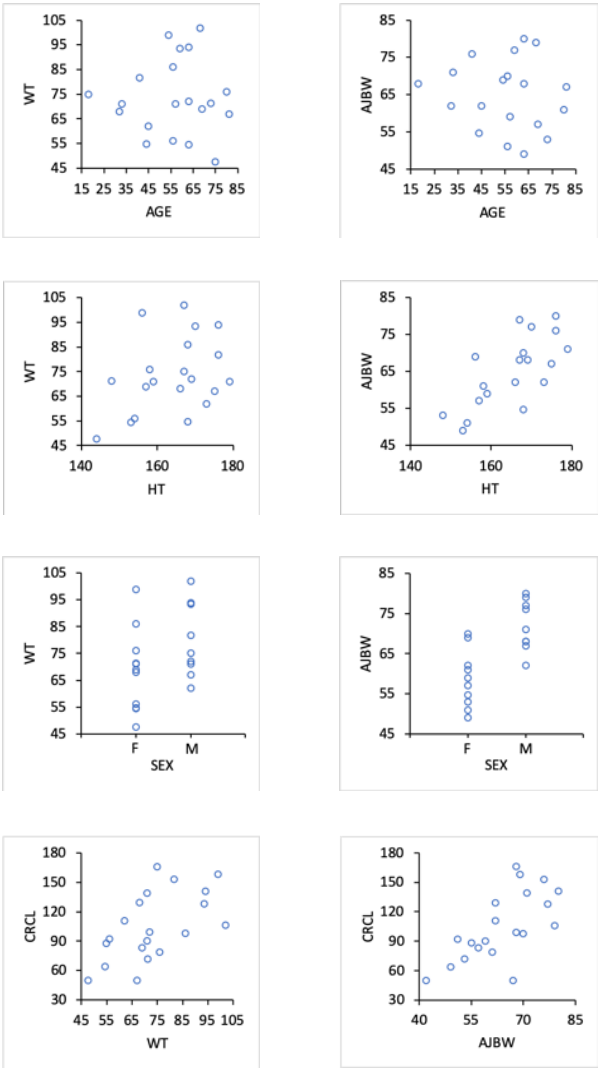


Figure 5 Scatter plots of weight versus age, height, sex, and creatinine clearance versus weight (left panel) and adjusted body weight versus age, height, sex, and creatinine clearance versus adjusted body weight (right panel).

Key: Blue circles are individual data points. WT, weight (kg); Age (years); HT, height (cm); sex (F female, M male); CRCL, creatinine clearance (ml/min); AJBW, adjusted body weight (kg).

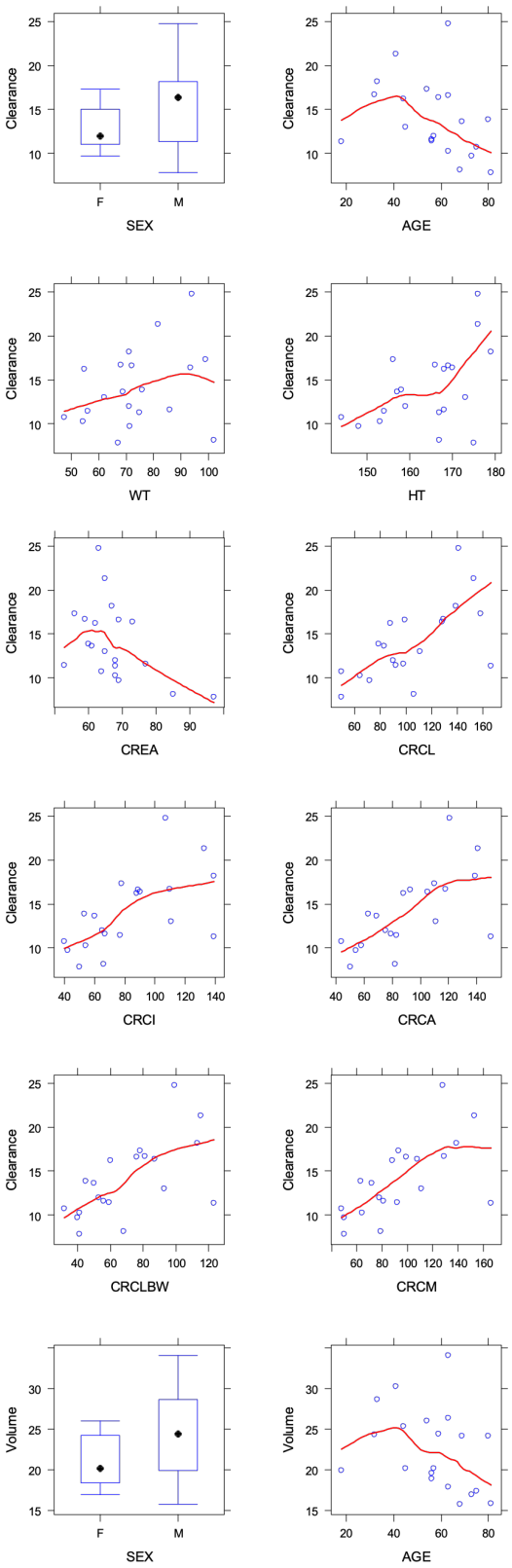


Figure 6 Scatter plots of individual estimates of amoxicillin clearance and volume of distribution versus clinical characteristics.

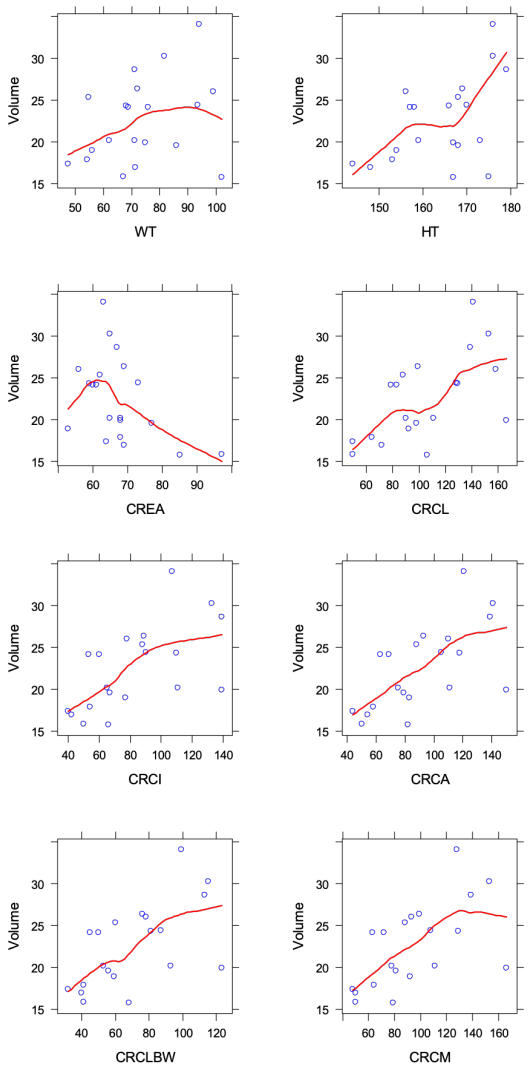


Figure 6 (continued) Scatter plots of individual estimates of amoxicillin clearance and volume of distribution versus clinical characteristics.

Key: Blue circles are individual data points, the red line is a smooth through the data. Sex (F female, M male); age (years); WT, weight (kg); HT, height (cm); Alb, albumin (g/L); CREA, creatinine concentration (µmol/L); CRCL, creatinine clearance (ml/min) based on total body weight; CRCI, creatinine clearance (ml/min) based on ideal body weight; CRCA, creatinine clearance (ml/min) based on adjusted body weight; CRCLBW, creatinine clearance (ml/min) based on lean body weight; CRCM, creatinine clearance (ml/min) based on maximum body weight.

Table 10 Summary of the key amoxicillin covariate models.

Model	CL	V	OFV	Model for comparison	ΔOFV
1	θ1	θ2	224.79		
2	θ1 x WT	θ2 x WT	224.30	1	0.49
3	θ1 x HT	θ2 x HT	220.96	1	3.83
4	θ1 x LBW	θ2 x LBW	223.73	1	1.06
5	θ1 x IBW	θ2 x IBW	219.24	1	5.55
6	θ1 x MBW	θ2 x MBW	219.00	1	5.79
7	θ1 x AJBW	θ2 x AJBW	218.84	1	5.95
8	θ1 x CRCA	θ2 x AJBW	223.33	1	1.46
9	θ1 x IBW + θ4 x CRCI	θ2 x IBW	212.97	5	6.27
10	θ1 x MBW + θ4 x CRCM	θ2 x MBW	212.81	6	6.19
11	θ1 x AJBW + θ4 x CRCA	θ2 x AJBW	212.53	7	6.31

Key: CL, clearance; V, volume of distribution; OFV, objective function value; ΔOFV, objective function value difference; WT, total body weight; HT, height; LBW, lean body weight; IBW, ideal body weight; MBW; maximum body weight; AJBW, adjusted body weight; CRCL, creatinine clearance based on total body weight; CRCI, creatinine clearance based on ideal body weight; CRCM, creatinine clearance based on maximum body weight; CRCA, creatinine clearance based on adjusted body weight.

Table 11 lists the PopPK parameters of the amoxicillin base and final models. The final model led to a small reduction in BSV in CL from 31.2% to 28.7% and in V from 23.7% to 17.9%. All estimates of shrinkage were below 25%. The convergence rate of the bootstrap procedure was 79.6%; the population parameter estimates were similar to the median of the bootstrap estimates and the 90% CI ranges for CL and V were narrow.

Table 11 Population pharmacokinetic parameters of the amoxicillin base and final models.

Model Parameter	Estimate (% RSE)		Shrinkage (%)	Bootstrap analysis
	Base model	Final model		Median (5th-95th percentile)
CL (L/h) θ_1	13.4 (7.0)	0.213 (6.6)	-	0.214 (0.188-0.236)
V (L) θ_2	22.0 (6.2)	0.353 (4.8)	-	0.356 (0.328-0.384)
BSV CL (%)	31.2 (27.0)	28.7 (40.4)	η_{sh} = 0.00	27.7 (16.2-37.7)
BSV V (%)	23.7 (27.5)	17.9 (32.2)	η_{sh} = 6.29	17.5 (12.3-22.7)
RE Proportional (% cv)	12.4 (11.9)	12.2 (11.7)	ε_{sh} = 16.1	12.2 (9.8-14.6)

Structure of final model: TVCL = θ_1 x AJBW; TVV = θ_2 x AJBW

Key: RSE, relative standard error; CL, clearance; V, volume of distribution; BSV, between-subject variability expressed as a % coefficient of variation (cv); RE, residual error; η_{sh}, shrinkage in eta; ε_{sh}, shrinkage in the residual error; TVCL, typical value of clearance (L/h); TVV, typical value of volume of distribution (L); AJBW, adjusted body weight (kg).

Figure 7 shows the measured versus the predicted concentrations for the base and final models. Overall, there is good agreement between the measured concentrations and the concentrations predicted by the PopPK model and the points are uniformly distributed along the line of identity with no obvious bias. There is a small improvement in the fit of the final population model compared to the base model. In both cases, there was a very close relationship between measured concentrations and concentrations based on individual parameter estimates. The ability of the final model to describe individual data is further demonstrated in Appendix 7, which shows profiles of measured, population predicted and individual predicted amoxicillin concentrations versus time for each patient. CWRES plots versus time after dose and population predicted concentrations are shown in Appendix 10. The data are evenly distributed about zero indicating no bias in either the structural or the error model. The VPC plot presented in Figure 8 shows that the percentiles based on the observed data are within the CI bands based on the simulated data and the measured concentrations are consistent with the final model predictions.

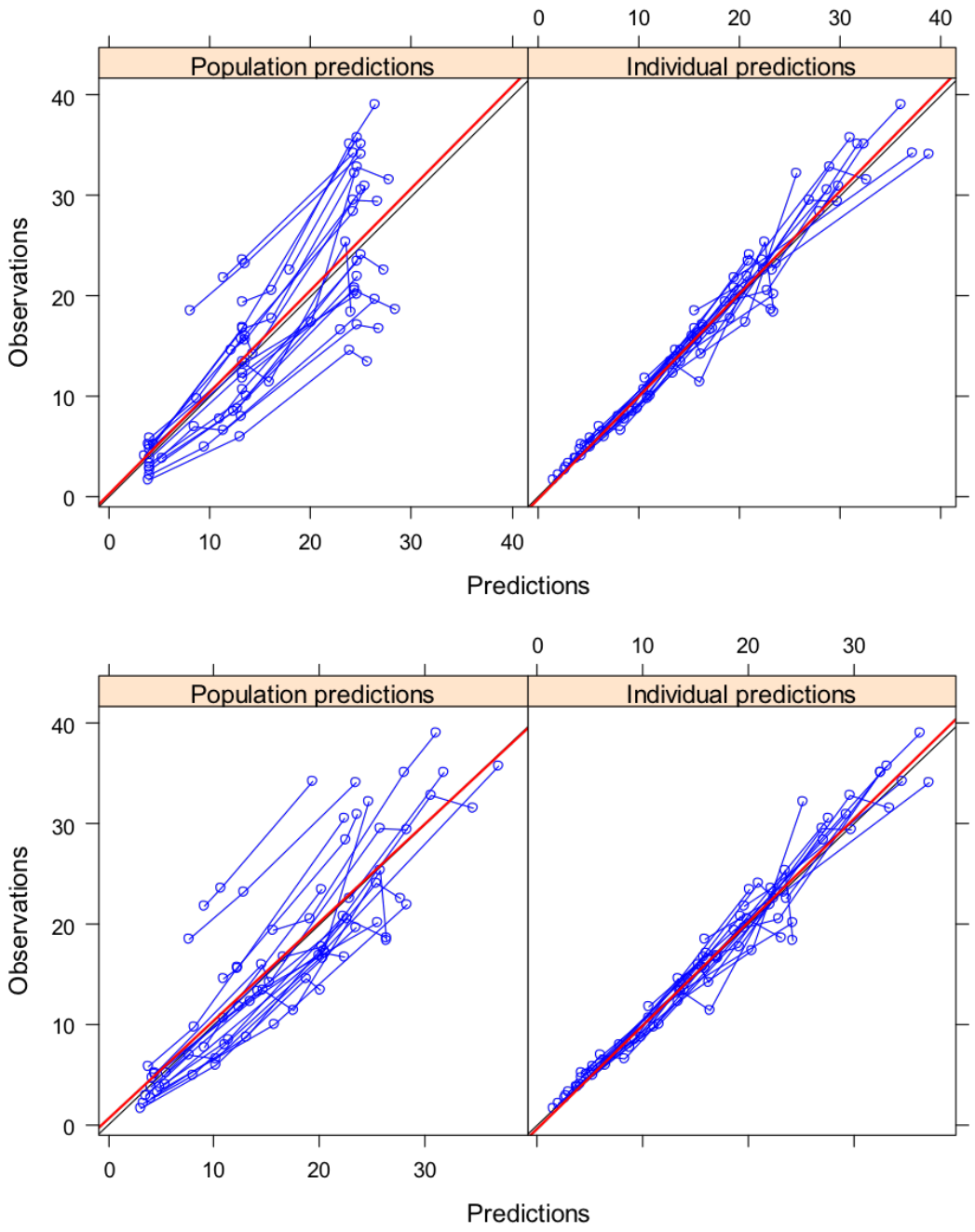


Figure 7 Plots of measured amoxicillin concentration versus population (left panel) and individual (right panel) predicted concentrations.

Key: The thin black line represents the line of identity; the thick red line is a smooth of the data. The top panel shows the results from the base model; the bottom panel shows the results from the final model. Individual profiles are represented by blue circles joined by a blue line.

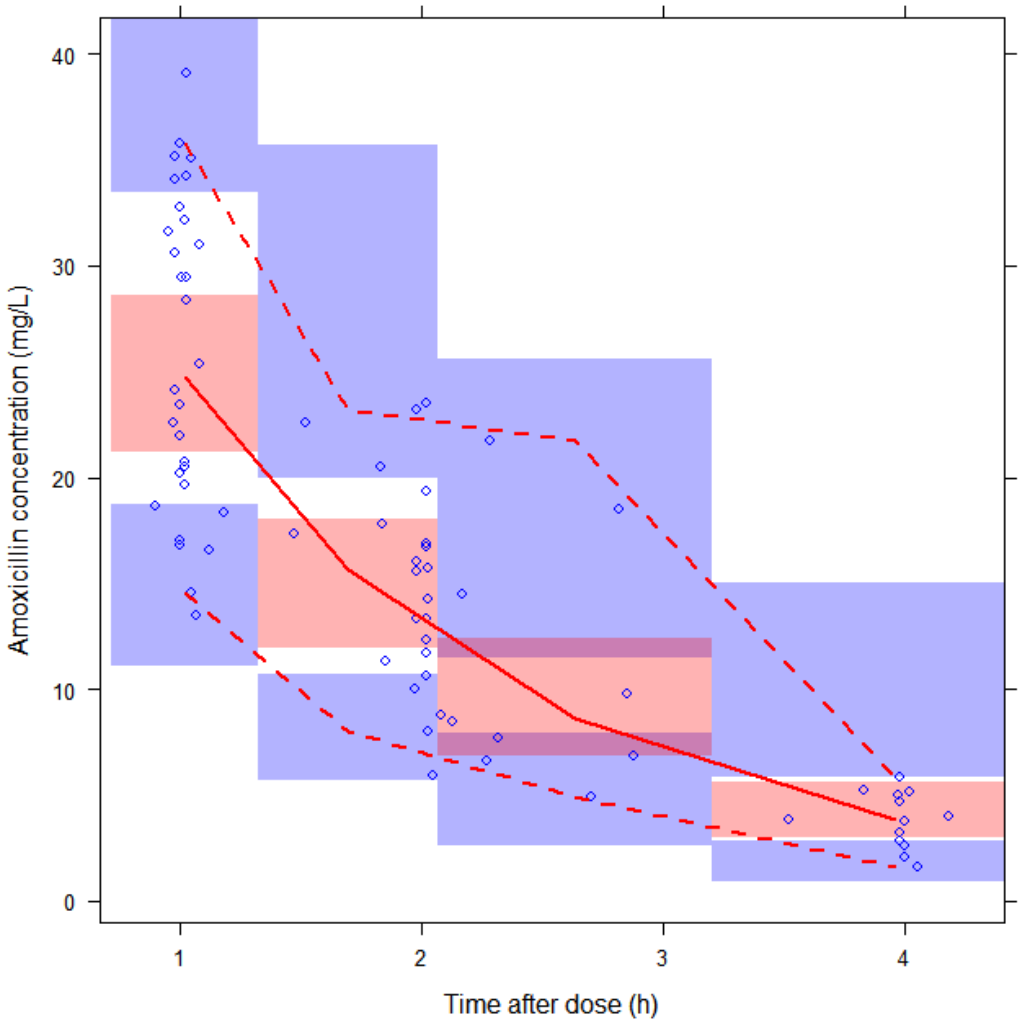


Figure 8 Visual predictive check of the final amoxicillin model.

Key: Observed concentration data are represented by blue circles. The solid red line represents the 50thpercentile of the observed data, the dashed red lines are the 5th, and 95thpercentiles of the observed data. The pink shaded areas represent the 95% confidence intervals of the 50thpercentile of the simulated data, the blue shaded areas are the 95% confidence intervals of the 5thand 95thpercentiles of the simulated data.

3.3.3 Pharmacokinetic parameters

The individual estimates of CL ranged from 7.9 to 25.2 L/h (mean 14.0 L/h); the individual estimates of V ranged from 15.7 to 35.3 L (mean 22.7 L); and the individual estimates of the elimination $t_{1/2}$ ranged from 1.0 to 1.5 h (mean 1.2 h). Individual parameter estimates for each patient are listed in Appendix 11.

3.4 Metronidazole data and analysis

3.4.1 Concentration-time profiles

A total of 99 serum metronidazole concentration measurements were available, with a median of 5 (range 3 – 6) measurements per patient. The pre-dose sample for each patient was used as a blank during drug quantification. Three samples were excluded from the final data set; one due to uncertainty regarding the sample time and two that were taken during the distribution phase. Individual concentration-time profiles are presented on linear and log-linear scales in figures 9a and b, respectively. The shape of the profiles in figure 9b suggests that the data would be adequately described using a one-compartment model.

Metronidazole concentrations at 1 hour after the first dose were available from 18 patients and ranged from 9.2 to 20.3 mg/L with a median of 14.1 mg/L. Concentrations at skin closure were available from 20 patients. Excluding the two patients who had a second dose of metronidazole, the skin closure sample times ranged from 1.6 to 6.9 hours (median 4.6 hours) and the concentrations ranged from 6.5 to 14.5 mg/L with a median of 9.7 mg/L.

3.4.2 Population analysis and covariate model

A one-compartment structural model was satisfactory to describe the individual concentration-time profiles. RE was initially described using a combined error model but the additive component was negligible and removal of this parameter had no influence on the OFV. A proportional error model was therefore used for the covariate analysis. Allowing covariance in BSV between CL and V did not lead to a significant reduction in OFV (0.13 points) and was therefore not included.

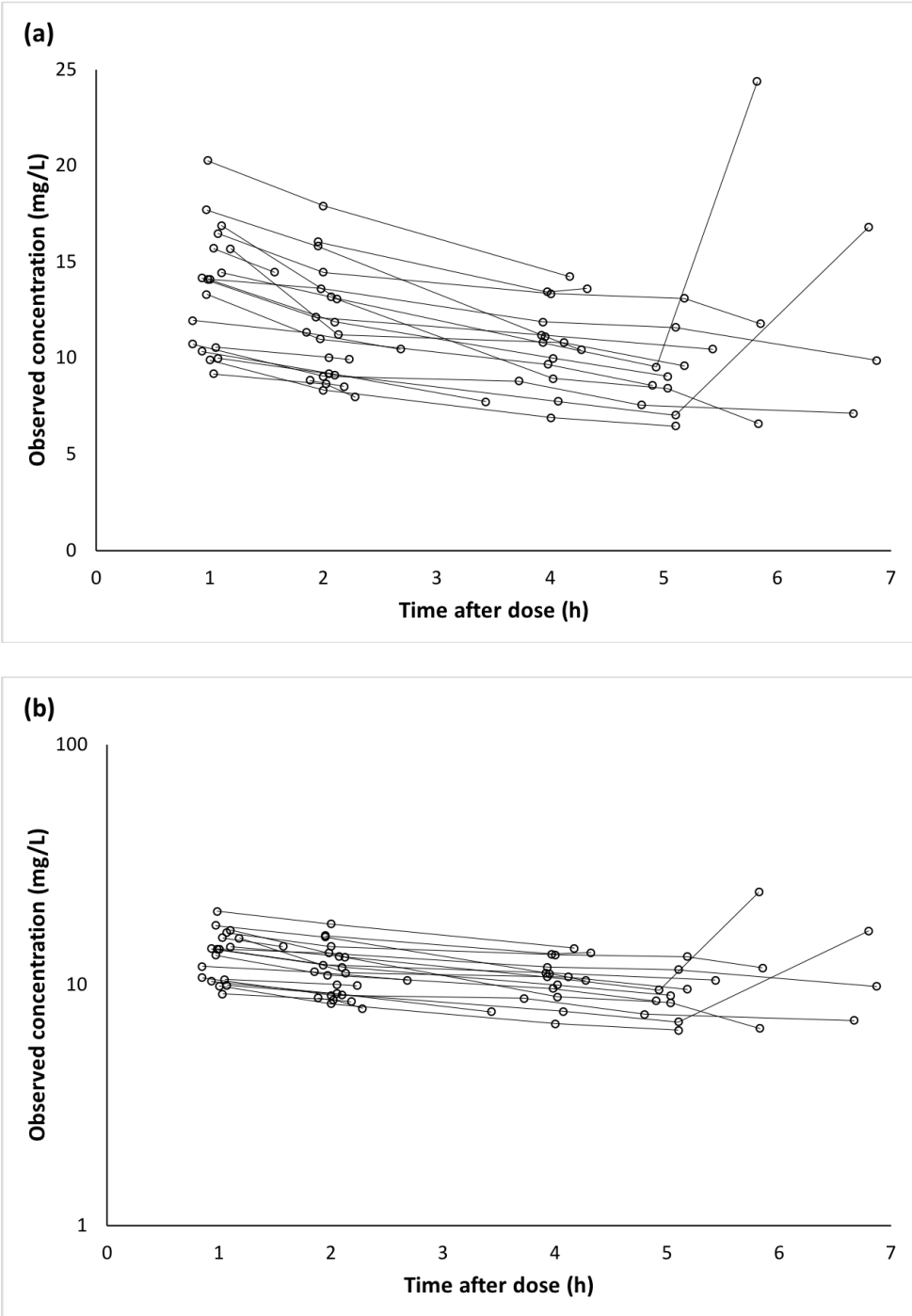


Figure 9 Metronidazole concentration measurements in 20 colorectal surgery patients following intravenous doses of 500 mg.

An additional dose of 500 mg was administered to two patients at 5 and 6 hours. (a) Linear concentration scale, (b) log-linear concentration scale.

Figure 10 shows scatter plots and box plots of individual estimates of metronidazole CL and V versus clinical characteristics. Box plots suggested that V estimates were higher in males while the scatter plots suggested that both CL and V increased with WT and V also increased with HT. There were no apparent relationships between CL and sex or HT, also, no apparent relationships were observed between CL or V and age, albumin concentrations (data not shown), creatinine concentrations, and CRCL.

Table 12 summarises the key covariate models that were tested during model development. Adding CRCL offered no improvement in fit (data not shown). There was an improvement in fit when WT, LBW and AJBW were included as descriptors of CL and V. The best model included an allometric relationship between CL and WT and a linear relationship between V and AJBW.

Table 13 lists the PopPK parameters of the metronidazole base and final models. The final model led to a reduction in BSV in CL from 31.3% to 26.7% and in V from 25.3% to 10.8%. All estimates of shrinkage were below 25%. The convergence rate of the bootstrap procedure was 99.8%; the population parameter estimates were similar to the median of the bootstrap estimates and the 90% CI ranges for CL and V were narrow.

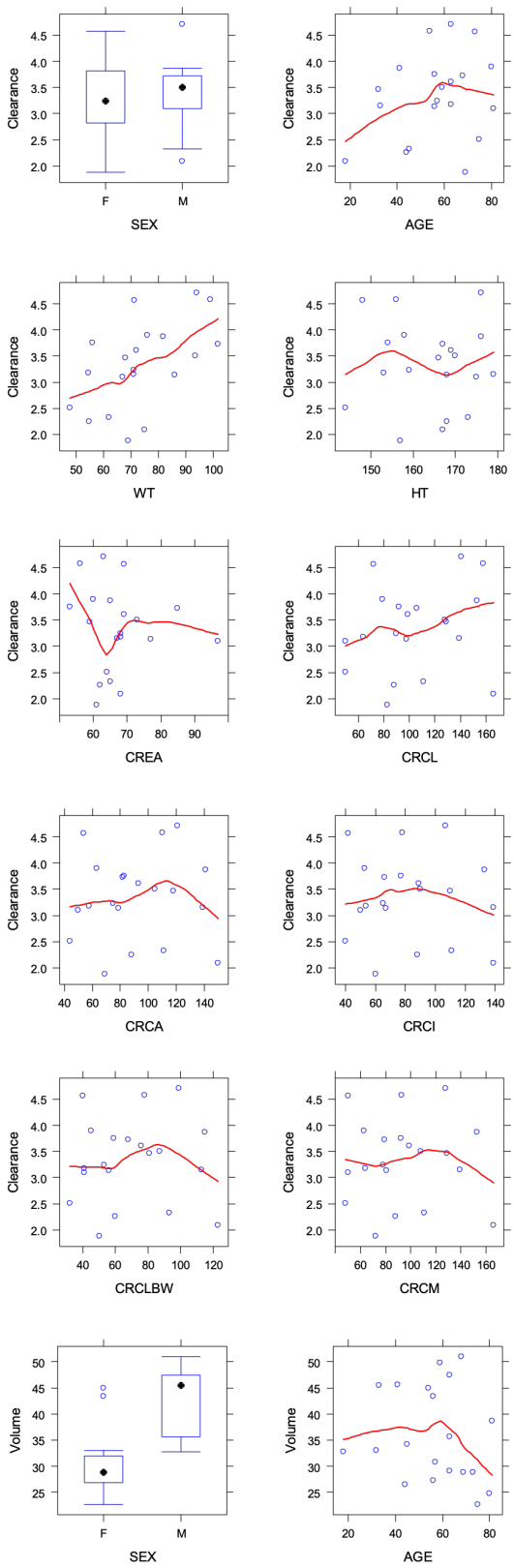


Figure 10 Scatter plots of individual estimates of metronidazole clearance and volume of distribution versus clinical characteristics.

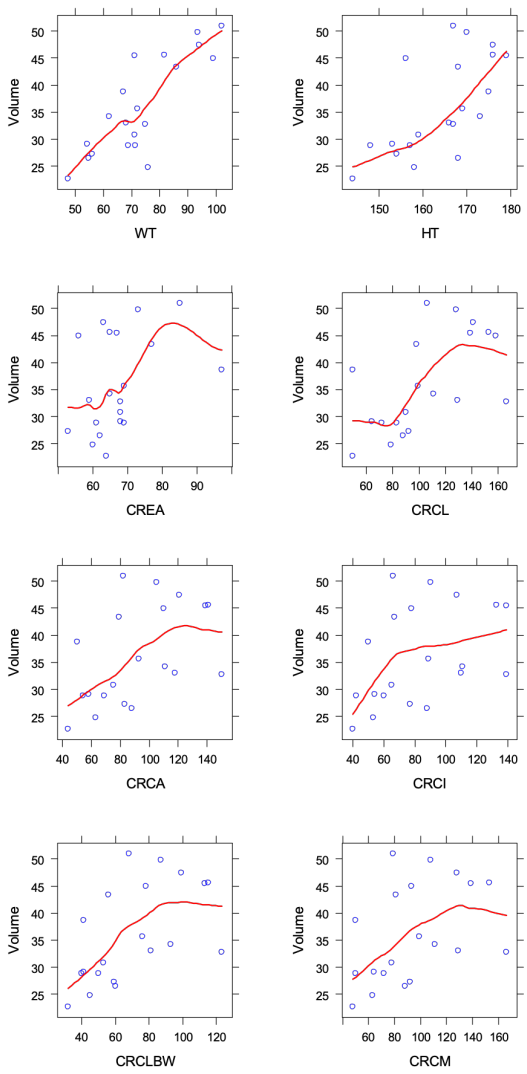


Figure 10 (continued) Scatter plots of individual estimates of metronidazole clearance and volume of distribution versus clinical characteristics.

Key: Blue circles are individual data points, the red line is a smooth through the data. Sex (F female, M male); age (years); WT, weight (kg); HT, height (cm); Alb, albumin (g/L); CREA, creatinine concentration (μmol/L); CRCL, creatinine clearance (ml/min) based on total body weight; CRCA, creatinine clearance (ml/min) based on adjusted body weight; CRCI, creatinine clearance (ml/min) based on ideal body weight; CRCLBW, creatinine clearance (ml/min) based on lean body weight; CRCM, creatinine clearance (ml/min) based on maximum body weight.

Table 12 Summary of the key metronidazole covariate models.

Model	CL	V	OFV	Model for comparison	ΔOFV
1	θ_1	θ_2	89.84		
2	θ_1 x WT	θ_2 x WT	63.26	1	26.58
3	θ_1 x WT	θ_2 x HT	75.45	1	14.39
4	θ_1 x AJBW	θ_2 x AJBW	58.72	1	31.12
5	θ_1 x IBW	θ_2 x IBW	76.52	1	13.32
6	θ_1 x LBW	θ_2 x LBW	61.48	1	28.36
7	θ_1 x MBW	θ_2 x MBW	70.71	1	19.13
8	θ_1 x $(WT/70)^{0.75}$	θ_2 x AJBW	54.42	4	4.30

Key: CL, clearance; V, volume of distribution; OFV, objective function value; ΔOFV, objective function value difference; WT, total body weight; HT, height; AJBW, adjusted body weight; IBW, ideal body weight; LBW, lean body weight; MBW, maximum body weight.

Table 13 Population pharmacokinetic parameters of the metronidazole base and final models.

	Estimate (% RSE)			Bootstrap analysis
Model Parameter	Base model	Final model	Shrinkage (%)	Median (5^{th}-95^{th} percentile)
CL (L/h) θ_1	3.19 (8.1)	3.22 (7.0)	-	3.24 (2.87-3.63)
V (L) θ_2	35.1 (5.5)	0.556 (2.5)	-	0.555 (0.533-0.577)
BSV CL (%)	31.3 (31.5)	26.7 (40.9)	η_{sh} = 13.8	25.6 (13.9-34.4)
BSV V (%)	25.3 (19.9)	10.8 (43.1)	η_{sh} = 6.14	10.3 (6.1-14.1)
RE Proportional (% cv)	4.50 (15.7)	4.46 (15.6)	ε_{sh} = 22.9	4.39 (3.32-5.57)

Structure of final model: TVCL = θ_1 x $(WT/70)^{0.75}$; TVV = θ_2 x AJBW

Key: RSE, relative standard error; CL, clearance; V, volume of distribution; BSV, between-subject variability expressed as a % coefficient of variation (cv); RE, residual error; η_{sh}, shrinkage in eta; ε_{sh}, shrinkage in the residual error; TVCL, typical value of clearance (L/h); TVV, typical value of volume of distribution; AJBW, adjusted body weight (kg).

Figure 11 shows the measured versus the predicted concentrations for the base and final models. Overall, there is good agreement between the measured concentrations and the concentrations predicted by the PopPK model and the points are uniformly distributed along the line of identity with no obvious bias. There is an improvement in the fit of the final population model compared to the base model. In both cases, there was a very close relationship between measured concentrations and concentrations based on individual parameter estimates. The ability of the final model to describe individual data is further demonstrated in Appendix 8, which shows profiles of measured, population predicted and individual predicted metronidazole concentrations versus time for each patient. CWRES plots versus time after dose and population predicted concentrations are shown in Appendix 10. The data are evenly distributed about zero indicating no bias in either the structural or the error model. The VPC plot presented in Figure 12 shows that the percentiles based on the observed data are within the CI bands based on the simulated data and the measured concentrations are consistent with the final model predictions.

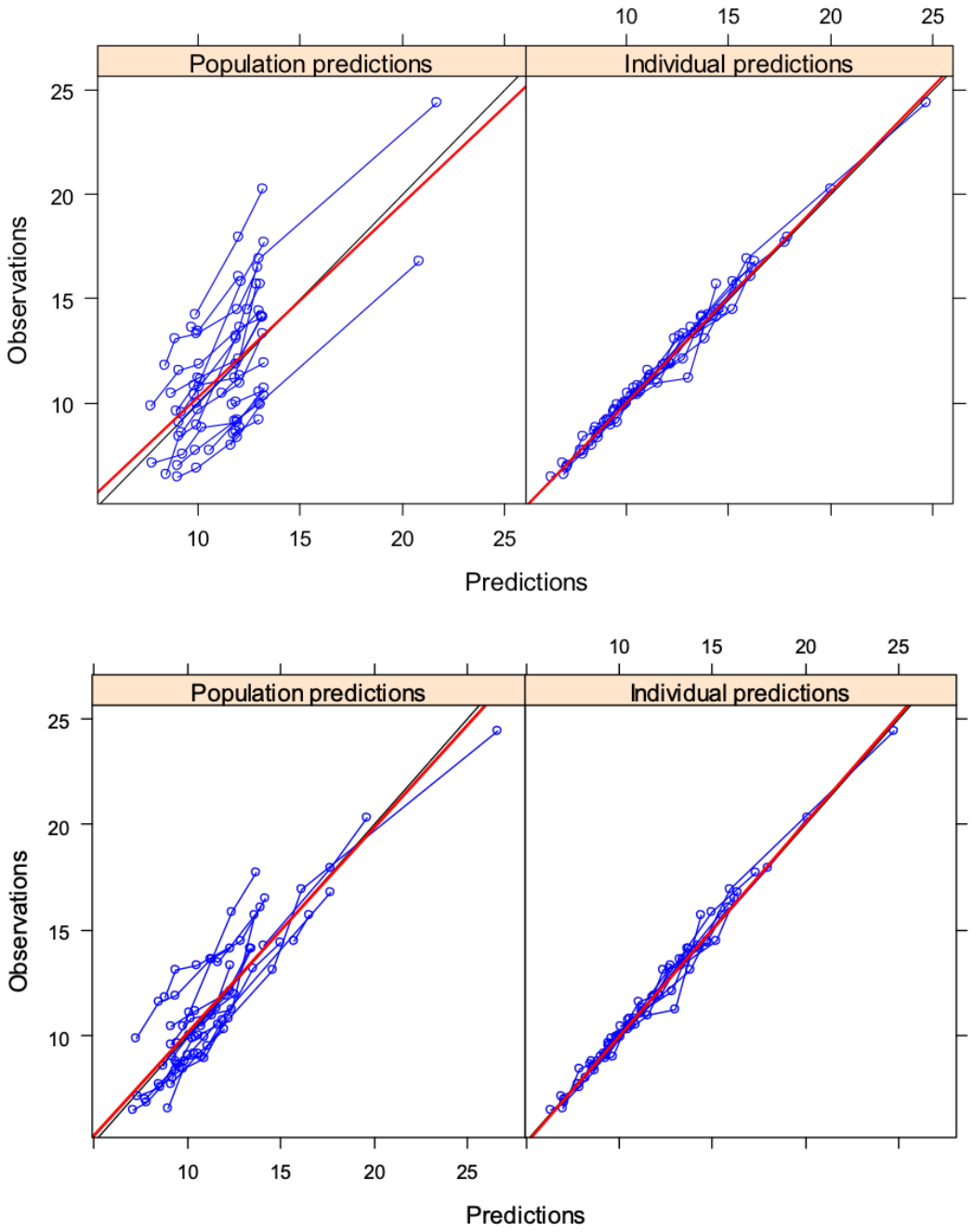


Figure 11 Plots of measured metronidazole concentration versus population (left panel) and individual (right panel) predicted concentrations.

Key: The thin black line represents the line of identity; the thick red line is a smooth of the data. The top panel shows the results from the base model; the bottom panel shows the results from the final model. Individual profiles are represented by blue circles joined by a blue line.

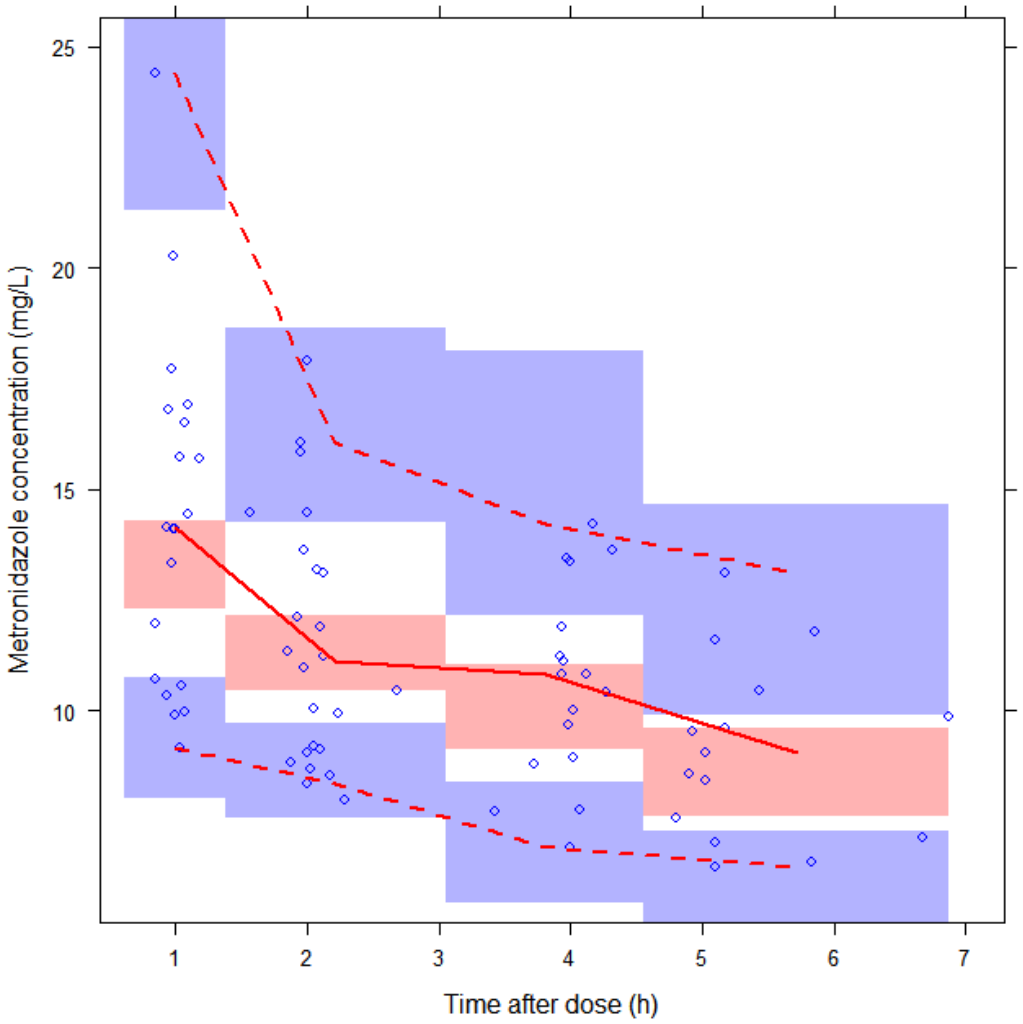


Figure 12 Visual predictive check of the final metronidazole model.

Key: Observed concentration data are represented by blue circles. The solid red line represents the 50thpercentile of the observed data, the dashed red lines are the 5th, and 95thpercentiles of the observed data. The pink shaded areas represent the 95% confidence intervals of the 50thpercentile of the simulated data, the blue shaded areas are the 95% confidence intervals of the 5thand 95thpercentiles of the simulated data.

3.4.3 Pharmacokinetic parameters

The individual estimates of CL ranged from 1.9 to 5.1 L/h (mean 3.5 L/h); the individual estimates of V ranged from 22.7 to 48.6 L (mean 35.7 L); and the individual estimates of the elimination $t_{1/2}$ ranged from 4.4 to 11.0 h (mean 7.5 h). Individual parameter estimates for each patient are listed in Appendix 11.

3.5 Gentamicin data and analysis

3.5.1 Concentration-time profiles

A total of 99 serum gentamicin concentration measurements were available, with a median of 5 (range 3 – 6) measurements per patient. The pre-dose sample for each patient was used as a blank during drug quantification. All samples were included in the final data set. Individual concentration-time profiles are presented on linear and log-linear scales in figures 13a and b, respectively. The shape of the profiles in figure 13b suggests that the data would be adequately described using a one-compartment model.

Gentamicin concentrations at 1 hour after the first dose were available from 20 patients and ranged from 7.8 to 13.7 mg/L with a median of 9.9 mg/L. Concentrations at skin closure were available from 20 patients. Excluding the two patients who had a second dose of gentamicin, the skin closure sample times ranged from 1.6 to 6.9 hours (median 4.6 hours) and the concentrations ranged from 1.6 to 9.7 mg/L with a median of 3.2 mg/L.

3.5.2 Population analysis and covariate model

A one-compartment structural model was satisfactory to describe the individual concentration-time profiles. RE was initially described using a combined error model but the additive component was negligible and removal of this parameter had no influence on the OFV. A proportional error model was therefore used for the covariate analysis. Allowing covariance in BSV between CL and V led to a reduction in OFV of 4.59 and was retained for subsequent analyses.

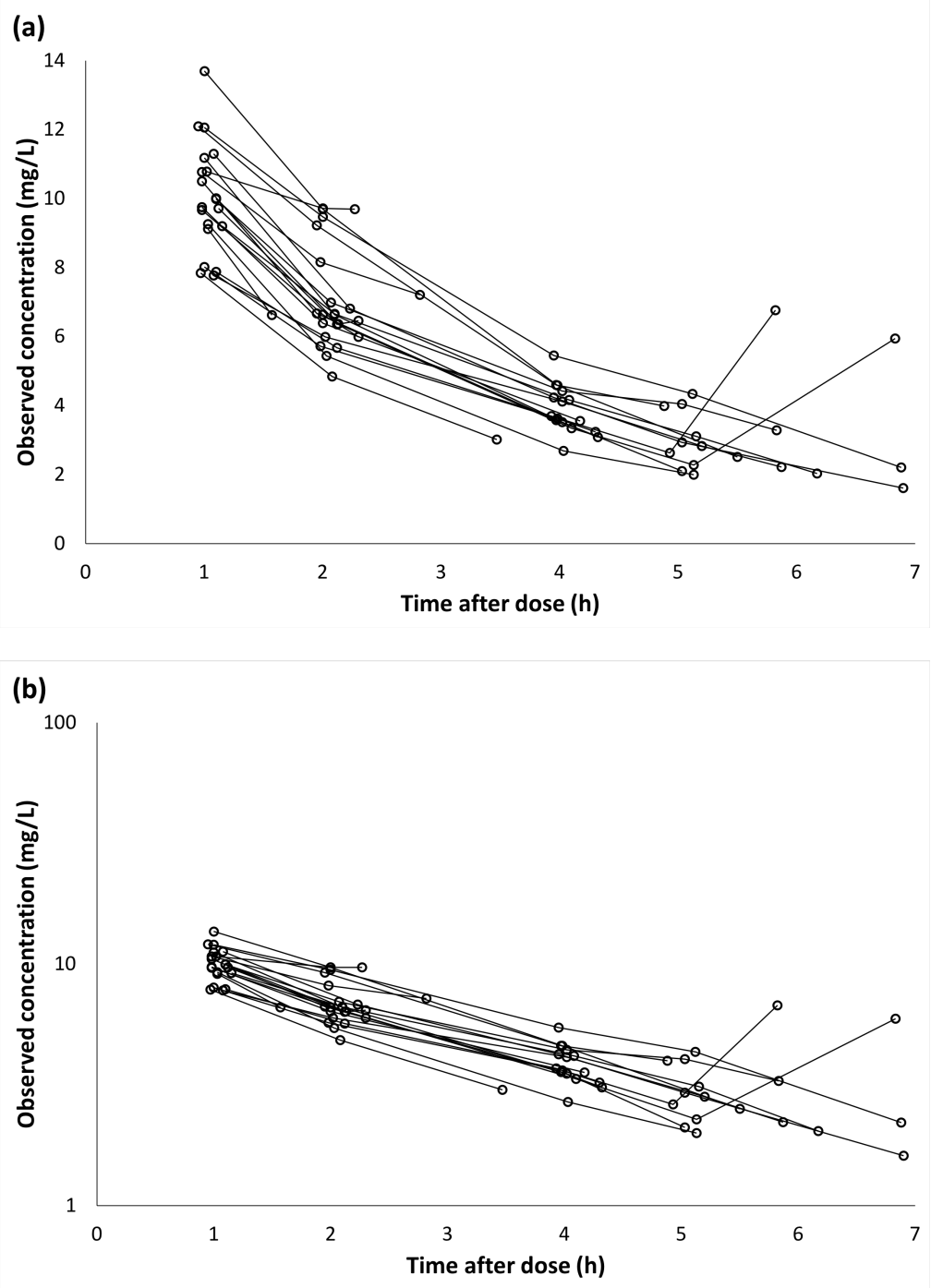


Figure 13 Gentamicin concentration measurements in 20 colorectal surgery patients following intravenous doses up to 300 mg; dose banded on height based on 3 mg/kg ideal body weight.

An additional dose was administered to two patients at 5 and 6 hours. (a) Linear concentration scale, (b) log-linear concentration scale.

Figure 14 shows scatter plots and box plots of individual estimates of gentamicin CL and V versus clinical characteristics. Box plots suggested that V estimates were higher in males while the scatter plots suggested that CL declined with age and increased with CRCL, and that both CL and V increased with WT and HT. There were no apparent relationships between CL and sex, and between V and age or CRCL, also, no apparent relationships were observed between CL or V and albumin (data not shown) or creatinine concentrations. The following characteristics were tested in the covariate model: age, WT, HT and CRCL. As the data set covered a wide range of BMI values, CRCL based on WT, IBW, AJBW, MBW, and LBW were also tested.

Table 14 summarises the key covariate models that were tested during model development. There was an improvement in fit when HT was included in the model. Models based on IBW, MBW, CRCA and AJBW also improved the fit. No improvement was observed when WT (linear or allometric) or LBW were included in the model. The best model included HT and CRCA.

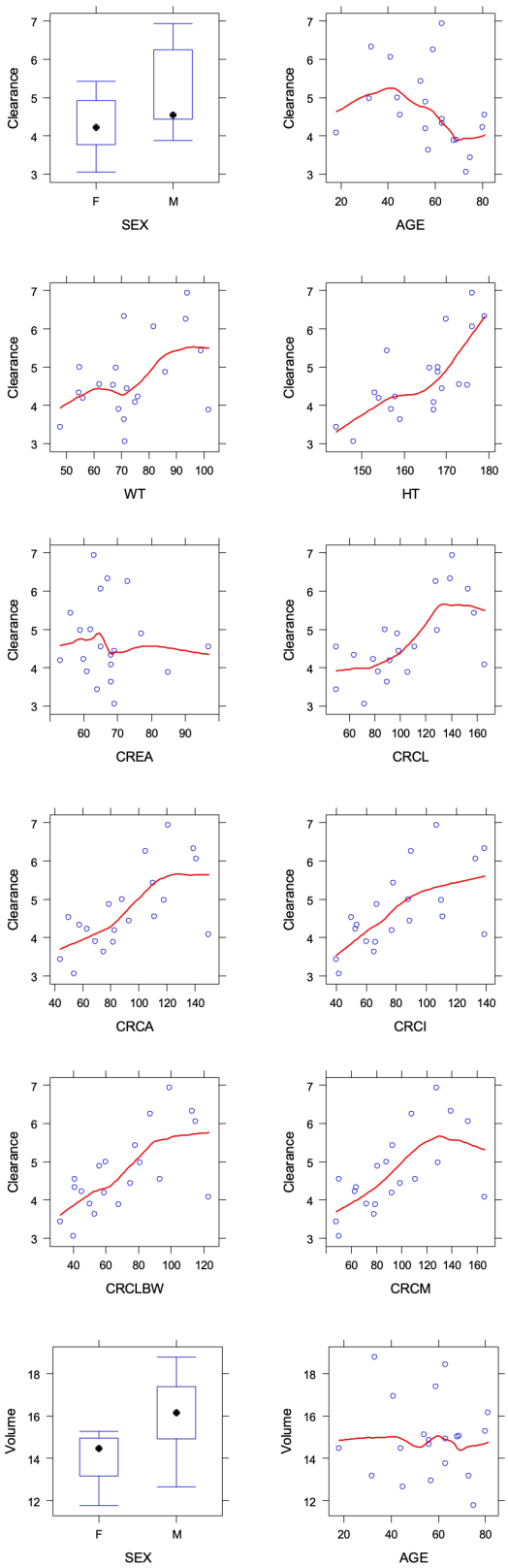


Figure 14 Scatter plots of individual estimates of gentamicin clearance and volume of distribution versus clinical characteristics.

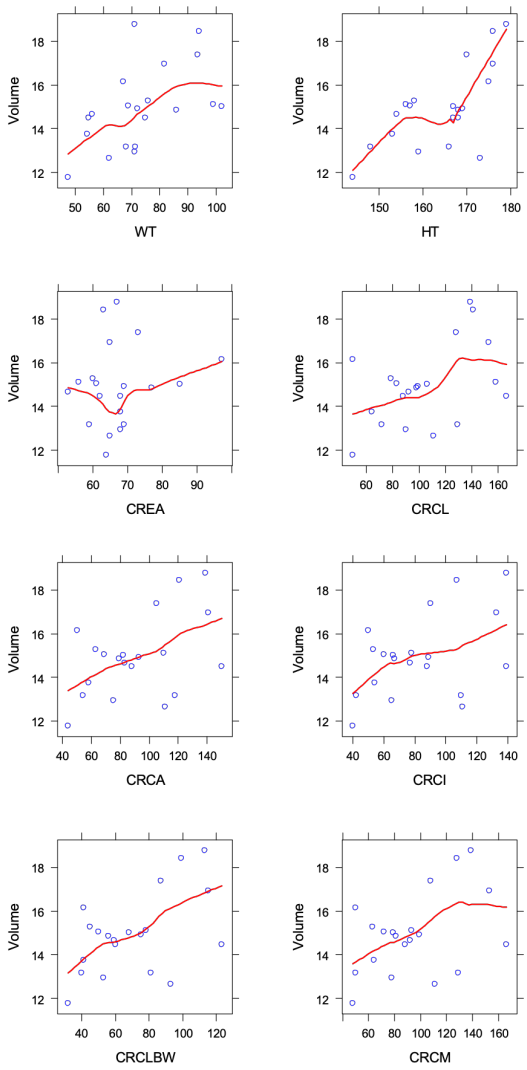


Figure 14 (continued) Scatter plots of individual estimates of gentamicin clearance and volume of distribution versus clinical characteristics.

Key: Blue circles are individual data points, the red line is a smooth through the data. Sex (F female, M male); age (years); WT, weight (kg); HT, height (cm); Alb, albumin (g/L); CREA, creatinine concentration (μmol/L); CRCL, creatinine clearance (ml/min) based on total body weight; CRCA, creatinine clearance (ml/min) based on adjusted body weight; CRCI, creatinine clearance (ml/min) based on ideal body weight; CRCLBW, creatinine clearance (ml/min) based on lean body weight; CRCM, creatinine clearance (ml/min) based on maximum body weight.

Table 14 Summary of the key gentamicin covariate models.

Model	CL	V	OFV	Model for comparison	ΔOFV
1	θ_1	θ_2	11.61		
2	θ_1 x HT	θ_2 x HT	2.27	1	9.34
3	θ_1 x WT	θ_2 x WT	15.66	1	-4.05
4	θ_1 x LBW	θ_2 x LBW	11.92	1	-0.31
5	θ_1 x MBW	θ_2 x MBW	1.71	1	9.90
6	θ_1 x IBW	θ_2 x IBW	3.12	1	8.49
7	θ_1 x AJBW	θ_2 x AJBW	0.70	1	10.91
8	θ_1 x CRCA	θ_2 x AJBW	7.08	1	4.53
9	θ_1 x AJBW + θ_4 x CRCA	θ_2 x AJBW	-5.75	7	6.45
10	θ_1 x HT + θ_4 x CRCA	θ_2 x HT	-6.58	2	8.85

Key: CL, clearance; V, volume of distribution; OFV, objective function value; ⊿OFV, objective function value difference; HT, height; WT, total body weight; AJBW, adjusted body weight; CRCA, creatinine clearance based on adjusted body weight.

Table 15 lists the PopPK parameters of the gentamicin base and final models. The final model led to a reduction in BSV in CL from 21.9% to 14.2% and in V from 13.5% to 10.5%. All estimates of shrinkage were below 25%. The parameters of the final CL model were poorly characterised with high coefficients of variation. However, the convergence rate of the bootstrap procedure was 89.3%; the population parameter estimates were similar to the median of the bootstrap estimates and the 90% CI ranges for CL and V were narrow.

Table 15 Population pharmacokinetic parameters of the gentamicin base and final models.

Model Parameter	Estimate (% RSE)		Shrinkage (%)	Bootstrap analysis
	Base model	Final model		Median (5th-95th percentile)
CL(L/h) θ_1	4.6 (5.0)	0.0179 (20.0)	-	0.0176 (0.0106-0.0237)
θ_4	-	0.0182 (39.1)	-	0.0189 (0.0067-0.0323)
V(L) θ_2	14.9 (3.4)	0.0915 (2.9)	-	0.0916 (0.0873-0.0959)
BSV CL (%)	21.9 (26.3)	14.2 (36.1)	η_{sh} = 2.80	13.4 (7.9-17.2)
BSV V (%)	13.5 (33.4)	10.5 (43.4)	η_{sh} = 14.8	10.2 (6.2-13.7)
RE Proportional (% cv)	6.69 (7.2)	6.68 (7.1)	ε_{sh} = 20.4	6.68 (5.89-7.50)

Structure of final model: TVCL = θ1 x HT + θ4 x CRCA; TVV = θ2 x HT

Key: RSE, relative standard error; CL, clearance; V, volume of distribution; BSV, between-subject variability expressed as a % coefficient of variation (cv); RE, residual error; η_{sh}, shrinkage in eta; ε_{sh}, shrinkage in the residual error; TVCL, typical value of clearance (L/h); TVV, typical value of volume of distribution; HT, height (cm); CRCA, creatinine clearance based on adjusted body weight (ml/min).

Figure 15 shows the measured versus the predicted concentrations for the base and final models. Overall, there is good agreement between the measured concentrations and the concentrations predicted by the PopPK model and the points are uniformly distributed along the line of identity with no obvious bias. There is a small improvement in the fit of the final population model compared to the base model. In both cases, there is a very close relationship between measured concentrations and concentrations based on individual parameter estimates. The ability of the final model to describe individual data is further demonstrated in Appendix 9, which shows profiles of measured, population predicted and individual predicted gentamicin concentrations versus time for each patient. CWRES plots versus time after dose and population predicted concentrations are shown in Appendix 10. The data are evenly distributed about zero indicating no bias in either the structural or the error model. The VPC plot presented in Figure 16 shows that the percentiles based on the observed data are within the CI bands based on the

simulated data and the measured concentrations are consistent with the final model predictions.

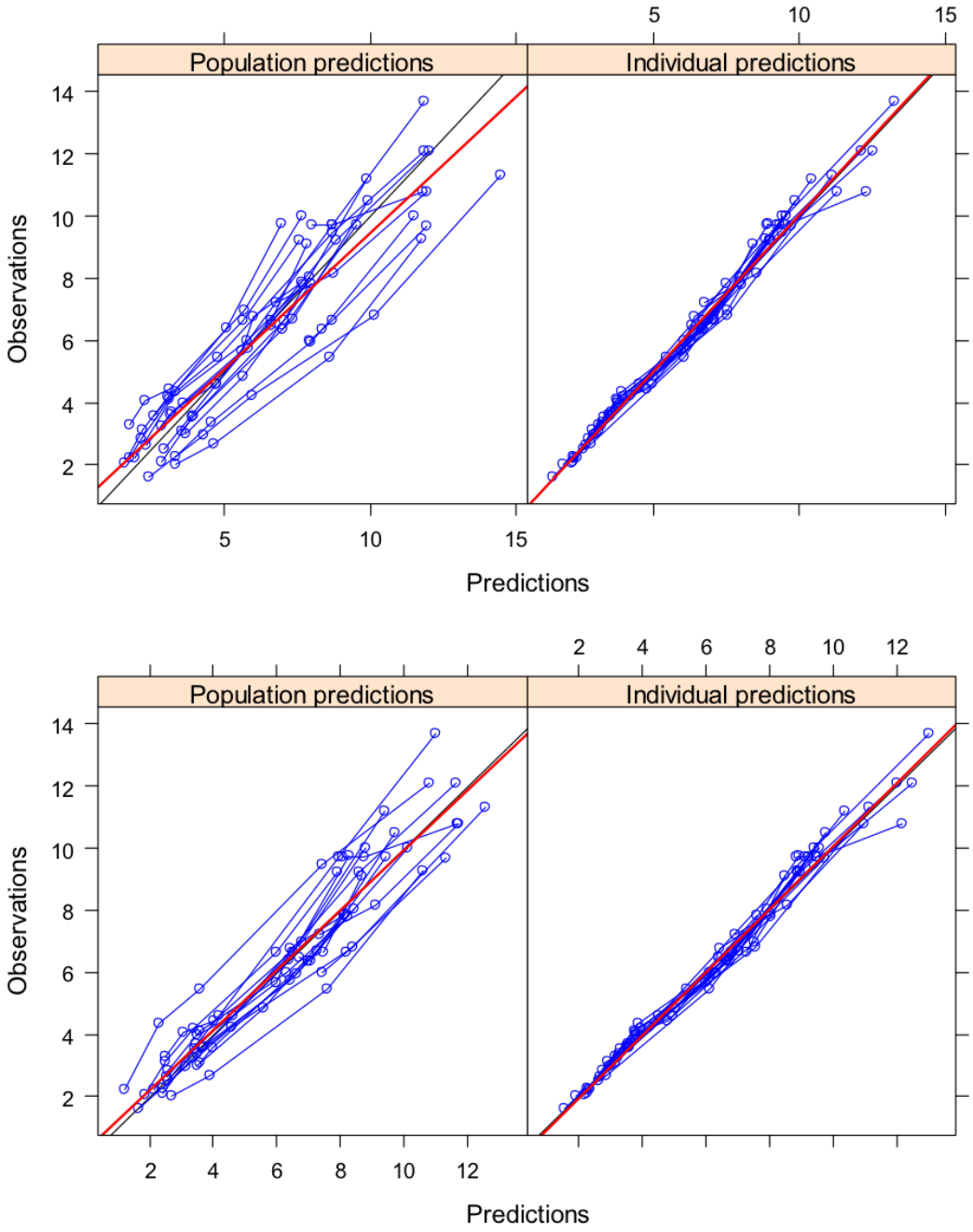


Figure 15 Plots of measured gentamicin concentration versus population (left panel) and individual (right panel) predicted concentrations.

Key: The thin black line represents the line of identity; the thick red line is a smooth of the data. The top panel shows the results from the base model; the bottom panel shows the results from the final model. Individual profiles are represented by blue circles joined by a blue line.

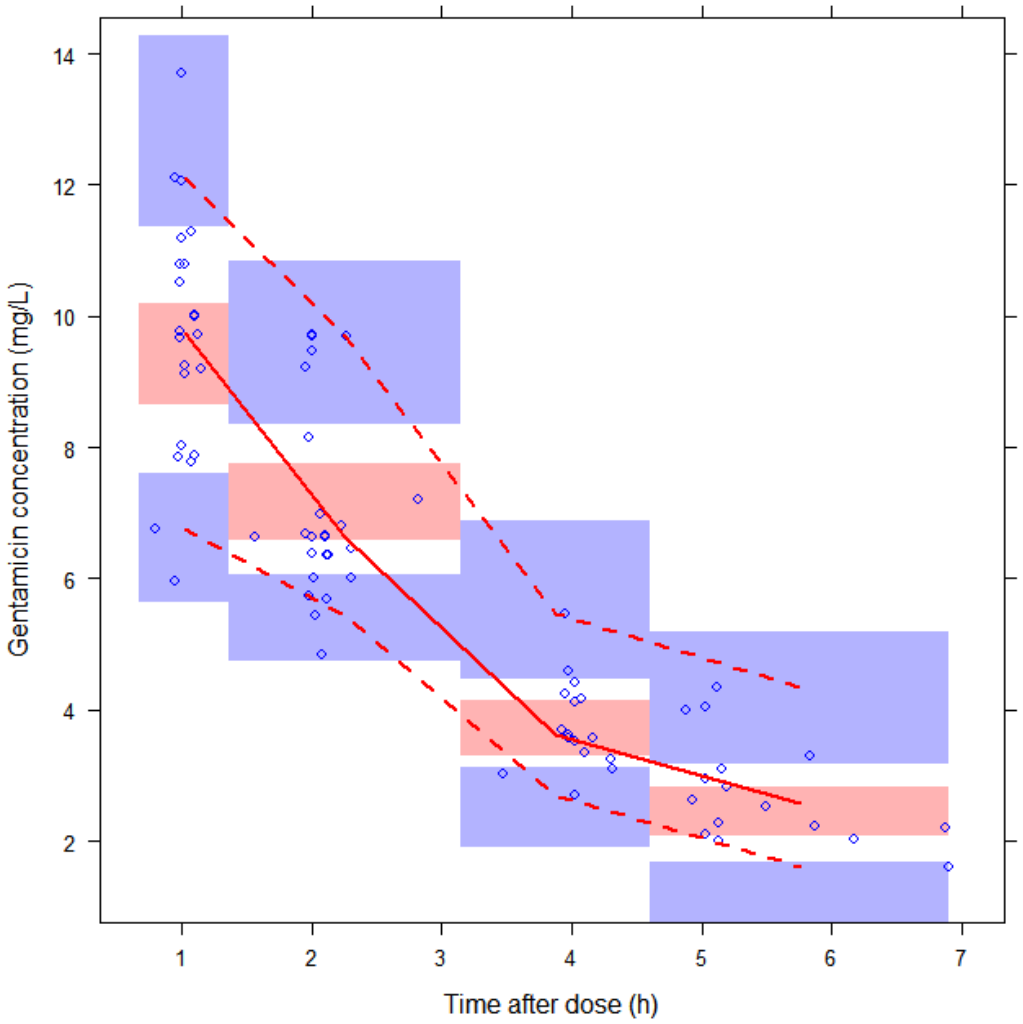


Figure 16 Visual predictive check of the final gentamicin model.

Key: Observed concentration data are represented by blue circles. The solid red line represents the 50thpercentile of the observed data, the dashed red lines are the 5th, and 95thpercentiles of the observed data. The pink shaded areas represent the 95% confidence intervals of the 50thpercentile of the simulated data, the blue shaded areas are the 95% confidence intervals of the 5thand 95thpercentiles of the simulated data.

3.5.3 Pharmacokinetic parameters

The individual estimates of CL ranged from 3.0 to 6.9 L/h (mean 4.7 L/h); the individual estimates of V ranged from 11.9 to 18.7 L (mean 15.1 L); and the individual estimates of the elimination $t_{1/2}$ ranged from 1.8 to 3.0 h (mean 2.3 h). Individual parameter estimates for each patient are listed in Appendix 11.

3.6 Pharmacodynamic analysis

3.6.1 Amoxicillin

3.6.1.1 Measured concentrations

The measured amoxicillin total concentrations and the calculated free concentrations at skin closure and at 4 hours are provided in Table 16. In all cases, concentrations at skin closure were above 0.5 mg/L, the typical MIC of the *Streptococcus anginosus* group. None of the patients was at high risk of IE, however, the calculated free concentrations at skin closure of patients 2, 13, 15 and 20 would have been below the MIC of enterococci (4 mg/L). Data regarding the 4 hour concentration, before re-dosing, were available from 12 patients. The total and calculated free concentrations were all above 0.5 mg/L but the calculated free concentrations at 4 hours in 8 (40%) patients would have been below the MIC of enterococci.

3.6.1.2 Simulated data

In order to explore the impact of failing to re-dose at 4 hours on the %*f*T>MIC, free drug concentration versus time profiles from 100 studies of 20 patients were simulated. The distributions of predicted amoxicillin free concentrations versus time are presented in Figure 17 and the probability of achieving the target, %*f*T>MIC at different times after the dose is presented in Figure 18. The probability of achieving a %*f*T>MIC of 100% for the *Streptococcus anginosus* group started declining at 4.5 hours. For enterococci this decline started at 2 hours. Assuming no intra-operative re-dosing, the simulations showed a drop in percentage probability to 73% for the *Streptococcus anginosus* group if surgery lasted 6.5 hours and to 59% for enterococci if surgery lasted 3.5 hours.

Table 16 Measured total concentrations and calculated free concentrations of amoxicillin at 4 hours and at skin closure.

Patient no	Sample time (h)	Total conc (mg/L)	Free conc (mg/L)	Skin closure sample time (h)	Skin closure total conc (mg/L)	Skin closure free conc (mg/L)
1	4.0	2.6	2.1	5.0	22.6	18.8
2	4.0	4.6	3.9	4.0	4.6	3.9
3	-	-	-	2.3	21.7	18.0
4	4.0	5.9	4.9	6.9	9.78	8.1
5	4.0	2.9	2.4	6.9	6.9	5.7
6	3.8	5.2	4.3	5.7	17.8	14.8
7	-	-	-	5.0	18.7	15.5
8	-	-	-	2.8	18.5	15.4
9	-	-	-	1.5	22.6	18.8
10	-	-	-	5.8	20.5	17.0
11	4.0	2.1	1.7	6.7	4.9	4.1
12	4.1	1.6	1.3	5.2	13.4	11.1
13	-	-	-	3.5	3.8	3.2
14	4.0	5.0	4.1	6.1	14.2	11.8
15	4.2	4.0	3.3	4.2	4	3.3
16	4.0	3.8	3.1	5.5	17.4	14.4
17	-	-	-	2.2	14.5	12.0
18	4.0	5.1	4.2	5.9	11.3	9.4
19	-	-	-	2.3	7.7	6.4
20	4.0	3.2	2.7	4.0	3.2	2.7

Key: no, number; conc, concentration. Free concentrations were calculated assuming 83% of the total concentration is unbound.

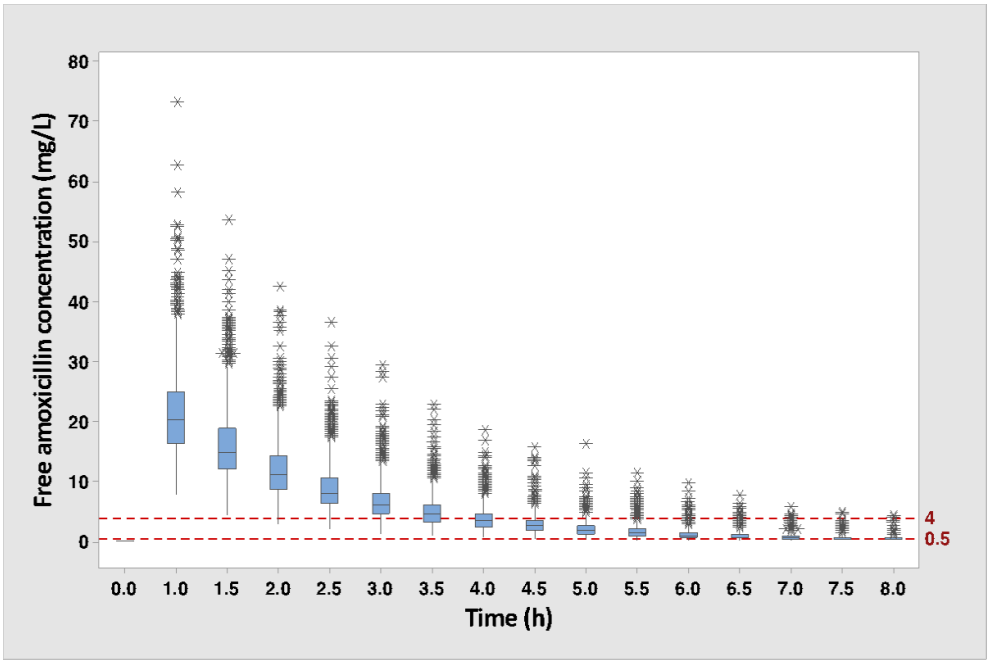


Figure 17 Boxplots of the predicted amoxicillin free concentrations versus time from 100 simulated studies of 20 patients given a single dose of 1000 mg.

Key: The dashed lines indicate the MIC for the Streptococcus anginosus group (0.5 mg/L) and the MIC for enterococci (4 mg/L).

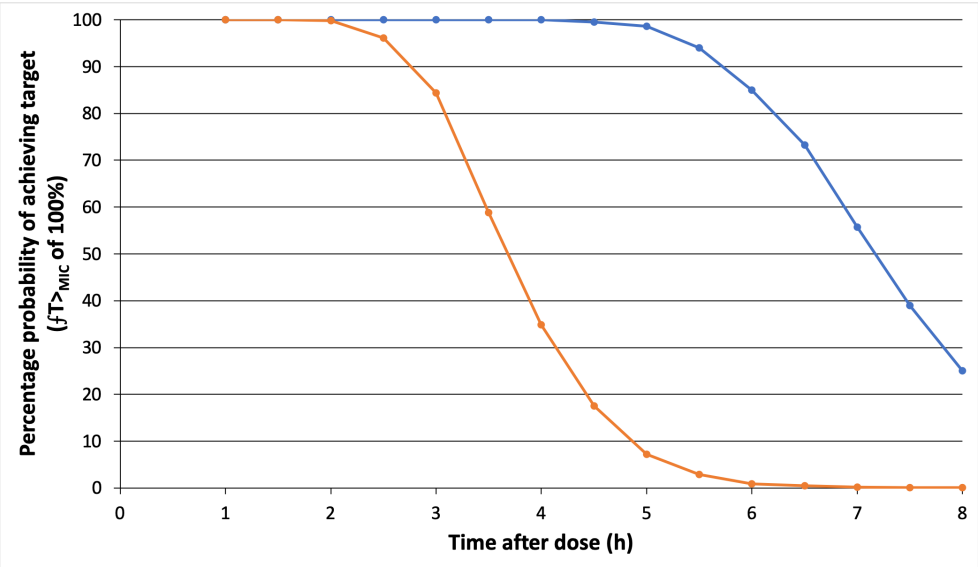


Figure 18 Percentage probability of achieving target *f*T>MIC of 100% versus time after the first dose of 1000 mg amoxicillin.

Key: Blue line, above an MIC of 0.5 mg/L (Streptococcus anginosus group); orange line, above an MIC of 4 mg/L (enterococci).

3.6.1.3 Simulated re-dosing data

The distributions of predicted amoxicillin free concentrations versus time following redosing at 2 hours are presented in Figure 19 (500 mg dose) and Figure 20 (1000 mg dose). The probability of achieving the target, %*f*T>MIC with no re-dosing and with re-dosing (using both doses) is presented in Figure 21. The probability of achieving a %*f*T>MIC of 100% for enterococci started declining at 3.5 hours with the 500 mg dose and 4 hours with the 1000 mg dose. The simulations showed a drop in percentage probability to 65%, with a 500 mg dose, if surgery lasted 5 hours and to 77%, with a 1000 mg dose, if surgery lasted 5.5 hours.

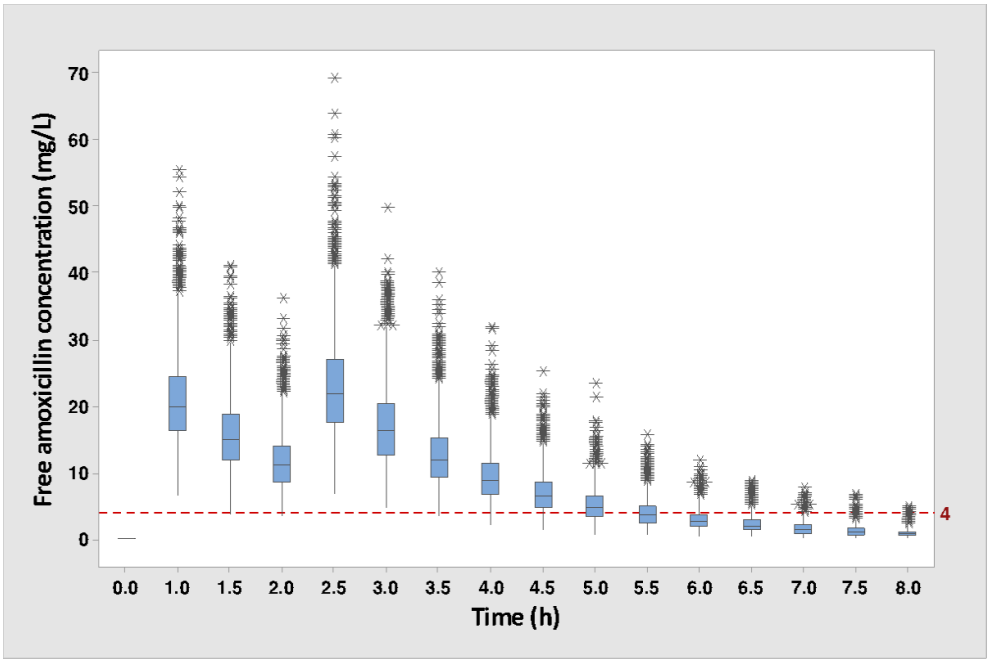


Figure 19 Boxplots of the predicted amoxicillin free concentrations versus time from 100 simulated studies of 20 patients (after an additional dose of 500 mg at 2 hours).

Key: The dashed line indicates the MIC for enterococci (4 mg/L).

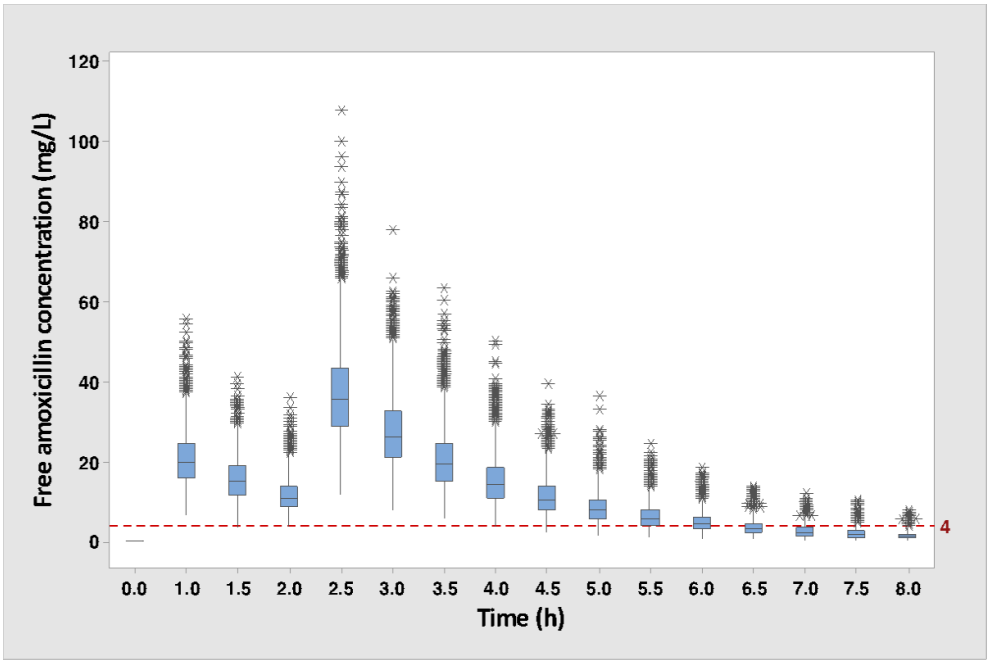


Figure 20 Boxplots of the predicted amoxicillin free concentrations versus time from 100 simulated studies of 20 patients (after an additional dose of 1000 mg at 2 hours).

Key: The dashed line indicates the MIC for enterococci (4 mg/L).

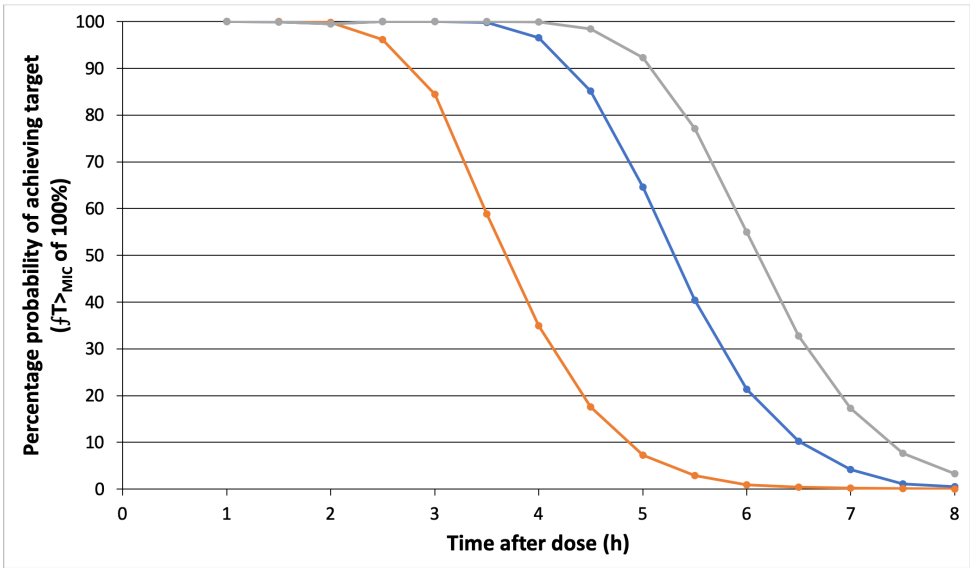


Figure 21 Percentage probability of achieving target *f*T>MIC of 100% for enterococci versus time after the dose using 3 different dosage regimens.

Key: Orange line, with no re-dosing; blue line, re-dosing at 2 hours with a 500 mg dose; grey line, re-dosing at 2 hours with a 1000 mg dose.

3.6.2 Metronidazole

3.6.2.1 Measured and calculated concentrations

The measured total concentrations of metronidazole, the calculated free concentrations at skin closure and the predicted concentrations at 8 hours are provided in Table 17. Skin closure concentrations from patients 6 and 11 were excluded due to re-dosing. The measured total and calculated free concentrations at skin closure were all above the typical MIC of the *Bacteroides fragilis* group (4 mg/L). At 8 hours, two of the predicted free concentrations were below 4 mg/L.

3.6.2.2 Simulated data

The distributions of predicted free concentrations of metronidazole versus time are presented in Figure 22 and the probability of achieving the target, %*f*T>MIC at different times after the dose is presented in Figure 23. The probability of achieving a %*f*T>MIC of 100% for the *Bacteroides fragilis* group started declining at 4.5 hours and dropped to 90% at 8 hours (the end of the re-dosing interval). The simulation data identified two free serum concentration (out of 2000) below the *Bacteroides fragilis* group MIC at 4.5 hours (patient 3). At 5.5 hours, 12 free serum concentrations were below this target (patients 3, 4, 10, 12, 13, 14, and 19).

Table 17 Measured total concentrations and calculated free concentrations of metronidazole at skin closure and predicted at 8 hours.

Patient no	Skin closure sample time (h)	Skin closure total conc (mg/L)	Skin closure free conc (mg/L)	Predicted 8 h total conc (mg/L)	Predicted 8 h free conc (mg/L)
1	5.0	9.1	7.7	6.8	5.8
2	4.3	10.4	8.9	6.3	5.4
3	2.3	8.0	6.8	4.6	3.9
4	6.9	9.9	8.4	9.1	7.7
5	6.7	7.1	6.1	6.4	5.5
6	-	-	-	6.6	5.6
7	4.9	8.6	7.3	6.6	5.6
8	2.7	10.5	8.9	6.7	5.7
9	1.6	14.5	12.3	7.9	6.7
10	5.8	6.6	5.6	4.8	4.1
11	-	-	-	5.4	4.6
12	5.1	6.5	5.5	4.9	4.1
13	3.4	7.7	6.6	4.5	3.8
14	5.2	9.6	8.2	7.7	6.6
15	4.2	14.2	12.1	9.5	8.1
16	5.4	10.5	8.9	8.8	7.4
17	2.2	9.9	8.5	5.7	4.9
18	5.9	11.8	10.0	10.5	8.9
19	2.2	8.5	7.2	4.8	4.1
20	4.3	13.6	11.6	10.1	8.6

Key: no, number; conc, concentration; h, hours. Free concentrations were calculated assuming 85% of the total concentration is unbound.

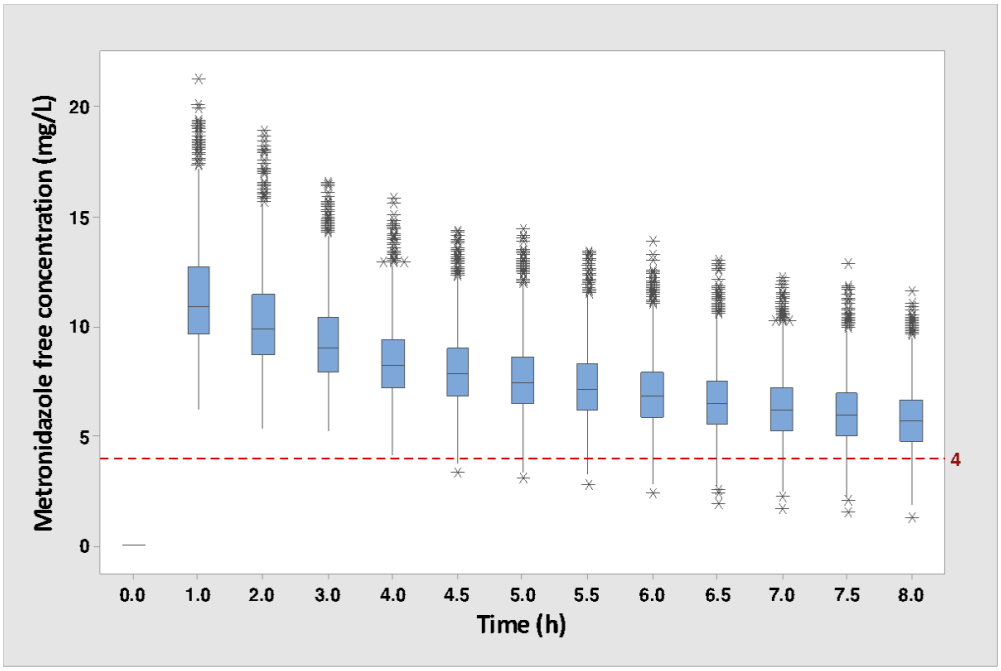


Figure 22 Boxplots of the predicted metronidazole free concentrations versus time from 100 simulated studies of 20 patients given a single dose of 500 mg.

Key: The dashed line indicates the MIC for the Bacteroides fragilis group (4 mg/L).

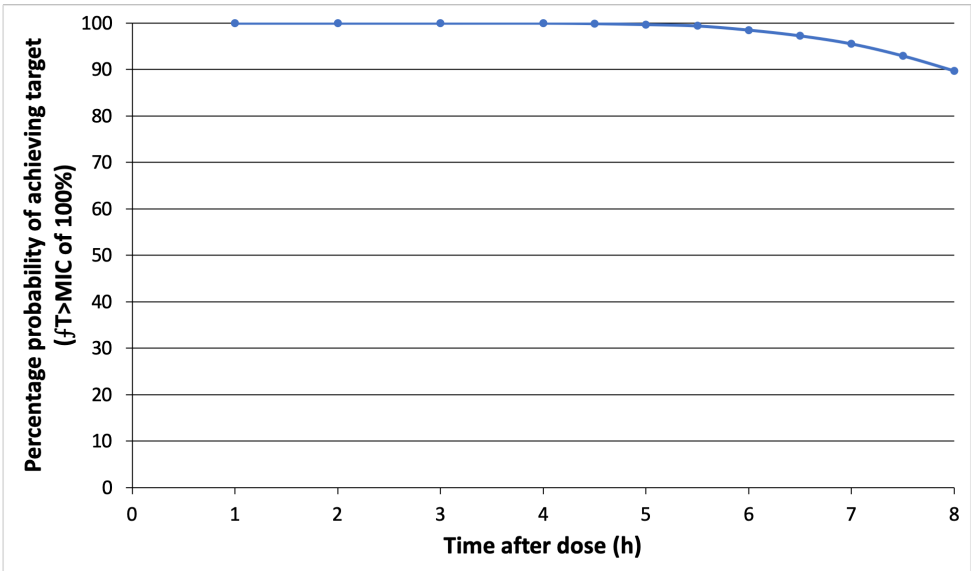


Figure 23 Percentage probability of achieving target *f*T>MIC of 100% versus time after a 500 mg dose of metronidazole.

Key: Blue line, above an MIC of 4 mg/L (Bacteroides fragilis group).

3.6.3 Gentamicin

3.6.3.1 Measured concentrations

The measured gentamicin total concentrations at skin closure and predicted at 8 hours are provided in Table 18. Skin closure concentrations from patients 6 and 11 were excluded due to re-dosing. The total concentrations at skin closure were all above 1 mg/L, the typical MIC of MSSA, and only patient 5 had a concentration below the MIC of *Escherichia coli* (2 mg/L). That patient had an EBL greater than 1.5 L and was re-dosed with gentamicin immediately after the sample was taken. At 8 hours, 19 (95%) of the predicted total concentrations were below the MIC of *Escherichia coli* and two (25%) were below the MIC of MSSA.

3.6.3.2 Simulated data

The distributions of predicted gentamicin total concentrations versus time are presented in Figure 24 and the probability of achieving the target, %*f*T>MIC at different times after the dose is presented in Figure 25. The probability of achieving a %*f*T>MIC of 100% for MSSA started declining at 5 hours. For *Escherichia coli* this decline started at 4 hours. The simulations showed a drop in percentage probability to 65% for MSSA if surgery lasted 8 hours and to 78% for *Escherichia coli* if surgery lasted 5.5 hours.

Table 18 Measured total concentrations of gentamicin at skin closure and predicted at 8 hours.

Patient no	Skin closure sample time (h)	Skin closure total conc (mg/L)	Predicted 8 h total conc (mg/L)
1	5.0	2.1	0.8
2	4.3	3.2	1.2
3	2.3	9.7	1.8
4	6.9	2.2	1.6
5	6.9	1.6	1.1
6	-	-	1.1
7	4.9	4.0	1.5
8	2.8	7.2	1.9
9	1.6	6.6	1.3
10	5.8	3.3	2.0
11	-	-	0.8
12	5.1	2.0	0.7
13	3.5	3.0	0.6
14	6.2	2.0	1.4
15	4.2	3.6	1.3
16	5.5	2.5	1.1
17	2.3	6.5	1.2
18	5.9	2.2	1.3
19	2.3	6.0	0.8
20	4.3	3.1	1.0

Key: no, number; conc, concentration; h, hours.

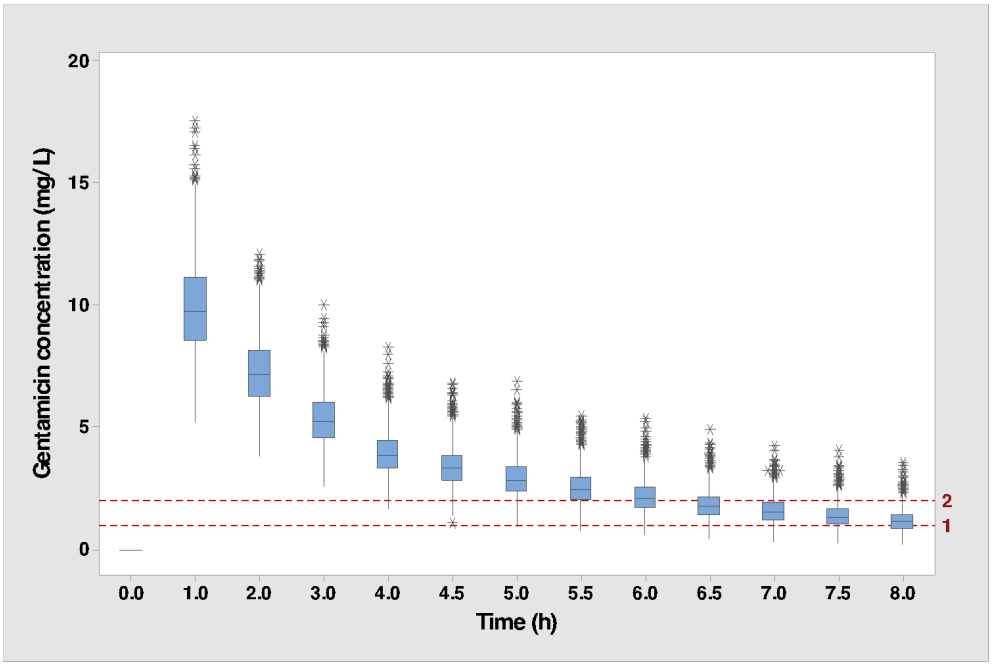


Figure 24 Boxplots of the predicted gentamicin total concentrations versus time from 100 simulated studies of 20 patients given a dose that is banded on height and based on 3 mg/kg ideal body weight.

Key: The dashed lines indicate the MIC for MSSA (1 mg/L) and the MIC for Escherichia coli (2 mg/L).

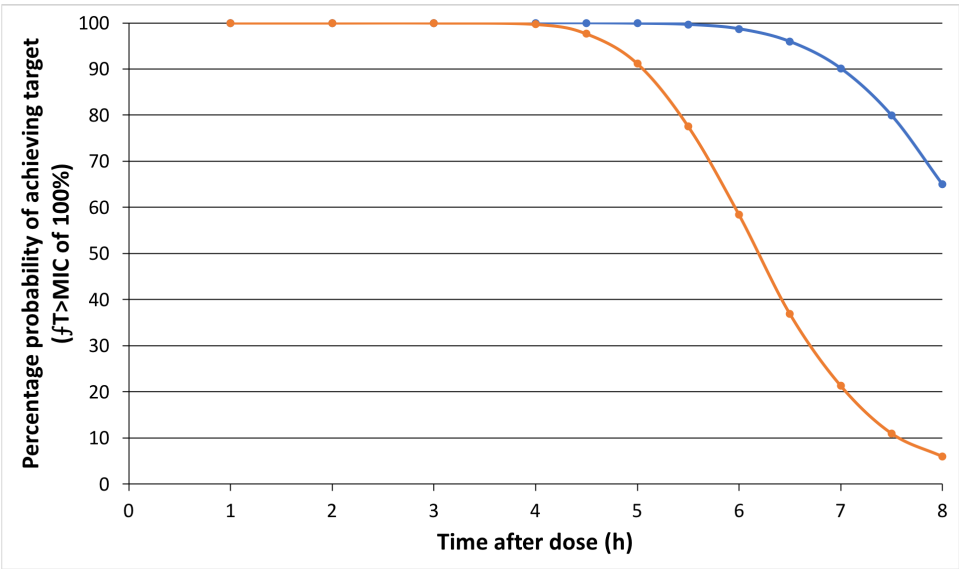


Figure 25 Percentage probability of achieving target *f*T>MIC of 100% versus time (dose banded on height based on 3 mg/kg ideal body weight).

Key: Blue line, above an MIC of 1 mg/L (MSSA); orange line, above an MIC of 2 mg/L (Escherichia coli).

3.6.3.3 Simulated higher dosage regimens

The distributions of predicted gentamicin total concentrations versus time following higher doses are presented in Figure 26 (5 mg/kg WT dose), Figure 27 (5 mg/kg MBW dose), and Figure 28 (dose banded on HT based on 5 mg/kg IBW). The probability of achieving the target, %*f*T>MIC for MSSA and *Escherichia coli* with all dosage regimens is presented in Figures 29 and 30 respectively. With the higher dosage regimens, the probability of achieving a %*f*T>MIC of 100% for MSSA started declining at 6.5 hours and fell to approximately 94% at 8 hours. For *Escherichia coli*, the probability of achieving a %*f*T>MIC of 100% started declining at 4.5 – 5 hours and fell to approximately 76% if surgery lasted 7 hours.

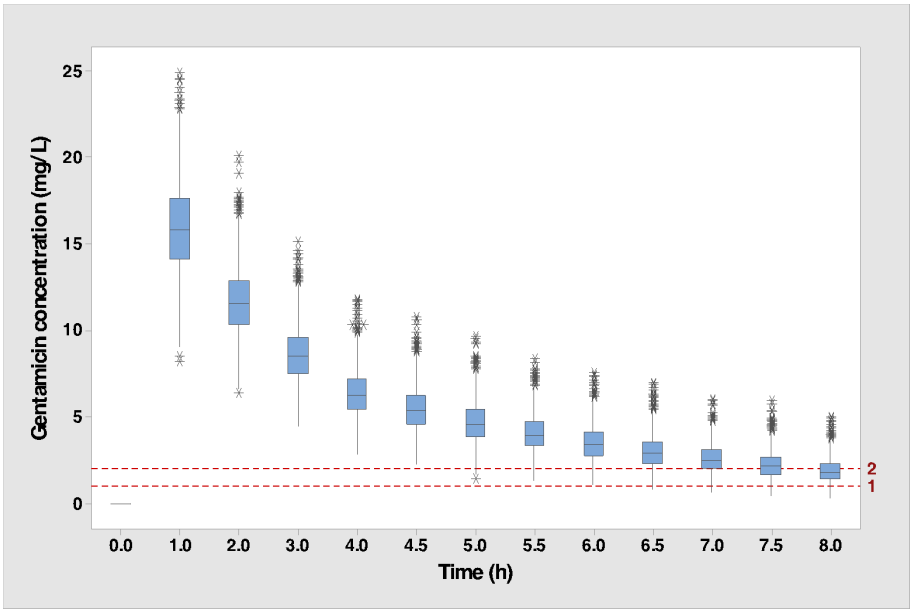


Figure 26 Boxplots of the predicted gentamicin total concentrations versus time from 100 simulated studies of 20 patients given a dose of 5 mg/kg total body weight.

Key: The dashed lines indicate the MIC for MSSA (1 mg/L) and the MIC for Escherichia coli (2 mg/L).

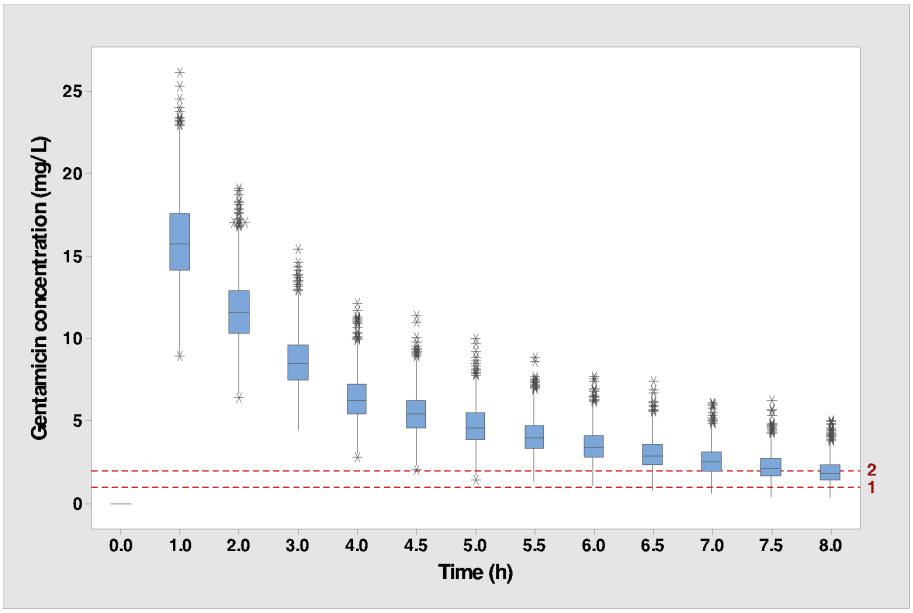


Figure 27 Boxplots of the predicted gentamicin total concentrations versus time from 100 simulated studies of 20 patients given a dose of 5 mg/kg maximum body weight.

Key: The dashed lines indicate the MIC for MSSA (1 mg/L) and the MIC for Escherichia coli (2 mg/L).

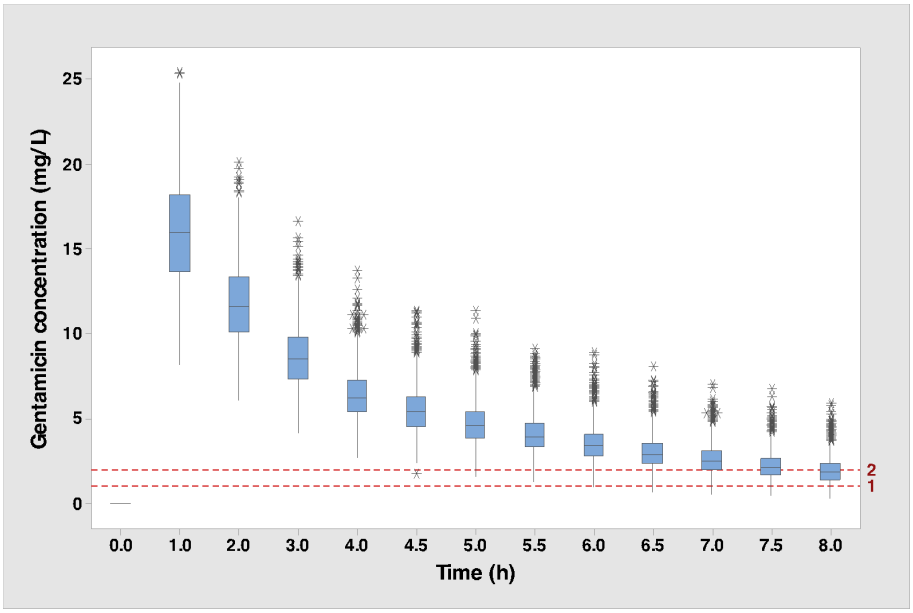


Figure 28 Boxplots of the predicted gentamicin total concentrations versus time from 100 simulated studies of 20 patients given a dose that is banded on height and based on 5 mg/kg ideal body weight.

Key: The dashed lines indicate the MIC for MSSA (1 mg/L) and the MIC for Escherichia coli (2 mg/L).

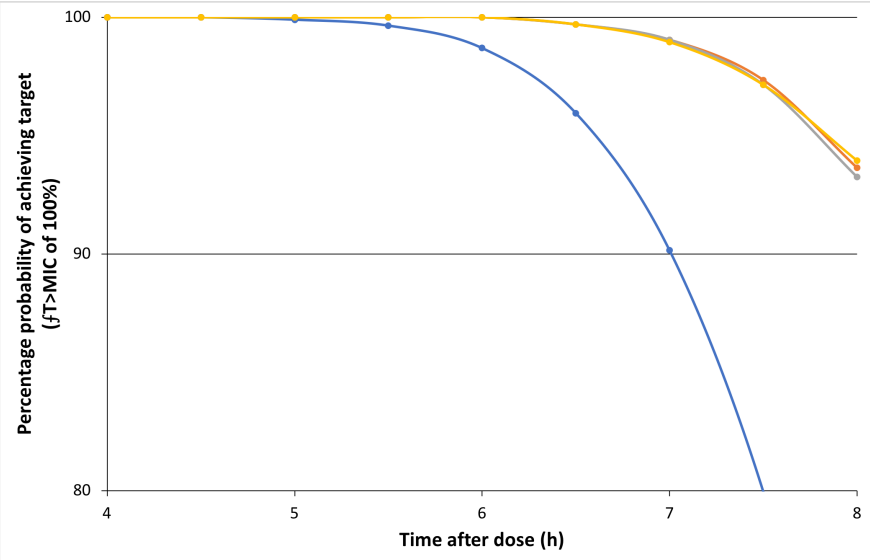


Figure 29 Percentage probability of achieving target *f*T>MIC of 100% for MSSA versus time after the dose.

Key: The blue line represents the dose banded on height based on 3 mg/kg ideal body weight; the yellow line represents the dose banded on height based on 5 mg/kg ideal body weight; the grey line represents a dose of 5 mg/kg maximum body weight; the orange line represents a dose of 5 mg/kg total body weight.

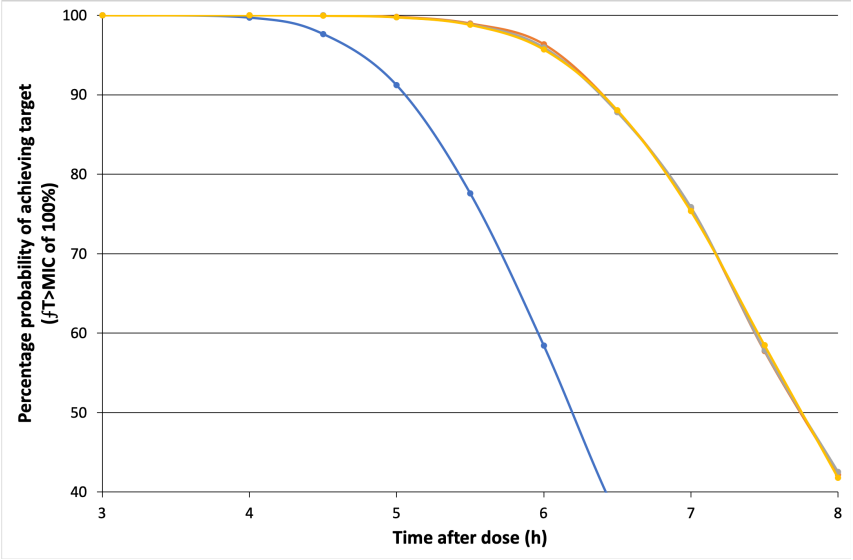


Figure 30 Percentage probability of achieving target *f*T>MIC of 100% for *Escherichia coli* versus time after the dose.

Key: The blue line represents the dose banded on height based on 3 mg/kg ideal body weight; the yellow line represents the dose banded on height based on 5 mg/kg ideal body weight; the grey line represents a dose of 5 mg/kg maximum body weight dosing; the orange line represents a dose of 5 mg/kg total body weight.

3.7 Surgical site infection follow-up

One of the 20 patients died and was excluded from the follow-up as the cause of death was not related to SSI. Of the remaining patients, only patient 13 (5.3%) developed an SSI within 30 days of surgery. None of the antibiotic concentrations in this patient was below the defined MIC breakpoints of the target organisms at skin closure. The patient was readmitted with discharge from the upper portion of her abdominal wound. The abdominal fluid culture yielded *Escherichia coli* and a diagnosis of organ/space SSI was made by the surgeon.

4 DISCUSSION

4.1 Surgical site infection and antibiotic prophylaxis

SSI is one of the most frequent HAIs in Scotland and has a significant clinical and financial burden to hospitals and society. The risk of SSI is multifactorial; it includes the *inoculum* of bacteria that contaminates the surgical site and various patient and procedure-related factors. Colorectal surgery is associated with the highest SSI rate among elective operations (Public Health England 2017, Health Protection Scotland 2013) and antimicrobial prophylaxis is one of the most effective measures available to prevent SSIs (Song and Glenny 1998). NHSGGC guidelines recommend a combination of amoxicillin, metronidazole and gentamicin. This is consistent with the recommendation that the ideal regimen of antimicrobial prophylaxis in colorectal surgery should provide good polymicrobial cover against bowel and skin bacteria. In addition to providing adequate antimicrobial coverage, the timing of administration is of major importance for effective prophylaxis (Classen et al. 1992, Burke 1961). NHSGGC guidelines follow the Antibiotic Prophylaxis in Surgery guideline, SIGN 104, which recommends administration of antibiotics within 1 hour of skin incision (Scottish Intercollegiate Guidelines Network 2014).

Studies that couple PopPK and MCS to determine the PTA against organisms frequently associated with colorectal surgery SSIs are scarce. The validity of the NHSGGC dosage regimen has not previously been evaluated. In addition to the questionable adequacy of the amoxicillin re-dosing interval of 4 hours, it was not clear whether the recommended doses of gentamicin and metronidazole would be able to maintain adequate serum concentrations in prolonged surgery. Accordingly, this study assessed the PK of amoxicillin, metronidazole and gentamicin in 20 patients undergoing elective colorectal surgery and used the resulting population models to assess how patient, surgical, and microbiological factors influenced the exposure to these antibiotics.

In surgical antibiotic prophylaxis, the aim is to maintain free antibiotic concentrations in plasma and tissue that exceed the MICs of organisms commonly associated with SSIs until skin closure. In the case of colorectal surgery, the

uncertainty of the size of bacterial *inoculum* and the time of resection and extraction of the colonic specimen, which typically occurs at the end of surgery, is likely to result in a higher risk of infection if antibiotic concentrations are inadequate. This is particularly important for antibiotics with a short elimination $t_{1/2,}$ such as amoxicillin. Depending on the types of organisms and the duration of the procedure, the rapid decline in amoxicillin concentrations after a single pre-operative dose could compromise effective prophylaxis. A study by Scher (1997) in clean-contaminated surgery confirmed the clinical benefit of intra-operative re-dosing when the duration of the procedure exceeded twice the elimination $t_{1/2}$ of cefazolin (1.8 h) while Ohge et al. (1999) concluded that a second dose of cefazolin was required 3 hours after the first dose to maintain adequate serum and tissue concentrations in patients undergoing pancreatectomy. The clinical benefit of maintaining adequate concentrations for the whole duration of the procedure has also been demonstrated in both colorectal and cardiac surgery (Zelenitsky et al. 2002, Zanetti et al. 2001, Morita et al. 2005). Although these studies highlight the importance of assessing prophylactic antibiotic regimens, information regarding the timing of re-dosing by surgery type and antibiotic is scarce. Consequently, further research in this area was identified as especially important in The Antibiotic Prophylaxis in Surgery Guideline, SIGN 104 (Scottish Intercollegiate Guidelines Network 2014).

The findings of the current research on the PK/PD of amoxicillin, metronidazole and gentamicin, when used for prophylaxis in colorectal surgery, are discussed in the following sections.

4.2 Pharmacokinetic results

Although other authors have found that two-compartment models provided a better fit of their data, serum concentration-time profiles of all three antimicrobials were adequately described using a one-compartment model in the present study. For amoxicillin, Arancibia et al. (1980) described a short distribution phase (mean $t_{1/2}$ of 0.3 h) and rapid elimination phase (mean $t_{1/2}$ of 1.1 h) in nine healthy subjects

while Gjerloff and Arnold (1982) described a distribution $t_{1/2}$ of metronidazole in five patients that ranged from 0.1 to 0.3 h, followed by a slower elimination phase with a $t_{1/2}$ of 4.7 to 15.8 h. A study of gentamicin in 11 healthy subjects, reported a mean distribution $t_{1/2}$ of 0.4 h and 0.7 h after doses of 2 mg/kg and 7 mg/kg, respectively (Demczar et al. 1997). The authors suggested saturation of the tissue uptake mechanism as a possible explanation for this difference. In adults from a general population, gentamicin elimination $t_{1/2}$ has been reported in the range of 2 – 3 h (Thummel et al. 2011). The differences between previous studies and the present study probably reflect the sampling strategy that was used. In the clinical setting of this study, the number of samples was limited and the distribution phases of all three antibiotics were avoided by restricting sample times to a minimum of 1 hour after the dose. Although the distribution half-lives reported in the previous studies might suggest that some of the initial samples may have been drawn during the distribution phase, the observed concentration-time profiles displayed a monoexponential decline so this was not considered to be a major problem. The long terminal $t_{1/2}$ of gentamicin (7 to 10 days) (Schentag et al. 2006), was also considered not relevant in view of the duration of surgery.

4.2.1 Amoxicillin

After the first 1000 mg dose of amoxicillin, the 4 hour post dose concentrations of 1.6 to 5.9 mg/L were generally higher than the values of 1.3 – 2.8 mg/L reported by Hill et al. (1980) in seven healthy males aged 23 to 40 years. These differences may reflect different analytical methodologies as the present study used an LC-MS assay whereas the previous study used a microbiological assay. Alternatively, the results in the present study could reflect a lower drug CL in the patient group compared to CL in the younger, healthy subjects, who participated in the previous study.

The means of the individual estimates of CL (14.0 L/h), V (22.7 L, 0.314 L/kg) and elimination $t_{1/2}$ (1.2 h) were similar to the values identified by Arancibia et al. (1980), who reported a mean CL of 13.3 L/h, V of 0.30 L/kg and $t_{1/2}$ of 1.1 h in nine healthy subjects after an IV dose of 500 mg. In contrast, Carlier et al. (2013)

estimated a lower CL of 10.0 L/h, and a higher V of 27.4 L in 13 critically ill patients. These differences could be explained by the altered and variable physiology in certain groups of critically ill patients (e.g. patients with sepsis, burns, or end-organ dysfunction) (Muller et al. 2018).

Although the present study initially identified an influence of CRCL on the PK of amoxicillin, which is consistent with its known excretion by glomerular filtration and tubular secretion (Bryskier 2005), the parameters of the CRCL model had high coefficients of variation and the bootstrap procedure was not successful. The simpler model, based on weight, was therefore chosen. The observed effect of body weight rather than of CRCL on CL was likely to be related to the narrow range of creatinine concentrations (53 – 97 μmol/L) in the patient group. In these circumstances, CRCL estimates, by the Cockcroft-Gault formula, depend mostly on body weight and age. In contrast, a PopPK study of amoxicillin and clavulanic acid in 13 critically ill patients by Carlier et al. (2013) identified CRCL as the only covariate that influenced the CL of both compounds. Differences in the ranges of renal function between the two patient groups is the most likely explanation for the inability to characterise the influence of renal function on amoxicillin PK in the present study. Although the median CRCL in the critically ill patients of 102 ml/min was similar to the median of 99 ml/min in the present study, the interquartile range was much wider at 50 – 157 ml/min compared to 82 – 132 ml/min.

As the present data set covered a wide range of BMI values and more than half the patients were overweight or obese, different size descriptors were tested in the population model. AJBW provided the best fit. It is known that obesity results in physiological changes that can cause variable PK alterations (Meng et al. 2017) and obesity is a risk factor for SSI following colorectal surgery (Bratzler et al. 2013, Fry 2013). Amoxicillin is soluble in water (Bryskier 2005) therefore its distribution should not be significantly influenced by excess adipose tissue, despite this, it would seem reasonable to use an AJBW for dosing in obesity as fat contains 30% water which will lead to a higher V (Medico and Walsh 2010). There are no data available

in terms of dosing of amoxicillin in obesity using different size descriptors. For penicillins, UK guidance recommends using a dose at the upper limit of the recommended treatment ranges in obese patients (UK Medicines Information 2017). Although the numbers of obese and severely obese patients in the present study, six and one, respectively, were probably insufficient to fully determine which body size descriptor should be used for dosing in obesity, the population model suggests that AJBW may be the most appropriate. This is an area that warrants further research.

4.2.2 Metronidazole

After the first 500 mg dose of metronidazole, the 1 hour post dose concentrations ranged from 9.2 to 20.3 mg/L (median 14.1 mg/L) and are comparable to the values reported by Hobbiss et al. (1988) in 10 patients undergoing colorectal surgery. Based on the plot of concentrations shown by the authors, the values ranged from around 9 – 23 mg/L (median around 13 mg/L). Although the mean of the individual estimates of CL (3.5 L/h) is identical to the value reported by Asin-Prieto et al. (2015b) in 63 patients undergoing colorectal surgery, the mean V (35.7 L, 0.49 L/kg) is higher than their result of 27.7 L. This apparent difference may simply reflect the higher WT of the current patient group, whose average weight was 74 kg compared to 69 kg in the previous study. Despite the shorter sampling time (up to 7 hours in the present study, compared to 24 hours), which may compromise the ability to characterise the terminal $t_{1/2}$, the estimates of elimination $t_{1/2}$ were similar, 7.5 h in the present study and 8.8 h in the previous study.

A study by Ventura et al. (2008) in 33 patients undergoing colorectal surgery, reported a similar CL (3.2 L/h) and higher V (0.68 L/kg) and elimination $t_{1/2}$ (11.8 h) compared to the present study. However, their average V and elimination $t_{1/2}$ data showed high variation (SD of 0.20 L/kg and 5.1 h, respectively, compared to 0.07 L/kg and 1.9 h). The average WT and age were comparable in both studies. The high variation may be explained by the sampling methodology as the collection of the initial samples in the study by Ventura and colleagues was undertaken during the

distribution phase of metronidazole and the data were analysed using a one-compartment model.

As expected, no relationship was identified between CRCL and CL, since metronidazole is extensively metabolised in the liver (Dubreuil 2005). Body weight was the only covariate that was found to influence the PK of metronidazole and, similarly to amoxicillin, AJBW provided the best fit when a linear relationship was examined. However, in contrast to amoxicillin, an allometric relationship between metronidazole CL and WT provided the best overall fit of the data. Asin-Prieto et al. (2015b) also found that body weight was the most important factor influencing the PK of metronidazole, however, they did not examine different size descriptors. Metronidazole is slightly lipophilic (Dubreuil 2005) and, consequently, will have a higher V compared to hydrophilic drugs, and this V will be further increased in the obese. Therefore using higher doses in obese patients would seem reasonable and although there are no data available regarding which size descriptor to use in obesity, the present study suggests that either using an allometric WT relationship or AJBW may be the most appropriate until future studies are conducted to clarify the ideal approach.

4.2.3 Gentamicin

Gentamicin concentrations from the present study could not be compared to other studies due to differences in dosing regimens. The mean of the individual estimates of V (0.211 L/kg) was in accordance with the values of 0.22 – 0.26 L/kg previously reported for gentamicin when used for prophylaxis in colorectal surgery (Ventura et al. 2008, Zelenitsky et al. 2000, Markantonis et al. 2004). It is difficult to compare the results directly as the study by Zelenitsky and colleagues neither specified the WT of the 34 patients included in the PK analyses, nor the time the first samples were drawn. The average WTs in the studies by Ventura and colleagues (73 kg) and Markantonis and colleagues (75 kg) were comparable to the present study (74 kg), however, their administration and sampling strategies were different. The V of the present and previous colorectal surgery studies were lower than the value of 0.31

L/kg reported in adults from a general population (Thummel et al. 2011). Since gentamicin is distributed in extracellular fluid, the lower estimates of V in the present and other studies may reflect pre-operative fasting with subsequent dehydration and a reduced body water content. However, interpretation of these values is difficult due to the lack of information regarding patient demographics and sampling times in other studies. For example, the initial samples in the study by Ventura et al. (2008) were taken 15 minutes after a 15 minute infusion and may have included some element of distribution, leading to a lower estimate of V when a one-compartment model was used to analyse their data.

As gentamicin CL depends on renal function, CL estimates will differ from study to study. The mean of the individual estimates of CL (4.7 L/h, 0.065 L/h/kg) was comparable to the values reported by Ventura et al. (2008) (4.7 L/h) and Markantonis et al. (2004) (5.31 L/h) but lower than the estimate of 0.091 L/h/kg observed in the study by Zelenitsky et al. (2000). CRCL data were not reported in that study. The mean elimination $t_{1/2}$ of gentamicin in the present study was 2.3 h, which was within the reported range of 2 – 3 h in adults from a general population (Thummel et al. 2011).

Gentamicin is excreted by glomerular filtration and, as expected, the present study identified an influence of CRCL on gentamicin CL. This finding is consistent with a recent study which reviewed 14 PopPK studies of gentamicin in adults and found that the most common covariate to have an influence in CL was renal function and on V was body size (Llanos-Paez et al. 2017). In the present study, the best fit was obtained with a model that combined HT with renal function calculated using AJBW (CRCA). However, the parameters of the final CL model were poorly characterised with high coefficients of variation. The small number of patients with renal impairment were likely to be contributing factors; only two patients had a mild to moderate reduction in glomerular filtration rate (both with a CRCL of 50 ml/min).

The extent to which a particular drug distributes into tissues is affected by its lipid solubility and protein binding. Gentamicin is hydrophilic with very low protein

binding and is characterised by a low V (Schentag et al. 2006). Therefore, it is largely confined to the intravascular compartment and obesity would be expected to have less influence on its V. However, not all the excess weight in obesity is adipose tissue, so dosing according to IBW might lead to concentrations that are too low. The population model identified in the present study suggests that AJBW may be the most appropriate size descriptor for obese patients, which is consistent with the findings of Bauer et al. (1983), who investigated the influence of weight on aminoglycoside PK. Despite high variability in the AJBW correction factor, the authors also suggested the use of AJBW with a correction factor of 0.4 for initial dosing of aminoglycosides in morbidly obese patients. In the present study, using AJBW to estimate CRCL by the Cockcroft-Gault formula, provided the best fit; this finding is consistent with a study by Leader et al. (1994) in 100 obese patients. They also found that gentamicin CL values were better predicted when estimating CRCL using AJBW.

4.3 Pharmacodynamic analysis

PK/PD principles and PopPK are increasingly being used in the development and individualisation of antimicrobial dosage regimens to optimise the treatment of infections, as well as in surgical prophylaxis (Asin-Prieto et al. 2015a). By combining PopPK and MCS the adequacy of antibiotic prophylaxis can be assessed while accounting for patient (e.g. renal function, weight), surgical (e.g. duration), and microbiological (e.g. organism and MIC breakpoint) factors.

The final PopPK models of amoxicillin, metronidazole and gentamicin were used to assess the probability of achieving adequate target exposures with a range of different dosing regimens. Similar approaches have been used in colorectal surgery previously. Moine and Fish (2013) and Zelenitsky et al. (2016) evaluated different surgery prophylaxis regimens with the aim to identify optimal antibiotics and optimise dosing regimens. Asin-Prieto et al. (2015b) assessed the adequacy of cefuroxime plus metronidazole, while Isla et al. (2012) evaluated cefoxitin. None of

these studies included amoxicillin or the dosing regimens of metronidazole and gentamicin used in the present study.

4.3.1 Amoxicillin

Amoxicillin is included in the colorectal surgery antibiotic prophylaxis regimen to cover the *Streptococcus anginosus* group. The current dosing regimen of a pre-operative dose of 1000 mg repeated intra-operatively 4 hours later, successfully met the PTA at the defined MIC breakpoint of 0.5 mg/L. It was observed that the PTA started to decline at 4.5 hours and if a further dose was not administered, the PTA dropped to 73% at 6.5 hours, emphasising the importance of re-dosing amoxicillin in prolonged surgery. Re-dosing guidelines usually recommend repeating the prophylactic dose when the duration of the surgical procedure exceeds twice the elimination $t_{1/2}$ of the relevant antibiotic (Bratzler et al. 2013). In the case of amoxicillin, this would mean repeating the dose at around 2.5 hours as the elimination $t_{1/2}$ in the present study averaged 1.2 h. However, in this particular case, re-dosing at 4 hours was acceptable due to the low MIC breakpoint of the *Streptococcus anginosus* group. In the present study, both the measured and simulated concentrations were above the defined MIC at the recommended re-dosing interval.

Antibiotic cover against enterococci is not normally required when antibiotic prophylaxis is used in colorectal surgery, however, it is recommended for patients at high risk of IE (Gould et al. 2006, Habib et al. 2015). Although current guidelines for prophylaxis of IE do not include specific re-dosing advice, it would seem reasonable to use a dosage regimen that achieves concentrations above the MIC breakpoint of 4 mg/L for the whole duration of the surgical procedure. The present study found that for enterococci, the PTA with the current dosing regimen started to decline at 2 hours and by 3.5 hours it was only 59%. If a second dose of 500 mg was given 2 hours after the first dose, the PTA started to decline at 3.5 hours and if no further dose was administered, the PTA dropped to 65% at 5 hours. Even if the patient was given a second dose of 1000 mg 2 hours after the first dose, the PTA

only remained acceptable until 4 hours and dropped to 77% at 5.5 hours. Consequently, to maintain adequate cover for patients at high risk of IE, the results indicate that 1000 mg amoxicillin should be administered every 2 hours during the surgical procedure.

4.3.2 Metronidazole

Metronidazole was included in the colorectal surgery antibiotic prophylaxis regimen to cover anaerobic organisms. The current dosing regimen of 500 mg pre-operatively and repeated intra-operatively 8 hours after the initial dose, did not successfully meet the PTA at the defined MIC breakpoint of 4 mg/L for the *Bacteroides fragilis* group. The PTA started to decline at 4.5 hours, falling to 90% at 8 hours. Asin-Prieto et al. (2015b) also performed a PK/PD study to assess the adequacy of cefuroxime and metronidazole in 63 patients undergoing colorectal surgery. Their metronidazole dose was 1500 mg pre-operatively and their defined MIC breakpoint was 8 mg/L. The authors found that the PK of metronidazole was influenced by WT and consequently evaluated three specific WTs (50, 68.5 and 90 kg). They concluded that the patients with a body weight of 90 kg required an additional dose of 1500 mg 4 hours after the first dose in order to maintain free drug concentrations above the MIC value (up to 8 hours). Although the present study used both a lower prophylactic dose and MIC breakpoint, there was a similar trend towards underdosing in patients with higher WT. The simulated concentrations that were below the MIC 4.5 hours after the dose were generally associated with overweight or obese patients (BMI $\geq$25 kg/m^2). Dosing information for metronidazole in obesity is limited. Mastrobattista et al. (2008) studied the influence of BMI on the treatment of bacterial vaginosis in 738 pregnant woman and concluded that 2000 mg of metronidazole had a similar efficacy across the different BMI categories, suggesting that size was not a critical factor when a high dose is used. The low number of severely/morbidly obese patients in the present study population (two patients) limited the opportunity to conduct further simulations to determine the exposure of different dosage regimens in obese

patients. However, the lower concentrations found in overweight or obese patients suggest that 4 hour re-dosing or using a higher pre-operative dose would be reasonable in this patient group to successfully achieve a PTA of 100% in surgical procedures longer than 4 hours. The results also indicate that in patients with normal weight (BMI <25 kg/m^2), free concentrations above the MIC are maintained up to 8 hours after the dose.

4.3.3 Gentamicin

Gentamicin was included in the colorectal surgery antibiotic prophylaxis regimen to cover MSSA and Gram-negative organisms. The current dose, banded on HT and administered pre-operatively and repeated intra-operatively 8 hours after the initial dose, did not maintain the PTAs at the defined MIC breakpoints of 1 mg/L for MSSA and 2 mg/L for *Escherichia coli*. As expected, the exposure increased when higher doses were simulated. The three options that were considered, 5 mg/kg WT, 5 mg/kg MBW, and HT-based doses that approximated to 5 mg/kg IBW, achieved similar PTAs. MCS were also used in a previous study that assessed different gentamicin regimens when used for prophylaxis in abdominal surgery (Zelenitsky et al. 2016). The authors determined the CTA by integrating PTA values and MIC distributions and found that without intra-operative re-dosing, the CTA at 6 hours remained above 90% for *Escherichia coli* with a gentamicin dose of 5 mg/kg and fell below 90% after 5 hours with a dose of 3 mg/kg. Since 90% of their colorectal procedures lasted less than 5 hours, the authors recommended the 3 mg/kg dosing regimen due to concerns about the risk of aminoglycoside-related toxicity. In contrast, only 60% of the colorectal procedures in the present study lasted less than 5 hours, also, the dose banded on HT based on 3 mg/kg IBW achieved a PTA of 100% for only up to 4 hours. Therefore, the results suggest that 5 mg/kg would be more appropriate for longer surgical procedures as the desired PTA, against *Escherichia coli*, would be maintained for up to 5 hours, and only fell below 90% at 6.5 hours. Since the results were similar for all 5 mg/kg dose options, the table of

doses based on HT, which approximates to 5 mg/kg IBW, is recommended to simplify dose calculations in a theatre setting.

4.4 Surgical site infection rate

Although the present study was not designed to investigate a causal association between SSIs and antibiotic concentrations, SSIs that were identified within 30 days of surgery were recorded and antibiotic concentrations were examined. One patient (5.3%) developed an SSI; this incidence was consistent with previous NHSGGC surveillance data of SSIs following large bowel surgery (rate of 3.6%), however, these rates are lower than previously reported national and international rates of 9-10% (Public Health England 2017, Health Protection Scotland 2013). Different protocols to collect surveillance data or implementation of local strategies to prevent SSIs could explain this difference. Also, national data have yet to be published by Health Protection Scotland to enable comparison with other boards.

The abdominal fluid culture in the patient who developed an SSI yielded *Escherichia coli.* None of the antibiotic concentrations in this patient were below the defined MIC breakpoints of the target organisms at skin closure. Furthermore, antibiotic concentrations measured at skin closure were above the defined MIC breakpoints for all patients except one, whose gentamicin concentration at 6.9 hours was 1.6 mg/L (below the MIC of *Escherichia coli* of 2 mg/L). However, that patient had an EBL of 1.8 L and had approximately 6 L of fluid replacement. It is well established that antibiotic concentrations fall in patients with excessive blood loss and fluid replacement (Levy et al. 1990, Swoboda et al. 1996, Markantonis et al. 2004) and the SIGN 104 guideline for antibiotic prophylaxis in surgery emphasises the need for re-dosing in these circumstances (Scottish Intercollegiate Guidelines Network 2014). As discussed previously, the risk of SSI is multifactorial and cases of SSI with adequate skin closure serum concentrations are therefore not surprising.

4.5 Dosage guidelines

Dosage guidelines for antibiotic prophylaxis in colorectal surgery developed from the final amoxicillin, metronidazole and gentamicin population models are presented in Table 19. The gentamicin dosing schedule, based on HT, is presented in Table 20. The re-dosing times were based on the PTA simulations. A PTA of 100%, against the *Streptococcus anginosus* group and enterococci, was maintained up to 4.5 hours and 2 hours, respectively, after 1000 mg of IV amoxicillin. Following 500 mg of IV metronidazole, a PTA of 100%, against the *Bacteroides fragilis* group, was maintained up to 4.5 hours in patients with a BMI $\geq$25 kg/m^2. For gentamicin, the dose banded on HT based on 5 mg/kg IBW, maintained a PTA of 100%, against MSSA and *Escherichia coli*, up to 6.5 hours and 5 hours, respectively.

Table 19 Recommended doses and re-dosing intervals for amoxicillin, metronidazole, and gentamicin for colorectal surgery prophylaxis.

Antibiotic	Recommended dose	Recommended re-dosing interval (from initiation of pre-operative dose)
Amoxicillin	1000 mg	4 h (2 h – if risk of infective endocarditis)
Metronidazole	500 mg	8 h (4 h – if BMI $\geq$25 kg/m^2)
Gentamicin	See prophylaxis dosing table	5 h (if CRCL >60 ml/min)

Key: BMI, body mass index; CRCL, creatinine clearance estimated by the Cockcroft-Gault formula (Cockcroft and Gault 1976) using adjusted body weight.

Table 20 Prophylactic gentamicin dosing table - dose banded on height based on 5 mg/kg ideal body weight (Devine 1974) and capped at 500 mg.

Height ranges (Feet and Inches)	Height ranges (cm)	Gentamicin Dose (mg)	
		Males	Females
4’ 8” – 4’ 10”	142 – 147	240	220
4’ 11” – 5’ 3”	148 – 160	300	260
5’ 4” – 5’ 10”	161 – 178	400	340
5’ 11” – 6’ 2”	179 – 188	500	440
≥ 6’ 3”	≥ 189	500	500

Recent surveillance data from NHSGGC (unpublished) from October 2017 to April 2018 showed that 52.1% of prescription charts or anaesthetic sheets had no documented evidence of re-dosing of antibiotic prophylaxis. Although this could be just a documentation issue, it highlights the need for effective implementation of guidelines, as poor compliance with re-dosing recommendations is also a reason for unsuccessful prophylaxis (Goede et al. 2013).

4.6 Limitations of the study

A possible limitation of the present study was choosing the %*f*T >MIC of 100% as the PK/PD target. There is a lack of published data on the ideal target in surgical antibiotic prophylaxis, particularly for concentration-dependent antibiotics such as gentamicin and metronidazole. Zelenitsky et al. (2002) identified that a gentamicin concentration at skin closure above 1.6 mg/L was required for effective prophylaxis in colorectal surgery. This finding informed the target of %*f*T >MIC of 100% that was assumed for both time-dependent and concentration-dependent antibiotics.

Another limitation of the present study was that the population models were based on serum concentrations of antibiotics rather than tissue concentrations. Although

gentamicin, metronidazole, and amoxicillin distribute well into extracellular fluids, tissue penetration studies are limited and variable. A study of metronidazole PK and tissue penetration in colorectal surgery by Martin et al. (1991) determined a tissue/serum drug concentration ratio of 0.42 for colonic wall at anastomosis (mean time 2.6 hours) and 0.13 for both abdominal wall fat and epiploic fat at skin closure (mean time 4.0 hours). These ratios are lower than the ratios determined by Kling and Burman (1989) of 0.76 for colonic mucosa and 0.21 for adipose tissue, 1 – 4 hours after the infusion. These conflicting results make it difficult to extrapolate serum concentrations to the interstitial space fluid.

Due to the lack of renal and liver impairment in the study population, the results cannot be extrapolated to these patient groups as their antibiotic clearances could not be characterised. This also applies to morbidly obese patients (BMI $\geq$40 kg/m^2). Based on this lack of variability, 100 simulations of the original dataset were considered acceptable, although 500 – 1000 simulations are more typical in this type of analysis. Ideally, the study would have been conducted in a larger patient group with a more diverse set of characteristics that might have been more representative of the patient population.

It is also important to note that the metronidazole PK/PD analysis did not include any in vitro activity of the hydroxyl metabolite, which has been reported to have 30 to 65% the antimicrobial activity of metronidazole (Lau et al. 1992). By only considering the MIC of the parent drug, overall efficacy may have been underestimated.

The unbound serum concentrations were calculated by using protein binding values published in the literature and the actual unbound concentrations may differ from the ones obtained in this study, particularly in patients with malignancy. However, the majority of the study patients had normal albumin values and since the protein binding of all three antibiotics is low, small variations are likely to have a negligible impact.

The significance of deviations in PTA for simulations based on the upper and lower confidence interval limit versus those based on the mean of the PK parameters estimates was not determined by sensitivity analysis. Furthermore, the PTA values from the present study were based on the EUCAST clinical MIC breakpoints without including the MIC distributions, and also may not reflect local or changing antimicrobial susceptibility.

It should also be highlighted that PK/PD relationships may be different in immunosuppressed patients as a competent immune system is required to achieve optimal antibiotic response. Consequently, higher drug exposures may be required in this patient population.

4.7 Further research

Although there was no evidence to suggest a difference in the PK parameters of any of the antibiotics between patients who received open versus laparoscopic surgery, further research would be required to address this issue as the present study was not designed to evaluate the influence of the type of surgery on PK parameters.

The dosage recommendations are based on normal renal and liver function, consequently further research would be welcome to assess the adequacy of the recommended dosing regimens in patients with renal and hepatic impairment. Furthermore, any unintended consequences of the higher gentamicin doses e.g. impact on nephrotoxicity or ototoxicity should be assessed. PopPK studies in the obese population would also be required. Finally, clinical validation of these findings would also be welcome.

4.8 Conclusions

In conclusion, the present study demonstrated the value of PopPK modelling in the area of surgical prophylaxis by establishing dosing recommendations for an antimicrobial prophylaxis regimen in colorectal surgery. The PopPK model results for amoxicillin, metronidazole, and gentamicin, were combined with clinical MIC

breakpoints of the most frequent organisms associated with SSIs to determine the probability of maintaining free drug concentrations above the MICs with the current and alternative dosing regimens.

The findings of this study showed that the current dosing regimens maintained a PTA of 100% (over the re-dosing interval) for the *Streptococcus anginosus* group but not for MSSA, *Escherichia coli,* enterococci (in patients at high risk of IE), and the *Bacteroides fragilis* group (in patients with a BMI $\geq$25 kg/m^2). The proposed dosage guidelines offer an improved profile for all three antibiotics and should maintain these PTAs over the re-dosing interval.

5 REFERENCES

Aarons, L. (1991) 'Population pharmacokinetics: theory and practice'. *British Journal of Clinical Pharmacology,* 32(6), pp. 669-670.

Anon. (2003) 'SOP 14: Population pharmacokinetic analysis'. *Onkologie,* 26(Suppl 6), pp. 60-66.

Arancibia, A., Guttmann, J., Gonzalez, G. and Gonzalez, C. (1980) 'Absorption and disposition kinetics of amoxicillin in normal human subjects'. *Antimicrobial Agents & Chemotherapy,* 17(2), pp. 199-202.

Asin-Prieto, E., Rodriguez-Gascon, A. and Isla, A. (2015a) 'Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents'. *Journal of Infection & Chemotherapy*, 21(5), pp. 319-329.

Asin-Prieto, E., Sorulace, A., Troconiz, I.F., Cimarras, E.C., de Ugarte Sobron, J.S., Rodriguez-Gascon, A. and Isla, A. (2015b) 'Population pharmacokinetic models for cefuroxime and metronidazole used in combination as prophylactic agents in colorectal surgery: Model-based evaluation of standard dosing regimens'. *International Journal of Antimicrobial Agents*, 45(5), pp. 504-511.

Bauer, L.A., Edwards, W.A., Dellinger, E.P. and Simonowitz, D.A. (1983) 'Influence of weight on aminoglycoside pharmacokinetics in normal weight and morbidly obese patients'. *European Journal of Clinical Pharmacology,* 24(5), pp. 643-647.

Bearden, D.T. and Rodvold, K.A. (2000) 'Dosage adjustments for antibacterials in obese patients: applying clinical pharmacokinetics'. *Clinical Pharmacokinetics*, 38(5), pp. 415-426.

Bernard, H.R. and Cole, W.R. (1964) 'The prophylaxis of surgical infection: the effect of prophylactic antimicrobial drugs on the incidence of infection following potentially contaminated operations'. *Surgery*, 56, pp. 151-157.

Bratzler, D.W., Dellinger, E.P., Olsen, K.M., Perl, T.M., Auwaerter, P.G., Bolon, M.K., Fish, D.N., Napolitano, L.M., Sawyer, R.G., Slain, D., Steinberg, J.P. and Weinstein, R.A. (2013) 'Clinical practice guidelines for antimicrobial prophylaxis in surgery'. *American Journal of Health-System Pharmacy,* 70(3), pp. 195-283.

Broex, E.C.J., van Asselt, A.D.I., Bruggeman, C.A. and van Tiel, F.H. (2009) 'Surgical site infections: how high are the costs?'. *Journal of Hospital Infection,* 72(3), pp. 193-201.

Bryskier, A. (2005) 'Penicillins', in Bryskier, A. (ed.) *Antimicrobial Agents*. Washington: ASM Press, pp. 113-162.

Burke, J.F. (1961) 'The effective period of preventive antibiotic action in experimental incisions and dermal lesions'. *Surgery*, 50, pp. 161-168.

Carlier, M., Noe, M., De Waele, J.J., Stove, V., Verstraete, A.G., Lipman, J. and Roberts, J.A. (2013) 'Population pharmacokinetics and dosing simulations of amoxicillin/clavulanic acid in critically ill patients'. *Journal of Antimicrobial Chemotherapy*, 68(11), pp. 2600-2608.

Classen, D.C., Evans, R.S., Pestotnik, S.L., Horn, S.D., Menlove, R.L. and Burke, J.P. (1992) 'The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection'. *New England Journal of Medicine,* 326(5), pp. 281-286.

Cockcroft, D.W. and Gault, M.H. (1976) 'Prediction of creatinine clearance from serum creatinine'. *Nephron,* 16(1), pp. 31-41.

Coello, R., Charlett, A., Wilson, J., Ward, V., Pearson, A. and Borriello, P. (2005) 'Adverse impact of surgical site infections in English hospitals'. *Journal of Hospital Infection,* 60(2), pp. 93-103.

Culver, D.H., Horan, T.C., Gaynes, R.P., Martone, W.J., Jarvis, W.R., Emori, T.G., Banerjee, S.N., Edwards, J.R., Tolson, J.S. and Henderson, T.S. (1991) 'Surgical wound infection rates by wound class, operative procedure, and patient risk index. National Nosocomial Infections Surveillance System'. *American Journal of Medicine,* 91(3B), pp. 152S-157S.

Demczar, D.J., Nafziger, A.N. and Bertino, J.S.Jr.(1997) 'Pharmacokinetics of gentamicin at traditional versus high doses: implications for once-daily aminoglycoside dosing'. *Antimicrobial Agents and Chemotherapy*, 41(5), pp. 1115-1119.

DeRyke, C.A., Lee, S.Y., Kuti, J.L. and Nicolau, D.P. (2006) 'Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: impact on the development of resistance'. *Drugs,* 66(1), pp. 1-14.

Devine, B.J. (1974) 'Gentamicin therapy'. *Drug Intelligence and Clinical Pharmacy,* 8(11), pp. 650-655.

Dong X., Ding L., Cao X., Jiang L. and Zhong, S. (2013) 'A sensitive LC-MS/MS method for the simultaneous determination of amoxicillin and ambroxol in human plasma with segmental monitoring'. *Biomedical Chromatography*, 27(4), pp. 520-526.

Dubreuil, L. (2005) '5-Nitroimidazoles', in Bryskier, A. (ed.) *Antimicrobial Agents*. Washington: ASM Press, pp. 930-940.

Dubrovskaya, Y., Tejada, R., Bosco, J.3rd., Stachel, A., Chen, D., Feng, M., Rosenberg, A. and Phillips, M. (2015) 'Single high dose gentamicin for perioperative prophylaxis in orthopedic surgery: Evaluation of nephrotoxicity'. *SAGE Open Medicine*, 3: 2050312115612803.

Electronic Medicines Compendium. (2018a) Amoxil Vials for Injection 1g. Available at: https://www.medicines.org.uk/emc/product/93/smpc (Accessed: 22 July 2018).

Electronic Medicines Compendium. (2018b) Gentamicin 40 mg/ml Injection. Available at: https://www.medicines.org.uk/emc/product/6531 (Accessed: 22 July 2018).

Electronic Medicines Compendium. (2018c) Metronidazole 500 mg / 100 ml Intravenous Infusion. Available at: https://www.medicines.org.uk/emc/product/1842/smpc (Accessed: 22 July 2018).

Erstad, B.L. (2004) 'Dosing of medications in morbidly obese patients in the intensive care unit setting'. *Intensive Care Medicine*, 30(1), pp. 18-32.

European Centre for Disease Prevention and Control (2019) *Healthcare-associated infections: surgical site infections*. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2017-SSI.pdf (Accessed: 25 January 2020).

European Committee on Antimicrobial Susceptibility Testing (2010) *Metronidazole: Rationale for the EUCAST clinical breakpoints, version 1.0*. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Metronidazole_rationale_1.0.pdf (Accessed: 10 July 2018).

European Committee on Antimicrobial Susceptibility Testing (2018) *Clinical breakpoints – bacteria*. Available at: http://www.eucast.org/clinical_breakpoints/ (Accessed: 07 May 2018).

Fredricsson, B., Hagstrom, B., Nord, C.E. and Rane, A. (1987) 'Systemic concentrations of metronidazole and its main metabolites after intravenous oral and vaginal administration'. *Gynecologic & Obstetric Investigation,* 24(3), pp. 200-207.

Fry, D.E. (2013) 'The prevention of surgical site infection in elective colon surgery'. *Scientifica,* 2013: 896297.

Gaikwad A., Gavali S., Narendiran, Katale D., Bonde S. and Bhadane, R.P. (2013) 'An LC-MS-MS method for the simultaneous quantification of amoxicillin and clavulanic acid in human plasma and its pharmacokinetic application'. *Journal of Pharmacy Research,* 6(8), pp. 804-812.

Gjerloff, C. and Arnold, E. (1982) 'Pharmacokinetics of intravenous metronidazole in man'. *Acta Pharmacologica et Toxicologica,* 51(2), pp. 132-135.

Goede, W.J., Lovely, J.K., Thompson, R.L. and Cima, R.R. (2013) 'Assessment of prophylactic antibiotic use in patients with surgical site infections'. *Hospital Pharmacy,* 48(7), pp. 560-567.

Gordon, R.C., Regamey, C. and Kirby, W.M. (1972) 'Serum protein binding of the aminoglycoside antibiotics'. *Antimicrobial Agents & Chemotherapy,* 2(3), pp. 214-216.

Gould, F.K., Elliott, T.S.J., Foweraker, J., Fulford, M., Perry, J.D., Roberts, G.J., Sandoe, J.A.T., Watkin, R.W. and Working Party of the British Society for Antimicrobial Chemotherapy. (2006) 'Guidelines for the prevention of endocarditis: report of the Working Party of the British Society for Antimicrobial Chemotherapy'. *Journal of Antimicrobial Chemotherapy,* 57(6), pp. 1035-1042.

Habib, G., Lancellotti, P., Antunes, M.J., Bongiorni, M.G., Casalta, J., Del Zotti, F., Dulgheru, R., El Khoury, G., Erba, P.A., Iung, B., Miro, J.M., Mulder, B.J., Plonska-Gosciniak, E., Price, S., Roos-Hesselink, J., Snygg-Martin, U., Thuny, F., Tornos Mas, P., Vilacosta, I., Zamorano, J.L. and ESC Scientific Document Group. (2015) '2015 ESC Guidelines for the management of infective endocarditis: The Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM)'. *European Heart Journal,* 36(44), pp. 3075-3128.

Haley, R.W., Culver, D.H., White, J.W., Morgan, W.M., Emori, T.G., Munn, V.P. and Hooton, T.M. (1985) 'The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals'. *American Journal of Epidemiology,* 121(2), pp. 182-205.

Hayward, R.S., Harding, J., Molloy, R., Land, L., Longcroft-Neal, K., Moore, D. and Ross, J.D.C. (2018) 'Adverse effects of a single dose of gentamicin in adults: a systematic review'. *British Journal of Clinical Pharmacology,* 84(2), pp. 223-238.

Health Protection Scotland (2013) *Surveillance of Surgical Site Infection: Annual Report - For procedures carried out from: January 2008 - December 2012.* Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=709 (Accessed: 02 July 2018).

Health Protection Scotland (2014) *Healthcare Associated Infection Annual Report 2013.* Available at: http://www.documents.hps.scot.nhs.uk/hai/annual-report/healthcare-associated-infection-annual-report-2013.pdf (Accessed: 02 July 2018).

Health Protection Scotland (2017) *Surgical Site Infection Surveillance Protocol 7th Edition 2017.* Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6442 (Accessed: 03 July 2018).

Health Protection Scotland (2018) *Healthcare Associated Infection Annual Report 2017*. Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6574 (Accessed: 02 July 2018).

Hill, S.A., Jones, K.H. and Lees, L.J. (1980) 'Pharmacokinetics of parenterally administered amoxycillin'. *Journal of Infection,* 2(4), pp. 320-332.

Hobbiss, J.H., Carr, N.D. and Schofield, P.F. (1988) 'Are we using the correct dose of metronidazole in colorectal surgery?'. *Journal of the Royal Society of Medicine,* 81(2), pp. 95-96.

Houghton, G.W., Thorne, P.S., Smith, J., Templeton, R. and Collier, J. (1979) 'Comparison of the pharmacokinetics of metronidazole in healthy female volunteers following either a single oral or intravenous dose'. *British Journal of Clinical Pharmacology,* 8(4), pp. 337-341.

Humbert, G., Spyker, D.A., Fillastre, J.P. and Leroy, A. (1979) 'Pharmacokinetics of amoxicillin: Dosage nomogram for patients with impaired renal function'. *Antimicrobial Agents & Chemotherapy,* 15(1), pp. 28-33.

Idsoe, O., Guthe, T., Willcox, R.R. and de Weck, A.L. (1968) 'Nature and extent of penicillin side-reactions, with particular reference to fatalities from anaphylactic shock'. *Bulletin of the World Health Organization,* 38(2), pp. 159-188.

Isla, A., Troconiz, I.F., de Tejada, I.L., Vazquez, S., Canut, A., Lopez, J.M., Solinis, M.A. and Gascon, A.R. (2012) 'Population pharmacokinetics of prophylactic cefoxitin in patients undergoing colorectal surgery'. *European Journal of Clinical Pharmacology,* 68(5), pp. 735-745.

Janmahasatian, S., Duffull, S.B., Ash, S., Ward, L.C., Byrne, N.M. and Green, B. (2005) 'Quantification of lean bodyweight'. *Clinical Pharmacokinetics,* 44(10), pp. 1051-1065.

Jonsson, E.N. and Karlsson, M.O. (1999) 'Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM'. *Computer Methods & Programs in Biomedicine,* 58(1), pp. 51-64.

Karlsson, M.O. and Savic, R.M. (2007) 'Diagnosing model diagnostics'. *Clinical Pharmacology and Therapeutics*, 82(1), pp. 17-20.

Kathriarachchi, U.L., Vidhate, S.S., Al-Tannak, N., Thomson, A.H., da Silva Neto, M.J.J. and Watson, D.G. (2018) 'Development of a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography (HILIC)'. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences*, 1089, pp. 78-83.

Khuroo, A.H., Monif, T., Verma, P.R.P. and Gurule, S. (2008) 'Simple, economical, and reproducible LC-MS method for the determination of amoxicillin in human plasma and its application to a pharmacokinetic study'. *Journal of Chromatographic Science*, 46(10), pp. 854-861.

Kirkland K.B., Briggs J.P., Trivette S.L., Wilkinson W.E. and Sexton, D.J. (1999) 'The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs'. *Infection Control and Hospital Epidemiology : the Official Journal of the Society of Hospital Epidemiologists of America,* 20(11), pp. 725-730.

Kling, P.A. and Burman, L.G. (1989) 'Serum and tissue pharmacokinetics of intravenous metronidazole in surgical patients'. *Acta Chirurgica Scandinavica,* 155(6-7), pp. 347-350.

Latte, S., Sordillo, E.M. and Edberg, S.C. (2015) 'Applications, significance of, and methods for the measurement of antimicrobial concentrations' in Amsterdam D. (ed.) *Antibiotics in Laboratory Medicine*. Philadelphia: Wolters Kluwer, pp. 319-407.

Lau, A.H., Lam, N.P., Piscitelli, S.C., Wilkes, L. and Danziger, L.H. (1992) 'Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives'. *Clinical Pharmacokinetics,* 23(5), pp. 328-364.

Leader, W.G., Tsubaki, T. and Chandler, M.H. (1994) 'Creatinine-clearance estimates for predicting gentamicin pharmacokinetic values in obese patients'. *American Journal of Hospital Pharmacy,* 51(17), pp. 2125-2130.

Levy, M., Egersegi, P., Strong, A., Tessoro, A., Spino, M., Bannatyne, R., Fear, D., Posnick, J.C. and Koren, G. (1990) 'Pharmacokinetic analysis of cloxacillin loss in children undergoing major surgery with massive bleeding'. *Antimicrobial Agents & Chemotherapy,* 34(6), pp. 1150-1153.

Lindbom, L., Ribbing, J. and Jonsson, E.N. (2004) 'Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming'. *Computer Methods & Programs in Biomedicine,* 75(2), pp. 85-94.

Llanos-Paez, C.C., Hennig, S. and Staatz, C.E. (2017) 'Population pharmacokinetic modelling, Monte Carlo simulation and semi-mechanistic pharmacodynamic modelling as tools to personalize gentamicin therapy'. *Journal of Antimicrobial Chemotherapy,* 72(3), pp. 639-667.

Loft, S., Dossing, M., Poulsen, H.E., Sonne, J., Olesen, K.L., Simonsen, K. and Andreasen, P.B. (1986) 'Influence of dose and route of administration on disposition of metronidazole and its major metabolites'. *European Journal of Clinical Pharmacology,* 30(4), pp. 467-473.

Lovering, A.M. (1999) 'Aminoglycosides', in Reeves D.S., Wise R., Andrews J.M. and White L.O. (eds.) *Clinical Antimicrobial Assays*. Oxford: Oxford University Press, pp. 123-148.

Ludwig, K.A., Carlson, M.A. and Condon, R.E. (1993) 'Prophylactic antibiotics in surgery'. *Annual Review of Medicine,* 44, pp. 385-393.

MacGowan, A.P., Bedford, K.A., Blundell, E., Brown, N.M., Habib, F., Hows, J., Kirkpatrick, B., Korner, R.J., Slade, R.R. and White, L.O. (1994) 'The pharmacokinetics of once daily gentamicin in neutropenic adults with haematological malignancy'. *Journal of Antimicrobial Chemotherapy,* 34(5), pp. 809-812.

Mangram, A.J., Horan, T.C., Pearson, M.L., Silver, L.C. and Jarvis, W.R. (1999) 'Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee'. *Infection Control & Hospital Epidemiology,* 20(4), pp. 250-278.

Markantonis, S.L., Kostopanagiotou, G., Panidis, D., Smirniotis, V. and Voros, D. (2004) 'Effects of blood loss and fluid volume replacement on serum and tissue gentamicin concentrations during colorectal surgery'. *Clinical Therapeutics,* 26(2), pp. 271-281.

Martin C., Mallet M.N., Bantz P., Ruperti A., Sastre B., De Micco P. and Gouin, F. (1993) 'Pharmacokinetics and tissue penetration of a single dose of netilmicin as antibiotic prophylaxis in colorectal surgery'. *Drug Investigation,* 6(5), pp. 285-290.

Martin, C., Mallet, M.N., Sastre, B., Viviand, X., Martin, A., De Micco, P. and Gouin, F. (1995) 'Comparison of concentrations of two doses of clavulanic acid (200 and 400 milligrams) administered with amoxicillin (2,000 milligrams) in tissues of patients undergoing colorectal surgery'. *Antimicrobial Agents & Chemotherapy*, 39(1), pp. 94-98.

Martin, C., Sastre, B., Mallet, M.N., Bruguerolle, B., Brun, J.P., De Micco, P. and Gouin, F. (1991) 'Pharmacokinetics and tissue penetration of a single 1,000-milligram, intravenous dose of metronidazole for antibiotic prophylaxis of colorectal surgery'. *Antimicrobial Agents and Chemotherapy*, 35(12), pp. 2602-2605.

Mastrobattista, J.M., Klebanoff, M.A., Carey, J.C., Hauth, J.C., Macpherson, C.A., Ernest, J., Cotroneo, M., Leveno, K.J., Wapner, R., Varner, M., Iams, J.D., Moawad, A., Sibai, B.M., Miodovnik, M., Dombrowski, M., O'Sullivan, M.J., Vandorsten, J.P., Langer, O. and National Institute of Child Health and Human Development Maternal-Fetal Medicine Unit Networks. (2008) 'The effect of body mass index on therapeutic response to bacterial vaginosis in pregnancy'. *American Journal of Perinatology,* 25(4), pp. 233-237.

Mattila, J., Mannisto, P.T., Mantyla, R., Nykanen, S. and Lamminsivu, U. (1983) 'Comparative pharmacokinetics of metronidazole and tinidazole as influenced by administration route'. *Antimicrobial Agents & Chemotherapy,* 23(5), pp. 721-725.

Medico, C.J. and Walsh P. (2010) 'Pharmacotherapy in the critically ill obese patient'. *Critical Care Clinics*, 26(4), pp. 679-688.

Meng, L., Mui, E., Holubar, M.K. and Deresinski, S.C. (2017) 'Comprehensive Guidance for Antibiotic Dosing in Obese Adults'. *Pharmacotherapy:The Journal of Human Pharmacology & Drug Therapy*, 37(11), pp. 1415-1431.

Moine, P. and Fish, D.N. (2013) 'Pharmacodynamic modelling of intravenous antibiotic prophylaxis in elective colorectal surgery'. *International Journal of Antimicrobial Agents*, 41(2), pp. 167-173.

Morita, S., Nishisho, I., Nomura, T., Fukushima, Y., Morimoto, T., Hiraoka, N. and Shibata, N. (2005) 'The significance of the intraoperative repeated dosing of antimicrobials for preventing surgical wound infection in colorectal surgery'. *Surgery Today,* 35(9), pp. 732-738.

Mould, D.R. and Upton, R.N. (2012) 'Basic concepts in population modeling, simulation, and model-based drug development'. *CPT: Pharmacometrics & Systems Pharmacology,* 1, pp. e6.

Muller, A.E., Huttner, B. and Huttner, A. (2018) 'Therapeutic Drug Monitoring of Beta-Lactams and Other Antibiotics in the Intensive Care Unit: Which Agents, Which Patients and Which Infections?'. *Drugs,* 78(4), pp. 439-451.

National Institute for Health and Care Excellence (2008) *Surgical Site Infections: Prevention and Treatment*. Available at: https://www.nice.org.uk/guidance/cg74/resources/surgical-site-infections-prevention-and-treatment-pdf-975628422853 (Accessed: 03 July 2018).

Ohge, H., Takesue, Y., Yokoyama, T., Murakami, Y., Hiyama, E., Yokoyama, Y., Kanehiro, T., Itaha, H. and Matsuura, Y. (1999) 'An additional dose of cefazolin for intraoperative prophylaxis'. *Surgery Today,* 29(12), pp. 1233-1236.

Owen, J.S. and Fiedler-Kelly, J. (2014) 'The Practice of Pharmacometrics', in Owen, J.S. and Fiedler-Kelly, J. (eds.) *Introduction to Population Pharmacokinetic / Pharmacodynamic Analysis with Nonlinear Mixed Effects Model*. Hoboken: Wiley, pp. 1-8.

Poggio, J.L. (2013) 'Perioperative strategies to prevent surgical-site infection'. *Clinics in Colon & Rectal Surgery,* 26(3), pp. 168-173.

Public Health England (2017) *Surveillance of surgical site infections in NHS hospitals in England, 2016 to 2017*. Available at: https://www.gov.uk/government/publications/surgical-site-infections-ssi-surveillance-nhs-hospitals-in-england (Accessed: 12 April 2018).

Public Health England (2019) *Surveillance of surgical site infections in NHS hospitals in England, April 2018 to March 2019*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/854182/SSI_Annual_Report_2018_19.pdf (Accessed: 25 January 2020).

R Core Team (2014) *R: A language and environment for statistical computing.* Available at: https://www.r-project.org (Accessed: 19 September 2018).

Ramirez-Ronda, C.H., Holmes, R.K. and Sanford, J.P. (1975) 'Effects of divalent cations on binding of aminoglycoside antibiotics to human serum proteins and to bacteria'. *Antimicrobial Agents & Chemotherapy,* 7(3), pp. 239-245.

Sagan, C., Salvador, A., Dubreuil, D., Poulet, P.P., Duffaut, D. and Brumpt, I. (2005) 'Simultaneous determination of metronidazole and spiramycin I in human plasma, saliva and gingival crevicular fluid by LC–MS/MS'. *Journal of Pharmaceutical and Biomedical Analysis*, 38(2), pp. 298-306.

Schentag, J., Meagher, A. and Jelliffe, R. (2006) 'Aminoglycosides', in Burton M., Shaw L., Schentag J. and Evans W. (eds.) *Applied Pharmacokinetics and Pharmacodynamics: Principles of Therapeutic Drug Monitoring*. Philadelphia: Lippincott Williams and Wilkins, pp. 285-327.

Scher, K.S. (1997) 'Studies on the duration of antibiotic administration for surgical prophylaxis'. *American Surgeon,* 63(1), pp. 59-62.

Scottish Antimicrobial Prescribing Group (2016) *Recommendations for Re-dosing Antibiotics for Surgical Prophylaxis*. Available at: https://www.sapg.scot/media/2947/sapg_recommendations_for_re-dosing_antibiotics_for_surgical_prophylaxis.pdf (Accessed: 02 July 2018).

Scottish Intercollegiate Guidelines Network (2014) *Antibiotic Prophylaxis in Surgery (SIGN Guideline No. 104)*. Available at: http://www.sign.ac.uk/assets/sign104.pdf (Accessed: 02 July 2018).

Sender, R., Fuchs, S. and Milo, R. (2016) 'Revised Estimates for the Number of Human and Bacteria Cells in the Body'. *PLOS Biology,* 14(8): e1002533.

Silva M., Schramm S., Kano E., Koono E., Porta V. and Serra, C. (2009) 'Development and validation of a HPLC-MS-MS method for quantification of metronidazole in human plasma'. *Journal of Chromatographic Science,* 47(9), pp. 781-784.

Song, F. and Glenny, A.M. (1998) 'Antimicrobial prophylaxis in colorectal surgery: a systematic review of randomized controlled trials'. *British Journal of Surgery,* 85(9), pp. 1232-1241.

Sprandel, K.A., Drusano, G.L., Hecht, D.W., Rotschafer, J.C., Danziger, L.H. and Rodvold, K.A. (2006) 'Population pharmacokinetic modeling and Monte Carlo simulation of varying doses of intravenous metronidazole'. *Diagnostic Microbiology & Infectious Disease,* 55(4), pp. 303-309.

Spyker, D.A., Rugloski, R.J., Vann, R.L. and O'Brien, W.M. (1977) 'Pharmacokinetics of amoxicillin: dose dependence after intravenous, oral, and intramuscular administration'. *Antimicrobial Agents & Chemotherapy,* 11(1), pp. 132-141.

Sutherland, R., Croydon, E.A. and Rolinson, G.N. (1972) 'Amoxycillin: a new semi-synthetic penicillin'. *British Medical Journal,* 3(5817), pp. 13-16.

Swoboda, S.M., Merz, C., Kostuik, J., Trentler, B. and Lipsett, P.A. (1996) 'Does intraoperative blood loss affect antibiotic serum and tissue concentrations?'. *Archives of Surgery,* 131(11), pp. 1165-1171.

Thummel, K., Shen, D. and Isoherranen, N. (2011) 'Design and Optimization of Dosage Regimens: Pharmacokinetic Data', in Brunton L., Chabner B. and Knollmann B. (eds.) *The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, pp. 1891-1990.

Tresadern, J.C., Farrand, R.J. and Irving, M.H. (1983) 'Streptococcus milleri and surgical sepsis'. *Annals of the Royal College of Surgeons of England,* 65(2), pp. 78-79.

Unger S., Li W., Flarakos J. and Tse F.L.S. (2013) 'Roles of LC-MS Bioanalysis in Drug Discovery, Development, and Therapeutic Drug Monitoring', in Li W., Zhang J. and Tse F.L.S. (eds.) *Handbook of LC-MS Bioanalysis*. New York: Wiley, pp. 3-13.

UK Medicines Information (2017) *How should antibiotics be dosed in obesity?* Available at: https://www.sapg.scot/media/2918/howshouldantibioticsbedosedinobesity_2016_update.pdf (Accessed: 12 June 2019).

Ventura Cerda, J.M., Alos Alminana, M., Nomdedeu Guinot, J., Merino Sanjuan, V. and Salvador Sanchis, J.L. (2008) 'The pharmacokinetics of metronidazole and gentamicin in a single preoperative dose as antibiotic prophylaxis in colorectal surgery'. *Farmacia Hospitalaria,* 32(2), pp. 77-82.

Veyssier, P. and Bryskier, A. (2005) 'Aminocyclitol Aminoglycosides', in Bryskier, A. (ed.) *Antimicrobial Agents*. Washington: ASM Press, pp. 453-469.

Wagner, J.G. and Metzler, C.M. (1969) 'Prediction of blood levels after multiple doses from single-dose blood level data: data generated with two-compartment open model analyzed according to the one-compartment open model'. *Journal of Pharmaceutical Sciences,* 58(1), pp. 87-92.

Weinstein, M.J., Luedemann, G.M., Oden, E.M. and Wagman, G.H. (1963) 'Gentamicin, a new broad-spectrum antibiotic complex'. *Antimicrobial Agents & Chemotherapy,* 161, pp. 1-7.

Wen A., Hang T., Chen S., Wang Z., Ding L., Tian Y., Zhang M. and Xu, X. (2008) 'Simultaneous determination of amoxicillin and ambroxol in human plasma by LC-MS/MS: Validation and application to pharmacokinetic study'. *Journal of Pharmaceutical and Biomedical analysis,* 48(3), pp. 829-834.

White, L.O. and Andrews, J.M. (1999) 'β-Lactams', in Reeves D.S., Wise R., Andrews J.M. and White L.O. (eds.) *Clinical Antimicrobial Assays*. Oxford: Oxford University Press, pp. 93-121.

White, L.O. and Lovering, A.M. (1999) 'Non-microbiological assays', in Reeves D.S., Wise R., Andrews J.M. and White L.O. (eds.) Clinical Antimicrobial Assays. Oxford: Oxford University Press, pp. 45-63.

World Health Organization (2002) Diagnostic Imaging and Laboratory Technology. *Use of anticoagulants in diagnostic laboratory investigations*. Available at: https://apps.who.int/iris/handle/10665/65957 (Accessed: 17 January 2020).

World Health Organization (2018) *Protocol for surgical site infection surveillance with a focus on settings with limited resources*. Available at: https://www.who.int/infection-prevention/tools/surgical/SSI-surveillance-protocol.pdf (Accessed: 28 January 2020).

Yoon, K., Lee, S., Kim, W., Park, J. and Kim, H. (2004) 'Simultaneous determination of amoxicillin and clavulanic acid in human plasma by HPLC-ESI mass spectrometry'. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences,* 813(1-2), pp. 121-127.

Zanetti, G., Giardina, R. and Platt, R. (2001) 'Intraoperative redosing of cefazolin and risk for surgical site infection in cardiac surgery'. *Emerging Infectious Diseases*, 7(5), pp. 828-831.

Zelenitsky, S.A., Lawson, C., Calic, D., Ariano, R.E., Roberts, J.A., Lipman, J. and Zhanel, G.G. (2016). 'Integrated pharmacokinetic-pharmacodynamic modelling to evaluate antimicrobial prophylaxis in abdominal surgery'. *Journal of Antimicrobial Chemotherapy*, 71(10), pp. 2902-2908.

Zelenitsky, S.A., Silverman, R.E., Duckworth, H. and Harding, G.K. (2000) 'A prospective, randomized, double-blind study of single high dose versus multiple standard dose gentamicin both in combination with metronidazole for colorectal surgical prophylaxis'. *Journal of Hospital Infection,* 46(2), pp. 135-140.

Zelenitsky, S.A., Ariano, R.E., Harding, G.K.M. and Silverman, R.E. (2002) 'Antibiotic pharmacodynamics in surgical prophylaxis: an association between intraoperative antibiotic concentrations and efficacy'. *Antimicrobial Agents & Chemotherapy,* 46(9), pp. 3026-3030.

6 APPENDICES

Appendix 1: NHSGGC Clinical Guideline, Antibiotic Prophylaxis in Gastrointestinal and Vascular Surgery

NHS Greater Glasgow and Clyde recommendations for antibiotic prophylaxis in Gastro-intestinal and Vascular surgery

Single dose, IV prophylaxis ≤ 60mins prior to skin incision/ intervention. For **gentamicin**[#] dose see surgical prophylaxis dosing tables.

- If >1.5L blood loss replace fluid and repeat antibiotic dose: **amoxicillin, flucloxacillin, metronidazole**, and **gentamicin** (at half prophylaxis dose). Do not re-dose teicoplanin.
- If surgery >4hrs redose **amoxicillin, flucloxacillin;** >8hrs redose **amoxicillin, flucloxacillin metronidazole**, and if eGFR>60mls/min **gentamicin** (full prophylactic dose). No repeat dosing of **teicoplanin** if surgery prolonged.

MRSA: decolonise prior to procedure as per NHS GGC infection control guidelines and discuss with microbiology antibiotic choice.

Procedure	Comments	Antibiotic
General/GI Surgery Oesophageal surgery, Stomach and Duodenal surgery, Gastric bypass surgery Small intestine surgery Bile duct surgery Gall bladder surgery (open). Appendicectomy Colorectal surgery	**Prophylaxis routinely recommended**	IV **Gentamicin**[#]. + IV **Metronidazole** 500mg + IV **Amoxicillin** 1g If true penicillin / beta-lactam allergy or high MRSA risk, replace IV **Amoxicillin** 1g with IV **Teicoplanin** 400mg
Gall bladder surgery (laparoscopic)	**Not routinely recommended** (Consider if: Intraoperative Cholangiogram, bile spillage, conversion to laporotomy, acute cholecystitis / pancreatitis, jaundice, pregnancy, immunosuppression, insertion of prosthetic devices.	
Splenectomy	**Not routinely recommended**, unless immunosuppressed patient. Remember post splenectomy prophylaxis.	
Hernia repair +/- mesh, open or laparoscopic	**Not routinely recommended**. (Meta-analysis does not support routine prophylaxis. Consider in patients with mesh insertion if: obesity, diabetes, or other risk factors for SSI)	IV **Gentamicin**[#]. + IV **Metronidazole** 500mg + IV **Teicoplanin** 400mg
Breast surgery	**Not routinely recommended**	
Vascular surgery Lower limb amputation Arterial reconstruction/graft/ prosthetic	Perform MRSA screening prior to planned surgery. High risk if: previous/ current MRSA carriage, hospitalisation or antibiotic therapy in previous 4 weeks, poorly controlled diabetes, tissue loss or recent foot sepsis.	IV **Flucloxacillin** 2g + IV **Metronidazole** 500mg If true penicillin / beta-lactam allergy or high MRSA risk, replace **Flucloxacillin** 2g with IV **Teicoplanin** 400mg IV **Flucloxacillin** 2g + IV **Gentamicin**[#] If true penicillin / beta-lactam allergy or high MRSA risk, replace **Flucloxacillin** 2g with IV **Teicoplanin** 400mg

Antimicrobial Utilisation Committee and General Surgeons February 2018
Review date Feb 2021

Prophylactic IV Gentamicin Dosing Table

Avoid if eGFR <20mls/min/ 1.73m^2: seek advice on alternative from microbiology In renal transplant patients avoid Gentamicin and seek advice from microbiology or renal team			
Height ranges (Feet and Inches)	**Height ranges (cm)**	**Gentamicin Dose (mg)**	
		Males	**Females**
4' 8" - 4' 10"	142 - 147	**160**	**140**
4' 11" - 5' 3"	148 - 160	**180**	**160**
5' 4" - 5' 10"	161 - 178	**240**	**200**
5' 11" - 6' 2"	179 - 188	**300**	**260**
≥ 6' 3"	≥ 189	**300**	**300**

Appendix 2: Consent Form

Lister Department of Surgery, Glasgow Royal Infirmary
84 Castle Street, Glasgow, G4 0ET

Participant Identification Number:

NHS
Greater Glasgow and Clyde

CONSENT FORM

Title of Project: Serum concentrations of amoxicillin, metronidazole and gentamicin for antibiotic prophylaxis in colorectal surgery

Name of Chief Investigator: Mr Graham MacKay

Please initial box

1. I confirm that I have read the information sheet dated 16/05/2016 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by the research team where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I agree to take part in the above study. ☐

6. Do you wish to receive a summary of the study results? Yes ☐ No ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher file; 1 (original) to be kept in medical notes.
Version 2.0 16th May 2016

Appendix 3: Participant Information Sheet

NHS
Greater Glasgow and Clyde

Serum concentrations of amoxicillin, metronidazole and gentamicin for antibiotic prophylaxis in colorectal surgery

Name of Chief Investigator: Mr Graham MacKay
Lister Department of Surgery, Glasgow Royal Infirmary
84 Castle Street, Glasgow, G4 0ET
Tel No 01412320852

Name of Co-investigator: Michael da Silva Neto
Pharmacy Department, Glasgow Royal Infirmary
84 Castle Street, Glasgow, G4 0ET
Tel No 01412110588

Participant Information Sheet

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

You will receive antibiotics just before you go into theatre for your colorectal surgery. This is routine practice and reduces the chance of your wound becoming infected after your operation. At present, the ideal dose of each antibiotic you receive and the best time to give another dose of antibiotic during long operations is not clear. The aim of this study is to check how much antibiotic remains in your blood at different times during your operation. The results of the study will be used to help us review our antibiotic guidelines.

Why have I been invited?

You have been invited to take part in this study because you are going to have colorectal surgery.

Do I have to take part?

We are looking for a total of 20 people to take part in the study. It is up to you to decide if you want to participate. We will describe the study to you and go through this information sheet, which we will then give to you. If you decide to take part you will be asked to sign a consent form. You are free to withdraw at any time without giving a reason. This will not affect the standard of care you receive.

What does taking part involve?

Taking part in this study will involve you:

- Giving a small sample of blood at different times during the surgical procedure. No more than 25 mL will be removed in total (approximately 5 teaspoons). Samples will be taken through a line that will already be in place during your operation. No additional needles will be required.
- Agreeing to have the following information collected on the day of your surgical procedure: surgical procedure name, length of surgery, age, height, weight, sex, estimated blood loss, fluid volumes administered and routine blood test results. This information is routinely collected and will not involve any additional needles or blood samples.
- Agreeing to allow the study team to look at your medical records for the 30 days after your surgical procedure and to record whether or not you were readmitted to hospital with a wound infection during this time.

What happens to the information?

Only the research team will know your identity and personal information. The data collected for the study will remain confidential and will be held in accordance with the Data Protection Act, which means that we keep it safely and cannot reveal it to other people, without your permission.

What will happen to my blood samples?

Blood samples collected for the purposes of the study will be:

- Frozen and kept securely in the research freezer at Glasgow Royal Infirmary. They will be labelled with a unique study number and not your name or any personal information.
- Used to measure the concentrations of routinely given antibiotics. Your samples will be analysed in the analytical research laboratory within the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow.
- Used to identify metabolites related to the efficacy of the antibiotics.

What are the possible benefits of taking part?

There will be no immediate direct benefit to you should you participate. However, the information we get from this study may help us to improve the way we use antibiotics to prevent wound infections.

Are there any risks for me in joining the study?

The risk of suffering harm as a result of taking part in this study is minimal. Your antibiotics and other routine clinical care will not be affected by this study. The only difference is that we will take up to 25 mL of blood (approximately 5 teaspoons) from you during the surgical procedure. No additional needles will be used.

Results of the Study

At the end of the study the information collected will be analysed and your study doctor will be able to supply a summary of the results to you on request. The identity of the patients who took part in the study will remain confidential.

Who has reviewed the study?

This study has been reviewed and approved by the East Midlands Research Ethics Committee (REC Reference Number: 16/EM/0209).

Time to Consider

You should take at least 24 hours to decide if you wish to take part.

Who should you contact with questions?

We will give you a copy of the information sheet and signed consent form to keep. If you have any problems or questions about this study please contact:

Doctor: Mr Graham MacKay

Tel No 01412320852

Antimicrobial Pharmacist: Michael da Silva Neto

Tel No 01412114486

If you would like more information about the study and wish to speak to someone not closely linked to the study, please contact:

Doctor: Dr Ruth McKee

Tel No: 01412114286

What to do if you have a complaint about any aspect of the study?

If you have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you (Patient Advice & Support Service: complaints@ggc.scot.nhs.uk Telephone: 0141 201 4500). In addition the Citizens Independent Advice and Support Service is available to provide advice and information on the complaints process and to assist in progressing a complaint. They can be contacted by telephone on 0845 2311010 or via any Citizens Advice Office.

Thank you for your time and co-operation

Appendix 4: Case Report Form

NHS Greater Glasgow and Clyde

Case Report Form

Title of project: Serum concentrations of amoxicillin, metronidazole and gentamicin for antibiotic prophylaxis in colorectal surgery

Name of Chief Investigator: Mr Graham MacKay

Patient ID: _____ Date: _____

Consent: Yes ☐ No ☐ Surgical procedure: _____

Age (years): ____ Sex: M ☐ F ☐

Weight (kg)		Date
Height (cm)		Date
Albumin (g/L)		Date
Creatinine (µmol/L)		Date
Haemoglobin pre op (g/L)		Date
Haemoglobin post op (g/L)		Date

Antibiotic	Dose (mg)	Time of the start of admin (24 h clock)	*Redosing if applicable (mg)*	*Time of the start of admin (24 h clock)*
Amoxicillin (bolus)		:		:
Gentamicin (bolus)		:		:
Metronidazole (infusion)		:		:

Time of incision (24 h clock): ____:____

Blood sample	**1** Predose	**2** 1h after the start of antibiotic admin	**3** 2h after the start of antibiotic admin	*Immediately before redosing if applicable*	*1h after redosing if applicable*	At skin closure
Target time (24 h clock)		:	:	:	:	
Actual time (24 h clock)	:	:	:	:	:	:
Estimated blood loss (mL)						
IV fluids volumes (mL)						
Storage time (24 h clock)	:	:	:	:	:	:

Time of skin closure (24 h clock): ____:____ Follow-up of SSI: Yes ☐ No ☐

Other info:

_____ _____

Signature Date

Glasgow Royal Infirmary

Case Report Form
V2.0/Aug 2016

Appendix 5: Protocol for sample processing and storage within the Glasgow Clinical Research Facility (GCRF) laboratory

NHS
Greater Glasgow and Clyde

Protocol for sample processing and storage within the Glasgow Clinical Research Facility (GCRF) laboratory

Title of project: Serum concentrations of amoxicillin, metronidazole and gentamicin for antibiotic prophylaxis in colorectal surgery

Name of Chief Investigator: Mr Graham MacKay

- The anaesthetist will collect 3 mL blood samples in the operating theatre as per study protocol dated 04/02/2016 (version 1.0). The blood samples will be collected in 5 mL Vacuette tubes containing clotting accelerator and separation gel.

- The Vacuette tubes should be mixed by inversion 6-8 times and immediately placed on ice.

- The blood sample(s) should be transported on ice to the GCRF laboratory as soon as possible after collection (in a blood transport bag).

- At the GCRF laboratory the blood samples should be centrifuged at 3000 g for 10 minutes at 4°C

- After centrifugation the supernatant should be removed with a Pasteur pipette and placed in a container suitable for freezing.

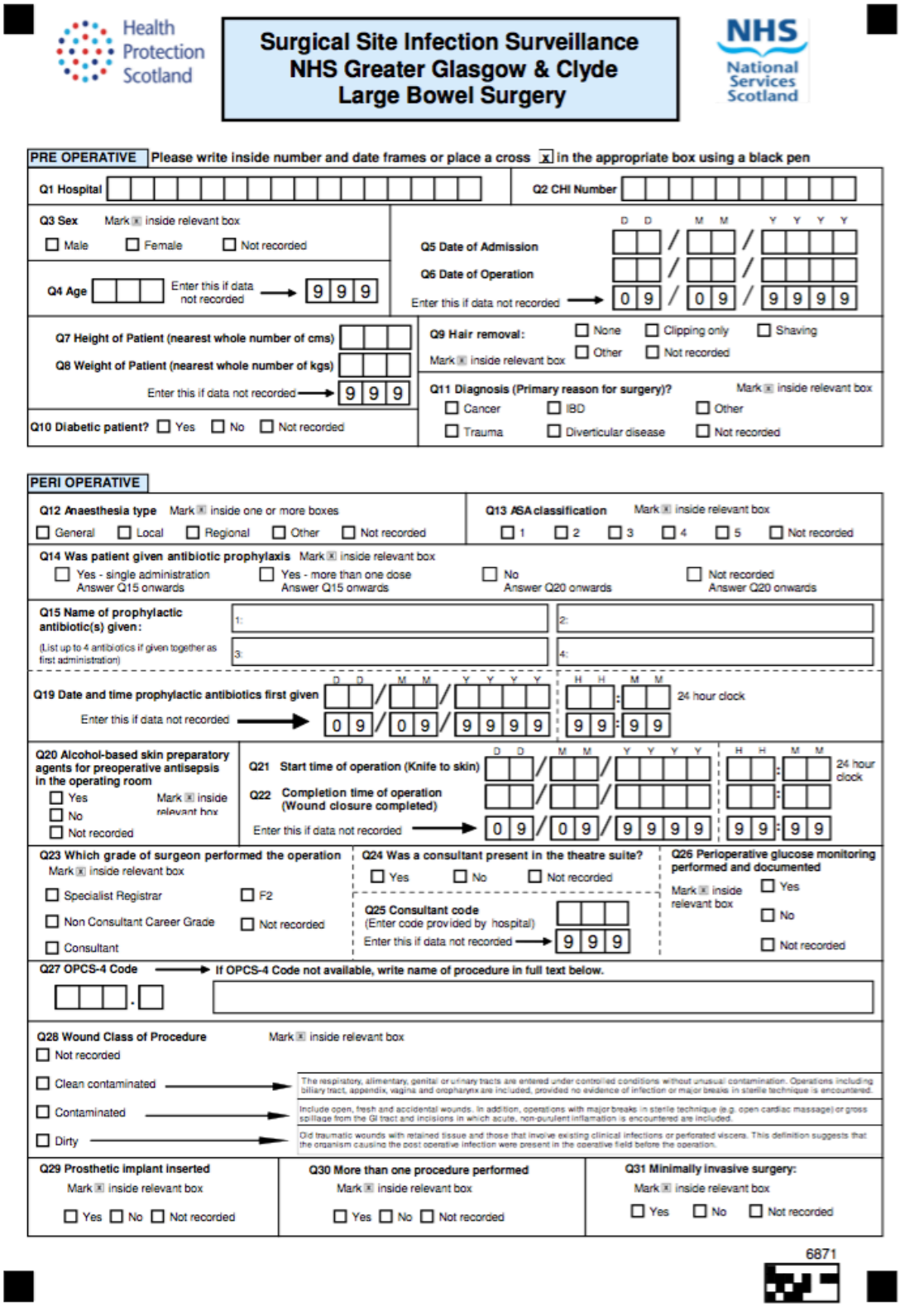
- The serum samples should be appropriately labelled and placed in the GCRF freezer at –80°C.

- The GCRF Freezer Sample Log should be completed accordingly.

- **Please note the GCRF sample storage timeline of 1 month.**

Protocol for sample processing and storage within the GCRF laboratory
V2.0/Jun 2016

Appendix 6: Surgical site infection surveillance, large bowel surgery, data collection form



Health Protection Scotland

Surgical Site Infection Surveillance
NHS Greater Glasgow & Clyde
Large Bowel Surgery

NHS National Services Scotland

PRE OPERATIVE Please write inside number and date frames or place a cross [X] in the appropriate box using a black pen

Q1 Hospital

Q2 CHI Number

Q3 Sex Mark [X] inside relevant box
☐ Male ☐ Female ☐ Not recorded

Q4 Age Enter this if data not recorded → 9 9 9

Q5 Date of Admission (D D / M M / Y Y Y Y)

Q6 Date of Operation (D D / M M / Y Y Y Y)

Enter this if data not recorded → 0 9 / 0 9 / 9 9 9 9

Q7 Height of Patient (nearest whole number of cms)

Q8 Weight of Patient (nearest whole number of kgs)

Enter this if data not recorded → 9 9 9

Q9 Hair removal: Mark [X] inside relevant box
☐ None ☐ Clipping only ☐ Shaving ☐ Other ☐ Not recorded

Q10 Diabetic patient? ☐ Yes ☐ No ☐ Not recorded

Q11 Diagnosis (Primary reason for surgery)? Mark [X] inside relevant box
☐ Cancer ☐ IBD ☐ Other ☐ Trauma ☐ Diverticular disease ☐ Not recorded

PERI OPERATIVE

Q12 Anaesthesia type Mark [X] inside one or more boxes
☐ General ☐ Local ☐ Regional ☐ Other ☐ Not recorded

Q13 ASA classification Mark [X] inside relevant box
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Not recorded

Q14 Was patient given antibiotic prophylaxis Mark [X] inside relevant box
☐ Yes - single administration Answer Q15 onwards
☐ Yes - more than one dose Answer Q15 onwards
☐ No Answer Q20 onwards
☐ Not recorded Answer Q20 onwards

Q15 Name of prophylactic antibiotic(s) given:
(List up to 4 antibiotics if given together as first administration)
1: 2: 3: 4:

Q19 Date and time prophylactic antibiotics first given (D D / M M / Y Y Y Y ; H H : M M 24 hour clock)
Enter this if data not recorded → 0 9 / 0 9 / 9 9 9 9 ; 9 9 : 9 9

Q20 Alcohol-based skin preparatory agents for preoperative antisepsis in the operating room
☐ Yes ☐ No ☐ Not recorded Mark [X] inside relevant box

Q21 Start time of operation (Knife to skin) (D D / M M / Y Y Y Y ; H H : M M 24 hour clock)

Q22 Completion time of operation (Wound closure completed)

Enter this if data not recorded → 0 9 / 0 9 / 9 9 9 9 ; 9 9 : 9 9

Q23 Which grade of surgeon performed the operation
Mark [X] inside relevant box
☐ Specialist Registrar ☐ F2 ☐ Non Consultant Career Grade ☐ Not recorded ☐ Consultant

Q24 Was a consultant present in the theatre suite?
☐ Yes ☐ No ☐ Not recorded

Q25 Consultant code
(Enter code provided by hospital)
Enter this if data not recorded → 9 9 9

Q26 Perioperative glucose monitoring performed and documented
Mark [X] inside relevant box
☐ Yes ☐ No ☐ Not recorded

Q27 OPCS-4 Code → If OPCS-4 Code not available, write name of procedure in full text below.
[ ][ ][ ].[ ]

Q28 Wound Class of Procedure Mark [X] inside relevant box
☐ Not recorded
☐ Clean contaminated → The respiratory, alimentary, genital or urinary tracts are entered under controlled conditions without unusual contamination. Operations including biliary tract, appendix, vagina and oropharynx are included, provided no evidence of infection or major breaks in sterile technique is encountered.
☐ Contaminated → Include open, fresh and accidental wounds. In addition, operations with major breaks in sterile technique (e.g. open cardiac massage) or gross spillage from the GI tract and incisions in which acute, non-purulent inflamation is encountered are included.
☐ Dirty → Old traumatic wounds with retained tissue and those that involve existing clinical infections or perforated viscera. This definition suggests that the organism causing the post operative infection were present in the operative field before the operation.

Q29 Prosthetic implant inserted
Mark [X] inside relevant box
☐ Yes ☐ No ☐ Not recorded

Q30 More than one procedure performed
Mark [X] inside relevant box
☐ Yes ☐ No ☐ Not recorded

Q31 Minimally invasive surgery:
Mark [X] inside relevant box
☐ Yes ☐ No ☐ Not recorded

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Health Protection Scotland

Surgical Site Infection Surveillance
NHS Greater Glasgow & Clyde
Large Bowel Surgery

NHS National Services Scotland

PERI OPERATIVE cont. Please write inside number and date frames or place a cross [x] in the appropriate box using a black pen

Q32 Laparoscopic-assisted approach:
☐ Yes ☐ No ☐ Not recorded Mark ☒ inside relevant box

Q33 Patient normothermia within one hour of the end of operation: (36-38°C (rectal) or 35,5-37,5 °C (non-rectal)):
☐ Yes ☐ No ☐ Not recorded Mark ☒ inside relevant box

POST OPERATIVE

Q34 Reintervention required within 24 hrs
Mark ☒ inside relevant box
☐ Yes - answer Q35 onwards →
☐ No - answer Q37 onwards ↓
☐ Not Recorded

Q35 Start time of reintervention (Knife to skin)
Q36 Completion time of reintervention (Wound closure time)
Enter this if data not recorded →

	D D	M M	Y Y Y Y	H H : M M (24 hour clock)
Q35				
Q36				
Enter this if data not recorded	0 9	0 9	9 9 9 9	9 9 : 9 9

Q37 Was patient given thromboprophylaxis? Mark ☒ inside relevant box
☐ Yes - mechanical only ☐ Yes - chemical only ☐ Yes - both mechanical and chemical ☐ No ☐ Not recorded

Q38 Date prophylactic antibiotics last given
Enter this if data not recorded →

D D	M M	Y Y Y Y
0 9	0 9	9 9 9 9

Time prophylactic antibiotics last given? (24 hour clock)
Enter this if data not recorded

H H : M M
9 9 : 9 9

Q39 Is patient receiving prophylactic antibiotics >24hrs following surgery? Mark ☒ inside relevant box
☐ Yes ☐ No ☐ Not recorded

Q40 If yes, reason why:

Q41 Was antibiotic prophylaxis in line with local guidelines? ☐ Yes ☐ No ☐ Don't know ☐ Not recorded Mark ☒ inside relevant box

Q42 Surgical Site Infection
Has the patient developed a surgical site infection? Mark ☒ inside relevant box
☐ Yes - answer Q43 onwards
☐ No - answer Q49 onwards
☐ Not recorded - answer Q49 onwards

Q43 Type of Surgical Site Infection
Mark ☒ inside relevant box
☐ Superficial ☐ Organ/Space
☐ Deep ☐ Not recorded

Q44 Site of SSI organ space
Mark ☒ inside relevant box
☐ Intra abdominal infection
☐ GI tract infection
☐ Not recorded

Q46 When was SSI detected?
Mark ☒ inside relevant box
☐ During the admission period
☐ On re-admission (within 30 days)
☐ Not recorded

Q47 Readmitted for SSI within 30 days?
Mark ☒ inside relevant box
☐ Yes ☐ No ☐ Not recorded

Q45 Criteria used to determine SSI - Record the diagnostic criteria that apply. Mark ☒ for all that apply
☐ Purulent Drainage ☐ Heat ☐ Incision is deliberately opened by surgeon
☐ Localised swelling ☐ Localised pain or tenderness ☐ Fever (temperature 38 degrees or more)
☐ Redness ☐ Incision spontaneously dehisces ☐ Not recorded
☐ Abscess/other evidence found during direct exam, a re-operation or radiology/histopathology
☐ Organisms isolated from an aseptically obtained culture of fluid, tissue, blood, bone or biopsy
☐ Diagnosis by surgeon or trained healthcare worker
Please specify: → ☐ Organisms isolated from swab ☐ Other
Extra Criteria Organ/Space Only
☐ Nausea ☐ Vomiting ☐ Jaundice ☐ Organisms seen on Gram stain
☐ Radiographic evidence of infection

	D D	M M	Y Y Y Y
Q48 Date of confirmed Surgical Site Infection			
Q49 Date of Discharge, Transfer or Death (post-op stay)			
If re-admitted to hospital: **Q50 Date of Re-admission to hospital**			
Q51 Date of Discharge, Transfer or Death (re-admission)			
Q52 Date Surveillance Discontinued			
Enter this if data not recorded →	0 9	0 9	9 9 9 9
Enter this if data not applicable →	1 0	1 0	9 9 9 9

Q53 Reason Surveillance Discontinued Mark ☒ inside relevant box
☐ End of 30 days surveillance
☐ Re-operation at same site
☐ Other, please specify below:
☐ Transfer ☐ Death → If patient dies, answer Q54
☐ Not recorded

Q54 Death related to SSI Mark ☒ inside relevant box
☐ SSI contributed to death
☐ Death not related to SSI
☐ SSI caused the death of the patient
☐ Not known if death related to SSI

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Health Protection Scotland

Surgical Site Infection Surveillance
NHS Greater Glasgow & Clyde
Large Bowel Surgery

NHS National Services Scotland

Microorganisms

Please write inside number and date frames or place a cross [x] in the appropriate box using a black pen

Q55 Microorganism 1	Q71 Microorganism 2	Q87 Microorganism 3
Microorganism code 1	Microorganism code 2	Microorganism code 3

Q56 Antimicrobial sensitivity 1			Q72 Antimicrobial sensitivity 2			Q88 Antimicrobial sensitivity 3		
Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant

Antimicrobials

Q103 Is the patient receiving antimicrobials? ☐ Yes ☐ No ☐ Not Recorded

Q104 Antimicrobial code 1	Q105 Antimicrobial code 2	Q106 Antimicrobial code 3

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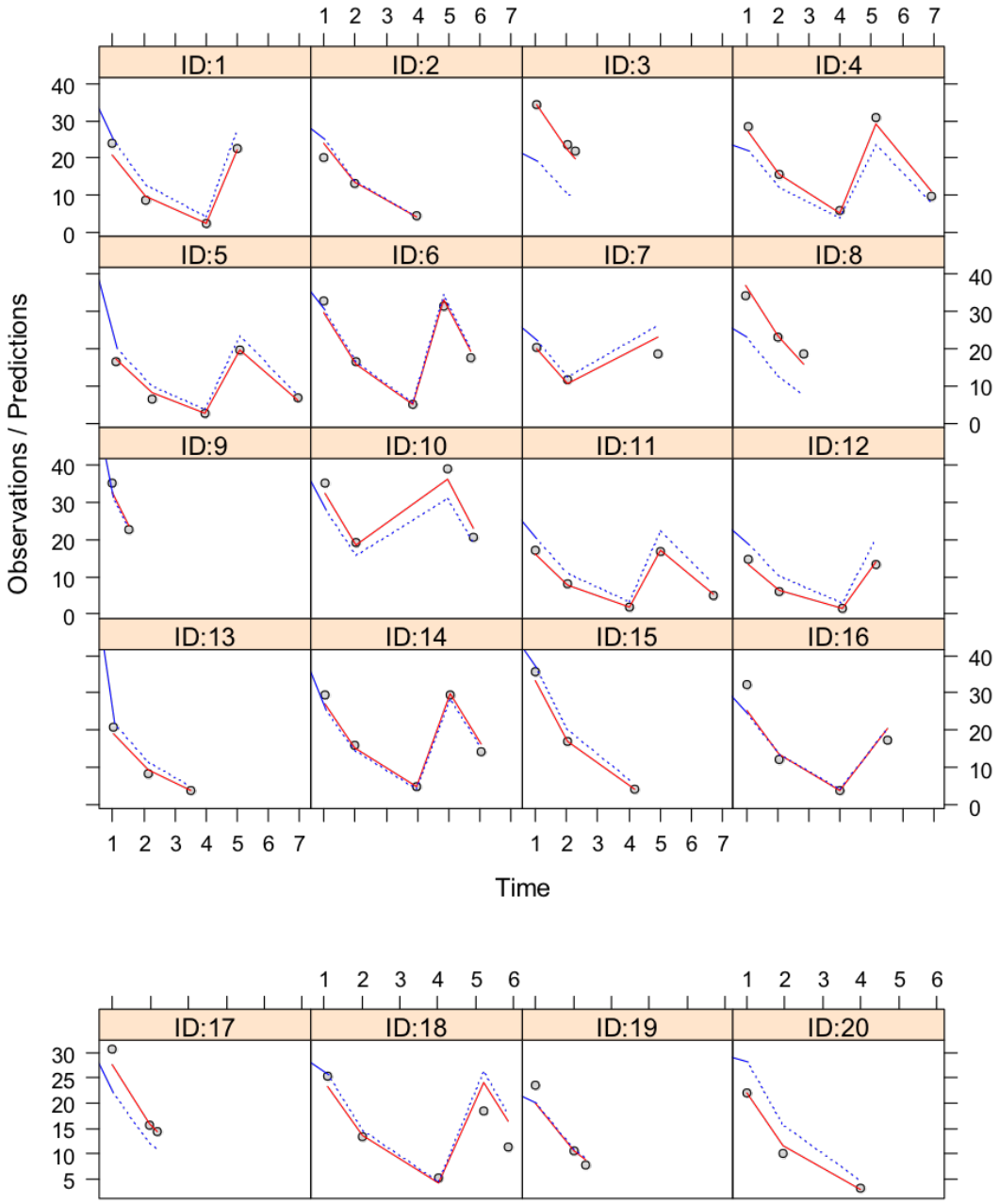
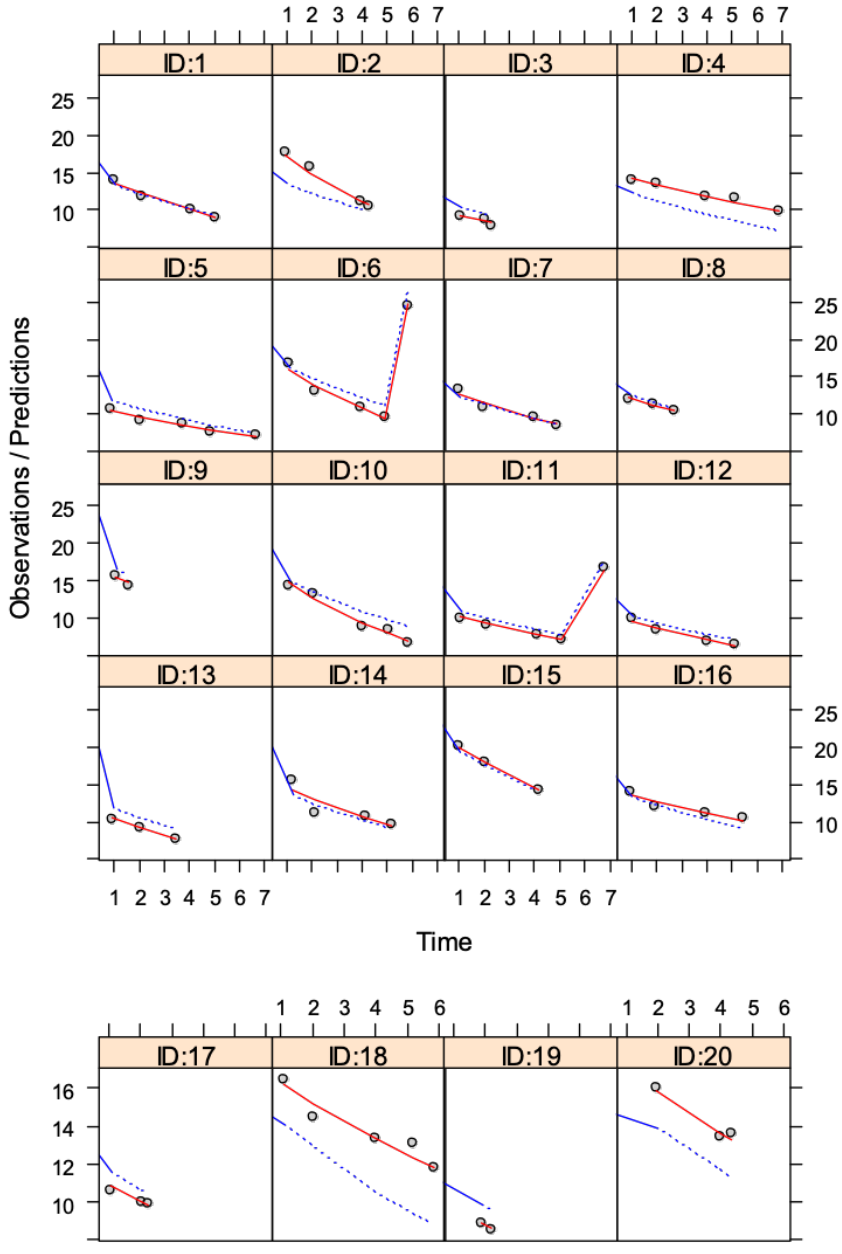
Appendix 7: Individual profiles of amoxicillin versus time with the final model



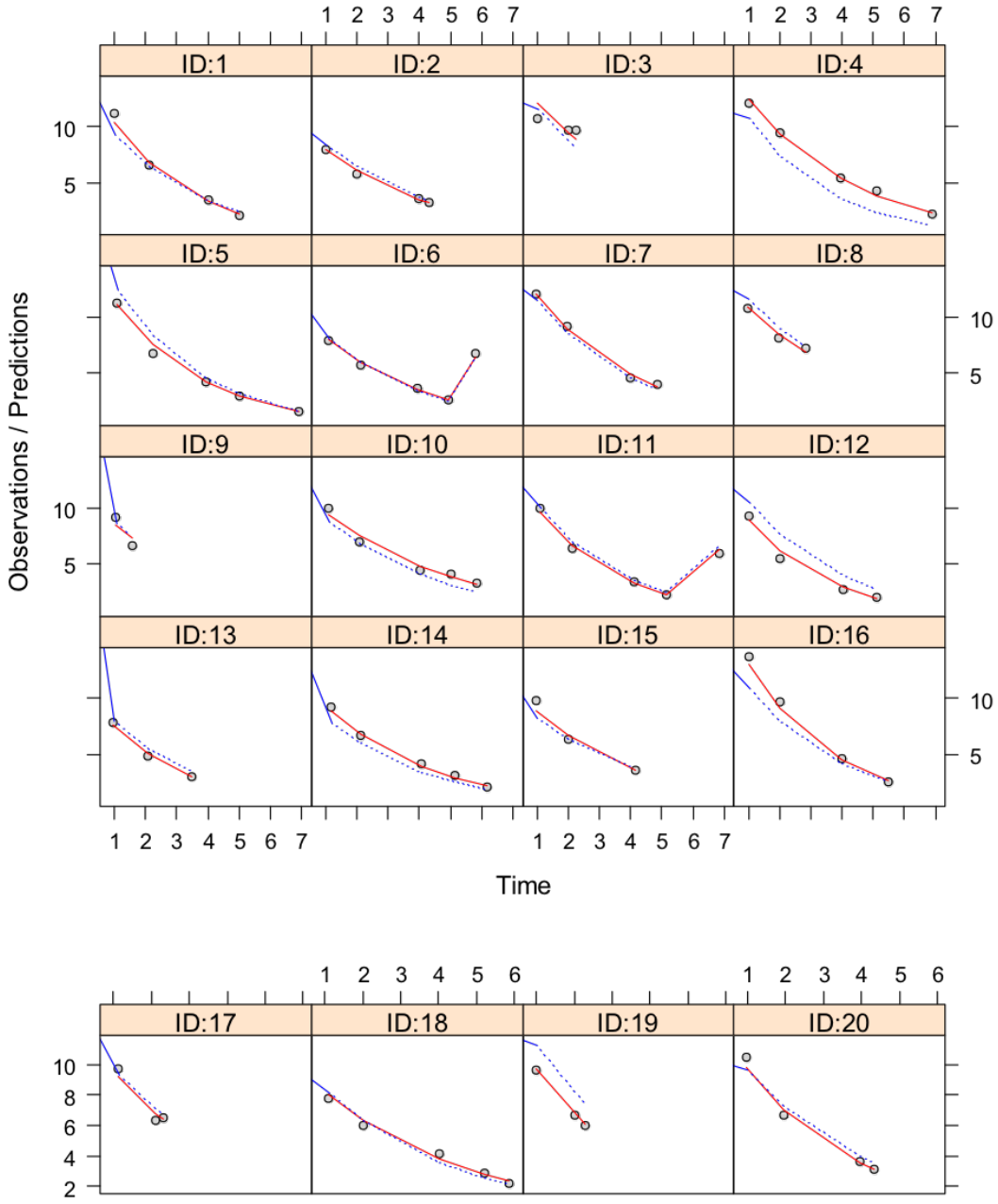
Key: Open circles are measured concentrations; blue dotted lines represent population predicted concentrations; red solid lines represent individual predicted concentrations.

Appendix 8: Individual profiles of metronidazole versus time with the final model



Key: Open circles are measured concentrations; blue dotted lines represent population predicted concentrations; red solid lines represent individual predicted concentrations.

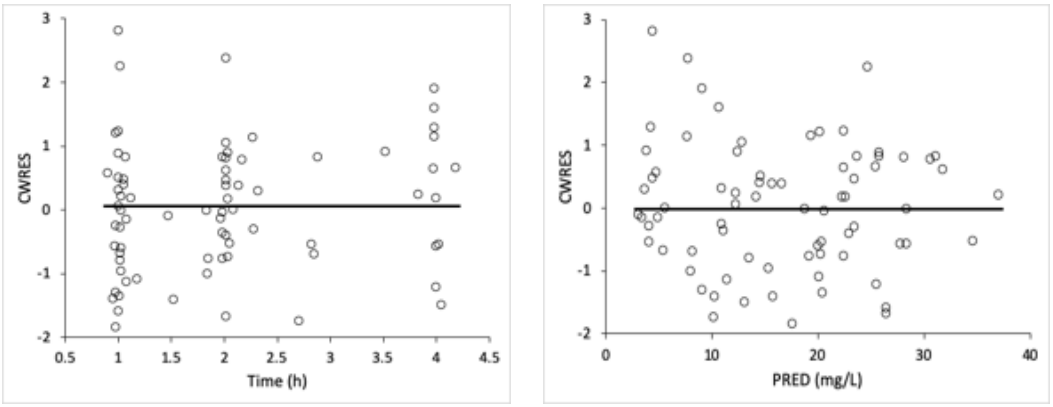
Appendix 9: Individual profiles of gentamicin versus time with the final model



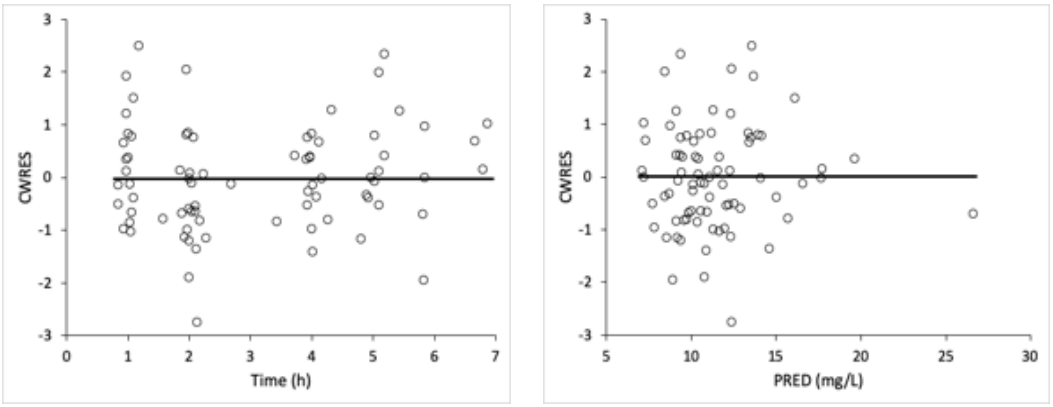
Key: Open circles are measured concentrations; blue dotted lines represent population predicted concentrations; red solid lines represent individual predicted concentrations.

Appendix 10: Conditional weighted residuals versus time and population predicted concentrations

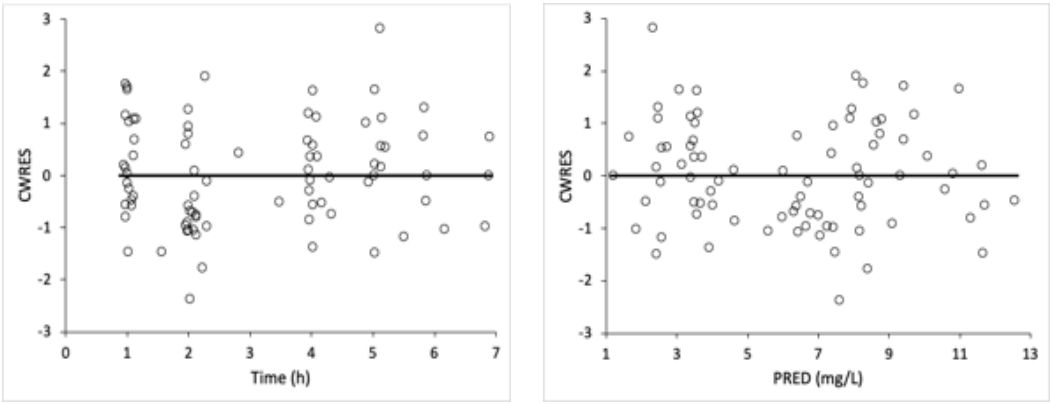
(a)



(b)



(c)



Key: CWRES, conditional weighted residuals versus time after dose (left panels) and versus PRED, population predicted concentrations (right panels). Solid lines indicate a CWRES of 0. (a) amoxicillin, (b) metronidazole, (c) gentamicin.

Appendix 11: Individual estimates of pharmacokinetic parameters of amoxicillin, metronidazole and gentamicin

	Amoxicillin			Metronidazole			Gentamicin		
Patient no	CL (L/h/kg)	V (L/kg)	$t_{1/2}$ (h)	CL (L/h/kg)	V (L/kg)	$t_{1/2}$ (h)	CL (L/h/kg)	V (L/kg)	$t_{1/2}$ (h)
1	0.246	0.357	1.0	0.050	0.489	6.7	0.074	0.195	1.8
2	0.178	0.303	1.2	0.049	0.338	4.7	0.055	0.202	2.5
3	0.081	0.179	1.5	0.047	0.477	7.0	0.038	0.149	2.7
4	0.153	0.278	1.3	0.028	0.440	11.0	0.055	0.192	2.4
5	0.257	0.404	1.1	0.045	0.634	9.7	0.089	0.264	2.1
6	0.201	0.324	1.1	0.066	0.488	5.1	0.075	0.259	2.4
7	0.231	0.367	1.1	0.050	0.500	7.0	0.062	0.210	2.4
8	0.118	0.255	1.5	0.046	0.577	8.6	0.064	0.257	2.8
9	0.188	0.313	1.2	0.053	0.536	7.0	0.074	0.259	2.4
10	0.136	0.236	1.2	0.064	0.404	4.4	0.043	0.185	3.0
11	0.265	0.382	1.0	0.049	0.553	7.9	0.074	0.207	1.9
12	0.268	0.375	1.0	0.051	0.502	6.8	0.073	0.195	1.8
13	0.177	0.269	1.1	0.052	0.438	5.9	0.055	0.148	1.9
14	0.168	0.284	1.2	0.045	0.436	6.7	0.051	0.184	2.5
15	0.216	0.329	1.1	0.051	0.477	6.4	0.071	0.250	2.4
16	0.212	0.335	1.1	0.037	0.552	10.2	0.074	0.208	1.9
17	0.135	0.245	1.3	0.043	0.489	7.9	0.055	0.178	2.2
18	0.193	0.329	1.2	0.027	0.420	10.6	0.057	0.219	2.7
19	0.179	0.284	1.1	0.049	0.502	7.1	0.066	0.187	2.0
20	0.285	0.426	1.0	0.038	0.499	9.0	0.091	0.268	2.0
Mean	**0.194**	**0.314**	**1.2**	**0.047**	**0.488**	**7.5**	**0.065**	**0.211**	**2.3**
SD	**0.054**	**0.062**	**0.1**	**0.010**	**0.066**	**1.9**	**0.014**	**0.037**	**0.4**

Key: no, number; CL, clearance; V, volume of distribution; $t_{1/2}$, elimination half-life; SD, standard deviation.