This is a peer-reviewed, accepted author manuscript of the following research article: da Silva Neto, MJJ., MacKay, G., Agaram, R., MacLeod, M., Watson, D. G., & Thomson, AH. (Accepted/In press). Evaluation of Amoxicillin, Metronidazole, and Gentamicin dosage regimes for use in antibiotic prophylaxis in colorectal surgery. *Journal of Antimicrobial Chemotherapy*.

EVALUATION OF AMOXICILLIN, METRONIDAZOLE AND GENTAMICIN DOSAGE REGIMENS FOR USE IN ANTIBIOTIC PROPHYLAXIS IN COLORECTAL SURGERY

MJJ da Silva Neto[1,2*], G MacKay[3], R Agaram[4], M MacLeod[5], DG Watson[2], AH Thomson[2]

1. Pharmacy Department, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK.
2. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK.
3. Lister Department of Surgery, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK.
4. Anaesthetic Department, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK.
5. Clinical Microbiology Department, Glasgow Royal Infirmary, 10-16 Alexandra Parade, Glasgow G31 2ER, UK.

Running title: Antibiotic prophylaxis for colorectal surgery

*Address for correspondence

Michael da Silva Neto

Pharmacy Department, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK

Tel: 00441412013246

Email: michael.dasilvaneto@ggc.scot.nhs.uk

ABSTRACT (243 words)

Objectives: To evaluate amoxicillin, metronidazole and gentamicin dosage regimens for antibiotic prophylaxis in colorectal surgery.

Methods: The study was conducted in 20 patients undergoing colorectal surgery. Patients received one or two doses of amoxicillin 1000 mg, metronidazole 500 mg and gentamicin 3 mg/kg ideal body weight, banded by height. Antibiotic concentrations were measured up to 7 h post dose. Population pharmacokinetic (PopPK) analysis with NONMEM followed by Monte Carlo simulation of different dosage regimens was used to estimate the PTA for potential organisms associated with surgical site infections (SSIs).

Results: A median of 5 (range 3 – 6) concentrations were available per patient. CL and V of all antibiotics were related to weight; gentamicin CL was also related to CL_{CR}. The administered doses maintained the desired PTA up to 8 h for the *Streptococcus anginosus* group but not for enterococci, *Bacteroides fragilis* group, MSSA, and *Escherichia coli*. An additional 500 mg amoxicillin every 4 h was sufficient to achieve the PTA for most relevant organisms but 2 hourly dosing was required for patients at risk of infective endocarditis. A metronidazole dose of 1000 mg was required for patients >85 kg. In patients with CL_{CR} >50 ml/min, 5 mg/kg gentamicin with an additional 2.5 mg/kg in prolonged surgery at 6 h, maintained PTA targets for >10 h.

Conclusions: PopPK analysis with Monte Carlo simulation identified prophylactic antibiotic regimens that would maintain the PTA for organisms associated with SSIs during short and long duration colorectal surgery.

INTRODUCTION (3610 words)

Surgical site infections (SSIs) increase post-operative morbidity, mortality and cost of treatment, causing a substantial burden to hospitals and society.[1] Within the UK and Europe, colorectal surgery is associated with the highest SSI rate among elective operations.[2] Antimicrobial prophylaxis reduces the risk of SSIs[3] and maintaining serum and tissue concentrations above the MICs of common organisms involved in SSIs from incision to skin closure has been found to reduce SSI rates in colorectal and cardiac surgery.[4,5] Intravenous (IV) gentamicin plus metronidazole is widely used first line in the UK for colorectal surgery prophylaxis[6] due to good Gram-negative and anaerobic cover and a low risk of *Clostridium difficile* infection. Amoxicillin may be added according to local microbiological advice. However, such antibiotic dosage regimens are largely empirical and it is not clear if they maintain adequate concentrations during prolonged surgical procedures.

Pharmacokinetic/pharmacodynamic (PK/PD) principles and population pharmacokinetics (PopPK) are increasingly being used to develop antimicrobial dosage regimens that optimise surgical prophylaxis and the treatment of infections.[7] By combining PopPK and Monte Carlo simulations, 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 aim of this study was to identify prophylactic antibiotic dosage regimens that would maintain plasma concentrations of amoxicillin, metronidazole and gentamicin above the MIC values of common organisms associated with SSIs in colorectal surgery.

METHODS

Adults undergoing colorectal surgery and who received amoxicillin, metronidazole and gentamicin were eligible for inclusion in the study, which was approved by the NHS East Midlands – Nottingham 1 Research Ethics Committee (16/EM/0209). All patients gave their written informed consent prior to entry into the study. The following antibiotics were administered within 1 h of skin incision according to current guidelines within NHS Greater Glasgow and Clyde: 1000 mg amoxicillin IV; 500 mg metronidazole IV; and an IV gentamicin dose banded according to height (HT) based on 3 mg/kg ideal body weight (IBW),[8] (Table S1). If the duration of surgery exceeded 4 h, an additional 1000 mg amoxicillin IV was administered at 4 h. If it exceeded 8 h, additional doses of 1000 mg amoxicillin IV, 500 mg metronidazole IV, and the banded gentamicin IV dose were administered at 8 h. Antibiotics were also re-dosed if the estimated blood loss was above 1.5 L. Blood samples were collected pre-dose then during surgery at 1 h and 2 h after dosing, and at skin closure. If additional doses were required, samples were also withdrawn before and 1 h after these doses.

Amoxicillin and metronidazole concentrations were determined simultaneously by LC-MS using an Orbitrap Exactive (Thermo Scientific). The method was linear in the range 0.1 to 6.4 mg/L and intra-run coefficients of variation (CVs) were <12% for amoxicillin and <5% for metronidazole at concentrations of 0.1, 0.8 and 6.4 mg/L.[9] Clinical samples were diluted to be within this range. Gentamicin concentrations were determined by a homogeneous particle-enhanced turbidimetric inhibition immunoassay (Architect, Abbott Laboratories) that was linear up to 10 mg/L and had inter-assay CVs of 2.2 – 4.5%.

PopPK parameters for each drug were estimated using NONMEM 7.4.2 (Icon Development Solutions, Ellicott City, MD) using first-order conditional estimation with interaction. Bootstrap analysis was performed using Perl-speaks-NONMEM version 4.6.0.[10] and visual predictive checks (VPC) were constructed using Wings for NONMEM version 750.[11] Preliminary analysis of the data indicated that a one-compartment model was adequate for all antibiotics. Between-subject variability (BSV) was assumed to be log-normally distributed; additive, proportional and combined models were compared for residual error. Patient age, sex, HT, total body weight (TBW), IBW, adjusted body weight (AJBW = IBW + 0.4 × (TBW−IBW)), allometric weight $(TBW/70)^{0.75}$, CL_{CR} and serum albumin were evaluated as covariates. CL_{CR} was estimated using the Cockcroft–Gault equation[12] based on TBW, IBW, AJBW, and lean body weight (LBW).[13] Potential relationships between PK parameters and covariates were examined graphically and then added individually and in combination to the population model in a stepwise manner. A decrease in the objective function value (OFV) of 3.84 ($p<0.05$) was considered statistically significant for forward selection of covariates and 6.63 ($p<0.01$) during backward elimination. Models were also evaluated using goodness-of-fit plots, a bootstrap sampling procedure with 1000 samples and a VPC based on 1000 simulations.

The final PopPK models were used within NONMEM to run Monte Carlo simulations of 1000 patients sampled from the patient dataset. Antibiotic concentration-time profiles were simulated according to current guidelines and with the following modifications: additional doses of 500 mg amoxicillin at 4 h only and at 2, 4 and 6 h; a single pre-operative metronidazole dose of 500 mg/1000 mg (if >85 kg); a single pre-operative metronidazole dose

of 1000 mg; a single pre-operative gentamicin dose of 3, 4 and 5 mg/kg TBW or AJBW (if TBW >IBW);[14] in patients with CL_{CR} >50 ml/min, a pre-operative gentamicin dose of 3 mg/kg TBW/AJBW with an additional dose of 3 mg/kg at 4 h and a pre-operative gentamicin dose of 5 mg/kg TBW/AJBW with an additional dose of 2.5 mg/kg at 6 h. Simulations were typically performed hourly up to 8 h but continued to 12 h after re-dosing of gentamicin. Free drug concentrations were estimated using published protein binding values for each antibiotic: 17% for amoxicillin;[15] 15% for metronidazole;[16] and 0% for gentamicin.[17] PTA was defined as the percentage probability of achieving free antibiotic concentrations above the MIC (%ƒT >MIC) and the following EUCAST MIC breakpoints were applied: amoxicillin, 0.5 mg/L for the *Streptococcus anginosus* group and 4 mg/L for enterococci; metronidazole, 4 mg/L for the *Bacteroides fragilis* group; gentamicin, 1 mg/L for MSSA and 2 mg/L for *Escherichia coli*.[18] A PTA of ≥90% was considered acceptable.

RESULTS

The characteristics of the 20 patients (11 female) who participated in the study are summarised in Table 1. Median age was 58 years and weight 71.2 kg; 12 patients had a BMI ≥25 kg/m^2. CL_{CR} ranged from 50 to 166 mL/min (median 99 mL/min). Laparoscopic procedures were conducted in 11 patients and 9 had open resections. Left colonic/rectal resection was the most common procedure (10 patients). The median duration of surgery was 4.5 h and the median volume of IV Hartmann's solution administered was 3.1 L; 2 patients required a blood transfusion. The median times from the beginning of the first dose of antibiotic prophylaxis to surgical incision were: amoxicillin, 22 min (range 3 – 38 min); metronidazole, 21 min (range 6 – 32 min); gentamicin, 21 min (range 6 – 36 min). Overall, 13 patients required an additional

dose of amoxicillin, 2 of which were also re-dosed with gentamicin and metronidazole due to blood loss. A total of 99 samples were withdrawn with a median of 5 (range 3 – 6) per patient. Of these, 4 amoxicillin and 3 metronidazole concentrations were removed from population analyses due to sampling errors.

PopPK analysis

The amoxicillin analysis included 75 concentrations, ranging from 1.6 to 39.1 mg/L. Including TBW in the model had no effect (OFV fell by 0.49) but relating both CL and V to AJBW reduced the OFV by 5.95, BSV in CL fell from 31.2% to 28.7% and in V from 23.7% to 17.9%. Adding CL_{CR} based on AJBW to this model reduced the OFV by a further 6.31 but the model parameters were poorly characterized and bootstrap analysis indicated a lack of stability. The final model had a typical CL of 0.213 L/h/kg AJBW and a V of 0.353 L/kg AJBW. Individual estimates of CL ranged from 7.9 to 25.2 L/h (mean 14.0 L/h), of V from 15.7 to 35.3 L (mean 22.7 L) and of elimination $t_{1/2}$ from 1.0 to 1.5 h (mean 1.2 h).

There were 76 metronidazole concentration measurements ranging from 6.5 to 24.4 mg/L. CL was best described by TBW and V by AJBW. This model reduced the OFV by 35.4 and had the following structure: CL (L/h) = $3.22 \times (TBW/70)^{0.75}$; V = 0.556 L/kg AJBW. Inclusion of these covariates reduced BSV in CL from 31.3% to 26.7% and in V from 25.3% to 10.8%. Individual estimates of CL ranged from 1.9 to 5.1 L/h (mean 3.5 L/h), of V from 22.7 to 48.6 L (mean 35.7 L) and of elimination $t_{1/2}$ from 4.4 to 11.0 h (mean 7.5 h).

159 There were 79 gentamicin concentration measurements ranging from 1.6 to 13.7 mg/L. CL
160 was best described by a combination of AJBW and CL_{CR} based on AJBW; V was best described
161 by AJBW. Inclusion of these covariates reduced the OFV by 17.4, BSV in CL from 21.9% to
162 16.1% and in V from 13.5% to 10.4%. The final model had the following structure: CL (L/h) =
163 $0.0449 \times AJBW + 0.0195 \times CL_{CR}$; V = 0.239 L/kg AJBW. Individual estimates of CL ranged from
164 3.0 to 7.0 L/h (mean 4.7 L/h), of V from 11.0 to 19.4 L (mean 15.2 L) and of elimination $t_{1/2}$
165 from 1.8 to 3.0 h (mean 2.3 h).
166
167 The parameter estimates of the final PopPK models for all three antibiotics and the results of
168 the bootstrap analyses are presented in Table 2. In all cases, residual error was best described
169 by a proportional error model. Both the VPCs for each antibiotic (Figure 1) and the bootstrap
170 analyses (Table 2) indicated that the parameters were well characterized and the models
171 described the data well. Figure S1 shows additional goodness-of-fit plots. Individual
172 parameter estimates for each patient for all three antibiotics are listed in Table S2.
173
174 **Pharmacodynamic analysis**
175 The PTA estimates for each antibiotic dosage regimen against susceptible organisms are
176 shown in Table 3. After a single dose of amoxicillin, the PTA for the *S. anginosus* group was
177 98.1% and 84.9% at 5 h and 6 h, respectively; with a second dose of 500 mg at 4 h the PTA
178 remained above 90% up to 8 h. For enterococci the PTA was 99.6% and 82.4% at 2 h and 3 h,
179 respectively; with additional doses of 500 mg at 2, 4 and 6 h, the PTA remained above 90%
180 up to 8 h. After a dose of 500 mg metronidazole, the PTA for the *B. fragilis* group dropped to
181 89.3% at 8 h. At 5 h, 40 concentrations (out of 20000) were below the MIC, mainly (90%) in

patients weighing >85 kg. Increasing the dose to 1000 mg (if >85 kg) maintained the PTA above 90% up to 8 h. For the gentamicin dose of 3 mg/kg banded on HT, the PTA for MSSA was 88.6% at 7 h and for *E. coli* it was 89.6% at 5 h. With 3 mg/kg TBW/AJBW, the PTA for MSSA was 84.6% at 7 h and for *E. coli* it was 85.8% at 5 h. Administration of an additional dose of 3 mg/kg at 4 h maintained the PTA for MSSA above 90% up to 11 h, while the PTA for *E. coli* was 76.4% at 10 h. With 4 mg/kg TBW/AJBW, the PTA for MSSA was 81.3% at 8 h and for *E. coli* it was 81.3% at 6 h. With 5 mg/kg TBW/AJBW, the PTA for MSSA remained above 90% up to 8 h, while the PTA for *E. coli* was 72.3% at 7 h. Administration of an additional dose of 2.5 mg/kg at 6 h maintained the PTA for MSSA above 90% up to 12 h, while the PTA for *E. coli* was 89.3% at 11 h. No data were available for patients with CL_{CR} <50 ml/min.

DISCUSSION

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. Monte Carlo simulations were used to calculate the PTA for each antibiotic and evaluate different dosage regimens.

Although two-compartment models have been used in other studies,[19-21] concentration-time profiles of all three antimicrobials were adequately described using a one-compartment model. This reflects the limited number of sample times and lack of information on distribution; the first sample was taken at least 1 h after the dose. The mean individual estimates of amoxicillin CL (14.0 L/h) and V (22.7 L, 0.314 L/kg) were similar to the values of

13.3 L/h and 0.30 L/kg reported by Arancibia *et al*.[19] in 9 healthy subjects and Carlier *et al*.[22] (CL 10.0 L/h, V 27.4 L) in 13 critically ill patients. Although Carlier *et al*.[22] found that CL_{CR} best described amoxicillin CL, which is consistent with its renal CL,[23] it was not possible to characterize a relationship between CL_{CR} and amoxicillin CL in the present study; the only covariate clearly identified was weight. This is likely to reflect the generally good renal function in the patient group; none of the patients had a CL_{CR} below 50 mL/min. Obesity can alter PK parameters[24] and is a risk factor for SSI following colorectal surgery.[14] The influence of obesity on amoxicillin PK has not previously been reported. In the present study, 35% of patients were obese (six) or severely obese (one) and AJBW provided a better description of CL and V than TBW.

Metronidazole is extensively metabolised in the liver[16] and body weight was the only covariate found to influence CL and V. The best model comprised an allometric relationship between metronidazole CL and TBW and a linear relationship between V and AJBW. Asin-Prieto *et al*.[25] found a linear relationship between body weight and both CL and V_1 but did not examine different size descriptors. The mean individual estimate of metronidazole CL (3.5 L/h) was consistent with values reported by Asin-Prieto *et al*.[25] (3.5 L/h) in 63 patients undergoing colorectal surgery and Cerda *et al*.[26] (3.2 L/h) in 33 patients undergoing colorectal surgery. The typical V of 0.49 L/kg in the present study was within the range of 0.40 L/kg[25] – 0.68 L/kg[26] previously reported. Differences in V may reflect different sampling strategies and inter-patient variability.[26]

As gentamicin CL depends on renal function, CL estimates would be expected to vary between studies, according to patient characteristics. Nevertheless, the mean individual CL estimate (4.7 L/h, 0.065 L/h/kg) was consistent with the values reported by Cerda *et al*.[26] (4.7 L/h) and Markantonis *et al*.[27] (5.31 L/h) although lower than the 0.091 L/h/kg reported by Zelenitsky *et al*.[28] As expected, CL_{CR} influenced gentamicin CL and V was related to patient size.[29] Using AJBW to estimate CL_{CR} by the Cockcroft-Gault formula, provided the best fit; this finding is consistent with a study by Leader *et al*.[30] in 100 obese patients. The mean individual estimate of gentamicin V (0.210 L/kg) was consistent with values of 0.22 – 0.26 L/kg previously reported for gentamicin prophylaxis in colorectal surgery[26-28] but lower than the value of 0.31 L/kg reported in adults from a general population.[31] Since gentamicin distributes into extracellular fluid, these lower values may reflect pre-operative fasting with subsequent dehydration and a reduced body water content. 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*.[32]

Pharmacodynamic analysis

A pre-operative dose of 1000 mg amoxicillin with an additional dose of 500 mg intra-operatively 4 h later, successfully met the PTA at the defined MIC breakpoint of 0.5 mg/L for the *S. anginosus* group. Without this additional dose, the PTA at 6 h dropped to 84.9%, emphasizing the importance of re-dosing amoxicillin in prolonged surgery. Guidelines usually recommend repeating the prophylactic dose when the surgical procedure extends beyond twice the elimination $t_{1/2}$ of the antibiotic.[14] In the case of amoxicillin, this would mean repeating the dose at around 2.5 h as the elimination $t_{1/2}$ averaged 1.2 h. However, an

additional dose of 500 mg at 4 h was acceptable due to the low MIC breakpoint of the *S. anginosus* group. Antibiotic cover against enterococci is not normally required for colorectal surgery except for patients at high risk of infective endocarditis (IE).[33] To maintain adequate cover for such patients, the results indicated that 500 mg amoxicillin should be administered every 2 h during the surgical procedure. A continuous infusion could also be considered.

The current dosage regimen of 500 mg metronidazole pre-operatively achieved a PTA for the *B. fragilis* group of 89.3% at 8 h, which was considered close enough to 90% to accept 8 hourly re-dosing. Asin-Prieto *et al*.[25] examined the PTA of a pre-operative dose of 1500 mg metronidazole against an MIC breakpoint of 8 mg/L in 63 patients undergoing colorectal surgery. They found that metronidazole PK was related to weight and concluded that patients with a TBW of 90 kg required an additional dose of 1500 mg 4 h after the first dose to maintain free drug concentrations above the MIC for up to 8 h. Although the present study used both a lower prophylactic dose and MIC breakpoint (4 mg/L) it also identified poor target attainment in heavier patients; simulated concentrations were typically below the MIC at 5 h in patients whose weight was >85 kg. A pre-operative dose of 500/1000 mg (if >85 kg) achieved a PTA of 95.8% at 8 h and would therefore be preferred. However, it is important to note that the metronidazole PK/PD analysis did not include the hydroxyl metabolite, which has been reported to have 65% of the antimicrobial activity of metronidazole against the *B. fragilis* group.[34] By only considering the MIC of the parent drug, overall efficacy may have been underestimated.

The current gentamicin dose of 3 mg/kg, banded on HT and repeated intra-operatively at 8 h, did not maintain the PTAs at the defined MIC breakpoints of 1 mg/L for MSSA and 2 mg/L for *E. coli*. The 3 mg/kg TBW/AJBW regimen achieved lower PTAs compared to the regimen banded on HT as the banding led to higher doses for some patients. As expected, the exposure increased when the higher doses were simulated. Monte Carlo simulations were also used in a previous study that assessed gentamicin regimens for prophylaxis in abdominal surgery.[35] The authors determined the cumulative target attainment (CTA) by integrating PTA values and MIC distributions and found that without intra-operative re-dosing, the CTA at 6 h remained above 90% for *E. coli* with a gentamicin dose of 5 mg/kg and fell below 90% after 5 h with a dose of 3 mg/kg. Since 90% of their colorectal procedures lasted less than 5 h, the authors recommended 3 mg/kg 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 h and the dose of 3 mg/kg TBW/AJBW achieved a PTA of 85.8% at 5 h for *E. coli* whereas the 5 mg/kg TBW/AJBW dose maintained the PTA above 90% until 6 h. These results suggest that 5 mg/kg would be acceptable for surgical procedures of up to 6 h. For procedures lasting more than 6 h, re-dosing with 2.5 mg/kg achieved a PTA of 89.3% at 11 h for *E. coli*. It would be reasonable to check the concentration before re-dosing to reduce the risk of toxicity. Furthermore, in patients with CL_{CR} <50 ml/min, lower doses might be safer and re-dosing unnecessary.

Dosage guidelines

The results of the PopPK and pharmacodynamic analyses suggested the following antibiotic doses to maintain a PTA ≥90% against organisms commonly encountered in colorectal

surgery: amoxicillin 1000 mg pre-operatively, with an additional 500 mg intra-operatively at 4 h in most cases and 2 hourly if there is a risk of IE; metronidazole 500 mg pre-operatively, increased to 1000 mg if the patient's weight is >85 kg and re-dosed at 8 h; gentamicin 5 mg/kg TBW, or AJBW if TBW >IBW, pre-operatively, re-dosed with 2.5 mg/kg intra-operatively at 6 h. Since the minimum CL_{CR} in the patient group was 50 ml/min, these doses would not apply to patients with lower estimates of CL_{CR}.

This study had some limitations. There is a lack of published data on the ideal targets for surgical antibiotic prophylaxis, particularly for concentration-dependent antibiotics, such as gentamicin and metronidazole. Zelenitsky *et al*.[5] reported that a gentamicin concentration at skin closure above 1.6 mg/L was required for effective prophylaxis in colorectal surgery, and this finding informed the target of %ƒT >MIC that was assumed for both time-dependent and concentration-dependent antibiotics. The PTA values from the present study were based on the EUCAST clinical MIC breakpoints rather than MIC distribution data and may not reflect local or changing antimicrobial susceptibility. Furthermore, the unbound serum concentrations were calculated from literature values of protein binding and may differ from actual unbound concentrations, particularly in patients with malignancy. However, most patients had normal albumin concentrations and since the protein binding of all three antibiotics is low, variations are unlikely to have a major impact.

It is recognised that the population models were based on serum rather than tissue antibiotic concentrations. Although all three antibiotics distribute well into extracellular fluids, tissue penetration studies are limited and variable. Although blood loss is known to reduce serum

antibiotic concentrations, estimated blood loss provides an unreliable quantification of peri-operative blood loss and was therefore not included in the PopPK analysis. In addition, due to the lack of renal and liver impairment, the results cannot be extrapolated to these patient groups or to patients weighing >100 kg. Nevertheless, the characteristics of the patients studied were typical of the patient population.

In conclusion, this study found that the current dosage regimens maintained the desired PTA over the re-dosing interval for the *S. anginosus* group but not for enterococci (in patients at high risk of IE), the *B. fragilis* group (in patients >85 kg), MSSA, and *E. coli*. The proposed dosage guidelines offer an improved profile for all three antibiotics and should maintain these PTAs for the likely duration of colorectal surgical procedures.

ACKNOWLEDGEMENTS

We would like to thank the patients who participated in the study. We would also like to thank the Glasgow Royal Infirmary Departments of Surgery and Anaesthetics, Biochemistry, Clinical Microbiology, and the Glasgow Clinical Research Facility for their support implementing this study.

FUNDING

MJJDSN was an MPhil student supported by the NHS Greater Glasgow and Clyde (NHSGGC) Learning & Education Bursary Scheme (2014254/2015083) and the NHSGGC Pharmacy Services Research & Development Endowment Fund. This study was supported by internal funding from the Strathclyde Institute of Pharmacy & Biomedical Sciences.

TRANSPARENCY DECLARATIONS

None to declare.

REFERENCES

1. Badia JM, Casey AL, Petrosillo N *et al*. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. *J Hosp Infect* 2017; **96**: 1-15.
2. European Centre for Disease Prevention and Control. *Healthcare-associated infections: surgical site infections – Annual epidemiological report for 2017*. ECDC, 2019. https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2017-SSI.pdf
3. Song F, Glenny AM. Antimicrobial prophylaxis in colorectal surgery: a systematic review of randomized controlled trials. *Br J Surg* 1998; **85**: 1232-41.
4. Zanetti G, Giardina R, Platt R. Intraoperative redosing of cefazolin and risk for surgical site infection in cardiac surgery. *Emerg Infect Dis* 2001; **7**: 828-31.
5. Zelenitsky SA, Ariano RE, Harding GKM *et al*. Antibiotic pharmacodynamics in surgical prophylaxis: an association between intraoperative antibiotic concentrations and efficacy. *Antimicrob Agents Chemother* 2002; **46**: 3026-30.
6. Joint Formulary Committee. *British National Formulary*. BMJ Group and Pharmaceutical Press, 2021. http://www.medicinescomplete.com
7. Asin-Prieto E, Rodriguez-Gascon A, Isla A. Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. *J Infect Chemother* 2015; **21**: 319-329.
8. Devine BJ. Gentamicin therapy. *Drug Intell Clin Pharm* 1974; **8**: 650-5.
9. Kathriarachchi UL, Vidhate SS, Al-Tannak N *et al*. Development of a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography (HILIC). *J Chromatogr B Analyt Technol Biomed Life Sci* 2018; **1089**: 78-83.
10. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. *Comput Methods Programs Biomed* 2004; **75**: 85-94.
11. Holford N. *Wings for NONMEM*. http://wfn.sourceforge.net
12. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41.
13. Janmahasatian S, Duffull SB, Ash S *et al*. Quantification of lean bodyweight. *Clin Pharmacokinet* 2005; **44**: 1051-65.
14. Bratzler DW, Dellinger EP, Olsen KM *et al*. Clinical practice guidelines for antimicrobial prophylaxis in surgery. *Am J Health Syst Pharm* 2013; **70**: 195-283.
15. Sutherland R, Croydon EA, Rolinson GN. Amoxycillin: a new semi-synthetic penicillin. *Br Med J* 1972; **3**: 13-6.
16. Dubreuil L. 5-Nitroimidazoles. In: Bryskier A, ed. *Antimicrobial Agents: Antibacterials and Antifungals*. ASM Press, 2005; 930-40.
17. Gordon RC, Regamey C, Kirby WM. Serum protein binding of the aminoglycoside antibiotics. *Antimicrob Agents Chemother* 1972; **2**: 214-6.
18. The European Committee on Antimicrobial Susceptibility Testing. *Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021*. https://www.eucast.org
19. Arancibia A, Guttmann J, Gonzalez G *et al*. Absorption and disposition kinetics of amoxicillin in normal human subjects. *Antimicrob Agents Chemother* 1980; **17**: 199-202.

20. Gjerloff C, Arnold E. Pharmacokinetics of intravenous metronidazole in man. *Acta Pharmacol Toxicol* 1982; **51**: 132-5.
21. Demczar DJ, Nafziger AN, Bertino JSJ. Pharmacokinetics of gentamicin at traditional versus high doses: implications for once-daily aminoglycoside dosing. *Antimicrob Agents Chemother* 1997; **41**: 1115-9.
22. Carlier M, Noe M, De Waele JJ *et al*. Population pharmacokinetics and dosing simulations of amoxicillin/clavulanic acid in critically ill patients. *J Antimicrob Chemother* 2013; **68**: 2600-8.
23. Bryskier A. Penicillins. In: Bryskier A, ed. *Antimicrobial Agents: Antibacterials and Antifungals*. ASM Press, 2005; 113-62.
24. Meng L, Mui E, Holubar MK *et al*. Comprehensive Guidance for Antibiotic Dosing in Obese Adults. *Pharmacotherapy* 2017; **37**: 1415-31.
25. Asin-Prieto E, Soraluce A, Troconiz IF *et al*. Population pharmacokinetic models for cefuroxime and metronidazole used in combination as prophylactic agents in colorectal surgery: Model-based evaluation of standard dosing regimens. *Int J Antimicrob Agents* 2015; **45**: 504-11.
26. Cerda JMV, Alminana MA, Guinot JN *et al*. The pharmacokinetics of metronidazole and gentamicin in a single preoperative dose as antibiotic prophylaxis in colorectal surgery. *Farm Hosp* 2008; **32**: 77-82.
27. Markantonis SL, Kostopanagiotou G, Panidis D *et al*. Effects of blood loss and fluid volume replacement on serum and tissue gentamicin concentrations during colorectal surgery. *Clin Ther* 2004; **26**: 271-81.
28. Zelenitsky SA, Silverman RE, Duckworth H *et al*. A prospective, randomized, double-blind study of single high dose versus multiple standard dose gentamicin both in combination with metronidazole for colorectal surgical prophylaxis. *J Hosp Infect* 2000; **46**: 135-40.
29. Llanos-Paez CC, Hennig S, Staatz CE. Population pharmacokinetic modelling, Monte Carlo simulation and semi-mechanistic pharmacodynamic modelling as tools to personalize gentamicin therapy. *J Antimicrob Chemother* 2017; **72**: 639-67.
30. Leader WG, Tsubaki T, Chandler MH. Creatinine-clearance estimates for predicting gentamicin pharmacokinetic values in obese patients. *Am J Hosp Pharm* 1994; **51**: 2125-30.
31. Thummel KE, Shen DD, Isoherranen N. Design and Optimization of Dosage Regimens: Pharmacokinetic Data. In: Brunton LL, Chabner BA, Knollmann BC, eds. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. McGraw-Hill, 2011; 1891-990.
32. Bauer LA, Edwards WA, Dellinger EP *et al*. Influence of weight on aminoglycoside pharmacokinetics in normal weight and morbidly obese patients. *Eur J Clin Pharmacol* 1983; **24**: 643-7.
33. Habib G, Lancellotti P, Antunes MJ *et al*. 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). *Eur Heart J* 2015; **36**: 3075-128.
34. Haller I. In vitro activity of the two principal oxidative metabolites of metronidazole against *Bacteroides fragilis* and related species. *Antimicrob Agents Chemother* 1982; **22**: 165-6.

35. Zelenitsky SA, Lawson C, Calic D *et al*. Integrated pharmacokinetic-pharmacodynamic modelling to evaluate antimicrobial prophylaxis in abdominal surgery. *J Antimicrob Chemother* 2016; **71**, 2902-8.

435 **Table 1** Summary of the demographic, clinical and surgery related characteristics of the 20

436 patients included in the study

Characteristic	Median	Range
Male/female	9/11	
Age (years)	58	18 – 81
Weight (kg)	71.2	47.6 – 101.9
Height (m)	1.67	1.44 – 1.79
BMI (kg/m^2)	26.7	19.4 – 40.7
Serum creatinine (µmol/L)	66	53 – 97
CL_{CR} (mL/min)	99	50 – 166
Albumin (g/L)	37.5	32 – 44
Laparoscopic/open	11/9	
IVs (L)	3.1	1.2 – 6.0
Dur (h)	4.5	1.5 – 8.0
Estimated blood loss (L)		<0.2 – 1.8

437 Key: IVs, intravenous Hartmann's solution volume administered during surgery; Dur,

438 duration of surgery from incision to skin closure.

439

Table 2 PopPK parameter estimates for amoxicillin, metronidazole and gentamicin in patients undergoing colorectal surgery

Parameter	Final estimate	RSE (%)	Bootstrap median (5^{th} and 95^{th} percentiles)
Amoxicillin			
θ_{CL}	0.213	6.6	0.214 (0.188-0.236)
θ_V	0.353	4.8	0.356 (0.328-0.384)
BSV CL (CV%)	28.7	40.4	27.7 (16.2-37.7)
Shrinkage ηCL	0.00%		
BSV V (CV%)	17.9	32.2	17.5 (12.3-22.7)
Shrinkage ηV	6.29%		
RE Proportional (CV%)	12.2	11.7	12.2 (9.8-14.6)
Shrinkage RE	16.1%		
Metronidazole			
θ_{CL}	3.22	7.0	3.24 (2.87-3.63)
θ_V	0.556	2.5	0.555 (0.533-0.577)
BSV CL (CV%)	26.7	40.9	25.6 (13.9-34.4)
Shrinkage ηCL	13.8%		
BSV V (CV%)	10.8	43.1	10.3 (6.1-14.1)
Shrinkage ηV	6.14%		
RE Proportional (CV%)	4.46	15.6	4.39 (3.32-5.57)
Shrinkage RE	22.9%		
Gentamicin			
θ_{AJBW}	0.0449	27.8	0.0442 (0.0239-0.0685)
$\theta_{CL_{CR}}$	0.0195	44.5	0.0195 (0.0036-0.0344)
θ_V	0.239	2.9	0.239 (0.227-0.250)
BSV CL (CV%)	16.1	41.8	14.6 (9.4-21.0)
Shrinkage ηCL	1.53%		
BSV V (CV%)	10.4	33.7	10.0 (6.7-12.7)
Shrinkage ηV	13.1%		
RE Proportional (CV%)	6.59	5.5	6.60 (5.89-7.24)
Shrinkage RE	20.0%		

Key: ηCL, individual variation in CL; ηV, individual variation in V; RE, residual error; CV%, coefficient of variation expressed as a percentage; RSE, relative standard error. Amoxicillin model CL (L/h) = $\theta_{CL} \times$ AJBW (kg), V(L) = $\theta_V \times$ AJBW (kg). Metronidazole model CL (L/h) = $\theta_{CL} \times (TBW(kg)/70)^{0.75}$, V(L) = $\theta_V \times$ AJBW (kg). Gentamicin model CL (L/h) = $\theta_{AJBW} \times$ AJBW (kg) + $\theta_{CL_{CR}} \times CL_{CR}$ (mL/min) using the Cockcroft-Gault equation[12] based on AJBW, V(L) = $\theta_V \times$ AJBW (kg).

Table 3 Summary of the PTA of amoxicillin, metronidazole and gentamicin for use in surgical prophylaxis against *S. anginosus* group, enterococci, *B. fragilis* group, MSSA and *E. coli* at susceptibility breakpoints recommended by EUCAST

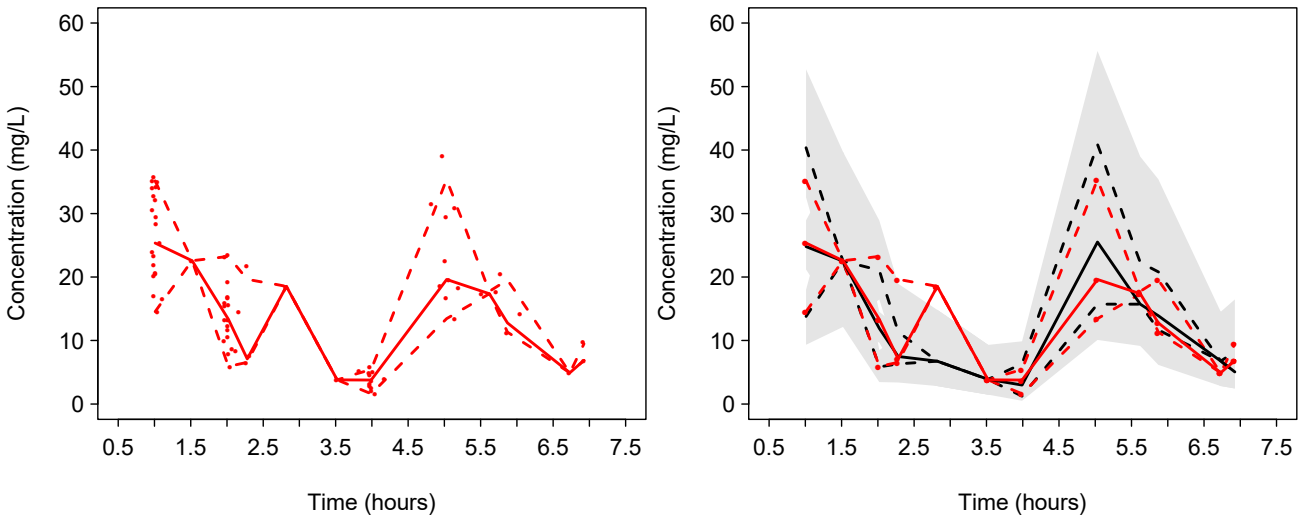
Antibiotic	Dose	PTA by organism (%)																					
		Time after dose (h)												Time after dose (h)									
		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
		S. anginosus group												Enterococci									
Amoxicillin[a]	1000 mg pre-op	100	100	100	100	98.1	84.9	54.2	24.5					100	99.6	82.4	34.9	7.1	0.8	0.1	0.0				
	1000 mg pre-op + 500 mg at 4 h	100	100	100	100	100	100	100	99.2																
	1000 mg pre-op + 500 mg at 2, 4 and 6 h													100	99.6	100	96.8	100	95.3	100	94.5				
		B. fragilis group																							
Metronidazole[b]	500 mg pre-op	100	100	100	100	99.8	98.7	95.4	89.3																
	500 mg/1000 mg (>85 kg) pre-op	100	100	100	100	100	99.7	98.5	95.8																
	1000 mg pre-op	100	100	100	100	100	100	100	99.9																
		MSSA												*E. coli*											
Gentamicin[c]	3 mg/kg IBW banded by height pre-op	100	100	100	100	100	98.6	88.6	64.8					100	100	100	99.4	89.6	58.5	24.9	7.8				
	3 mg/kg TBW/AJBW pre-op	100	100	100	100	99.9	97.6	84.6	59.8					100	100	100	99.2	85.8	52.2	20.6	5.5				
	3 mg/kg TBW/AJBW pre-op + 3 mg/kg TBW/AJBW at 4 h	100	100	100	100	100	100	100	100	99.9	99.1	92.9	77.4	100	100	100	99.0	100	100	100	99.8	95.3	76.4	47.7	22.3
	4 mg/kg TBW/AJBW pre-op	100	100	100	100	100	99.7	95.6	81.3					100	100	100	100	97.9	81.3	50.1	22.0				
	5 mg/kg TBW/AJBW pre-op	100	100	100	100	100	99.9	98.7	91.2					100	100	100	100	99.7	93.4	72.3	42.4				
	5 mg/kg TBW/AJBW pre-op + 2.5 mg/kg TBW/AJBW at 6 h	100	100	100	100	100	99.9	100	100	100	100	99.9	97.9	100	100	100	100	99.6	93.5	100	100	100	99.2	89.3	64.3

Key: pre-op, pre-operatively; [a] EUCAST breakpoints = 0.5 mg/L for *S. anginosus* group, 4 mg/L for enterococci; [b] EUCAST breakpoints = 4 mg/L for *B. fragilis* group; [c] EUCAST breakpoints = 1 mg/L for MSSA, 2 mg/L for *E. coli*.[18]

Figure 1 VPCs of the final population model describing (a) amoxicillin, (b) metronidazole and (c) gentamicin PK in patients undergoing colorectal surgery.

Key: Red and black lines represent the 5^{th} (dotted), 50^{th} (solid) and 95^{th} (dotted) percentiles of the observed data and predicted data, respectively. The shaded areas represent 95% CI for the percentiles of the predicted concentrations.

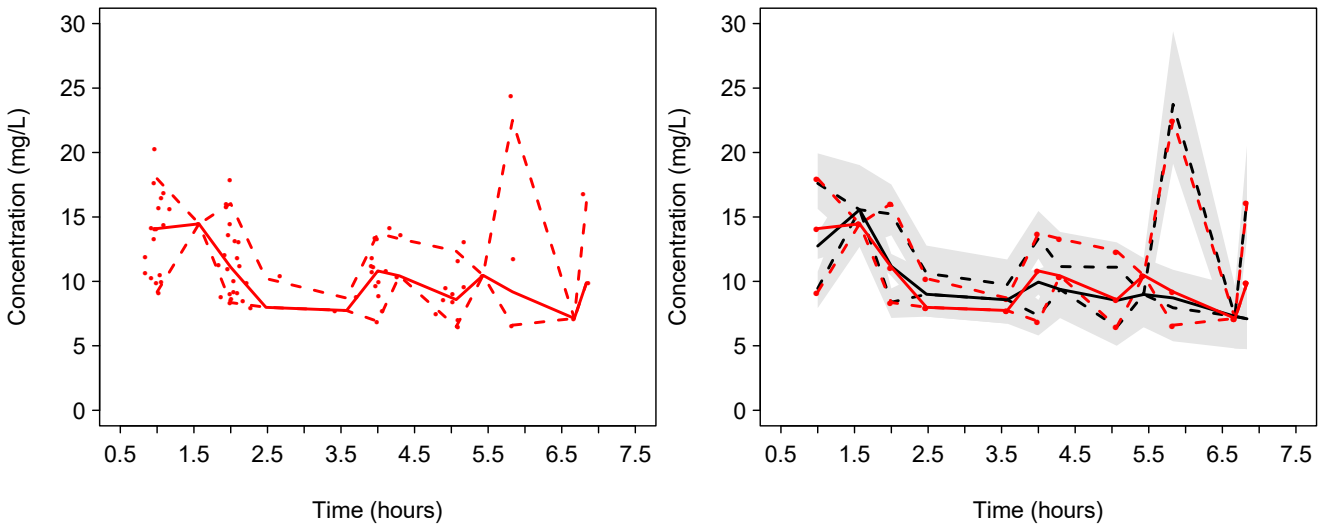
457 **Figure 1a**



458

459

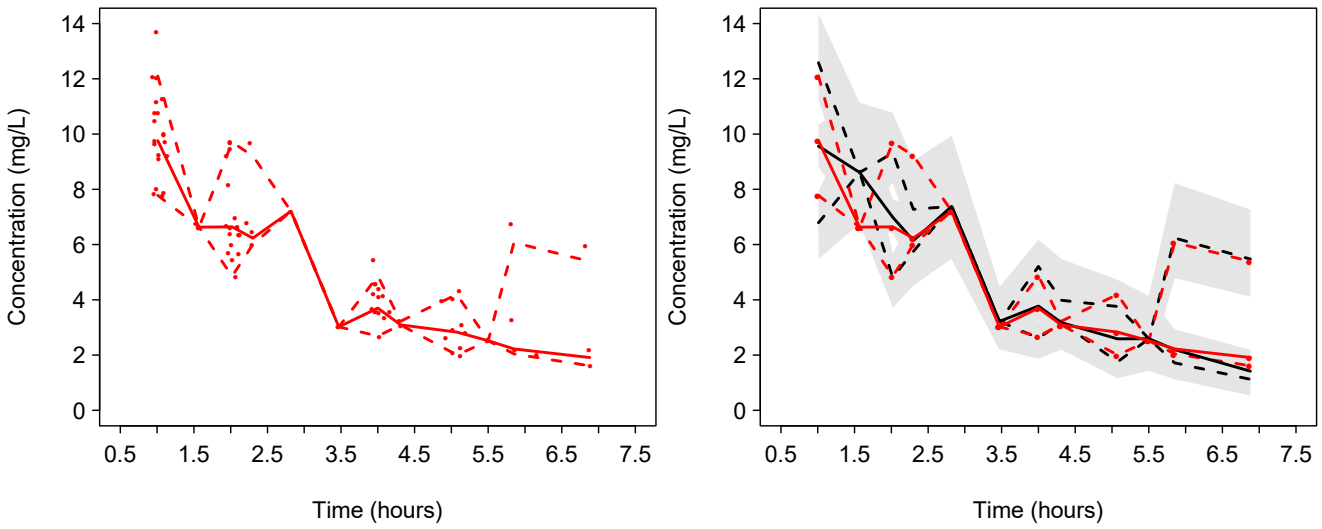
460 **Figure 1b**



461

462

463 **Figure 1c**



464