

**Therapeutic drug monitoring of gentamicin and
amikacin in hospitalised patients in a private
hospital, Western Cape**

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PREFACE

This mini-dissertation was written up in article format and the findings are presented in manuscript format in Chapter 3 (including a section on additional results). The manuscript was accepted for publication in the Ghana medical journal.

The manuscript follows the general formatting guidelines of the Ghana medical journal and references in the manuscript were cited according to the guidelines for the specific journal. The reference list at the end of the mini-dissertation is written according to the Harvard reference style required by the North-West University.

The following chapters are included in this mini-dissertation:

- Chapter 1 is an introductory chapter, which includes a summary of the research methodology used to conduct the study.
- Chapter 2 contains the literature review of therapeutic drug monitoring of aminoglycosides, a brief summary of antibiotics and guidelines for the dosing and monitoring of amikacin and gentamicin.
- Chapter 3 is the manuscript that contains the results and discussion. This chapter also contains additional results not addressed in the manuscript.
- Chapter 4 is the conclusion, recommendations and limitations that were drawn from this study.
- References and annexures follow at the end.

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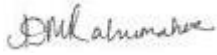
AUTHOR'S CONTRIBUTIONS TO MANUSCRIPT

The contributions of different authors with regard to the manuscript can be summarised as follows:

Author	Role in study
Mrs M du Toit	Study design and planning Collecting data Interpretation of results Writing the manuscript
Dr DM Rakumakoe (Study supervisor)	Conceptualised idea for manuscript and research design Guidance with data collection Guidance in data analysis and interpretation of results Approval of final manuscript
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Prof JR Burger (Co-supervisor)	Guidance with writing manuscript Guidance with interpretation of results Drafting of manuscript Revising of manuscript versions and approval of final manuscript.

Each co-author confirmed their role in the study and gave their permission for the manuscript to form part of this mini-dissertation by signing the following declaration:

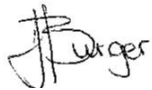
I declare that I approve of the manuscript as mentioned above and that my contributions are correctly reflected in the summary. I further give my consent that the work may be published as part of the MPharm study of Mariëtte du Toit.



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ABSTRACT

Background:

The burden of resistant bacteria is increasing and to ensure optimal treatment with the antibiotics currently available, therapeutic drug monitoring should be performed when prescribing aminoglycosides. Aminoglycosides are very effective in treating resistant gram-negative bacteria, but their use is limited by toxicity. Therapeutic drug monitoring (TDM) is essential to ensure that aminoglycoside peak concentrations are high enough for effective antimicrobial treatment and trough levels are low enough to minimise toxicity. Toxicity of aminoglycosides include reversible renal toxicity and irreversible ototoxicity. Inappropriate utilisation of TDM may lead to suboptimal therapy, toxicity and waste of resources that are already scarce in South Africa. The study aim was to investigate the standard of aminoglycoside TDM in a South African private hospital. The study determined whether dosage changes were made when the drug levels were outside the normal ranges, whether TDM was being done according to guidelines, and if samples were drawn at the correct times.

Method:

Retrospective data from November 2014 to October 2016 was used in this observational, descriptive, cross-sectional study, performed in a 221-bed private hospital in the Western Cape. All adult patients, older than 18 years, who were treated with intravenous amikacin or gentamicin for more than 48 hours, were included. A computerised database and patient files were used to obtain the information required for this study. Descriptive statistical analyses were used to describe and summarise data.

Results:

One hundred and three (103) patients were included: 65 patients on gentamicin and 38 on amikacin. Blood levels were performed on only 19 gentamicin (29.23%) and 22 amikacin (57.89%) patients. Trough levels were taken more than 2 hours before the next dose in 12 gentamicin (63.16%) and 12 amikacin (54.54%) patients. The majority of patients (96.92% on gentamicin and 84.21% on amikacin) received once daily doses. Therapeutic drug monitoring was performed in all patients with an estimated glomerular filtration rate (eGFR) lower than 60 mL/min/1.73m² and in 23.31% of gentamicin patients and 56.76% of amikacin patients with an eGFR higher than 60 mL/min/1.73m². All samples taken were trough levels and no peak levels were done. If a blood level was too high, the next dose was omitted.

Conclusions:

Incorrect sampling times and unnecessary levels taken in patients with normal renal function indicate a need for aminoglycoside treatment guidelines in the private hospital.

Key terms

Aminoglycosides, amikacin, gentamicin, private hospital, Western Cape, South Africa, therapeutic drug monitoring.

LIST OF DEFINITIONS

Absorption:	“The movement of a drug from its site of administration into the central compartment and the extent to which this occurs.” (Buxton, 2011:20).
Aerobic organisms:	Organisms requiring oxygen for the maintenance of life.
Anaerobic organisms:	Organisms that are able to survive or grow without oxygen.
Bacterial resistance:	Tolerance that certain bacterial strains develop toward a specific antibiotic or class of antibiotics.
Bactericidal:	Bacterial cells are killed by the antimicrobial agents (Kohanski <i>et al.</i> , 2010:423).
Bacteriostatic:	Antibiotics that merely inhibit the growth of micro-organisms (Kohanski <i>et al.</i> , 2010:423).
Bioavailability:	“The fraction of an administered drug reaching the systemic circulation.” (Smith <i>et al.</i> , 2012:1328).
Clearance:	“The volume of blood cleared of drug per time unit.” (Smith <i>et al.</i> , 2012:1328).
Community-acquired infection:	An infection acquired before admission to the hospital (infection already present at the time of admission). Symptoms will start within 24 hours of hospital admission (Henderson <i>et al.</i> , 2013:94).
Concentration-dependent killing:	“The higher the concentration, the greater is the rate at which bacteria are killed.” (MacDougall & Chambers, 2011:1507).
Empirical therapy:	Antibiotic treatment started, based on experience, and without the knowledge of the responsible organism.
Nosocomial infection:	Hospital acquired infection. An infection acquired at least 72 hours after hospitalisation (Henderson <i>et al.</i> , 2013:94).

Pharmacokinetics:	“The absorption, distribution, metabolism (biotransformation) and elimination of drugs are the processes of pharmacokinetics.” (Buxton 2011:17).
Post-antibiotic effect:	“That is, residual bactericidal activity persisting after the serum concentration has fallen below the minimum inhibitory concentration.” (MacDougall & Chambers, 2011:1507).
Therapeutic drug monitoring:	“Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drugs in plasma or blood at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimising individual dosage regimens.” (Roberts <i>et al.</i> , 2012:27).
Therapeutic index:	A ratio that compares the concentration at which a drug becomes toxic and the concentration at which the drug is effective.
Volume of distribution:	“This is a theoretical volume relating to the plasma concentration of the administered dose.” (Smith <i>et al.</i> , 2012:1328).

LIST OF ABBREVIATIONS

AD	<i>Anno Domini</i>
AKI	Acute kidney injury
AME	Aminoglycoside-modifying enzyme
ATP	Adenosine triphosphate
BBB	Blood brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
DHPS	Dihydropteroate synthetase
DNA	Deoxyribonucleic acid
eGFR	Estimated glomerular filtration rate
EML	Essential medicine list
ESBL	Extended spectrum β -lactamase
GFR	Glomerular filtration rate
GIT	Gastro-intestinal tract
HREC	Health Research Ethics Committee
IBW	Ideal body weight
ICU	Intensive Care Unit
MDRD	Modification of Diet in Renal Disease
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MUSA	Medicine Usage in South Africa

NSAID	Nonsteroidal anti-inflammatory drug
OHC	Outer hair cell
PABA	Para-aminobenzoic acid
PAE	Post-antibiotic effect
PBPs	Penicillin binding proteins
PD	Pharmacodynamics
PK	Pharmacokinetics
PTC	Proximal tubule cell
RNA	Ribonucleic acid
RTI	Respiratory tract infection
TB	Tuberculosis
TDM	Therapeutic drug monitoring
UTI	Urinary tract infection
Vd	Volume of distribution

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CHAPTER 1: OVERVIEW OF STUDY

1.1 Introduction

In this study the standard of therapeutic drug monitoring (TDM) practised by medical practitioners in patients over the age of 18 years who received the aminoglycoside antibiotics gentamicin and amikacin, in a private hospital, were investigated. The aim of the study was to determine whether dosage changes were made when the drug levels were outside the normal ranges and furthermore, whether TDM was being done according to guidelines and if samples were drawn at the correct times, were evaluated. The evaluation of therapeutic drug monitoring involved the comprehensive review of patient's prescription charts and laboratory data. This retrospective review detected patterns in prescribing therapeutic drug monitoring by medical practitioners and can serve as a means for developing guidelines and standards for future improvement in therapeutic drug monitoring practices.

1.2 Background and problem statement

Therapeutic drug monitoring is defined as the laboratory measurement of drug serum concentrations and adequate clinical interpretation of results to influence and individualise drug therapy in patients (Kovačević *et al.*, 2016:65). Drug dosing is individualised to maintain serum concentrations within a set range ensuring safety and efficacy of certain drugs for various clinical conditions (Kovačević *et al.*, 2016:65). Therapeutic drug monitoring improves patient outcomes and is of utmost importance in drugs with a narrow therapeutic index, high pharmacokinetic variability or in patients with hepatic or renal insufficiency (Kovačević *et al.*, 2016:66).

Aminoglycosides have a narrow therapeutic index, meaning there is a narrow margin between effective and toxic levels. An established concentration-effect relationship (toxicity), and drug monitoring is recommended for all patients treated with aminoglycosides. This class of antimicrobials includes streptomycin, kanamycin, gentamicin, tobramycin and amikacin (Roberts *et al.*, 2012:27).

Streptomycin was the first aminoglycoside to be discovered in 1944 (Shakil *et al.*, 2008:5). This was followed by the introduction of a series of milestone compounds, including kanamycin, gentamicin and tobramycin. In the 1970s, the semi-synthetic aminoglycosides dibekacin, amikacin and netilmicin were introduced demonstrating the possibility of synthesising compounds that were active against organisms that already developed resistance to older aminoglycosides. Streptomycin was isolated from a strain of *Streptomyces griseus* and gentamicin and netilmicin

were derived from *Micromonospora* spp., an actinomycete species (MacDougall & Chambers, 2011:1505). This established the usefulness of this class of antibiotics against gram-negative bacillary infections (Shakil *et al.*, 2008:6).

In contrast to most inhibitors of microbial protein synthesis that are bacteriostatic, this class of antibiotics has a bactericidal effect by ribosomal blockade, misreading in translation, membrane damage and irreversible uptake of the antibiotic (Shakil *et al.*, 2008:6). Aminoglycosides have bactericidal effects against aerobic gram-negative bacilli by binding irreversibly to the 30S subunit of the chromosome (McKinnon & Davis, 2004:271); this leads to misreading of the genetic code and inhibition of translocation (Kohanski *et al.*, 2010:425). The activity of aminoglycosides is sensitive to a change in pH and aminoglycosides are less effective at a lower pH. Lung and bronchial secretions have a low pH and this might lead to a decreased antimicrobial effect (MacDougall & Chambers, 2011:1507). Aminoglycosides cause bacterial cell death by achieving high concentrations at the binding site; this is called concentration-dependent killing. Concentrations more than 10 times the minimum inhibitory concentration (MIC) for the specific target organism have the best responses (Dobie *et al.*, 2006:253).

Aminoglycosides have a broad spectrum of antibiotic cover; many aerobic gram-negative bacteria, as well as mycobacteria, are susceptible to aminoglycoside activity. Aminoglycosides are not routinely used for infections caused by gram-positive infections such as *Staphylococcus aureus*, because in most cases it is not adequate monotherapy (Garrahan & Fallon, 2015). As the uptake of aminoglycosides into bacterial cells is oxygen-dependent, anaerobic organisms possess intrinsic resistance against aminoglycosides (Garrahan & Fallon, 2015).

The Therapeutic Drug Monitoring Special Interest Group of the South Australian Expert Advisory Group on Antimicrobials and Resistance reviewed the Therapeutic Guidelines for Antibiotics, version 15 (Antibiotic Expert Groups, 2014), which clearly delineate empirical and definitive treatment. According to these guidelines, empirical therapy with aminoglycosides should not continue for more than 48 hours and monitoring of plasma drug concentration is not required. Aminoglycosides (mostly in combination with other drugs) are used as empirical treatment for septicaemia, nosocomial respiratory tract infections (RTIs), complicated urinary tract and intra-abdominal infections, osteomyelitis and wound infection after open fractures (Hanberger *et al.*, 2013:162) because of their rapid bactericidal effect and low rates of resistance in community and healthcare settings (Antibiotic Expert Groups, 2014). Gentamicin is usually used against infections caused by gram-negative organisms, such as *Pseudomonas aeruginosa*, *Proteus* spp., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia marcescens* and *Citrobacter* spp.,

as well as against *Staphylococcus* spp. (both coagulase-positive and coagulase-negative) (Drew, 2014). Amikacin, with a wider bacterial coverage than gentamicin, in combination with an antipseudomonal beta-lactam or carbapenem, may be used to treat hospital-acquired pneumonia (Drew, 2014).

Due to the concern for ototoxicity and nephrotoxicity, aminoglycosides are not routinely used as first line treatment of susceptible organisms (Drew, 2014; Prayle *et al.*, 2010:655). Aminoglycosides can cause acute kidney injury (AKI) in 10 to 25% of therapeutic courses, even when patient monitoring is being done (Lopez-Novoa *et al.*, 2011:33). The potential to cause ototoxicity depends on the aminoglycoside used: neomycin is considered the most highly toxic, followed by gentamicin, kanamycin and tobramycin, with amikacin and netilmicin the least toxic (Xie *et al.*, 2011:30). Aminoglycosides are also associated with cochlear and vestibular toxicity, leading to hearing loss and disequilibrium respectively (Dobie *et al.*, 2006:253).

There are some scenarios where aminoglycoside dosages should be adjusted to prevent ototoxicity and nephrotoxicity. The following are some examples where dosage adjustments are recommended:

- Dosing weight: Ideal body weight (IBW) should be used, unless the patient is 20% over IBW (then use adjusted body weight instead).
- Burns (more than 20% of body surface), pregnancy, ascites or third spacing, haemodynamic instability, impaired renal function or cystic fibrosis (Nicolau *et al.*, 1995:1360).
- Renal function, when creatinine clearance is <60 ml/min (Department of Health, 2015:497).

Dosing is once-daily doses of 15 mg/kg/dose for amikacin and 5 to 7 mg/kg/dose for gentamicin and tobramycin (except when administered synergistically for gram-positive infections, where it is 1 mg/kg/dose for gentamicin and tobramycin, administered eight hourly) (Dobie *et al.*, 2006:253).

Trough levels should be determined before administration of the next dose and the desired trough level is <1 mg/L in the case of gentamicin or tobramycin and <5 mg/L in case of amikacin. For once-daily dosing of aminoglycosides a peak level can be determined for efficacy, which should be 10 to 12 times the MIC of the infecting organism (Dobie *et al.*, 2006:253).

To minimise toxicity and adverse drug reactions, but also ensure successful treatment and prevent antimicrobial drug resistance, it is important to monitor drug levels and optimise dosing (Avent *et al.*, 2011:444). According to Wong *et al.* (2014:288), there is significant variability in

therapeutic drug monitoring (TDM) practices (including patient selection, sampling time for monitoring of drug concentration, selection of pharmacokinetic and pharmacodynamics targets and dose optimisation strategies) among institutions. A study published in 2012 found that approximately 20% of gentamicin blood samples were collected at inappropriate times, or dosage administration times were not documented; both lead to incorrect results and ineffective dosing (Martin *et al.*, 2012:4). In the same study, 15% of doses were adjusted without monitoring and more doses were adjusted, despite drug concentrations being in the therapeutic range. Efficient monitoring of drug levels of aminoglycosides is therefore not always done and doses are not adjusted according to the drug levels.

In an attempt to assess the extent of research on TDM of aminoglycosides, a literature search was performed using the search terms in Google Scholar and PubMed searches (“therapeutic drug monitoring” OR “dose” OR “dosing” or “dosing strategy” AND “aminoglycosides” OR “gentamicin” OR “amikacin”) in titles or abstracts. The search language was English. Relevant articles from 2006 onwards were studied and details of authors, inception period, country where the study was done, study design, measurements, study sample and results are summarised in Table 1-1.

The following conclusions can be drawn from Table 1-1. All the studies were carried out on hospitalised adults on aminoglycoside treatment. From the studies, it is clear that aminoglycoside dosing and TDM is not always performed according to guidelines, and results are often interpreted incorrectly. Sampling times are often not correct and samples are frequently collected at incorrect times. None of the studies found during the search were performed in South Africa, therefore there is a definite lack of similar South African studies. This brought the following questions to mind:

- Are there any guidelines for therapeutic drug monitoring of aminoglycoside levels in a private hospital in the Western Cape?
- Is TDM being performed in a private hospital in the Western Cape?
- Were dosages of gentamicin and amikacin adjusted according to therapeutic drug monitoring (TDM) results?

Table 1-1: Summary of studies on TDM of aminoglycosides

Authors	Inception period	Country/ study setting	Study design	Measurements	Study sample, N, Age and Gender	Results
Al Za'abi <i>et al.</i> (2015)	Oct. 2013 – Jan. 2014	Oman	Prospective, cross-sectional study	TDM results. Appropriateness of TDM. Appropriateness of sampling time.	733 patients Mean $\pm$ SD age 25.38 $\pm$ 26.8 years 53.9% males	TDM results: Low: (n = 302; 41.2%) Within: (n = 310; 42.3%) High: (n = 121; 16.5%) TDM appropriate: Yes: (n = 573; 78.2%) No: (n = 160; 21.8%) Sampling time appropriate: Yes: (n = 209; 28.5%) No: (n = 468; 63.8%)
Allou <i>et al.</i> (2016)	Apr. 2015 – Dec. 2015	France	Prospective, observational study	Impact of mortality of amikacin concentrations of 60-80 mg/L in patients with sepsis or septic shock.	110 patients Age median (25 th -75 th percentile) 61(51-70) 70.9% males	Amikacin dose: Median 30 (29.2-36.6) mg/kg C_{max}: 60-80 mg/L for 46 patients (41.8%) TDM performed: 65 (68.2%) patients had trough levels done; 51 (78.5%) had trough concentrations >2.5 mg/L.

Authors	Inception period	Country/ study setting	Study design	Measurements	Study sample, N, Age and Gender	Results
Drusano and Louie. (2011).	Unknown	United States of America	Data from a prior population's pharmacokinetic analysis were used to generate Monte Carlo simulations.	To determine the probability of effect and toxicity at specific doses of aminoglycosides.		Toxicity: Less aminoglycoside toxicity was observed with once-daily dosing than with multiple daily dosing. Shorter period of treatment also showed lower toxicity.
Jenkins <i>et al.</i> (2016)	Unknown	United Kingdom	Retrospective systematic literature review	Amikacin dosage associated with good outcomes; determine amikacin doses causing oto- and nephrotoxicity.	1677 patients from 17 studies. Patients were older than 18 years	Amikacin doses: 9-15 mg/kg, but most studies had a dose of 15 mg/kg/day. Toxicity: Amikacin showed less nephrotoxicity than other aminoglycosides, but similar ototoxicity.
Kovačević <i>et al.</i> (2016)	Unknown	Srpska, Bosnia and Herzegovina	Prospective study	Assessment of dosage-appropriateness of gentamicin and amikacin in critically and non-critically ill patients.	31 patients on gentamicin Age: Mean $\pm$ SD 60.58 $\pm$ 18.018 years 72.7% males 16 patients on amikacin	Dosing: 1 patient on amikacin (9.1%) received a once-daily dose. TDM levels: 5/20 (25%) of critically ill patients had toxic aminoglycoside trough levels and 2/27 (7.4%) non-critically ill patients had toxic levels. Peak levels were within reference range in 81.8% critically ill

Authors	Inception period	Country/ study setting	Study design	Measurements	Study sample, N, Age and Gender	Results
					Age: Mean $\pm$ SD 59.44 $\pm$ 15.72 years 27.3% males.	patients on amikacin and 88.9% critically ill patients on gentamicin.
Leong <i>et al.</i> (2006)	1 Feb. – 12 Mar. 2004	Australia	Prospective descriptive study	Audit gentamicin usage – focused on initial dosing and TDM practices.	132 patients on gentamicin treatment.	Dosing: 82% were given once-daily doses. 66% initial doses not according to hospital guidelines. Most commonly prescribed dose was 240 mg once daily. TDM: 77% of patients who should have received TDM, did. 8.8% of the TDM was done according to guidelines.
Martin <i>et al.</i> (2012)	Unknown	Australia	Retrospective	Appropriateness of gentamicin prescribing and monitoring.	161 adult patients on gentamicin where at least one serum concentration was measured. Two hospitals with female: male (%) 40:60 and 49:51.	Sampling times: inappropriate in 19% of patients in hospital 1 and 23% patients in hospital 2. TDM and dosage changes: 16% of dosage changes were made without using TDM and 15% dosages were changed although drug concentrations were within range.

Authors	Inception period	Country/ study setting	Study design	Measurements	Study sample, N, Age and Gender	Results
					Age 55 ± 21 years and 49 ± 20 years.	
Namazi <i>et al.</i> (2016)	Apr. – Dec. 2011	Iran	Cross-sectional	Assessment of amikacin usage pattern.	63 patient older than 18 years on IV amikacin for more than three days.	TDM: 45% trough and 38% peak levels were within therapeutic ranges. Dose adjustments not done in 89% of patients where it was indicated. In 19% of patients, no optimal therapeutic effect was achieved.
Nezic <i>et al.</i> (2014)	2013	Switzerland	Prospective	Comparison of pharmacokinetic profiles and Bayesian calculations to monitor TDM of aminoglycosides.	14 patients on once-daily aminoglycoside doses for over three days.	Sampling of two blood levels: The ideal time points for sampling TDM are 1h after starting infusion and 8-10 hours after infusion for the second blood sample.
Tabah <i>et al.</i> (2015)	Unknown	Australia	Online questionnaire	A survey of dosing and monitoring of antimicrobials in ICUs.	Questionnaires were sent to 402 health professionals – 78% were Intensive Care specialists and 12% were pharmacists.	Aminoglycoside prescribed: 55% of patients received gentamicin, 40% amikacin and 5% tobramycin. TDM results: 80% would change dosages if the levels were outside of normal ranges. 79.2% of respondents measured trough levels and 37.9% measured peak levels.

1.3 Research aims and objectives

1.3.1 Research aim

The study aim was to investigate the standard of aminoglycoside TDM in a South African private hospital. The study determined whether dosage changes were made when the drug levels were outside the normal ranges, whether TDM was being done according to guidelines, and if samples were drawn at the correct times.

1.3.2 Specific research objectives

This research project had specific literature and empirical objectives.

The literature objectives were:

- To review guidelines to determine in which cases therapeutic drug monitoring should be conducted when administering intravenous aminoglycosides in different populations.
- To determine the time at which the trough or peak levels should be measured before the administration of the next dose.
- To determine normal reference ranges for gentamicin and amikacin trough or peak levels.
- To conceptualise aminoglycoside toxicity and describe the different mechanisms and consequences of toxicity.
- To determine the influence of aminoglycosides on serum creatinine levels in patients.

The empirical research objectives were:

- To determine the dosages and time intervals of aminoglycosides prescribed for the patients during the period of the study.
- To determine the percentage of patients on aminoglycosides whose drug levels were monitored.
- To calculate the percentage of patients with an abnormal renal function where therapeutic drug monitoring was done.

- To determine whether dosage adjustments were made in case of drug levels outside the normal reference range.

1.3.2.1 Literature review

A literature review was conducted by using internet searches in appropriate databases such as ScienceDirect, EBSCOHost, Scopus, Google Scholar or similar. Keywords were used to find appropriate articles to answer the research questions. Examples of key words and phrases used separately and in combination were:

- Therapeutic drug monitoring of aminoglycosides.
- Dosage adjustment for aminoglycosides.
- Minimum inhibitory concentration and aminoglycoside.
- Ototoxicity and nephrotoxicity and aminoglycosides.
- Pharmacokinetics of aminoglycosides.
- Different classes of antibiotics.

1.3.2.2 Empirical investigation

The research design and study setting will be explained in the following section.

1.4 Research methodology

1.4.1 Study design

In this study, an observational, descriptive, retrospective, cross-sectional design was followed.

Observational studies are defined as studies in which individuals are observed or certain outcomes are measured, whilst no attempt is made to affect the outcome (Mann, 2012:38). In this study, retrospective data were observed, with no active intervention from the researcher. Observations were made under precisely defined conditions in a systematic and objective manner to ensure the data were considered scientific.

Descriptive studies are observational studies in which the patterns of disease occurrence in relation to variables such as person, place and time are described. A descriptive study is done

to identify patterns or trends, without identifying the causal linkages among the elements (Business Dictionary, 2017). In this study, data were described in terms of the frequency of therapeutic drug monitoring in patients who received intravenous aminoglycosides while admitted in hospital during the research period from 1 November 2014 to 31 October 2016.

Retrospective data from patient files that have already been collected for other purposes were used. Retrospective is defined as “looking back on or dealing with past events or situations.”

1.4.2 Study setting

The empirical investigation took place in a private hospital in the Western Cape. The hospital consists of 221 beds, with roughly equal numbers of surgical and medical patients, as well as a 26-bed Intensive Care Unit. This hospital was chosen because the researcher worked in the facility and had access to data.

The hospital is the main medical centre for many patients living in the northern suburbs of Cape Town and neighbouring countryside towns.

1.4.3 Target and study population

The target population included all patients who received intravenous gentamicin or amikacin while admitted in the hospital during the period 1 November 2014 to 31 October 2016. The specific aminoglycosides were chosen because they are the most commonly used aminoglycosides in South Africa. The study population consisted of all patients meeting the inclusion criteria, after application of the exclusion criteria.

Inclusion criteria for the study included:

- All patients over the age of 18 years, who received intravenous amikacin or gentamicin for more than 48 hours while admitted in the hospital between 1 November 2014 and 31 October 2016.
- All patients over 18 years, who received intravenous gentamicin or amikacin while admitted in the emergency room, who were thereafter admitted to the hospital and received treatment with aminoglycosides between 1 November 2014 and 31 October 2016.

Exclusion criteria for the study included:

- Patients who received a single dose of gentamicin or amikacin as surgical prophylaxis (regardless of the route of administration of the medication). These patients were excluded from the study because therapeutic drug monitoring could not be done when only one dose was administered.
- Patients who received one dose of intravenous gentamicin or amikacin while admitted in the emergency centre before being discharged or transferred to another facility. These patients were excluded because they received only one dose, therefore, no therapeutic drug monitoring was possible.

1.4.4 Sampling

Data from all patients in the target population who fit the inclusion criteria were used for the research to obtain an accurate reflection of the therapeutic drug monitoring that was done in the facility during the study period. All-inclusive sampling was used in the study; therefore, no power analysis was necessary.

1.4.5 Data sources

The following data sources were used during the study:

- The hospital dispensing programme (The AS400 dispensing programme).
- Patient files.
- Pathology laboratory user website.

Permission was obtained from the research committee of the hospital group to conduct the study in the specific facility by using the AS400 dispensing programme and retrospective data from patient files (Refer to Annexure A – Permission from Corporate Office).

The AS400 hospital dispensing programme was used to identify patients who received gentamicin and amikacin during the study period. Patient files for these specific patients were then used to obtain information on:

- Patient's age.
- Patient's gender.

- Patient's weight.
- Dosage and frequency of gentamicin or amikacin prescribed.
- Time of day at which dosages were administered.
- Laboratory reports to determine whether peak and trough levels were measured and the time at which the blood samples were taken for measurement.
- Results from laboratory reports to determine whether the results were within the normal reference ranges.
- Dosage changes, if any, made after receiving results back from the laboratory.

The patient's weight, age and gender were important factors when calculating creatinine clearance and the appropriate dosage of aminoglycoside prescribed. The dosage and frequency of gentamicin and amikacin prescribed, as well as whether dosage changes were made, were factors used to determine the percentage of patients where TDM was done. The inclusion of the time of day at which dosages were administered, and the laboratory results, indicated whether TDM was done correctly (sampling time of measured trough level and time of administration of aminoglycoside).

The patient's renal function (measured by Modification of Diet in Renal Disease (MDRD), estimated glomerular filtration rate (eGFR) and serum urea and creatinine) was not recorded in the patient chart and was obtained from the pathology laboratory's user website. The researcher obtained a specific username and password from the laboratory and had access to each individual patient's laboratory results (Refer to Annexure B – Permission from laboratory manager). In the laboratory results, renal function is expressed as eGFR, which is calculated by using either the CKD-EPI or MDRD calculation (refer to paragraph 2.6.1 – General aminoglycoside guidelines).

1.4.6 Data collection tool

1.4.6.1 Development of data collection tool

The data collection tool (Annexure I – Data collection tool) consisted of an Excel document where all relevant data were captured.

All fields relevant to the dosing and monitoring of aminoglycoside levels (Hanberger *et al.*, 2013:170; Roberts *et al.*, 2012:27) were included in the data collection tool. The specific data points were chosen because they represented criteria that should be considered when prescribing, administering and monitoring aminoglycoside therapy. This included demographic information (i.e. patient's gender, weight and age), information about the dosage (dosage and time of day it was administered), therapeutic drug monitoring data (whether it was done, the results and whether a new dose was prescribed if the results were outside of the normal range) and the renal function of the patients.

Demographic information is important to be able to compare the patient population in the study with other studies performed. The pharmacologic advantages of once-daily dosing of aminoglycosides are widely known (Stankowicz *et al.*, 2015:1357). The information regarding dosing (the dose administered and time of day when it was administered) will therefore give an indication if dosage administration in the hospital was done according to international guidelines. The time of day when dosages were administered, together with laboratory data and time when the samples were taken for TDM, evaluates the appropriateness of sampling times; inappropriate sampling times might lead to a significant waste of resources and incorrect adjustment of dosages (Al Za'abi *et al.*, 2015:459). It is therefore of utmost importance that correct sampling times are adhered to, to ensure effective and optimal use of TDM. A study between 2013 and 2014 in Oman revealed that sampling times were inappropriate for 71.5% (N = 733) of patients where TDM were performed (Al Za'abi *et al.*, 2015:460). The measuring of renal function is important to determine the empirical dosage prescribed, as well as to monitor for nephrotoxicity (Avent *et al.*, 2011:443).

1.4.6.2 Validity of the data collection tool

Validity and reliability of measurements have an influence on the probability of study significance when completing the data analysis. The results, as well as the conclusion, are influenced thereby. Validity and reliability are concerned with how specific the indicators or measurements for the study were developed (Leedy & Ormrod, 2014:91). Internal validity is defined as the degree to

which the outcomes of experiments can be attributed to independent variables rather than to uncontrolled and unrelated factors. Any factor that influences the dependent variable holds a threat to validity (Brink *et al.*, 2012:109). External validity refers to the degree to which the results of a specific study can be generalised to other settings or other people (Brink *et al.*, 2012:111).

Validation of the data collection tool in this study was done using face validity and content validity.

1.4.6.2.1 Face validity

Face validity refers to the grade to which an instrument looks valid. Although face validity cannot be quantified or tested, an instrument is scrutinised by experts in the field to ensure a high degree of face validity (Pietersen & Maree, 2014:217). The experts in the field who determined whether the measurement tool adequately covered the content and represented a contrast of interest included study supervisors, who are experts on clinical pharmacy and pharmacology, and a statistician at the North-West University (NWU). The data collection tool was also sent to academics with hospital practice experience for evaluation.

1.4.6.2.2 Content validity

Content validity refers to the extent to which a measurement tool can cover the complete content of what it is supposed to measure (Pietersen & Maree, 2014:217). Content validity is demonstrated by conducting an extensive literature review of similar studies using specific measuring instruments. In this study, content validity was established by conducting an extensive literature review to select variables relevant to TDM of aminoglycosides (Refer to paragraph 2.3).

1.4.6.3 Reliability of the data collection tool

Reliability of a data collection tool ensures that consistent results would be obtained when different users apply the tool, or when the tool is used in different occasions. It refers to whether a result is consistent, stable and repeatable. Different types of reliability can be explained — stability, homogeneity and equivalence. The specific data collection tool displayed stability, due to the fact the same results would be obtained on repeated use. Equivalence is another type of reliability and refers to the inter-rater reliability — the level of agreement among researchers who use the same tool for data collection (Twycross & Shields, 2004b:36). To ensure reliability of the data collection tool in this study, the same data collection tool was used for all data collected and only the researcher completed the information in the data collection tool and therefore input errors

and variability due to several data capturers were minimised. After data were captured, however, random data checks and checks for data outliers were performed to assure data quality.

1.4.7 Data-collection process

Data collection started after the necessary permission to conduct the study was obtained from the hospital group ethical committee, hospital and pharmacy managers, laboratory and the Health Research Ethics Committee (HREC) from the North-West University (refer to paragraph 1.6). Data were collected from the hospital dispensing programme (AS400), patient files and the pathology laboratory's user website (refer to paragraph 1.4.5).

The hospital dispensing programme (AS400), which has the history of medications dispensed for a five-year period, contained data of all medicine dispensed to patients in the hospital for the two-year period from 1 November 2014 to 31 October 2016. The patient file numbers of those who received gentamicin and amikacin for the specific period were found by conducting a search for all gentamicin and amikacin dispensed from 1 November 2014 to 31 October 2016. This list contained only patient file numbers and not patient's demographical data, such as identity numbers and addresses.

The data were collected from files already filed in the hospital archive facility. The hospital filing clerk, who went to the archive facility as part of his daily duties, was given the specific file numbers for patients who received gentamicin and amikacin in order to collect and take the files to the pharmacy. The researcher requested files every Friday, to be able to collect data on the Friday evening after working hours, on the Saturday morning and during the following week. The researcher sent an e-mail to the filing clerk with specific file numbers, the reason for requesting the files, as well as the date and time when files were requested and would be returned.

The researcher then determined which files fit the inclusion criteria before collecting data. Data were extracted from the files over weekends and during the following week, ensuring that the files never left the hospital premises. Data collection from files took place in the pharmacy manager's office after working hours, when there were no other personnel in the pharmacy. Files were kept in a locked cupboard in the office, to which only the researcher and pharmacy manager had the keys. The filing clerk took the files back to the archive facility after one week. The filing clerk signed a confidentiality agreement stating that no information would be made known to any person outside the study (refer to Annexure F – Confidentiality agreement).

Data that could not be found in the patient files (refer to paragraph 1.4.5) were retrieved from the pathology laboratory's website. Written permission was obtained from the laboratory manager to collect and use the data (refer to Annexure B – Permission from laboratory manager). A specific username and password were used to access the information of the pathology user's website. Data were also collected outside of normal working hours when there were no other personnel in the pharmacy. Information regarding the therapeutic drug monitoring and drug levels were also extracted from this website. Data were captured onto the data collection tool.

In case of a patient being admitted more than once per year and treated with gentamicin or amikacin on more than one admission, it was regarded as a new patient every time and data were used for every admission as a separate case.

1.5 Data analysis

The statistical analysis was performed using the SPSS® programme, version 24 (IBM Corp., 2013).

Only descriptive statistical analysis was used to describe and summarise data. Categorical variables were expressed as frequencies and percentages and continuous variables were expressed as means and standard deviations.

The variables used in the study are described in Table 1-2.

Table 1-2: Study variables and description

Variable	Description
Age	Age of the patient during treatment, as indicated in the patient's hospital file.
Gender	Gender of the patient, as indicated in the patient's hospital file. Gender was categorised as male or female.
Weight	Weight of the patient during treatment, as indicated in the patient's hospital file (measured in kg).
Renal function	eGFR as calculated by the laboratory and indicated on the laboratory reports.

Variable	Description
Type of aminoglycoside prescribed	Gentamicin or amikacin prescribed.
Dose	Dose prescribed to the patient, in mg.
TDM requested	Whether TDM was requested by the physician (categorised as “Yes” or “No”), as was indicated in the physician’s notes in the patient prescription chart.
TDM result	Laboratory result with amikacin and gentamicin levels, as indicated on the laboratory website, measured in mg/L. The results were categorised as either within the normal ranges or outside of normal ranges as defined by the laboratory standards for peak or trough levels.
Sampling time	Time of day when blood was drawn for TDM, as indicated in patient prescription chart. This was measured together with the time of day when the dosage was administered to measure whether sampling time was within one hour of the next aminoglycoside dose for trough levels.

1.6 Ethical considerations

Permission to use data from patient files at the hospital where the research was done was obtained from:

- The ethics approval from the hospital group’s corporate office (refer to Annexure A)
- The hospital manager at the facility where the research was conducted (Annexure C)
- The pharmacy manager in the hospital (Annexure D)
- The laboratory manager (Annexure B)
- The Health Research Ethics Committee of the North-West University (HREC).

Goodwill permission was obtained from the ethics committee of the hospital group to conduct research at the specific hospital and from the manager of the laboratory to obtain and use data from the laboratory’s user website. Final approval was given after HREC approved the study (NWU-00363-15-S1) (Refer to Annexure E – Ethics approval certificate).

As only retrospective data were used and no patient information would be published, it was not required to obtain consent from individual patients.

Each participant was given a number (starting from one), and these numbers were used when collecting and analysing the data to ensure anonymity was maintained. No personal information was captured that could cause a patient to be identified during any stage of data collection. All possible efforts were made to ensure no patient information was known to any parties other than the researchers. A confidentiality agreement was signed by the filing clerk. No information regarding the prescribing medical practitioner was recorded and the name of the hospital will not be published, therefore the hospital or associated medical practitioners could not be identified. During the study period, data were kept on a password-protected laptop.

The study held medium risk and precautions were taken to ensure that all parties were protected against anticipated risks (refer to paragraph 4.3 – Strengths and limitations). The benefits of conducting this study outweighed the risks if anonymity and confidentiality were maintained.

1.7 Chapter summary

In this chapter, the background of the study was described, as well as research aims and the research methodology. The data collection tool and data-collection process were discussed and an overview of the statistical analysis and ethical considerations were provided. In the next chapter, the therapeutic drug monitoring of aminoglycosides will be discussed.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

In this literature review, the history, rationale, role and implementation of therapeutic drug monitoring (TDM) in hospitalised patients will be discussed. The discussion will then focus on the TDM of aminoglycosides. The background and history of different antibiotic classes, the difference between concentration- and time-dependent antibiotics, mechanism of action, spectrum, and uses of different antibiotic classes will then be discussed. The structure of aminoglycosides, mechanism of action, antimicrobial spectrum and uses, pharmacodynamics, toxicity and bacterial resistance will be explained and general and specific guidelines for the use of aminoglycosides will be reviewed.

2.2 Therapeutic drug monitoring

Therapeutic drug monitoring refers to the individualisation of drug dosage to maintain plasma or blood drug concentrations within the therapeutic window. Therapeutic drug monitoring is an established and useful clinical service if used correctly. Therapeutic drug monitoring was introduced as a new aspect of clinical practice in the 1960s, when pharmacokinetic studies were first linked to mathematical theories to improve patient outcome. In the beginning, TDM focused on adverse drug reactions and showed early on that by constructing therapeutic ranges, the incidence of toxicity of narrow therapeutic drugs, such as digoxin, phenytoin, lithium and theophylline, could be minimised. The increased awareness of drug concentration-response relationships, mapping of drug pharmacokinetics and the advancements in analytical technology encouraged the emergence of TDM over the years (Kang & Lee, 2009:1).

Therapeutic drug monitoring is an important part of the patient care plan, but an increase in demand for this service may lead to a direct increase in hospital cost and the need for more resources; for this reason it is important to conduct TDM in the correct manner (Al Za'abi *et al.*, 2014:460). Over the last 40 years, growing concerns over rising healthcare costs, forced the principles of pharmacoeconomics to be applied to TDM. Pharmacoeconomic principles are applied to ensure that costs are allocated optimally and effectively to ensure quality of life, patient satisfaction and satisfy patient preferences. Therapeutic drug monitoring as an intervention improves patient response to life-sustaining drugs and decreases adverse drug reactions. This means that the resources consumed by TDM practice, will likely be regained by positive outcomes, including decreased hospitalisations (Kang & Lee, 2009:7). In developing countries

with already limited resources, it is of utmost importance that TDM services are utilised appropriately. Guidelines are not always implemented and followed accurately (Al Za'abi *et al.*, 2014:460; Martin *et al.*, 2012:5). The benefit of TDM lies in the correctness of the data collected from the patients. An appropriate pharmacokinetic evaluation requires properly timed blood specimens and it is crucial that the TDM team must be informed as to when the plasma sample was obtained in relation to the last dose administered (Kang & Lee, 2009:6). The main benefit of TDM is that dosages can be individualised if a blood level concentration is available by the use of certain pharmacokinetic equations available in the literature.

It is important to remember that TDM is a combination of measured plasma or blood drug concentrations and some pharmacokinetic parameters and equations. Pharmacokinetics is defined as the study of the time course of drug absorption, distribution, metabolism and excretion. Clinical pharmacokinetics and TDM is the application of the pharmacokinetic principles to the safe and effective therapeutic management of drugs in an individual patient, taking into consideration the clinical condition of the patient (Buxton, 2011:14). Paragraphs 2.2.1-4 describe the pharmacokinetic parameters most often utilised in clinical pharmacokinetics, namely volume of distribution (Vd), clearance (Cl), elimination constant (k_e) and half-life ($t_{1/2}$).

2.2.1 Volume of distribution (Vd)

The apparent volume of distribution is not a real figure, but a figure that gives a theoretical size of the compartment that would be required if the complete amount of drug in the body was present at the same concentration as the measured sample. A small Vd normally indicates that the drug is water-soluble and prefers to remain in blood vessels and a large Vd that the drug distributes extensively outside vascular tissue, i.e. fat, muscle or red blood cells (Buxton, 2011:14). Volume of distribution is the parameter used to calculate loading dose (Buxton, 2011:14).

$\text{Volume of distribution (Vd)} = \frac{\text{Dose}}{\text{Plasma concentration (Cp)}}$

The equation for loading dose is (Buxton, 2011:14):

$$\text{Loading dose} = \frac{V_d * C_p}{S * F}$$

Where:

V_d = Volume of distribution.

C_p = Desired plasma level.

S = Salt factor.

F = Bioavailability.

These equations can be altered, at steady state, when more than one blood sample is available and that will be discussed under the Sawchuk-Zaske method (Paragraph 2.3.4.3) (Bauer, 2008:102).

2.2.2 Clearance (Cl) and steady-state (C_p^{-ss})

Clearance is the most important parameter when maintenance doses are calculated. Clearance refers to the volume of plasma from which a drug is completely removed per unit time; the units are mL/min. The total body clearance will be equal to renal + hepatic + lung clearance (Buxton, 2011:14). It is important to maintain steady-state concentrations within the therapeutic window. Steady state will be achieved when rate of drug elimination equals rate of drug administration as calculated using the formula (Buxton, 2011:14):

$$D = \frac{Cl * C_p^{-ss}}{F}$$

Where:

D = Dose in one interval.

Cl = Clearance.

C_p^{-ss} = Steady state plasma concentration.

F = Bioavailability.

2.2.3 Elimination rate constant (k_e)

The elimination rate constant (k_e) reflects the fraction of drug removed from the compartment per unit time. The elimination rate constant can be calculated using the formula (Buxton, 2011:14):

$$K_e = -\left[\frac{\ln C_1 - \ln C_2}{t_1 - t_2}\right]$$

Where:

C_1 = Plasma concentration 1.

C_2 = Plasma concentration 2.

t_1 = Time 1.

t_2 = Time 2.

For aminoglycosides:

$$K_e = 0.00293(CrCl) + 0.14$$

2.2.4 Half-life ($t_{1/2}$)

The half-life is the time it takes for the plasma concentration or the amount of drug to be reduced by 50% (Buxton, 2011:16). Half-life can be calculated as follows (Buxton, 2011:16):

$$Half\ life = \frac{0.693(Vd)}{Cl}$$

Several studies have shown that the inappropriate utilisation of TDM can lead to significant waste of resources and can even lead to incorrect dosing recommendations (Al Za'abi *et al.*, 2014:459) (Refer to Table 1-1: Summary of studies on TDM of aminoglycosides). Al Za'abi *et al.* (2014:459) documented that the sampling times were inappropriate in 71.5% of the samples. They concluded that the inclusion of pharmacists on ward rounds could increase the standard of TDM in hospitals. The collection of blood samples at incorrect times or failure to document times when the dosages were administered or when samples were taken were also documented by Martin *et al.* (2012:4). In this study, approximately 20% of gentamicin concentrations were collected at inappropriate times or had insufficient documentation of administration times. The authors stated that these

factors cause difficulty in interpreting the concentration and inevitably lead to ineffective dosing (Martin *et al.*, 2012:4). Use of inaccurate plasma drug concentration may have major consequences for the calculation of elimination half-life or clearance. Incorrect recording of times for the start and end of administration is another source of error that may lead to incorrect TDM (Touw *et al.*, 2009:84).

Inefficient TDM practices will lead to waste of resources in sample collection time, analytical costs, time to interpret results and unknown costs associated with increased hospital stay, more laboratory tests and treatment with a number of antimicrobials (Martin *et al.*, 2012:4). Conversely, therapeutic drug monitoring practices to optimise antimicrobial prescribing improves clinical outcome and reduces the development of antimicrobial resistance and toxicity (Ashwlayan & Singh, 2016:282; Roberts *et al.*, 2012:27). The impact of TDM is well noted in a study documented in the publication by Roberts *et al.* (2012:27). The study was performed in 232 hospitalised patients, where 'standard' TDM was compared to 'active' TDM, 'standard' TDM involved the physician writing prescriptions and TDM only done when requested, and 'active' TDM was the use of optimisation of dosage with PK strategies. The results showed that 'active' TDM strategies resulted in shorter hospitalisation and reduced nephrotoxicity (Roberts *et al.*, 2012:30).

According to Nwobodo (2014:1), the most crucial aspect of TDM is the expert clinical interpretation of drug concentration results, as the mere measurement of results without clinical interpretation is a waste of time and already limited resources in developing countries. In many healthcare facilities in developing countries, TDM is still only a "measure", performed by clinical chemistry laboratories, rather than a "monitor" with clinical interpretation (Nwobodo, 2014:1). Therapeutic drug monitoring services were only available in 45.1% of responding countries in Africa, compared to 93.3% in Europe and 95.8% in the Americas (Nwobodo, 2014:2). Performing TDM requires a multidisciplinary approach and only complete collaboration by a TDM team will result in meaningful and accurate monitoring of therapy (Kang & Lee, 2009:2). The ideal TDM team comprises laboratory scientists, clinicians, nursing staff and a clinical pharmacist, and excellent communication amongst these members will ensure the achievement of best practices in the team (Kang & Lee, 2009:2). The TDM service not only involves measuring of levels, but history taking, the clinical condition or diseases of the patient, sample collection and analysis. The clinical pharmacist will advise on compliance, dose adjustment, adverse drug reactions and drug-drug interactions (Nwobodo, 2014:2).

It is also important to collect the correct data from the patients and to have guidelines and protocols in place. The most important data to collect from patients are age, weight, dose, dosing

interval, time of last dose, time sample drawn and in the case of aminoglycosides, renal function (Roberts *et al.*, 2012:27). The measurement of levels is only one aspect of TDM, because therapeutic ranges are not absolutes and for that reason, expert clinical interpretation is invaluable (Nwobodo, 2014:2).

Considering all these factors, it can be concluded that TDM adds value to the patient care and improves patient outcomes. Primary outcome measures include mortality, length of hospital stay and days to cure of infection. Secondary outcome measures include blood drug concentrations within the predefined range, associated with maximum efficacy and minimal chance of toxicity. A pro-active TDM strategy with optimised dosing regimens has proved to reduce mortality, decrease toxicity and be cost-effective (Touw *et al.*, 2009:86).

2.3 Therapeutic drug monitoring of the aminoglycosides

Therapeutic drug monitoring is normally performed on drugs, i.e. aminoglycosides, with a large inter-subject variation, small therapeutic index and an established concentration-effect relationship (toxicity). Appropriate drug therapy in critically ill patients is of extreme importance to ensure quality care. Data regarding guidelines for treating critically ill patients are limited and clinicians need to understand pharmacokinetics and pharmacodynamics in order to provide optimal care for these patients (Smith *et al.*, 2012:1327). Therapeutic drug monitoring of aminoglycosides with the goal to minimise toxicity (trough) and maximise effectiveness (peak) has become routine practice in most healthcare facilities. Once-daily dosing of the aminoglycosides decreases the oto- and nephrotoxicity, compared to multiple daily dosing, but TDM is still necessary to monitor toxicity and prevent inadequate plasma levels, which can lead to treatment failure (Touw *et al.*, 2009:72).

To utilise TDM to the maximum with aminoglycosides, it is important to understand the pharmacokinetics of the drug, the susceptibility of the causative pathogen and the variation in pharmacokinetics in certain populations such as critically ill and obese patients (Roberts *et al.*, 2012:27). In this section, the pharmacokinetics of the aminoglycosides, with factors influencing these parameters, will be discussed. The pharmacodynamics of the aminoglycosides are discussed in section 2.5.4.

2.3.1 Absorption

Absorption is defined as the rate and extent a medication leaves the site of administration and moves into the circulatory system (Smith *et al.*, 2012:1328). Bioavailability describes the fraction of the drug that reaches the systemic circulation after administration (Smith *et al.*, 2012:1328). Intravenous medications have a bioavailability of 100%. Aminoglycosides are poorly absorbed after oral administration, and are therefore mostly used in hospital settings rather than on an outpatient basis via the intravenous route (Xie *et al.*, 2011:28); less than 1% of an aminoglycoside dose is absorbed after either oral or rectal administration. Aminoglycosides can also be administered via the intraventricular route for the treatment of central nervous system infections caused by multidrug-resistant and nosocomial organisms (LeBras *et al.*, 2016:492). Aminoglycosides have limited access to the central nervous system due to the blood brain barrier and blood-cerebrospinal fluid barrier. Intraventricular administration of aminoglycosides allows drug delivery directly into the site of action (LeBras *et al.*, 2016:493).

Aminoglycosides enter the organ (kidney or cochlea where it is toxic) by active transport. Due to the saturable- and concentration-dependent nature of this type of transport, the extent of uptake is unchanged at higher concentrations (Croes *et al.*, 2012:91). When the drug is given at regular intervals or infused continuously, the blood concentration maintains at a constant level, causing constant uptake in the cells and accumulation in the organ occurs (Croes *et al.*, 2012:91). Aminoglycosides accumulate in lysosomes and during repetitive dosing (as seen with conventional or multiple daily dosing), lysosomes continue to take up drugs, causing an increase in their size. The loss of membrane integrity, disruption and release of proteolytic enzymes and aminoglycosides into the cell cytosol causes cell necrosis and renal failure (Beaucaire, 2000:355). For this reason, aminoglycosides when administered once daily compared to multiple daily doses are less toxic (Croes *et al.*, 2012:91).

2.3.2 Volume of distribution (Vd)

Volume of distribution (Vd), as described in paragraph 2.2.1, describes the relationship between the drug dose and the resulting serum concentration. An important consideration with the Vd is the ability of the drug to penetrate or distribute into tissues (Smith *et al.*, 2012:1329). Generally, hydrophilic drugs, like the aminoglycosides, will remain in the plasma, resulting in a small Vd. The total plasma volume will therefore have a direct effect on the aminoglycoside concentration (Smith *et al.*, 2012:1330). The Vd of gentamicin and amikacin is 0.26 L/kg (Smith *et al.*, 2012:1330). Lipophilic medication will often have a much larger Vd. Although, plasma protein binding may

affect Vd, aminoglycosides are less than 10% protein bound therefore albumin and α -1-acid-glycoprotein levels will not have a direct effect on aminoglycoside levels (Smith *et al.*, 2012:1331).

Certain important factors that can influence the Vd of aminoglycosides are addressed in the following paragraphs.

2.3.2.1 Body weight

Aminoglycosides are hydrophilic molecules, which do not distribute well into adipose tissue, therefore gentamicin and amikacin should be dosed according to the patient's ideal body weight (IBW). When total body weight is used to calculate dosages, it is clear that significant obesity leads to an overestimation of renal elimination capacity, as well as volume of distribution of water-soluble drugs (Hanberger *et al.*, 2013:169). It also seems that the drug can distribute into the extracellular water within the adipose tissue. The following equations are used to calculate ideal body weight (Hanberger *et al.*, 2013:169):

$$IBW \text{ (males)} = 50\text{kg} + 0.9 \text{ kg per cm height over } 152 \text{ cm}$$

$$IBW \text{ (females)} = 46 \text{ kg} + 0.9 \text{ kg per cm height over } 152 \text{ cm}$$

For patients who are obese, the adjusted body weight should be used and for those weighing less than the IBW, actual body weight should be used to calculate the dose.

Adjusted body weight (ABW) is calculated as follows (Hanberger *et al.*, 2013:169):

$$ABW = IBW + 0.4 \times (\text{current weight} - IBW)$$

Monitoring changes in serum concentrations, and the interpretation thereof, is of particular importance in obese patients. It is important to note that the situation is not identical in patients with high body weight due to increased muscle mass or patients with extensive oedema due to chronic or acute disease. In both these cases, the volume of distribution is increased and the initial dose should be based on actual body weight. For these patients, the indication for serum drug concentrations to determine follow-up doses is of critical importance (Hanberger *et al.*, 2013:169).

2.3.2.2 Fluid resuscitation

Fluid resuscitation in critically ill patients may lead to a sudden increase in total body water, resulting in a decreased serum concentration of aminoglycosides. A further complication in patients with vasodilatory shock is capillary leak syndrome. When these two complications occur concurrently, there is a potential for increased third spacing and an unpredictable change in interstitial volume (Smith *et al.*, 2012:1331). Dasta and Armstrong (1988:327) observed an increase in the Vd of tobramycin and gentamicin in critically ill surgical patients.

2.3.2.3 Burn and sepsis patients

Alterations in the volume of distribution can be very large in patients with unstable fluid balances (sepsis or burns) resulting in a marked reduction in the aminoglycoside concentrations if doses are left unchanged (Roberts *et al.*, 2012:29). The extracellular fluid compartment in patients with severe burns and sepsis can be extremely large, resulting in this high volume of distribution and prolonged half-life.

2.3.2.4 Ascites

The volume of distribution is markedly increased in patients with ascites. An expanded extracellular fluid volume attributed to the ascites fluid explains the increase in volume of distribution (Bauer, 2008:103).

2.3.2.5 Cystic fibrosis

The volume of distribution increases dramatically in patients with cystic fibrosis. The larger Vd is mainly due to the increased amount of lean body mass per kg bodyweight, although increased tissue binding may also account for part of this.

2.3.2.6 Dialysis

In renal failure, the patients commonly have fluid imbalances that may decrease (underhydration) or increase (overhydration) the volume of distribution.

Therapeutic drug monitoring, optimal loading doses and optimisation of drug dosing are necessary to ensure adequate serum concentration in patients on haemodialysis or peritoneal dialysis where the Vd is altered (Radigan *et al.*, 2010:327). In developing countries, nutritional deficiencies may negatively influence drug pharmacokinetics and require consideration in aminoglycoside use in critically ill patients (Nwobodo, 2014:2).

2.3.3 Clearance (Cl)

Total body clearance is the total of hepatic, renal and lung clearance. Drug metabolism can occur in the kidneys, liver, gastro-intestinal tract (GIT), lungs and brain. The liver is the predominant site for drug metabolism. The cytochrome P450 enzymes are responsible for the majority of Phase 1 metabolic activity and various enzyme inducers or inhibitors cause drug interactions by affecting these enzymes. Phase 2 metabolism occurs when large polar molecules are added to the parent drug through glucuronidation, sulfation or acetylation reactions, making the compound more water-soluble and enhancing urinary drug elimination (Smith *et al.*, 2012:1332).

The aminoglycosides are not metabolised in the liver and are practically eliminated (more than 90%) unchanged by the urine, primarily by glomerular filtration. Acute kidney injury (AKI) can occur in critically ill patients and can lead to a reduction in the glomerular filtration rate (GFR) and therefore a reduction in the clearance of drugs excreted in the urine. Aminoglycosides are freely filtered by the glomerulus, and renal clearance therefore is linearly proportional to the creatinine clearance (Smith *et al.*, 2012:1333). Aminoglycoside antibiotics accumulate in the proximal tubular cells of the kidney, decrease the ability of the kidney to concentrate urine and, ultimately, decrease glomerular filtration (Bauer, 2008:99).

The total daily dose of aminoglycosides needs to be adjusted in patients with impaired renal function. However, a dosage reduction could cause a sub-therapeutic peak dose and associated treatment failure. Prolonged dosing intervals, with a higher dose, could ensure that bactericidal peak levels are reached with every dose and lower trough levels should reduce the risk of toxicity (Hanberger *et al.*, 2013:169).

2.3.3.1 Renal function

Renal function is the most important factor to consider with the parameter clearance. Trough steady-state concentration (usually within 30 minutes before the next dose) must be below 1 µg/ml (also equals 1 mg/L) for gentamicin and below 5 µg/ml (also equals 5 mg/L) for amikacin to reduce the incidence of nephrotoxicity and ototoxicity (Hanberger *et al.*, 2013:170). An increase over time in the trough levels can be an indication of a decline in renal function.

Serum creatinine (a blood measurement) is an important indicator of renal health due to ease of measuring the by-product of muscle metabolism excreted unchanged by the kidneys. **Serum creatinine**, produced in the muscle, is the waste product of creatinine and creatinine phosphate. It is excreted entirely by the kidneys, just like aminoglycosides, and is therefore an indication of

the excretion rate of the drug. In patients with normal renal function the rate of creatinine production is equal to the rate it is excreted. Serum creatinine values are used to calculate creatinine clearance (Cl_{cr}). The normal values for estimated **glomerular filtration rate** in non-obese adults are >90 mL/min and <120 mL/min. When kidney function slows down it could have an impact on the creatinine level, which would rise. Increased levels of creatinine indicate a slowing down of the **glomerular filtration rate (GFR)**. The advantage of serum creatinine is that it is a once off measurement.

Although creatinine clearance is an indication of kidney function, normal serum creatinine levels alone should not be used to rule out decreased renal function. Serum creatinine will rise higher than normal values only once 50% of glomerular filtration has already been lost (Delanaye *et al.*, 2010:1). An estimated glomerular filtration rate is calculated by laboratories to give a more accurate indication of renal function (Levey *et al.*, 2009:606). There are a number of equations to determine eGFR in patients over 18 years of age, the Cockcroft and Gault method uses weight in the equation, and the Schwartz formula for children uses height, however, the problem arises with patients in ICU as weight is not always available. The Cockcroft and Gault equation (Rossiter *et al.*, 2014:15), can be calculated as follows:

$$eGFR (mL/min) = \frac{[(140 - age)(Weight)]}{SCr}$$

Where:

Age = patient's age in years

Weight = Patient's weight in kilogram (kg)

SCr = serum creatinine in $\mu\text{mol/L}$

* For females multiple the GFR by 0.85.

* Weight is measured in kg

** Conversion factor from mg/dl to $\mu\text{mol/L}$ = 88.42

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) equations do not require weight or height variables because the results are reported normalised to 1.73m² body surface area, and accepted average adult surface area (Levey *et al.*, 2009:606). Both these equations include variables for age, gender and race. The CKD-EPI equation is (Levey *et al.*, 2009:606):

$$eGFR = 141 * \min\left(\frac{S_{Cr}}{k, 1}\right)^{\alpha} * \max\left(\frac{S_{Cr}}{k, 1}\right)^{-1.209} * 0.993^{Age} * 1.018 \text{ (if female)} * 1.159 \text{ (if black)}$$

Where:

eGFR = (estimated glomerular filtration rate) = mL/min/1.73 m²

S_{Cr} = serum creatinine in mg/dL

K = 0.7 for females and 0.9 for males

α = -0.329 for females and -0.411 for males

Min indicates the minimum of S_{Cr}/k or 1

Max indicates the maximum of S_{Cr}/k of 1

The MDRD equation is calculated as follows:

$$GFR = 175 * S_{Cr}^{-1.154} * Age^{-0.203} * (0.742 \text{ if female}) * 1.212 \text{ (if African-American)}$$

Stage 3 chronic kidney disease is defined as an estimated GFR of lower than 60mL/min/1.73m² (Delanaye *et al.*, 2010:1). For patients with very abnormal basal creatinine production, creatinine clearance should be measured from 24-hour urine collections. This might be the case with patients of extreme muscle mass or body size (malnourished, obese, paraplegics, amputees), or those with unusual dietary protein intake (vegan or patients on creatine supplements) (Delanaye *et al.*, 2010:2).

Aminoglycosides are removed with relevant efficiency in both peritoneal dialysis and haemodialysis. In both cases, a dose of 1 to 2 mg/kg of aminoglycoside may be administered during the final hour of dialysis. In between dialysis sessions, the drug elimination will be extremely slow, and the half-life up to two days. Although this lower dose may not reach a peak high enough to be bactericidal, the exposure over time will be higher for patients on haemodialysis than for patients with normal renal function (Hanberger *et al.*, 2013:169). Most patients in an ICU

setting would receive continuous renal replacement therapy (CRRT) rather than intermittent haemodialysis. In these cases, normal doses of aminoglycosides can be administered, because the flow rate correlates with a normal clearance rate of 100 to 120 ml/min (Hanberger *et al.*, 2013:169).

The administration of aminoglycosides in patients with renal dysfunction is extremely difficult, because the balance between high peak levels required for maximum effect and low trough levels to prevent toxicity is not clear. In patients with renal dysfunction it is often required to extend the dosing interval, rather than to decrease individual doses.

2.3.4 The individualisation of aminoglycoside dosing

Therapeutic drug monitoring was previously only used as a tool to minimise toxicity of aminoglycosides, but is currently used increasingly to optimise antimicrobial dosing and outcomes of severe infections in the critically ill (Wong *et al.*, 2014:1). The South African guidelines do not make provision for the measurement of post-dose peak levels, except in obese patients (Department of Health, 2015:497), but the South African Medicines Formulary (SAMF) recommends peak levels (Rossiter, 2014:274). Measurement of the peak concentrations, however, ensures the sufficient maximum drug concentration above MIC of the pathogen and is important to determine clinical efficacy of treatment (Nezic *et al.*, 2014:830).

In patients with compromised pharmacokinetics, i.e. obesity, burn wounds, sepsis and renal failure, the pharmacokinetics and especially the volume of distribution can be unpredictable and constantly changing (Roberts *et al.*, 2012:32). In these patients, it becomes important to draw two levels in the elimination phase to calculate pharmacokinetic parameters for volume of distribution and clearance. In critically ill patients, variable volumes of intravenous fluids may be administered and body water and renal function are subject to change. All of the above will influence drug disposition. Methods used to individualise aminoglycoside therapy must: (1) accurately estimate volume of distribution (Vd) and clearance (Cl); (2) be able to predict changes in aminoglycosides pharmacokinetics that occur with disease states; (3) be easy to implement; (4) be cost-effective (Denaro & Ravenscroft, 1989:37).

A few methods to calculate the patient's own pharmacokinetic parameters to predict desirable peak and trough levels follows in subsequent paragraphs.

2.3.4.1 Linear Pharmacokinetic Method

The Linear Pharmacokinetic Method describes drug pharmacokinetics where the steady-state drug concentration changes proportionally with the dose (Bauer, 2008:134). Aminoglycosides follow linear, dose-proportional pharmacokinetics at steady state and therefore the following equation could be used (Bauer, 2008:134):

$$\frac{D_{new}}{Cp^{-ss}_{new}} = \frac{D_{old}}{Cp^{-ss}_{old}} \quad \text{or} \quad D_{new} = \frac{Cp^{-ss}_{new}}{Cp^{-ss}_{old}} * D_{old}$$

Where:

D = dose (new or old)

Cp^{-ss} = Steady-state level peak or trough

The disadvantage of this method is that the levels must be at steady state, and levels are constantly changing in critical ill patients.

2.3.4.2 Hartford nomogram

The Hartford nomogram is very popular with clinicians (quoted by Martin *et al.*, 2012:1). Concentrations are measured at the 6 to 14 hour time point and plotted on the nomogram, which indicates the minimum and maximum plasma concentrations expected at the given time. When concentrations lie outside of this range, a new dose will be calculated. These methods are popular due to ease of use and expert knowledge from a clinical pharmacist is not required for interpretation. This method can only be used in adult patients with normal renal function. Critically ill patients often have unpredictable renal function and kinetics, making this method unsuitable for use in this patient population (Martin *et al.*, 2012:1). Another limitation of this method is the accuracy; nomogram based recommendations often lead to sub-therapeutic treatment of infection, even in patients with normal renal function (Martin *et al.*, 2012:4).

2.3.4.3 Sawchuk-Zaske method

The least squares method, also known as the Sawchuk Zaske method or SZ method, was proposed in 1976 by Sawchuk and Zaske (Sawchuk & Zaske, 1976:183) and is used for steady state levels, and to calculate individual doses (Nezic *et al.*, 2014:835). This method is superior to nomogram methods and physician intuition for aminoglycoside therapy (Denaro & Ravenscroft, 1989:38). The usual procedure is to measure the aminoglycoside plasma concentration at two

time points — the first up to four hours after the end of the infusion and the second 6 to 14 hours after the end of the infusion (Nezic *et al.*, 2014:835). The rate constant for elimination (k_e) is calculated from a least squares regression. Volume of distribution and clearance are calculated from a one-compartment model equation and real maximum and minimum concentrations can be calculated (Denaro & Ravenscroft, 1989:38). The C_{max} can be calculated more accurately if the first time point is chosen early (one hour after infusion ended) and the second time point 6 to 8 hours after the first (Nezic *et al.*, 2014:835). If a drug is repeatedly administered to a patient, the drug and its metabolites will accumulate in the body. When the amount administered equals the amount being eliminated, a steady state or equilibrium is reached. The time required for this steady state depends on the half-life of the drug. After five half-lives, steady state will be achieved (Kang & Lee, 2009:6).

The steps to follow for the Sawchuk-Zaske method at steady state (Sawchuk & Zaske, 1976:183) are depicted in Figure 2-1.

Since the patient is at steady state, the measured trough level obtained before the dose was given can be extrapolated to the next dosing time to compute the elimination constant. Calculate k_e and $t_{1/2}$.

$$k_e = \ln \frac{C_{\max \text{ measured}}}{C_{\min \text{ measured}} \text{ time}}$$

$$t_{1/2} = \frac{0.693}{k_e}$$

a) Establish "true" peak. (The measured concentration is not always the peak concentration.)

$$C_0 = \frac{Ct}{e^{-kt}}$$

$$C_{\max} = \frac{C_{\max \text{ measured}}}{e^{-k(t_{\text{peak}} - T)}}$$

Where:

$Ct = C_{\max \text{ measured}}$

t = time between $C_{\max}$ measured and true peak

T = infusion time

t_{peak} = time from beginning of infusion until $C_{\max}$ measured

(b) Establish "true" trough. (The measured concentration is not always the trough concentration.)

$$C_{(t)} = C_0 e^{-kt}$$

$$C_{\min} = C_{\max} e^{(-k)(\tau - T)}$$

Where:

$C_0 = C_{\min \text{ measured}}$

t = time between $C_{\min}$ measured and true trough

T = infusion time

(c) Calculate V_d (Volume of distribution) USING TRUE PEAK AND TROUGH

$$V_d = \left(\frac{D(1 - e^{-kT})}{kT} \right) (C_{\max} - C_{\min} e^{-kT})$$

(d) Calculate dosing interval (τ)

$$\tau = - \left\{ \left[\frac{\ln \left(\frac{C_{\min}}{C_{\max}} \right)}{k} \right] \right\} + T$$

(e) Calculate ideal maintenance dose (MD) in mg

$$MD = T * k * V_d * C_{\max} \left[\frac{(1 - e^{-kT})}{(1 - e^{-k\tau})} \right]$$

(f) Calculate expected peak and trough levels on new dose

$$C_{\max} = MD \left(\frac{D(1 - e^{-kT})}{k} \right) * T * V_d (1 - e^{-k\tau})$$

$$C_{\min} = C_{\max} e^{(-k)(\tau - T)}$$

Figure 2-1: The steps to follow for the Sawchuk-Zaske method at steady state

2.3.4.4 Bayesian approach

By taking the trough level after approximately five half-lives, (aminoglycosides all have half-lives of about 2 to 4 hours) a more accurate actual trough level ($C_{\min}$) can be calculated using linear regression or Bayesian approaches (Nezic *et al.*, 2014:836; Wong *et al.*, 2014:4).

The Bayesian approach is currently considered the gold standard in TDM. The role of Bayesian estimation is to provide an individualised patient model, based on patient demographics such as renal function, as well as drug dosage and plasma drug concentration data (Touw *et al.*, 2009:83). With this method, initial parameter estimates are calculated by using pre-developed population PK models (clearance, volume of distribution, etc.). Subsequent plasma concentrations are used to individualise the PK model further (Martin *et al.*, 2012:2). Traditional methods require two blood samples, but recent Bayesian software requires only one plasma drug concentration; this saves time for nursing, pharmacy and laboratory staff and money for the patient (Martin *et al.*, 2012:2). One advantage of the Bayesian approach is that once the patient-specific PK model has been achieved, concentrations can be sampled at any time post dose (Martin *et al.*, 2012:4). The Bayesian approach, however, requires computational assistance and assumptions are usually based on pharmacokinetic data originating from different patient populations than the individual patients studied (Nezic *et al.*, 2014:836). When using the Bayesian approach, it is not necessary to wait for steady state condition before performing TDM (Touw *et al.*, 2009:84). In the case of aminoglycosides, with a well-described dose-dependent activity and toxicity, it is better to be aware of the pharmacokinetic parameters of a patient as soon as possible in order to adjust dosage and avoid treatment failure or toxicity. In clinical practice, where it is difficult to wait for two to three doses before taking blood samples, and the elimination half-life of the drug is known beforehand, the second sample can be drawn 12 to 15 hours after the first dose (Touw *et al.*, 2009:84).

2.3.5 Guidelines for the monitoring of aminoglycoside levels

The minimum inhibitory concentration (MIC) is an important component of the pharmacokinetics and pharmacodynamics of the aminoglycosides. When prescribing aminoglycosides, a target maximum drug concentration to minimum inhibitory concentration ($C_{\max}/MIC$) ratio of 8 to 10 mg/L is suggested (Roberts *et al.*, 2012:29). If a bacterial pathogen was isolated and the MIC is 0.5 mg/L, then a $C_{\max}$ of 5 mg/L is suggested to achieve the optimal PK/PD and increasing the likelihood of effective bactericidal effect (Roberts *et al.*, 2012:29). Hanberger *et al.* (2013:170) also suggest that post-dose concentrations (peak concentrations) should be monitored more

regularly in unstable patients to ensure that antimicrobial targets are reached, with a $C_{\max}/\text{MIC}$ ratio >10 mg/L.

Aminoglycosides are small, hydrophilic molecules with a volume of distribution quite similar to the extracellular volume and clearance proportional to eGFR, alterations in volume of distribution can be very large in certain conditions (sepsis or severe burn injuries). This will result in reduced peak concentrations and suboptimal treatment if TDM is not performed to monitor serum drug concentrations (Roberts *et al.*, 2012:29). With concentration dependant antibiotics, an increased volume of distribution will decrease the likelihood that the antibiotic will achieve the required $C_{\max}$ concentration. To ensure the required $C_{\max}$ target is achieved, the peak level can be measured 30 minutes to one hour after intravenous administration of the antibiotic (Roberts *et al.*, 2012:29).

Many hospitalised patients may have impaired renal function and standardised doses may cause toxicity due to reduced aminoglycoside clearance. In such patients, TDM should be performed to ensure the clearance is sufficient and trough levels taken just before administration of the next dose are low enough to prevent toxicity.

A summary of guidelines regarding the monitoring of aminoglycoside levels follows. In South Africa, the Sawchuk-Zaske method can be used by qualified clinical pharmacists to calculate and individualise aminoglycoside dosages. When clinical pharmacists are not available, dosages are simply adjusted according to trough levels without any calculations.

(1) The Department of Health (2015) guidelines in South Africa for haemodynamically stable patients:

- Serum creatinine levels should be monitored three times per week to determine renal function.
- Pre-dose amikacin or gentamicin levels (just before the next dose) should be done three times per week.
- Treatment should be discontinued if vestibular or cochlear symptoms develop.

For patients on once daily dosages:

A trough level of <5 mg/L for amikacin and <1 mg/L for gentamicin:

- In patients with normal renal function — it is not necessary to wait for amikacin trough levels before administration of the next dose —the level should be adjusted the following day if necessary.
- Patients with impaired renal function — wait for aminoglycoside trough levels before administration of the next dose. Only give the next dose if trough levels are <5 mg/L for amikacin and below 1 mg/L for gentamicin.
- In obese patients, it is important to measure the peak concentration immediately after completion of infusion (Department of Health, 2015:497).

(2) The South African Medicines Formulary (SAMF 12th Edition) (Rossiter, 2014:303):

For patients on once-daily dosages

A trough level of <1 mg/L for both amikacin and gentamicin. A peak level of >30 mg/L for amikacin and >8 mg/L for gentamicin.

- Trough levels: 48 to 72 hours after commencement of therapy, immediately prior to next dose.
- Peak levels: 1 hour after commencement of an IV infusion (given over 15 to 30 minutes) or 1 hour after IM or IV bolus injection. Adequate peak levels are necessary for effective bacterial kill. Confirm adequate dosing by obtaining a peak level after the second dose.

(3) Stanford Hospital and Clinics Aminoglycoside monitoring guidelines (Mui, 2017):

- For **once-daily gentamicin** dosing, the peak level should be taken 30 minutes after the end of the intravenous infusion of the second dose. The target peak concentration post dose should be 20 to 25 mg/L for a daily dose of 7 mg/kg and the target trough for this dose should be <1 to 2 mg/L.
- For an eight hourly dose of 1 mg/kg, the target peak should be 3 to 5 mg/L and the target trough level <1 mg/L.
- Amikacin administered at 5 to 7.5 mg/kg 12-hourly should have a target peak level of 20 to 35 mg/L and a target trough of <5 to 8 mg/L.

- When amikacin is administered at 15 mg/kg **once daily**, the target peak level is 35 to 50 mg/L and the target trough <4 to 8 mg/L.

(4) Swedish Reference Group for Antibiotics (Hanberger *et al.*, 2013:170):

- Trough samples should be taken less than one hour before the next dose is administered, and the peak dose should be measured 30 minutes after intravenous infusion or injection.
- There are no clear guidelines for measuring of trough levels in multiple daily dosing strategies.

(5) Healthcare NHS trust (NHS) guidelines (Surrey and Sussex Healthcare NHS Trust, 2015):

- A trough level <5 mg/L for amikacin and <1 mg/L for gentamicin for once-daily doses.
- Routine peak level monitoring not necessary.
- Pre-level (18-24 hours) before next dose. Clearly marked on the request from time of sample taken and last dose.
- Daily serum creatinine and urea is recommended for patients on IV dosages.
- If renal function is poor or deteriorates, await assay result and only give dose if trough level is lower than threshold.
- Monitor renal function carefully, 2 to 3 times a week, if courses are longer than 5 days.

The information on the different guidelines together with the pharmacokinetic knowledge is important to give a patient the best care regarding aminoglycoside therapy.

2.4 Background and history of different antibiotic classes, with emphasis on the aminoglycosides

The discovery of antibiotics is probably one of the most important moments in the history of medicine. Without antibiotics, the control of infectious diseases would be impossible and the morbidity and mortality due to infections would be much higher.

2.4.1 History of antibiotics and aminoglycosides

Traces of tetracyclines have been found in the skeletal remains of humans from ancient Sudanese Nubia, dating back to 350 to 550 AD (Aminov, 2010:1). This can be explained by exposure to tetracycline-containing materials in the diet of these people. The hypothesis is that grain stored in mud bins during the period 350 to 550 AD provided the medium for cultivation of *Streptomyces*, which is the bacteria nonsynthetic tetracyclines are produced from (Basset *et al.*, 1980:1533). Even though the Sudanese Nubian population did not take the tetracycline as therapeutic agents, it possibly had a protective antibacterial effect, which might explain the extremely low infectious disease rates found among this population (Basset *et al.*, 1980:1534).

The beginning of the ‘modern antibiotic era’ started with Paul Erlich (Aminov, 2010:2). Erlich believed that chemical compounds could be synthesised to “be able to exert their full action exclusively on the parasite harboured within the organism” (Aminov, 2010:2). In 1904, Erlich found a drug effective against syphilis (caused by the organism *Treponema palladium*). The drug, arsphenamine, marketed as Salvarsan®, was the most frequently prescribed drug until it was replaced by penicillin in the 1940s. Until this day, the mechanism of action of arsphenamine is unknown, but it was known to convert to an active form in the body where it was toxic for spirochetes and other parasites (Zaffiri *et al.*, 2012:68).

On September 3, 1928, Alexander Fleming noticed something quite unusual — the growth of *Staphylococcus aureus* on an old culture plate was inhibited by the presence of a contaminating blue mould (Zaffiri *et al.*, 2012:68). Fleming was not able to demonstrate clinical value of penicillin, due to the instability of the substance. Howard Florey and Ernest Chain published a paper in the early 1940s, describing purification of penicillin quantities sufficient for clinical testing. This led to mass production of penicillin and distribution from 1945 (Aminov, 2010:2).

In 1932 prontosil was discovered while two chemists, Klarer and Mietzich, synthesised sulfonamidochrysoidine as part of research to show the antibacterial effects of dye (Zaffiri *et al.*, 2012:68). It was later proven that prontosil was a prodrug that was metabolised to sulphanilamide, the first sulphonamide antibiotic to be discovered (Zaffiri *et al.*, 2012:68).

In July 1943, a fungus, identified as *Cephalosporium acremonium*, was isolated by Guiseppe Brotzo from sewer water in Cagliari, Italy. Brotzo found that this fungus inhibited the growth of certain gram-negative organisms (*Salmonella typhi*, *Yersinia pestis*, *Vibrio cholera*) and *Staphylococcus aureus* (Bo, 2000:6). After many years of work, the first semi-synthetic

cephalosporin released for clinical work in 1964, was cephalotin. The drug was marketed by Eli Lilly and Company, and available only for parenteral use (Zaffiri *et al.*, 2012:68).

Streptomycin was the first aminoglycoside to be isolated, in 1944, and the first antibiotic against tuberculosis (Vakulenko & Mobashery, 2003:430; Zaffiri *et al.*, 2012:68). Over the next 20 years, other aminoglycosides were isolated from soil bacteria. Most aminoglycosides are naturally occurring substances that are produced by actinomycetes of either the genus *Streptomyces* or *Micromonospora* (Vakulenko & Mobashery, 2003:430).

During the late 1980s, the molecular target of aminoglycosides was identified as the 16S ribonucleic acid (RNA) component of the bacterial ribosome. High-resolution structures have only become available for aminoglycosides and their specific ribosomal RNA targets during the late 1990s and early 2000s (Hermann, 2005:357). Gentamicin has been known as the 'work horse' of the aminoglycosides and has been used for the treatment of serious gram-negative infections since the early 1960s (Antibiotic Expert Groups, 2014). Gentamicin is also on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic healthcare system (WHO, 2017).

In 1948 the first macrolide, erythromycin, was discovered and it was introduced to the market in 1951 (Lewis, 2013:371).

In the 1950s, there were few options to treat penicillin-resistant staphylococcal infections and Eli Lilly initiated a programme to discover antibiotics against these pathogens. In 1952, vancomycin was produced from a soil sample found in Borneo. This drug was the first glycopeptide to be discovered (Levine, 2006:42).

Rifampicin (also known as rifampin) was discovered in 1957 in Milan, Italy, and introduced for the parenteral treatment of infections due to gram-positive organisms of the biliary tract (Sensi, 1983:402).

Five years after the discovery of rifampicin, trimethoprim was discovered in 1961 (Lewis, 2013:371).

Quinolones and fucidic acid were also discovered in 1961. Ciprofloxacin was the first of the quinolones to be introduced to the market in 1968 (Lewis, 2013:371).

The late 1960s marked the emergence of beta-lactamases, threatening the use of penicillin. Thienamycin, the first 'carbapenem,' is still considered the parent drug for carbapenems (Papp-

Wallace *et al.*, 2011:4943). The first true carbapenem, imipenem, was discovered in 1976, and in 1985 was the first carbapenem available for use against cephalosporin-resistant gram-positive and gram-negative organisms (Papp-Wallace *et al.*, 2011:4944).

Linezolid, an oxazolidinone, was discovered in 1955, but was not immediately available for medicinal use. The emergence of new antibiotic-resistant gram-positive organisms created a substantial need for new antimicrobial agents. This led to the research programme that eventually, in 2000, led to the marketing of linezolid (Ford *et al.*, 2001:181).

Daptomycin, a lipopeptide antibiotic, was discovered in 1986 and marketing started in 2003. Even though this drug was not available to the market then, resistance was already present in 1987 (Lewis, 2013:371).

The years between the 1950s and 1980s are referred to as the golden era of antibiotic discovery, with no novel antibiotic classes discovered since then (Zaffiri *et al.*, 2012:68).

2.4.2 Mechanism of action, spectrum and uses of different antibiotic classes

The pharmacodynamics of antibiotics can be described as either concentration-dependent or time-dependent interactions of the antibiotic against pathogens in the host (Barger *et al.*, 2003:896):

1. Concentration-dependent antibiotics (including aminoglycosides, amphotericin B, daptomycin, metronidazole and quinolones): How much higher the antibiotic concentration exceeds the minimum inhibitory concentration (MIC) of the organism, the more effective the antimicrobial activity will be, irrespective and independent of the time the concentration exceeds the MIC. A ratio of concentration to MIC of 10:1 is required for this type of action. A dose regimen should therefore be chosen to ensure tissue or serum concentration of at least 10 times the MIC of the organism. Failure to reach sufficient levels will result in clinical and bacteriological failure and is likely to induce bacterial resistance. Due to this action, it is advised that aminoglycosides be dosed at a once-a-day dosing to obtain high peak concentration levels (Cotta *et al.*, 2015:567). For concentration-dependent antibiotics, the pharmacological indices AUC/MIC and C_{max}/MIC are used (Barger *et al.*, 2003:893) (refer to paragraph 2.4.5 – Pharmacodynamics of aminoglycosides).
2. Time-dependent antibiotics (including penicillins, cephalosporins, carbapenems and macrolides): The time the antibiotic exceeds the MIC is crucial in predicting clinical outcome and cure. Concentrations of time-dependent antibiotics should exceed the MIC at least

50% of the dosing interval. In resistant bacterial infections, it is advised that the time above MIC is increased even more. The role of continuous and extended infusions may play an important role here to ensure the maximum time above MIC in critically ill patients with resistant gram-negative organisms. More frequent dosing of time-dependent antibiotics is required to ensure maximum effectiveness (Cotta et al., 2015:565). For time-dependent antibiotics, the pharmacological indices T_{max}/MIC is used (Barger et al., 2003:893) (refer to paragraph 2.4.5 – Pharmacodynamics of aminoglycosides).

Sulphonamides are bacteriostatic antimetabolites, which inhibit the folate synthesis pathway. They are para-aminobenzoic acid (PABA) analogues and are competitive dihydropteroatesynthetase (DHPS) inhibitors. By blocking folate synthesis, deoxyribonucleic acid (DNA), RNA and protein synthesis are inhibited (MacDougall & Chambers, 2011:1464).

Sulphonamides have a broad spectrum of activity against both gram-positive and gram-negative bacteria (Zaffiri *et al.*, 2012:68).

Penicillins contain a beta-lactam ring structure that is essential for antimicrobial activity. They inhibit the formation of peptidoglycan crosslink in the cell wall of bacteria by attaching to bacterial enzymes called penicillin-binding proteins (PBPs). This leads to cell wall autolysis (Zaffiri *et al.*, 2012:69).

The spectrum of activity of penicillins varies from narrow-spectrum (natural penicillin), very-narrow spectrum (penicillinase resistant penicillins), extended-spectrum (aminopenicillins) and broad-spectrum (anti-pseudomonal penicillins) (Zaffiri *et al.*, 2012:69).

Cephalosporins are bactericidal, as with all beta-lactam antibiotics. The mechanism of action involves interference with bacterial cell wall synthesis by inhibiting transpeptidase enzyme in the bacterial cell wall.

Cephalosporins are classified into generations (first to fifth generation) and grouped according to *in vitro* activity. Progressive generations exhibit more gram-negative activity than first and second-generation cephalosporins, but often with decreased gram-positive activity (MacDougall & Chambers, 2011:1493). The exception is ceftaroline, which shows activity against both gram-positive organisms (including MRSA) and gram-negative organisms (Laudano, 2011:iii11).

Macrolides exhibit broad-spectrum antibacterial activity by binding reversibly to the 50S ribosomal subunit of bacteria and inhibits protein synthesis (Lewis, 2013:371).

Since introduction to the market in 1958, glycopeptides have been widely used for their rapid antibacterial activity against gram-positive organisms, despite adverse effects such as nephrotoxicity (Lewis, 2013:371). Vancomycin is used in hospitals for the treatment of nosocomial infections caused by MRSA. Vancomycin acts by inhibiting cell wall biosynthesis (Lewis, 2013:371).

Rifampicin acts by binding to RNA polymerase β -subunit (Lewis, 2013:371). It is used in South Africa as part of the first line of treatment against tuberculosis (TB) (Department of Health, 2015:240) and also to treat infections where prosthetic material is involved, as it has the ability to penetrate biofilm efficiently (Department of Health, 2015:104).

Ciprofloxacin, the first quinolone to be introduced to the market, inhibits DNA synthesis and has broad-spectrum activity (Lewis, 2013:371). Quinolones mainly inhibit topoisomerase IV in gram-positive bacteria and DNA gyrase in gram-negative microbes (MacDougall & Chambers, 2011:1470). It is used widely in South Africa to treat community acquired urinary tract infections (UTIs), but resistance develops rapidly and this drug is one of those that can cause Methicillin-resistant *Staphylococcus aureus* (MRSA) infections, as well as *Clostridium difficile* associated diarrhoea (Stewardson *et al.*, 2014). Fucidic acid has activity against gram-positive organisms and used often in topical preparations to treat wound infections (Lewis, 2013:371).

Carbapenems are also a class of beta-lactams, which bind to penicillin-binding proteins (PBPs) to inhibit the cell wall of bacteria (Papp-Wallace *et al.*, 2011:4946). It exhibits broad-spectrum activity against both gram-positive and gram-negative organisms. The key to efficacy of the carbapenems is the fact they can bind to multiple different PBPs (Papp-Wallace *et al.*, 2011:4946).

Linezolid, the only oxazolidinone available in South Africa, has activity against gram-positive bacteria and acts by binding of 50S ribosomal subunit. This is one of the agents used to treat MRSA infections (Lewis, 2013:371). Linezolid is bacteriostatic, which means the drug inhibits the growth of bacteria, but does not rapidly kill bacterial cells (Lewis, 2013:371).

Daptomycin, a lipopeptide, depolarises the cell membrane and is bactericidal against gram-positive bacteria. The drug is inactivated due to surfactant present in the lungs and can therefore not be used to treat infections of the respiratory tract (Lewis, 2013:371).

2.5 Aminoglycosides

2.5.1 Structure and chemical properties

Understanding the structure of the aminoglycosides is important to understand its chemical properties. Aminoglycosides are multi-functional hydrophilic sugars and possess several amino and hydroxyl functionalities (Kotra *et al.*, 2000:3250). The molecules in the aminoglycoside group have a backbone structure consisting of an aminocyclitol ring saturated with amine and hydroxyl substitutions. In most aminoglycosides, the aminocyclitol component is streptamine or 2-deoxystreptamine. The only exception to this rule is streptomycin, possessing a streptidine molecule. The aminocyclitol nucleus is connected through glycosidic linkages to various amino sugars (Jana & Deb, 2006:141). Based on the position of their glycoside linkages, aminoglycosides can be divided into three structural groups:

- 4,6-disubstituted 2-deoxystreptamines (gentamicin, tobramycin, amikacin and netilmicin);
- the 4,5-disubstituted 2-deoxystreptamines (neomycin and paromomycin);
- others (streptomycin and spectinomycin) (Shakil *et al.*, 2008:5).

Aminoglycosides are basic, strongly polar compounds that are cationic (positively charged). Their cationic structure, depending on the number of amino groups on their distribution within the molecule, plays an important role in the toxicity, mostly affecting renal and hearing tissues where the molecules accumulate (Lopez-Novoa *et al.*, 2011:33). These molecules are highly water-soluble, but relatively insoluble in lipids, with enhanced antimicrobial activity in alkaline environments, and reduced activity in acidic environments. This means that aminoglycosides are poorly absorbed from the gastro-intestinal tract, cross the blood-brain barrier poorly and are not very effective to treat infections in the lungs. The positive charge of the molecules adds to the nephrotoxicity, ototoxicity and neuromuscular blockade (Shakil *et al.*, 2008:6).

2.5.2 Mechanism of action

Aminoglycosides bind electrostatically to the negatively charged bacterial cell membranes of gram-negative organisms. This is a passive, non-energy dependent process (Shakil *et al.*, 2008:7). Aminoglycosides diffuse through outer membrane porin channels and move into the periplasmic space, whereafter the molecules are transported via active, oxygen and energy dependent transport into the cell cytosol (Mandell *et al.*, 2010:592). This transport requires metabolic energy from the electron transport system and is a rate-limiting step. The necessity for

oxygen in the transport of aminoglycosides into the cell cytosol explains why aminoglycosides are only active in aerobic environments. This energy-dependent phase is also inhibited by a reduction in pH, hyperosmolality and divalent cations (Shakil *et al.*, 2008:7). After being transported into the cell, aminoglycosides bind to the aminoacyl site of 16S ribosomal DNA within the 30S ribosomal subunit, where translation is prevented and cell death follows (Shakil *et al.*, 2008:7). Secondary effects aminoglycosides have on bacterial cells include metabolic perturbations, such as translation, membrane damage, altered cellular ionic concentrations and disturbances in DNA and RNA synthesis (Shakil *et al.*, 2008:7).

Aminoglycosides are concentration-dependent bactericidal antibiotics and it is important to achieve peak plasma levels of 10 to 12 times the MIC ratio (C_{max}/MIC ratio of 10-12) to prevent bacterial resistance (Avent *et al.*, 2011:444). Sub-MIC levels at trough levels may be allowed, because these drugs exhibit a post-antibiotic effect against both gram-positive and gram-negative organisms (Pea & Viale, 2006:1765). The post-antibiotic effect means that aminoglycosides demonstrate a persistent suppression of bacterial growth after short exposure to the drug (Avent *et al.*, 2011:444). Persistent antimicrobial growth suppression for up to 7.5 hours after the drug has been cleared has been described for both gram-negative bacilli and *Staphylococcus aureus*, but not for other gram-positive cocci (Durante-Mangoni *et al.*, 2009:201). For this reason, it makes sense to administer once-daily doses to achieve the required peak plasma level and minimise possible adverse effects or toxicities.

Aminoglycosides are chemically stable, have a rapid bactericidal effect, act synergistically with beta-lactam antibiotics, show little bacterial resistance and are relatively low in cost. This makes this group of antibiotics a useful choice against bacteria resistant to other antibiotics (Lopez-Novoa *et al.*, 2011:33).

2.5.3 Antimicrobial spectrum and indications for aminoglycosides

Aminoglycosides have a broad antimicrobial spectrum and is active against most gram-negative bacteria, including Enterobacteriaceae, *Pseudomonas* spp., *Acinetobacter* spp., *E. coli*, *Klebsiella*, *Serratia*, *Salmonella* and *Shigella* species. It also provides activity against most strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. They are inactive against anaerobes, which are unable to take up the aminoglycosides. Most strains of enterococci are resistant against aminoglycosides as monotherapy, but when used in combination with penicillins, may be effective treatment for enterococcal endocarditis (Drew, 2014). The synergistic effect of the penicillins is due to the disruption of the cell wall synthesis. The disruption of the bacterial cell

wall increases the concentration of aminoglycoside within the cell and leads to an increased bactericidal effect of aminoglycosides (Drew, 2014).

With the introduction of newer antimicrobial agents with a more favourable safety profile (anti-pseudomonal penicillins, third generation cephalosporins and carbapenems), there has been a shift away from prolonged administration of aminoglycosides due to the improved safety and pharmacokinetic parameters of these agents. According to the latest edition of the Therapeutic Guidelines for antibiotics (Antibiotic Expert Groups, 2014), there is still an important place for aminoglycoside treatment as empirical and directed therapy.

The mortality rate from gram-negative bloodstream infections in children was more than double that of malaria. Aminoglycosides, especially gentamicin, retains activity against many gram-negative bacteria and are often lifesaving medications (Ashwlayan & Singh, 2016:283). With the emergence of multidrug-resistant bacteria in urinary tract infections, tuberculosis and visceral leishmaniasis, aminoglycosides once again have an important role to play especially in hospital-acquired infections (Xie *et al.*, 2011:29). Aminoglycosides have recently been re-evaluated for use with colistin, a polymyxin antibiotic. In serious infections caused by extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae (*Klebsiella*, *E coli* and *Acinetobacter* spp.) and *Pseudomonas aeruginosa*, including healthcare associated urinary tract infections, aminoglycosides may be used in combination with colistin to treat the infections (Durante-Mangoni *et al.*, 2009:202). In addition, they are cost-effective, which is an important consideration in the South African environment.

Aminoglycosides have a rapid bactericidal action and the ability to act synergistically with other drugs, making them a good choice of drug for serious nosocomial infections (Shakil *et al.*, 2008:6). With a few exceptions, the discovery rate of new antibiotics is declining, while antimicrobial resistance is increasing. Drug combinations offer potential strategies to control the evolution of antimicrobial resistance (Bollenbach, 2015:1).

Antibiotic synergism occurs when two or more antibiotics exert an inhibitory effect on an organism that is greater than the additive effects of the individual antibiotics. The use of initial combination therapy may broaden the empiric coverage, exploit synergy observed *in vitro* between two antimicrobials thus improving outcome, and prevent the emergence of antimicrobial resistance (Tamma *et al.*, 2012:451). The benefits of synergistic combinations include lower toxicity and more rapid killing of bacteria (Tamma *et al.*, 2012:452). Synergy between two antimicrobials is defined as a greater-than-2-log increase in bactericidal activity compared to bactericidal activity of each individual agent (Tamma *et al.*, 2012:452).

Beta-lactam antibiotics act by inhibiting the bacterial cell wall synthesis. This causes enhanced uptake of aminoglycosides into the cell, where the aminoglycoside acts on the ribosomal subunit. Proven synergism exists with ampicillin against enterococci where ampicillin enhances the uptake of aminoglycosides by disrupting the peptidoglycan cell wall, which is normally impermeable to aminoglycosides (Durante-Mangoni *et al.*, 2009:203). Therefore, synergism between the two classes is used clinically to treat enterococcal endocarditis (ampicillin and gentamicin) and resistant pseudomonal infections (beta-lactam and aminoglycoside of choice) (Department of Health, 2015:103).

It is important to note that not all combination strategies are clinically acceptable. There exists antagonism between aminoglycosides and glycopeptides (vancomycin and teicoplanin) in MRSA infections due to the induction of aminoglycoside acetyltransferase and phosphotransferase enzymes (Durante-Mangoni *et al.*, 2009:203).

Amikacin may be the drug of choice in severe neonatal sepsis, and other aminoglycosides are often used to treat complicated urinary tract infections, intra-abdominal infections and osteomyelitis. There are also indications that the spectrum against the gram-negative aerobes is wider than with gentamicin (Shakil *et al.*, 2008:11).

Gentamicin is the aminoglycoside used most often because of the reliable activity against most gram-negative aerobes (Rougier *et al.*, 2004:156). Tobramycin may be the best choice of aminoglycoside against *Pseudomonas aeruginosa*, because it exhibits greater *in vitro* activity than other aminoglycosides and amikacin is particularly effective against organisms that show resistance to other aminoglycosides due to its chemical structure making it less susceptible to inactivating enzymes (Peloquin *et al.*, 2004:1543).

According to Durante-Mangoni and colleagues, aminoglycosides reach very high concentrations in the urinary tract (25 to 100-fold that of serum) and approximately 99% of the administered dose is excreted unchanged in the urine. Therefore, aminoglycosides are very potent antibiotics in the treatment of complicated urinary tract infections, even infections caused by organisms that are more resistant. In an era where ESBL-producing organisms are often the cause of UTIs, aminoglycosides could prove significant value in treatment (Durante-Mangoni *et al.*, 2009:203).

Aminoglycosides are no longer preferred antimicrobials against lower respiratory tract infections, because of three major reasons: (1) the drugs do not reach high concentrations in the alveolar lining of the lungs; (2) aminoglycosides are inactivated by the acidic pH of inflamed lung tissue;

(3) there is a high risk of nephrotoxicity in already critically ill patients (Durante-Mangoni *et al.*, 2009:203).

2.5.4 Pharmacodynamics

Drug pharmacodynamics is defined as the study of the biochemical and physiological effects of drugs and their mechanism of action, including the interactions of pharmacologically active molecules with their target site of action (Avent *et al.*, 2011:444). It is, therefore, the relationship between the drug concentration and its biological effect. One of the most important goals of pharmacodynamics is to determine the proper dose to get the desired effect, while keeping the adverse effects or toxicity to a minimum (Avent *et al.*, 2011:444).

The pharmacodynamic parameter used to rationalise antibiotic treatment, is mainly the MIC of the organism. For concentration-dependent antibiotics, the AUC/MIC and $C_{\max}/\text{MIC}$ are used. AUC/MIC, which is defined as the area under the concentration-time curve, is divided by the MIC; the $C_{\max}/\text{MIC}$ is the peak concentration divided by the MIC. This index is used to predict or describe the antibacterial effect of concentration dependent antibiotics. For time-dependent antibiotics, the parameter $T_{>\text{MIC}}$ is mainly used. This parameter is the cumulative percentage of time over a 24-hour period that the drug concentration exceeds the MIC. It was suggested in 1971 that the efficacy of an antibiotic in a particular infection could be defined in terms of the *in vivo* drug concentration and the antibacterial activity of a substance determined by the MIC (Barger *et al.*, 2003:893).

2.5.5 Toxicity

The most significant clinical toxicities of aminoglycosides include nephrotoxicity, ototoxicity and less often, neuromuscular toxicity (Burton *et al.*, 2007:290).

Hypersensitivity reactions, nausea and vomiting, headache, tremor, arthralgia and hypotension have also been reported (Durante-Mangoni *et al.*, 2009:202).

Patient factors that may influence the risk of toxicity include pre-existing diseases, severity of illness, concomitant drug administration and genetic factors. Prolonged aminoglycoside therapy and high doses are independent risk factors for the development of toxicity (Burton *et al.*, 2007:290).

The Swedish Reference Group for Antibiotics (SRGA) (Hanberger *et al.*, 2013:162), suggest that aminoglycosides are contraindicated in the following cases:

- Chronic renal impairment or renal failure.
- Known hearing loss.
- Genetic predisposition to aminoglycoside-induced hearing loss.
- Concomitant treatment with other nephrotoxic or ototoxic drugs.

According to the SAMF (Rossiter *et al.*, 2014:303), aminoglycosides are contraindicated in patients with myasthenia gravis. Even though not listed as contraindications, caution should be taken in patients with renal insufficiency and drug interactions are listed with other ototoxic and nephrotoxic drugs, as well as general anaesthetics or neuromuscular blocking agents. The SAMF also states that aminoglycosides should be used with caution in elderly patients (Rossiter *et al.*, 2014:303).

The mechanism of toxicity, clinical findings, laboratory test changes and ways to prevent toxicity, is discussed in subsequent paragraphs.

2.5.5.1 Nephrotoxicity

Aminoglycoside nephrotoxicity manifests as reversible, non-oliguric renal failure, with a slow increase in serum creatinine, urea and other metabolic products and a hypo-osmolar urine output after several days of therapy (Lopez-Novoa *et al.*, 2011:34). Clinical findings may also include proteins, enzymes, amino-acids and glucose in the urine and electrolyte alterations: hypercalciuria, hypermagnesuria, hypocalcemia and hypo-magnesemia (Banday *et al.*, 2008:450). Nephrotoxicity was reported in between 6% and 10% of patients treated with aminoglycosides and there is conflicting reports regarding the difference in nephrotoxicity between gentamicin, amikacin and tobramycin in the literature (Banday *et al.*, 2008:452). Nephrotoxicity due to aminoglycoside therapy is unlikely to occur before 3 to 5 days of therapy with proper dosing. That is why TDM and blood level monitoring is so important. Keeping the peak and trough levels in the suggested ranges, decreases the likelihood of these adverse effects. It is also important to remember that duration of therapy exceeding 14 days, large total cumulative doses, and concurrent therapy with other nephrotoxic drugs, such as vancomycin, can predispose patients to these effects (Bauer, 2006:99).

Nephrotoxicity caused by aminoglycosides can be divided into three groups — tubular effects, glomerular effects and vascular effects (Lopez-Novoa *et al.*, 2011:34).

The tubular toxicity of aminoglycosides presents as death of tubular epithelial cells, associated with a very important inflammatory component and the nonlethal, functional alteration of key cellular components involved in water and solute transport (Lopez-Novoa *et al.*, 2011:34). Aminoglycosides, in their cationic form, bind to the brush-border membrane of tubular cells via the phospholipids. Thereafter aminoglycosides are transferred to megalin, a transmembrane protein, where they become internalised in endosomes. Megalin is expressed in renal tubular epithelium, as well as epithelial cells in the inner ear and retina and is probably responsible for the selective uptake and toxicity of aminoglycosides by these cells. Once aminoglycosides are inside the tubules, tubular cell death occurs due to apoptosis (El Mouedden *et al.*, 2000:665), as well as focal necrosis of the tubular epithelium (Edwards *et al.*, 2007:2). After a few days of treatment with therapeutic doses of aminoglycosides, changes in lysosomes of the proximal tubular cells occur, which is consistent with the accumulation of polar lipids. Other signs of tubular dysfunctions follow soon after that (decreased protein reabsorption, wasting of potassium, magnesium, calcium and glucose, phospholipiduria and excretion of cast cells). Fanconi's syndrome or a Bartter's-like syndrome has been observed on rare occasions (Lopez-Novoa *et al.*, 2011:35).

Tubular necrosis is most certainly the primary cause of the functional toxicity (Lopez-Novoa *et al.*, 2011:36).

The glomerulus is the first part of the nephron to be exposed to chemical substances (Lopez-Novoa *et al.*, 2011:38). Gentamicin alters filtration by various mechanisms: reduction in glomerular filtration rate and the ultrafiltration coefficient, stimulation of mesangial proliferation, slight morphological changes in the glomerulus (only in high doses gentamicin) and the loss of glomerular filtration barrier selectivity (Lopez-Novoa *et al.*, 2011:38).

Vascular effects include activation of the renin-angiotensin system causing an induced reduction in renal blood flow. This reduced renal blood flow is a consequence of an increase in the resistance of the renal vascular bed rather than of a lower perfusion pressure (Lopez-Novoa *et al.*, 2011:37). This very well explains the aggravating effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on the nephrotoxicity of aminoglycosides, since the NSAIDs inhibit the production of vasodilatory prostaglandin PGE₂. A decrease in renal blood flow will cause a decrease in glomerular filtration rate (GFR) and sensitisation of tubule cells leading to cell death by reduction of oxygen and ATP availability (Lopez-Novoa *et al.*, 2011:37).

The kidney has a unique capacity to compensate for tubular damage and ongoing cell death processes may remain undetected for a long time. Damaged cells regenerate, and these regenerating cells are less differentiated and susceptible to aminoglycoside damage (Taber & Mueller, 2006:358).

The prevention of aminoglycoside-induced nephrotoxicity is an important therapeutic objective that will improve the clinical utility of these drugs significantly. In many cases, nephrotoxicity is the most important limitation to the intensity and dosage of the therapeutic regimen and it may lead to serious health complications, and even death. About 25 of the 100 most used drugs in Intensive Care Units are potentially nephrotoxic, and this drug-induced nephrotoxicity is responsible for 10 to 20% of acute renal failure in these units (Taber & Mueller, 2006:358). Extreme caution should be taken when administering aminoglycosides with other nephrotoxic drugs, such as amphotericin B, vancomycin, colistin, diuretics, nonsteroidal anti-inflammatory drugs, cisplatin, cyclosporine and iodinated contrast agents (Hanberger *et al.*, 2013:166).

Besides drug monitoring, maintaining sufficient hydration and applying dialysis where clinically appropriate, there is little clinicians can do to prevent drug nephrotoxicity. In patients who were monitored and where sufficient hydration therapy was given, the renal function usually returns to normal after discontinuation of the drug (Xie *et al.*, 2011:35).

Studies are being performed to determine the usefulness of renoprotective drugs to minimise the toxic effects of aminoglycosides. For example, statins have been shown to reduce accumulation of gentamicin in the tubule cells and therefore reduce renal damage (Antoine *et al.*, 2010:647). Anti-oxidants may also alleviate aminoglycoside nephrotoxicity, but there is no clear evidence to support the administration of antioxidants to prevent nephrotoxicity (Lopez-Novoa *et al.*, 2011:41). Nephrotoxicity caused by aminoglycosides may be difficult to distinguish from acute kidney injury caused by generalised infection or sepsis, however measuring aminoglycoside plasma concentrations may assist in distinguishing between the two (Kang & Lee, 2009:3).

2.5.5.2 Ototoxicity

Aminoglycosides can cause permanent, irreversible vestibular and auditory ototoxicity. In the treatment of acute infections, which may last for five to seven days, hearing loss induced by aminoglycosides may occur in up to 20% of patients and balance might be affected in about 15% (Xie *et al.*, 2011:30). Exceeding the recommended peak concentrations for the aminoglycosides leads to an increase risk of ototoxicity. Ototoxicity may be overlooked because it may only start at the end of aminoglycoside treatment and develop slowly thereafter. Aminoglycosides may

enter the inner ear fluids via the bloodstream and cause intracellular morphological and biochemical changes of the cochlear outer hair cells (OHC) (Xie *et al.*, 2011:30). Aminoglycosides form an oxidative compound after binding with iron. This contributes to the formation of free radicals, which may cause tissue damage due to oxidative activities with proteins and other targets (Xie *et al.*, 2011:31). Ototoxicity caused by aminoglycosides, present initially as an irreversible bilateral sensorineural hearing loss beginning at loss of high frequencies (>4000 Hz); this is because of damage to the OHCs and supporting cells at the basal end of the cochlea, where high frequencies are transduced. With prolonged use, damage may progress to the apical cochlear portion responsible for low frequencies and further ototoxicity may occur (Petersen & Rogers, 2015:1). Vertigo, nausea, vomiting, nystagmus and ataxia (vestibulotoxicity) may also occur (Xie *et al.*, 2011:30). Often, the first sign of auditory ototoxicity is tinnitus. Vestibular ototoxicity results in the loss of balance. Unlike with nephrotoxicity, there is a difference in severity of potential ototoxicity between different aminoglycosides. Neomycin is considered the most highly toxic, followed by gentamicin, kanamycin and tobramycin, whereas amikacin and netilmicin are considered the least ototoxic (Xie *et al.*, 2011:30). Elevated peak and trough levels, increased age, duration of therapy, concurrent treatment with loop diuretics or vancomycin, underlying diseases and previous aminoglycoside treatment are risk factors increasing the incidence of ototoxicity (Xie *et al.*, 2011:30).

Due to the permanent nature of hearing loss, it is recommended that all patients receiving aminoglycosides be screened and that hearing testing be done to detect early onset of cochleotoxicity before it affects the patient's ability to communicate. It is not sufficient to rely on patients to report symptoms of hearing loss, as that would be too late and too much damage would have been done (Petersen & Rogers, 2015:3).

2.5.5.3 Neuromuscular blockade

Neuromuscular blockade and apnoea is an unusual toxic reaction attributed to the aminoglycosides. Neuromuscular toxicity is not as common as nephrotoxicity and ototoxicity, but can occur in patients who receive concomitant anaesthetics, have pre-existing renal failure or neuromuscular disease. Patients with myasthenia gravis are particularly susceptible to neuromuscular blockade after aminoglycoside administration (MacDougall & Chambers, 2011:1514). Neuromuscular blockade generally occurs after high doses of aminoglycosides administered by the intrapleural or intraperitoneal route, but the reaction can follow intravenous or intramuscular administration (MacDougall & Chambers, 2011:1514). The action is the result of inhibition of prejunctional acetylcholine release, and reduced postsynaptic sensitivity. High

doses of aminoglycosides may cause neuromuscular blockade by competing with serine, but not with calcium chloride, in terms of blocking the neuromuscular junction (Xie *et al.*, 2011:34). The mechanism of toxicity is the inhibition of pre-junctional release of acetylcholine and reduction of postsynaptic sensitivity to the transmitter. As calcium can overcome this effect, the intravenous administration of a calcium salt is the preferred treatment for aminoglycoside-induced neuromuscular blockade (MacDougall & Chambers, 2011:1514). Inhibitors of acetylcholinesterase (neostigmine) have also been used with varying degrees of success (MacDougall & Chambers, 2011:1514). Careful monitoring is essential in patients receiving aminoglycosides and neuromuscular blockers during peri-operative periods (Avent *et al.*, 2011:443).

Table 2-1 gives a summary of the toxicities of the four most commonly prescribed aminoglycosides (Ashwlayan & Singh, 2016:286).

Table 2-1: Comparative toxicities of aminoglycosides

Aminoglycoside	Nephro	Cochlear	Vestibular	Neuromuscular
Amikacin	++	++	+	+
Gentamicin	++	++	++	+
Streptomycin	+	+	+++	++
Tobramycin	++	++	++	+

Streptomycin causes the most vestibular and neuromuscular toxicity, but is least likely to cause nephrotoxicity and cochlear toxicity. The toxicity of amikacin and gentamicin is very similar, with gentamicin being more likely to cause vestibular toxicity. According to Ashwlayan and Singh (2016), tobramycin has similar toxicities than gentamicin.

Adverse effects after intraventricular administration include hearing loss, aseptic meningitis, seizures and eosinophilic cerebrospinal fluid pleocytosis (LeBras *et al.*, 2016:502). Administration of aminoglycosides directly into the intraventricular space may theoretically reduce toxicity, due to limited systemic exposure (LeBras *et al.*, 2016:493).

2.5.6 Mechanisms of bacterial resistance

Resistance to aminoglycosides can be endogenous or required. Bacteria exhibiting endogenous resistance include *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. Acquired resistance can occur in almost all gram-negative organisms (Avent *et al.*, 2011:443).

There are four main mechanisms of acquired aminoglycoside resistance (Magnet *et al.*, 2005:481):

- Deactivation of the aminoglycoside by *N*-acetylation, adenylation or *O*-phosphorylation (enzymatic modification). This causes diminished affinity for the ribosomal A-site target and a loss of antibacterial activity.
- Changes in outer membrane permeability, decreased inner membrane transport, active efflux and drug trapping, all lead to a reduction of the intracellular concentration of aminoglycoside due to the fact the bacterial uptake of the drug is poor. The bacterial efflux pump is an energy-dependent adenosine triphosphate (ATP) pump, which is a major cause of bacterial resistance. This is often the mechanism of bacterial resistance in multidrug-resistant nosocomial infections. *Pseudomonas aeruginosa*, *Burkholderia pseudomallei*, *Acinetobacter baumannii* and *E. coli* are known organisms that make use of efflux pumps as intrinsic or acquired bacterial resistance methods.
- Alteration of the 30S subunit of the bacterial ribosome by mutation. Ribosomal target mutation is only relevant for streptomycin resistance in *Mycobacterium tuberculosis*. A single mutation can lead to the production of a homogeneous population of mutant ribosomes and, thus, result in resistance (Jana & Deb, 2006:146).
- Methylation of the aminoglycoside binding site. This mechanism results in high-level resistance to gentamicin, tobramycin and amikacin (Avent *et al.*, 2011:444). Clinical isolates of *Pseudomonas aeruginosa* and *Serratia marcescens* have been found with genes encoding for this mechanism of resistance.

Genes encoding for aminoglycoside-modifying enzymes (AMEs) are often located in plasmids. This permits cell-to-cell dissemination of the resistance trait by horizontal gene transfer (conjugation) (Zarrilli *et al.*, 2005:829). Most resistance to aminoglycosides is caused by inactivation of the drug by intracellular bacterial enzymes. Due to its unique structure, amikacin

is not inactivated by the common enzymes that inactivate gentamicin and tobramycin (Watanabe *et al.*, 2004:423).

2.6 Dosing strategies

Once-daily dosing regimens for aminoglycosides are as effective as, and less toxic than multiple daily doses (Beaucaire, 2000:355).

The standard method of dosing for patients with normal renal function has previously been the administration of a weight-based dose eight hourly, but during the 1980s, a once-daily dosing (also referred to as high-dose extended interval dosing) started gaining popularity (Stankowicz *et al.*, 2015:1357). A study in 2014 found that once-daily dosing was used in 95% of certified cystic fibrosis treatment centres in the United States (Stankowicz *et al.*, 2015:1357). Peak concentrations after administration of high-dose extended interval dosing were noticeably higher than after multiple daily dosages and trough levels before administration of the next dose were lower. The desired ratio of maximum concentration (C_{max}) over the MIC can be reached much easier with once-daily dosing (Nezic *et al.*, 2014:830). This suggests that effective antimicrobial effect was maintained with a lower risk of toxicity (Stankowicz *et al.*, 2015:1358). As the kidney is not able to excrete the total aminoglycoside dose within the dosage interval when administered eight-hourly, a once-daily dosing has the advantage of minimised repeat exposure and therefore potentially less nephrotoxicity (Beaucaire, 2000:355).

Further advantages of once-daily dosing include decreased nursing time, decreased laboratory costs, decreased laboratory personnel time and straightforward TDM calculations, all resulting in decreased total cost to the patient (Nezic *et al.*, 2014:830).

2.7 Guidelines for the use of aminoglycosides

2.7.1 General aminoglycoside guidelines

According to the Johns Hopkins Antibiotics Guide (2015-2016), aminoglycosides can be used in the following cases:

- For the treatment of pelvic inflammatory disease and endometriosis in patients who are allergic to penicillin, gentamicin is administered with clindamycin.
- Gentamicin is also used to treat native valve endocarditis with vancomycin or penicillin.

- Patients with healthcare-associated pneumonia (not ventilator-associated pneumonia) who are allergic to penicillin may be treated with vancomycin plus gentamicin.
- Due to the rapid bactericidal effect and broad spectrum, gentamicin plus piperacillin/tazobactam, or gentamicin combined with cefepime, is the recommended empirical treatment for sepsis without a clear source. Cultures for microbiology and susceptibility should be sent right before commencing antimicrobial treatment to de-escalate to narrow spectrum treatment as soon as indicated.
- Gentamicin is also indicated in treatment of urinary tract infections where patients are allergic to penicillin.

The Swedish Reference Group for Antibiotics (Hanberger *et al.*, 2013:164), suggest the following aminoglycoside treatment regimens:

- For severe sepsis and septic shock (gram-negative bacteraemia), aminoglycoside monotherapy is not recommended, but an aminoglycoside combined with a beta-lactam is recommended.
- Patients with pyelonephritis and other foci of infection can be treated with aminoglycoside monotherapy, especially in urinary tract infections. It is unknown whether monotherapy with aminoglycosides treatment for pyelonephritis and complicated UTI requires longer courses of treatment. However, urine concentrations of aminoglycosides may remain above the MIC for up to four days after the last dose, favouring shorter treatment courses with fewer adverse effects.
- Aminoglycoside therapy for endocarditis is always administered in combination with a beta-lactam antibiotic. According to Hanberger *et al.* (2013), the Endocarditis Working Group (EWG) of the Swedish Society of Medicine has updated guidelines in 2012, suggesting a once-daily dosing of aminoglycosides for endocarditis, except twice-daily dosing in difficult-to-treat cases, such as enterococcal endocarditis, and the involvement of prosthetic valves. When treating *S. aureus* endocarditis, a dose of 5 mg/kg of gentamicin once daily is recommended (Hanberger *et al.*, 2013:166).

2.7.2 South African guidelines

2.7.2.1 Guidelines for the use of gentamicin

According to the Standard Treatment Guidelines and Essential Medicine List for South Africa (Department of Health, 2015:66), gentamicin is used as first-line treatment for patients with febrile neutropenia. Gentamicin is administered with a 3rd generation cephalosporin as empirical therapy within 48-hours of admission. Once cultures are available, treatment is adjusted to the most appropriate narrow spectrum agent.

Gentamicin is the first-line therapy for native valve endocarditis, combined with cloxacillin. It is important to note that when gentamicin is combined with a penicillin, as in this case, the dose is 1.5 mg/kg 12-hourly (a total daily dose of 3 mg/kg/day), and not a once-daily dose as normal. When a patient has prosthetic valve endocarditis, gentamicin is combined with vancomycin and rifampicin as first-line treatment regime.

Patients with severe pelvic inflammatory disease and penicillin allergy may receive gentamicin combined with clindamycin. Once the patient clinically improves, treatment should change to oral clindamycin and ciprofloxacin.

Gentamicin is used as empirical therapy for acute pyelonephritis with symptoms of severe sepsis and brucellosis.

Gentamicin is not often the agent of choice as a surgical prophylaxis, but for gastro-intestinal surgery, clean-contaminated urology procedures, hysterectomy and vaginal repairs gentamicin will be added to clindamycin to provide adequate gram-negative cover (Department of Health, 2015:66).

2.7.2.2 Guidelines for the use of amikacin

Even though aminoglycosides are not first-line therapy for respiratory tract infections, amikacin is used in combination with ceftriaxone for hospital-acquired pneumonia where there are no risk factors for multidrug-resistant (MDR) infection. Amikacin is also often used to treat catheter associated urinary tract infections. As hospital-acquired infections are often more resistant than community-acquired infections, amikacin is a suitable drug of choice for empirical therapy. As soon as cultures are available, treatment can be adjusted to the most appropriate narrow spectrum agent (Department of Health, 2015:236).

Amikacin is used as non-first line tuberculosis treatment when treatment failure due to adverse effects or non-compliance occurs (Department of Health, 2015:262).

2.8 Chapter summary

This chapter focused on the literature review. Section 2.2 discussed therapeutic drug monitoring and section 2.3 gave a brief overview of the background and history of different antibiotic classes, including aminoglycosides. Section 2.4 focused on the structure, chemical properties, mechanism of action, spectrum and indications for the use of antibiotics. In this section, the pharmacokinetics and pharmacodynamics, toxicity and mechanisms of bacterial resistance were also discussed. Dosing strategies were discussed in section 2.5, followed by South African and international guidelines for the use of aminoglycosides in section 2.6. To conclude this chapter, dosing considerations, including weight and renal function, were discussed.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Introduction

The results of the empirical investigation are presented in this chapter in the form of a manuscript and additional results. The empirical research objectives and cross-references are summarised in Table 3-1.

Table 3-1: Summary of research objectives

Objective	Cross-reference
To determine the dosages and time intervals of aminoglycosides prescribed for the patients during the period of the study.	Manuscript (Section 3.2), Table II – Dosage and blood level results
To determine the percentage of patients on aminoglycosides whose drug levels were monitored.	Manuscript (Section 3.2), Table II – Dosage and blood level results
To calculate the percentage of patients with an abnormal serum creatinine level where therapeutic drug monitoring was done.	Manuscript (Section 3.2), Table II – Dosage and blood level results
To determine whether dosage adjustments were made in case of drug levels outside the normal reference range.	Section 3.3 – research objectives not addressed in the manuscript

The title of the manuscript is “Standards of aminoglycoside therapeutic drug monitoring in a South African private hospital: perspectives and implications.” The manuscript was accepted for publishing on 15th October 2018 (refer to Annexure H for the approval letter from the Journal).

The manuscript was structured according to the specific guidelines of the Ghana Medical Journal for an original research study, in English (UK). Referencing was done according to International Committee of Medical Journal Editors (ICEMJ) recommendations, as specified in the journal guidelines (Annexure G – Ghana Medical Journal Guidelines). Although the guidelines require authors to use Arial size 10 with a line spacing of 1.0, the manuscript included as section 3.2 in this dissertation was changed, for the purpose of uniformity, to Arial point size 11, with a line spacing of 1.5.

3.2 Manuscript

Title

Standards of aminoglycoside therapeutic drug monitoring in a South African private hospital: perspectives and implications.

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Conflicts of interest

None to declare.

Keywords

Therapeutic drug monitoring; aminoglycosides; dosing considerations; sampling times; South Africa.

Abstract

Background: Therapeutic drug monitoring (TDM) is essential to ensure that aminoglycoside peak concentrations are high enough for effective antimicrobial treatment and trough levels are low enough to minimise toxicity. Inappropriate utilisation of TDM may lead to suboptimal therapy, toxicity and waste of resources. This study aimed to investigate the standard of aminoglycoside TDM performed in adult hospitalised patients.

Method: An observational, descriptive, cross-sectional study was performed in a 221-bed private hospital, using retrospective data from November 2014 to October 2016. All patients, older than 18 years, who were on intravenous aminoglycosides for more than 48 hours were included. A computerised database and patient files were used to obtain the information required for this study. Descriptive statistical analysis was used to describe and summarise data.

Results: One hundred and three (103) patients were included: 65 on gentamicin and 38 on amikacin. Blood levels were performed in only 19 gentamicin (29.23%) and 22 amikacin (57.89%) patients. Trough levels were taken more than 2 hours before the next dose in 12 gentamicin (63.16%) and 12 amikacin (54.54%) patients. The majority of patients (96.92% on gentamicin and 84.21% on amikacin) received once daily doses. TDM was performed in all patients with an estimated glomerular filtration rate (eGFR) lower than 60 mL/min/1.73m² and in 23.31% of gentamicin patients and 56.76% of amikacin patients with an eGFR higher than 60 mg/min/1.73m².

Conclusions: Incorrect sampling times and unnecessary levels taken in patients with normal renal function indicate a need for aminoglycoside treatment guidelines in the private hospital.

Introduction

Therapeutic drug monitoring (TDM) is defined as a measured drug concentration combined with adequate medical interpretation to influence the drug therapy in patients¹ and an important tool in pharmacotherapy. The purpose of TDM is to individualise the therapeutic regimen of the patient to gain optimal benefit with the lowest toxicity. Research has shown blood samples for TDM purposes are not always taken correctly and protocols do not exist, or are not always followed,² which can lead to incorrect dosing regimens and waste of time and resources, especially in developing countries.² Measurement of aminoglycoside concentrations and dosage individualisation should be standard practice for all critically ill patients treated with aminoglycosides.³

Aminoglycoside antibiotics are concentration-dependent — a higher antibiotic concentration ($C_{\max}$) to minimum inhibitory concentration (MIC) ratio ($C_{\max}:\text{MIC}$) will exhibit a greater antimicrobial activity. Aminoglycosides also exhibit a post-antibiotic effect, meaning that a persistent suppression of bacterial growth is demonstrated even after drug concentration drops below the MIC.⁴

The hydrophilic nature of aminoglycosides can lead to changes in volume of distribution in certain conditions, such as sepsis, which can lower peak concentrations and the effect of the drug.³

Aminoglycosides are mainly excreted in the kidneys and many critically ill patients present with impaired renal function. In patients with impaired renal function, two samples (peak and trough) are recommended to individualise the regimen by using the Sawchuk-Zaske calculations or Bayesian approaches.⁵ Guidelines to perform TDM become even more important in Intensive Care Units (ICUs), where the weights of the patients may be unknown and their pharmacokinetics can change daily.

The once daily dosing regimen adopted over the past few years is based on the assumption that the peak concentration will be adequate for most infections and peak levels are not routinely requested. There is, however, evidence indicating that the length of hospital stay is decreased and nephrotoxicity reduced by aminoglycoside dosage individualisation in critically ill patients;^{1,3} therapeutic drug monitoring, therefore also improves overall patient outcome. The South African Medicines Formulary (SAMF) recommends that trough levels be obtained 48 to 72 hours after commencement of therapy, immediately prior to the next dose, and dose or interval adjustments

made if necessary. It also recommends a peak level for effective bacterial kill after the second dose.

There is limited data on TDM of aminoglycosides in South Africa therefore, this study was performed to evaluate the standard of this service in a private hospital.

Methods

Study design -and population

The study was an observational, descriptive, retrospective, cross-sectional study conducted in a 221-bed private hospital located in the Western Cape, South Africa. The target population included all patients who received gentamicin and amikacin, for longer than 48 hours, during the research period. The study population consisted of all patients, aged 18 years and older, who received aminoglycosides whilst admitted in the hospital during the research period from November 2014 to October 2016. Patients from the emergency centre, or who received aminoglycosides as surgical prophylaxis were excluded from the study.

The measurements taken were to determine the dosages of aminoglycosides prescribed, the percentage of patients where drug levels were monitored, the percentage of patients with abnormal renal function where TDM was done and whether dosages were adjusted in case of drug levels outside of the normal range.

Data collection

Patient files and a computerised database were used to collect data at a specific time point. The hospital dispensing programme was used to find patient numbers for patients who received gentamicin or amikacin treatment. Data collected from the patient's files included the patient's age, gender and weight, the dosage of aminoglycoside prescribed and frequency, as well as time of day when gentamicin or amikacin were prescribed.

Laboratory reports were used to determine the peak and trough levels in cases where blood samples were taken for TDM. The laboratory results were also used to determine whether the results were within normal reference ranges. The following was documented: dosages

prescribed, patient weight, age and gender, time of collection of blood sample, whether the drug levels were within reference ranges and patient renal function.

Data analysis

The statistical analysis was performed using the SPSS programme.⁶ Descriptive statistical analysis was used to describe and summarise data. Categorical variables were expressed as frequencies and percentages, and continuous variables were expressed as mean and standard deviations (SDs).

Ethical considerations

Permission to conduct the study was obtained from the Health Research Ethics Committee of North-West University (NWU-00363-15-A1). Goodwill permission was obtained from the hospital group's corporate office, the hospital manager and manager of the laboratory.

Results

This study comprised 178 files of patients who received aminoglycosides while admitted to the private hospital during the study period of 1 November 2014 and 31 October 2016. Of these, 103 patient files met the inclusion criteria. The 75 patients excluded from the study either received aminoglycosides as surgical prophylaxis, or were treated for less than 48 hours. Sixty-five patients received gentamicin and 38 amikacin.

Demographic characteristics of the patients

The demographic characteristics of the patients are shown in Table I. Of the 65 patients on gentamicin (mean age 57.38 ± 15.16 years), 63.10% ($n = 41$) were males. Patients on amikacin were marginally younger at 54.29 ± 22.85 years (Cohen's $d = 0.14$) and of these, 60.53% ($n = 23$) were males.

Only two patients on gentamicin had an estimated glomerular filtration rate (eGFR) of lower than 60 mL/min/1.73m², compared to one patient on amikacin. Most of the patients, 66.10% in the gentamicin and 55.26% in the amikacin group, had eGFR values higher than 90 mg/min/1.73m².

Table I. Patient demographics

	Gentamicin (N = 65)	Amikacin (N = 38)
Age (years), mean $\pm$ SD	57.38 $\pm$ 15.16	54.29 $\pm$ 22.85
Gender, n (%)		
Male	41 (63.10)	23 (60.53)
Female	24 (36.90)	15 (39.47)
Renal function (eGFR), n(%)		
< 60 mL/min/1.73m ²	2 (3.08)	1 (2.63)
60-90 mL/min/1.73m ²	15 (23.08)	15 (39.48)
> 90 mL/min/1.73m ²	43 (66.15)	21 (55.26)
Renal function not tested	5 (7.69)	1 (2.63)

Dosages and blood level results of the patients

Table II shows the dosages and blood level results according to ideal trough level concentrations and renal functions, and sampling times for patients on gentamicin and amikacin, respectively. The sampling time refers to the time when blood was drawn to measure aminoglycoside levels. In all patients, levels taken were trough levels.

Only two patients (3.08%) on gentamicin received an eight-hourly dose, 63 patients received a once daily dose and 38 (60.32%) received a dose of 240 mg daily. Blood levels were drawn in only 19 (29.23%) of the patients and 13 levels were lower and six higher than 1 mg/L. Therapeutic drug monitoring was performed in both patients with a renal function of lower than 60 mL/min/1.73m². The renal function was higher than 60 mL/min/1.73m² in 17 (89.47%) patients on gentamicin where TDM was performed.

In the amikacin group, six patients (N = 38) received a 12-hourly dose and 30 (78.95%) received a dose of 1000 mg daily. Blood levels were drawn in 22 (57.89%) of the patients and 19 levels were lower and three were higher than 5 mg/L. Therapeutic drug monitoring was performed for the one patient on amikacin with a renal function lower than 60 mL/min/1.73m².

Table II. Dosage and blood level results for the patients

	Gentamicin n(%)	Amikacin n(%)
Dosage		
240 mg/day	38 (58.5)	n/a
160 mg/day	10 (15.4)	n/a
400 mg/day	7 (10.8)	n/a
180 mg/day	5 (7.7)	n/a
320 mg/day	2 (3.1)	n/a
80 mg 8-hourly	2 (3.1)	n/a
480 mg/day	4 (1.5)	n/a
1000 mg/day	n/a	30 (78.95)
500 mg 12-hourly	n/a	5 (13.16)
750 mg/day	n/a	2 (5.26)
1000 mg 12-hourly	n/a	1 (2.63)
TDM results		
<1 mg/L	13 (20.00)	n/a
>1 mg/L	6 (9.23)	n/a
<5 mg/L	n/a	19 (50.00)
>5 mg/L	n/a	3 (7.89)
No TDM done	46 (70.77)	16 (42.11)
TDM for eGFR< 60 mL/min/1.73m²		
TDM done	2 (100)	1 (100)
TDM not done	0	1
TDM for eGFR> 60 mL/min/1.73m²		
TDM done	17 (29.31)	21 (56.76)
TDM not done	41 (70.69)	16 (43.24)
TDM done where eGFR not tested		
TDM done	0	0
TDM not done	5 (100)	0
Sampling times		
0-1 hour before next dose	1 (5.26)	5 (22.73)
1-2 hours before next dose	6 (31.58)	5 (22.73)

	Gentamicin n(%)	Amikacin n(%)
> 2 hours before next dose	12 (63.16)	12 (54.54)
TDM = Therapeutic drug monitoring n/a = Dosage range not applicable to specific drug		

Discussion

To our knowledge, this was the first study where the standard of therapeutic drug monitoring of aminoglycosides, gentamicin and amikacin were investigated in a private hospital in the Western Cape, South Africa, and resulted in a number of key findings.

Firstly, in this study the male to female ratio was the same for gentamicin and amikacin groups, with approximately 60% males and 40% females. It is important to remember that this is a general hospital and the study only investigated the patients on aminoglycosides, therefore it is not a reflection of all the patients admitted to the hospital. Kushner *et al.* (2016),⁷ reported a higher percentage of women on gentamicin in patients younger than 50 years. Interestingly, in the population older than 55 years, the same age as the patients in this study, the usage of gentamicin was comparable with the results of this study.

Secondly, we found that approximately 97% of patients in the gentamicin group and 84% in the amikacin group received once-daily doses of the drug, which is in line with literature recommendations for treatment⁸, as well as the South African guidelines for the use of aminoglycosides.⁹ In a survey performed in 2015 by Tabah *et al.*, 94% of patients received once-daily doses of gentamicin and amikacin.¹⁰ Once daily dosing of aminoglycosides is associated with similar efficacy, but with fewer adverse effects due to the concentration dependent nature of the drug.¹¹

Thirdly, we determined that trough levels only were performed for 29.23% of patients on gentamicin and 57.89% on amikacin treatment. The higher percentage of trough level measurements with amikacin compared to gentamicin needs further exploration. Although nephrotoxicity is an adverse effect with all aminoglycosides, literature indicates that gentamicin concentrates in the kidneys to a higher degree than the other aminoglycosides and therefore is more prone to toxicity than amikacin.¹²

Trough levels are sufficient to determine the likelihood of adverse effects, but to calculate individual doses per patient to optimise treatment, it is necessary to measure both trough and peak levels. With these calculations, it can be ensured the desired MIC for the organism is

reached, and trough levels are below threshold levels to prevent adverse effects.¹³ Peak levels become very important in critically ill patients with abnormal pharmacokinetics and in organisms with higher MIC levels. When individualised patient dosages are calculated by measuring peak and trough levels, a greater percentage of patients will achieve the targeted concentration compared to those who receive fixed doses. Improved clinical outcomes are achieved in patients who attained targeted therapeutic serum concentrations early in treatment.⁴ To ensure optimal treatment of organisms, it is suggested that a $C_{\max}/MIC$ ratio of 8 to 10 is achieved. Whether this is the case in individual patients can only be determined if a peak level is measured.³ In patients with abnormal renal function (renal insufficiency) it is of utmost importance to take two levels to determine true peak and trough levels and individualise treatment for each patient; this has not been necessary in our study. This could lead to treatment failure in infections caused by organisms with higher MIC, because the peak level is not measured and not known to be 8 to 10 times the MIC. In critically ill patients, a peak and trough level can be used to individualise dosages and ensure optimal treatment.³

Fourthly, sampling time was not consistently within one hour before administration of the next dose. Of the six patients (9.23%) in the gentamicin group, where the gentamicin levels were > 1 mg/L, none of the samples was taken within one hour before administration of the next dose. In the amikacin group, there were three cases where the amikacin levels were > 5 mg/L, and none of these was taken within one hour before administration of the next dose; of these values, none are therefore reliable and true trough levels. Trough levels should be measured within one hour before the next dose and the peak levels 30 minutes after completing the intravenous injection.¹⁴ Incorrect sampling time will lead to incorrect trough levels, as actual levels just before administration of the next dose will be lower than the levels measured more than two hours earlier. A trough level, without the knowledge of the sampling time, can be mistaken for toxicity.

The South African Department of Health's guidelines suggest that trough levels for amikacin and gentamicin be measured three times per week in haemodynamically stable patients.⁹ In our study, TDM was performed in all patients with $eGFR < 60 \text{ mL/min/1.73m}^2$, but in patients with $eGFR > 60 \text{ mL/min/1.73m}^2$ it seems that TDM was done randomly and no pattern of practice could be found. In the gentamicin group, TDM was performed for 29.13% of patients with $eGFR > 60 \text{ mL/min/1.73m}^2$ and in the amikacin group, TDM was performed for 56.67%. In the gentamicin group, there were five patients where $eGFR$ was not tested at all and no TDM was done either. Similar results were found in a study by Al Za'abi *et al.*, at a teaching hospital in Oman.² The authors ascribed their results to incorrect sampling times and the lack of policy.² Active TDM

strategies (performed according to policy for all patients and not only when prescribed by the medical practitioner) result in reduced nephrotoxicity as well as a shorter hospital stay.³

Finally, we found that dosages were not calculated per patient according to weight. This might be because many of the patients were in ICU and could not be weighed.

Limitations of this study

This study reflects only the situation in a private hospital in South Africa and does not indicate the standard of TDM practices in other private or public hospitals in the country. As only retrospective data were used, the patient outcomes could not be determined. All information required to determine the standard of TDM in the private hospital was not available, as many patient's weights were not recorded in patient charts.

Conclusion

The study has shown that aminoglycoside levels are drawn, but the results are underutilised due to the lack of clear TDM guidelines. In this small population, most of the patients had normal renal function and most of the trough levels were within the recommended range, although levels were not always taken at the correct times and sample times were not always documented. Blood levels were also more frequently taken in patients on amikacin than gentamicin. If hospital policy is compiled in line with international guidelines, it can ensure that trough levels improve therapy and reduce the probability of toxicity, especially in patients with unstable or impaired renal function. The policy can also include peak levels in patients with resistant organisms and compromised pharmacokinetics to individualise therapy even further.

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References

1. Kovačević T, Avram S, Milaković D, Špirić N, Kovačević P. Therapeutic drug monitoring of amikacin and gentamicin in critically and non-critically ill patients. *J Basic Clin Pharm.* 2016 Jun;7(3):65-9.
2. Al Za'abi M, Muqbal J, Al-Waili K. Sampling time and indications appropriateness for therapeutically monitored drugs at a teaching university hospital in Oman. *Saudi Pharm J.* 2015 Sep;23(4):458-62.
3. Roberts JA, Norris R, Paterson DL, Martin JH. Therapeutic drug monitoring of antimicrobials. *Br J Clin Pharmacol.* 2012 Jan;73(1):27-36.
4. Avent ML, Rogers BA, Cheng AC, Paterson DL. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. *Intern Med Journal.* 2011 Jun;41(6):441-9.
5. Nežić L, Derungs A, Bruggisser M, Tschudin-Sutter S, Krähenbühl S, Haschke M. Therapeutic drug monitoring of once daily aminoglycoside dosing: comparison of two methods and investigation of the optimal blood sampling strategy. *Eur J Clin Pharmacol.* 2014 Jul;70:829-37.
6. IBM Corp. IBM SPSS Statistics for Windows. Version 24.0. Armonk,: IBM Corp; 2013.
7. Kushner B, Allen PD, Crane BT. Frequency and demographics of gentamicin use. *Otol Neurotol.* 2016 Feb;37(2):190-5.
8. Beaucaire, G. Does once-daily dosing prevent nephrotoxicity in all aminoglycosides equally? *Clin Microbiol Infect.* 2000 Jul;6(7):355-60.
9. South Africa. Department of Health. *Hospital level adults standard treatment guidelines and essential medicine list for South Africa.* 4th ed. Pretoria: National Department of Health; 2015.
10. Tabah A, De Waele J, Lipman J, Zahar JR, Cotta MO, Barton G, et al. The ADMIN-ICU survey: a survey on antimicrobial dosing and monitoring in ICUs. *J Antimicrob Chemother.* 2015 Sep;70(9):2671-7.
11. Stankowicz MS, Ibrahim J, Brown DL. Once-daily aminoglycoside dosing: An update on current literature. *Am J Health Syst Pharm.* 2015 Aug;72(16):1357-64.
12. Sweileh WM. A prospective comparative study of gentamicin and amikacin induces nephrotoxicity in patients with normal baseline renal function. *Fundam Clin Pharmacol.* 2009 Aug;23(9):515-20.
13. Touw DJ, Westerman EM, Sprij AJ. Therapeutic drug monitoring of aminoglycosides in neonates. *Clin Pharmacokinet.* 2009;48(2):71-88. Erratum in: *Clin Pharmacokinet.* 2009;48(3):209-10.

14. Hanberger H, Edlund C, Furebring M, G Giske C, Melhus A, Nilsson LE, et al. Rational use of aminoglycosides – review and recommendations by the Swedish Reference Group for Antibiotics (SRGA). *Scand J Infect Dis*. 2013 Mar;45(3):161-75.

3.3 Research objectives not addressed in the manuscript

The last research objective as stated in paragraph 1.3.2 was not addressed in the manuscript. The objective was to determine whether dosage adjustments were made in case of drug levels outside the normal reference range.

Results of the analysis showed that of the six patients who received gentamicin and where the trough levels were above the normal reference value (> 1 mg/L), no dosage adjustments were made, but the doses on the day TDM was performed, were omitted in these patients (i.e. no aminoglycosides were administered). The Department of Health (2015:497) guidelines suggest that the following dose is only administered once trough levels reach the desired levels. In these patients, however, it was not recorded whether follow-up trough levels were performed to determine whether trough levels were below the desired level before administration of the next dose. In one patient, the trough level was 0.3 mg/L, but the dose was still decreased from 240 mg daily to 160 mg daily. No reasons for this decrease in dose were documented in the patient's folder.

Three patients on amikacin had trough levels above 5 mg/L and in two of the cases, the doses on the day that therapeutic drug monitoring was performed, were omitted. In the case of one patient, the dose was decreased from 1000 mg daily to 500 mg daily according to the trough level of 6.9 mg/L. One patient had a trough level of 4.6 mg/L, but the dose was still omitted on the day TDM was performed. From these results, it is clear there is no definitive guideline in the hospital to suggest what should be done when trough levels are higher than normal levels. In some patients, dosages were simply not given and in others, dosages were decreased. The South African Medicines Formulary (SAMF) (Rossiter, 2014:303) suggests that trough levels for amikacin should be <1 mg/L before administering the next dose. Other guidelines, for example the guidelines by the South African Department of Health (2015:497), suggest trough levels of <5 mg/L for amikacin and <1 mg/L for gentamicin (this is applicable to once daily dosing). Having two different sets of guidelines in the country makes it more complicated to compile a hospital-specific guideline for the therapeutic drug monitoring practice in the hospital. A study by Leong *et al.* (2006:39) revealed that even though 77% of patients who required TDM to be done, had levels drawn, only 8.8% of TDM in their study population at The Royal Melbourne Hospital (N = 132) was done according to guidelines. This hospital has specific guidelines that are accessible from every ward computer, yet guidelines are still not followed. According to Leong *et al.* (2006:38), education of doctors, nurses and phlebotomists need to be emphasised to ensure

appropriate timing of blood tests. The study further suggested that placing the TDM services in the responsibility of clinical pharmacists could resolve these problems.

3.4 Chapter summary

This chapter contained the results of the empiric investigation in the form of a manuscript that was accepted for publishing in the Ghana Medical Journal and a discussion of research objectives not addressed in the manuscript (section 3.3). The next chapter concludes the study, focusing on the study's conclusions, strengths and limitations, and suggested recommendations for future studies.

CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

4.1 Introduction

This chapter provides a brief overview of the content of the mini-dissertation and a summary of the findings. It also includes conclusions drawn from the specific objectives discussed in Chapter 1. Limitations and strengths identified during the study are also discussed, and based on the findings possible recommendations for future practice will be considered to conclude the chapter.

The study aim was to investigate the standard of aminoglycoside TDM in a South African private hospital. The study determined whether dosage changes were made when the drug levels were outside the normal ranges, whether TDM was being done according to guidelines, and if samples were drawn at the correct times.

Conclusions drawn from literature and empirical research are summarised in paragraphs 4.2 and 4.3.

4.2 Conclusions based on the literature review

4.2.1 Review of guidelines to determine in which cases therapeutic drug monitoring should be conducted when administering intravenous aminoglycosides in different populations

From the literature study, it can be concluded that two sets of guidelines exist for the therapeutic drug monitoring of aminoglycosides in South Africa: The South African guidelines determined by the Department of Health (2015) and the SAMF guidelines (Rossiter, 2014:303). Because the study was conducted in South Africa, South African guidelines were used as the standard and international guidelines were used as reference. The South African guidelines determined by the Department of Health (2015:497) suggest that TDM should be performed three times per week in haemodynamically stable patients (refer to paragraph 2.3.5 – Guidelines for the monitoring of aminoglycoside levels). These guidelines also suggest that only trough levels should be performed. In haemodynamically stable patients the serum creatinine levels should also be measured three times per week and treatment should be adjusted when the serum creatinine levels increase, trough levels are above the maximum levels or if renal or ototoxicity appear. In patients who are not haemodynamically stable, trough levels should be measured every day and the dose should only be administered once the trough levels are below the target concentration. The SAMF guidelines (Rossiter, 2014:303) suggest measuring peak levels one hour after

commencement of intravenous infusion at the second dose of an aminoglycoside to determine whether sufficient peak levels are reached. This guideline also suggests that trough levels are measured immediately prior to administering the next dose. Having different guidelines in a country, or the lack of clear guidelines in a hospital, may make it difficult for physicians to follow specific guidelines. It is also important to note that private hospitals and specialists are not required to follow national guidelines and based on our results, this demonstrates the need for specific guidelines for private hospitals to ensure that all medical practitioners follow the same guidelines.

4.2.2 Determining the time at which the trough and peak levels should be measured before the administration of the next dose

It was clear from the literature that the guidelines differ regarding the ideal time to measure trough and peak levels. International guidelines, including the Stanford guidelines (Mui, 2017) and Swedish guidelines (Hanberger *et al.*, 2013:170) recommend taking a trough level less than one hour before administration of the following dose and the South African guidelines in the SAMF (Rossiter, 2014:303) recommends taking the first trough sample 48 to 72 hours after commencement of treatment, immediately prior to administration of the next dose (Refer to paragraph 2.3.5 – Guidelines for the monitoring of aminoglycoside levels). The South African Department of Health recommends taking pre-levels (trough levels) immediately before administration of the next dose. From all the literature it seems that it is of utmost importance that trough levels are taken to minimise toxicity and that samples to determine these levels are drawn as close to administration of the next dose as possible, but definitely not more than two hours before administration of the next dose. When measuring trough and peak levels and individualising dosing by using the Sawchuk Zaske method (Sawchuk & Zaske, 1976:183), trough and peak levels can be measured at any time point, because the levels and the time at which the levels were taken will be taken into consideration when doing the calculation. This method, however, is not often used to calculate dosages and suggested dosage adjustments, due to the complicated nature of calculations and lack of clinical pharmacists to perform these calculations (Denaro & Ravenscroft, 1989:38).

4.2.3 Determining the normal reference ranges for gentamicin and amikacin trough and peak levels

There are different reference ranges in literature for trough levels for amikacin and gentamicin. According to the South African Department of Health (2015:497) the normal trough levels for gentamicin should be <1 mg/L and amikacin <5mg/L. The SAMF (Rossiter, 2014:303) recommends trough levels <1 mg/L for both gentamicin and amikacin. From the literature, it became clear that the peak level depends on the susceptibility of the organism. When prescribing aminoglycosides, a target maximum drug concentration to minimum inhibitory concentration (C_{max}/MIC) ratio of 8-10 or even a ratio >10 is suggested. The importance of once daily dosing, which will result in a high peak level, was well documented in the literature covered for this study.

Aminoglycosides are often used as second line therapy or when there is resistance to other antibiotics and in these cases, it is even more important to ensure that dosages are sufficient and peak levels reach a C_{max}/MIC ratio of 10 times the MIC of the organism (Antibiotic Expert Groups, 2014). In this study, most of the patients received once daily doses, but no peak levels were done to determine whether the peak levels were sufficient for bactericidal activity against pathogens. The SAMF (Rossiter, 2014:303) recommended a peak level >30 mg/l for amikacin and >8 mg/l for gentamicin, which is a valuable tool if MIC concentrations are not available.

4.2.4 Conceptualising aminoglycoside toxicity

The most important toxic effects of aminoglycosides are nephrotoxicity and ototoxicity. Nephrotoxicity may be reversible, but ototoxicity is irreversible (Xie *et al.*, 2011:30). Neuromuscular toxicity is a rare, but serious adverse effect and patients that are on concomitant neuromuscular blockers or anaesthetic medications may experience neuromuscular toxicity. In patients with pre-existing conditions, concomitant use of other ototoxic or nephrotoxic medications and prolonged treatment with aminoglycosides increases the risk for toxicity. The importance of monitoring aminoglycoside plasma levels to monitor toxicity were discussed in the literature (Refer to section 2.5.5 – Toxicity). Nephrotoxicity caused by aminoglycosides is unlikely to occur before 3 to 5 days when correct dosages are administered. This emphasises the importance of TDM and blood level monitoring. Ensuring that trough levels are within the suggested ranges decreases the likelihood of adverse effects. It is also important to remember that therapy exceeding a duration of 14 days, large total cumulative doses, and concurrent therapy with other nephrotoxic drugs such as vancomycin can predispose patients to these effects (Bauer, 2006:99). The literature linked both oto- and nephrotoxicity to an increase in trough levels (Bauer, 2006:99). As mentioned in sections 2.3.5 and 2.5.5, TDM is required for all patients who receive

aminoglycoside treatment for more than 48 to ensure that the risk of adverse effects is kept to a minimum and treatment is effective.

4.2.5 Determining the influence of aminoglycosides on serum creatinine levels in patients

The role that aminoglycosides play in the development of nephrotoxicity and the relationship with serum creatinine levels was discussed in depth in the literature review (Refer to 2.2.2 – Clearance, 2.3.3 – Clearance and 2.5.5 – Toxicity). It was also explained in section 2.3.3 that serum creatinine is an important indicator of renal health because it is an easily measured by-product of muscle metabolism excreted unchanged by the kidneys (Smith *et al.*, 2012:1333). Serum creatinine, a waste product of creatinine, is produced in muscle and excreted entirely by the kidneys, just like aminoglycosides. It is therefore a good indication of the rate of excretion of the drug. Serum creatinine values are used to calculate creatinine clearance (Cl_{cr}). A decrease in creatinine clearance (or an increase in serum creatinine) may indicate a decrease in renal function and aminoglycoside toxicity (Refer to section 2.3.3 – Clearance).

Aminoglycoside nephrotoxicity presents as non-oliguric, reversible renal failure, which results in an increase in serum creatinine levels over several days (Refer to section 2.5.5 – Toxicity).

4.3 Conclusions based on results of the empirical investigation

The discussion below is based on the results discussed in the manuscript and additional results. The study population consisted of all patients over the age of 18 years who received intravenous amikacin or gentamicin for more than 48 hours while admitted in the hospital between 1 November 2014 and 31 October 2016. Patients who received a single dose of gentamicin or amikacin as surgical prophylaxis, or who received a single dose in the emergency centre before being discharged or transferred to another facility, were excluded from the study.

The study was performed in a 221-bed private hospital in the Western Cape, which has roughly equal numbers of medical and surgical patients. No sampling was done and all patient files from patients who fit the inclusion criteria were included in the study. Data were analysed using descriptive statistics. Paragraphs 4.3.1 to 4.3.4 present the conclusions derived at following the analysis.

4.3.1 Determining the dosages and time intervals of aminoglycosides prescribed for the patients during the period of the study

The majority of the patients received once daily doses: 97% of patients on gentamicin (N = 66) and 84% on amikacin (N = 32). These observations (also discussed in the manuscript, Table II) correspond with the results from the literature study (Refer to section 2.6 – Dosing strategies). The high percentage of patients who received once daily dosages of amikacin and gentamicin proves that national and international dosing guidelines are adhered to by medical practitioners at the hospital (Department of Health, 2015:66, Hanberger *et al.*, 2013:164, Nezic *et al.*, 2014:830). Once daily doses ensure less toxicity and a higher peak level for better efficacy (Beaucaire, 2000:355). Aminoglycoside dosing is weight-based and a main limitation of the study was that the weight of the patients was either not measured or not recorded therefore the aminoglycoside dosages (in mg/kg) could not be calculated. It was not possible to calculate whether dosages prescribed were according to guidelines, suggesting that amikacin dosage should be 15 mg/kg and gentamicin 5 to 7 mg/kg (Mui, 2017). However, most patients received a standard dose of 240 mg gentamicin (60%, N = 38) once daily and amikacin 1 g (79%, N = 30) once daily.

4.3.2 Determining the percentage of patients on aminoglycosides whose drug levels were monitored

Drug levels were only measured for 58% of patients on amikacin (N = 22) and 29% of patients on gentamicin (N = 19). All levels taken were trough levels, and the times they were taken varied (Refer to 3.2 – Manuscript, Table II). The trough levels were only taken at the recommended time of 1 hour before the next dose for six patients (1 on gentamicin and 5 on amikacin). Incorrect sampling times lead to inaccurate results and unnecessary dosage adjustments or omissions. This does not benefit the patients and leads to a waste of resources. No peak levels were determined and the data were only recorded once. It was not possible to determine whether TDM was done every third day, as recommended by the Department of Health (2015:497), or if it was done only once. It is possible that trough levels were monitored every day for patients with normal renal function and this is a waste of resources, which combined with incorrect sampling times, will lead to unnecessary dosage adjustments and financial losses to the patient. This is a limitation of the study, because the results only showed whether TDM was performed. No further information was available to determine whether TDM was only performed once for a patient and whether trough levels were taken every day before administration of the following dose.

4.3.3 Calculating the percentage of patients with an abnormal serum creatinine level where therapeutic drug monitoring was done

Therapeutic drug monitoring was done for all patients with abnormal or impaired renal function (two patients on gentamicin and one patient on amikacin had eGFR levels below 60 mL/min/1.73m²). This was done in agreement with the South African guidelines for the therapeutic drug monitoring of aminoglycosides (Department of Health, 2015:497), which suggests that TDM should be performed for all patients with impaired renal function (Refer to section 2.3.5 – Guidelines for the monitoring of aminoglycoside levels).

4.3.4 Determining whether dosage adjustments were made in case of drug levels outside the normal reference range

In cases where the trough levels were higher than 1 mg/L for gentamicin or 5 mg/L for amikacin, the following dose was omitted (Refer to section 3.3 – Research objectives not addressed in the manuscript). No dosage adjustments were made, except for one patient on amikacin where the dose was reduced, but the aminoglycoside doses were omitted until trough levels were within the normal ranges again.

Even though TDM is sometimes performed in the hospital where the research was conducted, it seems to be done randomly for patients with normal renal function. This leads to unnecessary services being delivered and is a potential waste of scarce resources. Therapeutic drug monitoring was done for all patients with impaired renal function. Samples were not taken at the right times, making it impossible to interpret results and come to conclusions regarding correct dosing.

4.4 Strengths and limitations

The strengths of the study can be summarised as follows:

- It is the first study to look into the standard of aminoglycoside TDM in a private hospital in South Africa.
- The study highlighted the lack of a comprehensive set of guidelines for the specific hospital, which can improve the standard of TDM. Even though guidelines exist in South Africa, they are not uniform and those for TDM differ between different sets of guidelines. Guidelines that are more specific can make TDM more cost-effective and that can be to the benefit of both

the hospital and patient. Although the study was only conducted at one private hospital, the results can form the basis of more such studies.

The limitations of this study must be kept in mind when interpreting findings. Data were derived from one private hospital and the findings cannot be generalised to other provinces, private or public hospitals in South Africa. A major limitation of this study is the fact that only the first trough levels were used to determine whether TDM was performed. The patient charts used as data source only indicated whether the prescribing doctor requested TDM once and did not indicate whether follow-up TDM levels were requested and if it were performed. It was, therefore, not possible to indicate how often TDM was performed and whether follow-up levels were requested in cases where trough levels were outside of the normal reference ranges.

Another limitation was the retrospective nature of the study. Patient outcomes could not be measured and there was no indication whether there was a difference in outcomes between patients who received TDM and those who did not. Due to the retrospective nature of the study there was no follow-up in terms of toxicity, therefore, it was not possible to determine whether any of the patients experienced toxicity from aminoglycosides when trough levels were higher than the normal reference ranges.

The weights of patients were not always documented in patient charts and it was not possible to determine whether dosages were correct according to the individual's weight.

4.5 Recommendations

Based on the findings of this study, the following recommendations can be made:

- The lack of a detailed TDM protocol in the study setting caused incorrect sampling times and inappropriate utilisation of TDM. It is important to have a hospital protocol in order to use TDM optimally. A detailed protocol can save money for the hospital and ensure optimisation of patient treatment. The protocol should also indicate when peak levels in certain cases can be of benefit to the patient and when these levels should be included in TDM.
- It was clear from this study that samples taken were not always true trough levels. Ensuring that levels are taken at correct times is extremely important when the eGFR is below 60 mL/min/1.73m². In these cases, the aminoglycoside level is an indication of renal toxicity and that can be misleading if the level is too high. The correct time of a trough sample must be indicated in the protocol and adhered to. The information obtained from this study can give

an indication of therapeutic drug monitoring (TDM) practices in other private hospitals in the country and need to be explored further.

- Future interventional studies can investigate the role of the clinical pharmacist in TDM of aminoglycosides and the implications thereof in treatment costs and patient outcomes. Having an expert that can interpret peak and trough levels and make recommendations with regard to dosage adjustments can improve overall outcomes for patients.

4.6 Chapter summary and reflection

This chapter provided a summary of the conclusions drawn from the literature review and empirical study that were performed as part of this research project. Additionally, the chapter contained the limitations, strengths and recommendations for further studies. Hereby the objectives set for the study have been achieved.

From this empirical investigation, it is clear that even though TDM is being done in the hospital where the research was performed, there is no consistency in the way it is done or the type of patients where TDM is performed. Therapeutic drug monitoring was performed for all patients with impaired renal function, as literature suggests, but sampling times were inappropriate in the majority of patients.

REFERENCES

- Al Za'abi, M., Al Muqbali, J. & Al-Waili, K. 2015. Sampling time and indications appropriateness for therapeutically monitored drugs at a teaching university hospital in Oman. *Saudi pharmaceutical journal*, 23(4):458-462.
- Allou, N., Bouteau, A., Allyn, J., Snauwaert, A., Valance, D., Jabot, J., Bouchet, B., Galliot, R., Corradi, L., Montravers, P. & Augustin, P. 2016. Impact of high loading dose of amikacin in patients with severe sepsis or septic shock. *Annals of intensive care*, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5093100/pdf/13613_2016_Article_211.pdf. Date of access: 24 Mar. 2017.
- Aminov, R.I. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in microbiology*, 1:134. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109405/pdf/fmicb-01-00134.pdf>. Date of access: 17 Oct. 2017.
- Antibiotic Expert Groups. 2014. Therapeutic guidelines: antibiotic. Version 15. <http://www.tg.org.au/?sectionid=41> Date of access: 3 Dec. 2015.
- Antoine, D.J., Srivastava, A., Pirmohamed, B. & Park, B.K. 2010. Statins inhibit aminoglycoside accumulation and cytotoxicity to renal proximal tubule cells. *Biochemical pharmacology*, 79(4):647-654.
- Ashwlayan, V.D. & Singh, G. 2016. Analysis of aminoglycosides. *International journal of pharmaceutical science review and research*, 39(1):282-293.
- Avent, M.L., Rogers, B.A., Cheng, A.C. & Paterson, D.L. 2011. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. *International medicine journal*, 41(6):441-449.
- Banday, A.A., Farooq, N., Priyamvada, E., Yusufi, A.N. & Khan, F. 2008. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissue. *Life sciences*, 82(9-10):450-459.
- Barger, A., Fuhst, C. & Wiedemann, B. 2003. Pharmacological indices in antibiotic therapy. *Journal of antimicrobial chemotherapy*, 52(6):893-898.

- Basset, E.J., Keith, M.S., Armelagos, G.J., Martin, D.L. & Villanueva, A.R. 1980. Tetracycline-labeled human bone from ancient Sudanese Nubia (A.D. 350). *Science*, 209(4464):1532-1534.
- Bauer, L.A. 2006. The aminoglycoside antibiotics. (In Bauer, L.A., ed. Applied clinical pharmacokinetics. 2nd ed. New York, NY: McGraw-Hill. p. 97-206).
- Beaucaire, G. 2000. Does once-daily dosing prevent nephrotoxicity in all aminoglycosides equally? *Clinical microbiology and infection*, 6(7):355-360.
- Bo, G. 2000. Guiseppe Brotzo and the discovery of cephalosporins. *Clinical microbiology and infection*, 6(Suppl. 3):6-8.
- Bollenbach, T. 2015. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. *Current opinion in microbiology*, 27(10):1-9.
- Brink, H., van der Walt, C. & van Rensburg, G., eds. 2012. Fundamentals of research methodology for healthcare professionals. 3rd ed. Cape Town: Juta.
- Burton, M.E., Shaw, L.M., Schentag, J.J. & Evans, W.E. 2007. Applied pharmacokinetics and pharmacodynamics. 4th ed. Philadelphia, PA: Lippincott, Williams & Wilkins.
- Business Dictionary. 2017. Descriptive study. Businessdictionary.com/definition/descriptive-study.html Date of access: 27 Sep. 2017.
- Buxton, I.L.O. 2011. Pharmacokinetics and pharmacodynamics. (In Brunton, L.L., Lazo, J.S. & Parker, K.L., eds. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York, NY: McGraw-Hill. p. 17-40).
- Cotta, M.O., Roberts, J.A. & Lipman, J. 2015. Antibiotic dose optimization in critically ill patients. *Medicina intensiva*, 39(9):563-572.
- Croes, S., Koop, A.H., van Gils, S.A. & Neef, C. 2012. Efficacy, nephrotoxicity and ototoxicity of aminoglycosides, mathematically modelled for modelling-supported therapeutic drug monitoring. *European journal of pharmaceutical sciences*, 45(1-2):90-100.
- Dasta, J.F. & Armstrong, D.K. 1988. Variability in aminoglycoside pharmacokinetics in critically ill surgical patients. *Critical care medicine*, 16(4):327-330.

Delanaye, P., Cavalier, E., Mariat, C., Maillard, N. & Krzesinski, J.M. 2010. MDRD or CKD-EPI study equations for estimating prevalence of stage 3 CKD in epidemiological studies: which difference? Is this difference relevant?. *BioMedCentral nephrology*, 11(8):
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2891733/pdf/1471-2369-11-8.pdf>. Date of access: 13 Aug. 2018.

Denaro, C.P. & Ravenscroft, P.J. 1989. Comparison of Sawchuk-Zaske and Bayesian forecasting for aminoglycosides in seriously ill patients. *British journal of clinical pharmacology*, 28(1):37-44.

Department of Health **see** South Africa. Department of Health.

Dobie, R.A., Black, F.O., Pezsnecker, S.C. & Stallings, V.L. 2006. Hearing loss in patients with vestibulotoxic reactions to gentamicin therapy. *Archives of otolaryngology – head & neck surgery*, 13(3):253-257.

Drew, R. 2014. Aminoglycosides.
<https://hpm.healthsoc.co.za/contents/aminoglycosides?topicKey=ID%2f483&elapsedarticles>.
Date of access: 5 Jun. 2015.

Drusano, G.L. & Louie, A. 2011. Optimization of aminoglycoside therapy. *Antimicrobial agents and chemotherapy*, 55(6):2528-2531.

Durante-Mangoni, E., Grammatikos, A., Utili, R. & Falagas, M.E. 2009. Do we still need the aminoglycosides? *International journal of antimicrobial agents*, 33(3):201-205.

Edwards, J.R., Diamantakos, E.A., Peuler, J.D., Lamar, P.C. & Prozialeck, W.C. 2007. A novel method for evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney using ethidium homodimer. *BioMedCentral physiology*, 7(1).
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1810561/pdf/1472-6793-7-1.pdf>. Date of access: 14 Sep. 2017.

El Mouedden, M., Laurent, G., Mingot-Leclercq, M.P., Taper, H.S., Cumps, J. & Tulkens, P.M. 2000. Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. *Antimicrobial agents and chemotherapy*, 44(3):665-675.

Ford, C.W., Zurenko, G.E. & Barbagyn, M.R. 2001. The discovery of linezolid, the first oxazolidinone antibacterial agent. *Current drug targets. Infectious disorders*, 1(2):181-199.

Garraghan, F. & Fallon, R. 2015. Gentamicin: dose regimens and monitoring. *The pharmaceutical journal*. <http://www.pharmaceutical-journal.com/learning/learning-article/gentamicin-dose-regimens-and-monitoring/20069096.article> Date of access: 6 Aug. 2015.

Hanberger, H., Edlund, C., Furebring, M., Giske, C.G., Melhus, A., Nilsson, L.E., Petersson, J., Sjölin, J., Ternhag, A., Werner, M. & Eliasson, E. 2013. Rational use of aminoglycosides — review and recommendations by the Swedish Reference Group for Antibiotics (SRGA). *Scandinavian journal of infectious diseases*, 45(3):161-175.

Henderson, K.L., Muller-Pebody, B., Johnson, A.P., Sharland, M. & Gilbert, R. 2013. Community-acquired, healthcare-associated and hospital-acquired bloodstream infection definitions in children: a systematic review demonstrating inconsistent criteria. *Journal of hospital infections*, 85(2):94-105.

Hermann, T. 2005. Drugs targeting the ribosome. *Current opinion in structured biology*, 15(3):355-366.

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Jana, S. & Deb, J.K. 2006. Molecular understanding of aminoglycoside action and resistance. *Applied microbiology and biotechnology*, 70(2):140-150.

Jenkins, A., Thompson, A.H., Brown, N.M., Semple, Y., Sluman, C., MacGowan, A., Lovering, A.M. & Wiffen, P.J. 2016. Amikacin use and therapeutic drug monitoring in adults: do dose regimens and drug exposures affect either outcome or adverse events? A systematic review. *Journal of antimicrobial chemotherapy*, 71(10):2754-2759.

Johns Hopkins antibiotics guide.

https://www.hopkinsguides.com/hopkins/index/Johns_Hopkins_ABX_Guide/All_Topics/A. Date of access: 27 Mar. 2017.

Kang, J. & Lee, M. 2009. Overview of therapeutic drug monitoring. *The Korean journal of internal medicine*, 24(1):1-10.

Kohanski, M.A., Dwyer, D.J. & Collins, J.J. 2010. How antibiotics kill bacteria: from targets to networks. *Nature reviews microbiology*, 8(6):423-435.

- Kotra, L.P., Haddad, J. & Mobashery, S. 2000. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrobial agents and chemotherapy*, 44(12):3249-3256.
- Kovačević, T., Avram, S., Milaković, D., Špirić, N. & Kovačević, P. 2016. Therapeutic drug monitoring of amikacin and gentamicin in critically and noncritically ill patients. *Journal of basic and clinical pharmacy*, 7(3):65-69.
- Kushner, B., Allen, P.D. & Crane, B.T. 2016. Frequency and demographics of gentamicin use. *Otology & neurology*, 37(2):190-195.
- Laudano, J.B. 2011. Ceftaroline fosamil: a new broad-spectrum cephalosporin. *Journal of antimicrobial chemotherapy*, 66(Suppl. 3):iii11-iii18.
- LeBras, M., Chow, I., Mabasa, V.H. & Ensom, M.H.H. 2016. Systematic review of efficacy, pharmacokinetics, and administration of intraventricular aminoglycosides in adults. *Neurocritical Care Society*, 25(3):492-507.
- Leedy, P.D. & Omrod, J.E. 2016. Practical research: planning and design. 11th ed. Upper Saddle River, NJ: Pearson.
- Leong, C.L., Buising, K., Richards, M., Robertson, M. & Street, A. 2006. Providing guidelines and education is not enough: an audit of gentamicin use at The Royal Melbourne Hospital. *Internal medicine journal*, 36(1):37-42.
- Lerner, A., Reyes, M. & Cone, L. 1983. Randomised, controlled trial of the comparative efficacy, auditory toxicity and nephrotoxicity of tobramycin and netilmicin. *The lancet*, 1(8334):1123-1134.
- Levey, A.S., Stevens, L.A., Schmid, C.H., Zhang, Y.L., Castro, A.F. & Feldman, H.I. 2009. A new equation to estimate glomerular filtration rate. *Annals of internal medicine*, 150(9):604-612.
- Levine, D.P. 2006. Vancomycin: a history. *Clinical infectious diseases*, 42(Suppl. 1):S5-S12.
- Lewis, K. 2013. Platforms for antibiotic discovery. *Nature reviews drug discovery*, 12(5):371-387.

- Lopez-Novoa, J.M., Quiros, Y., Vincente, L., Morales, A.I. & Lopez-Hernandez, F.J. 2011. New insights into the mechanisms of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney journal*, 79(1):33-45.
- MacDougall, C. & Chambers, H.F. 2011. Aminoglycosides. (In Brunton, L.L., Lazo, J.S. & Parker, K.L., eds. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York, NY: McGraw-Hill. p. 1505-1520).
- Magnet, S. & Blanchard, J.S. 2005. Molecular insights into aminoglycoside action and resistance. *Chemical reviews*, 105(2):477-498.
- Mandell, G.I., Bennett, J.E. & Dolin, R. 2010. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 7th ed. Philadelphia, PA: Churchill Livingstone/Elsevier.
- Mann, C.J. 2012. Observational research methods — cohort studies, cross sectional studies, and case-control studies. *African journal of emergency medicine*, 2(1):38-46.
- Martin, J., Barras, M., Ah Yui, N., Kirkpatrick, C., Kubler, P. & Norris, R. 2012. Gentamicin monitoring practices in teaching hospitals: time to undertake the necessary randomised controlled trial. *Journal of clinical toxicology*, 2(8):1-5.
- McKinnon, P. & Davis, S. 2004. Pharmacokinetic and pharmacodynamics issues in the treatment of bacterial infectious diseases. *European journal of microbiology and infectious diseases*, 23(4):271-288.
- Mui, E. 2017. Stanford Health Care aminoglycoside dosing guideline. http://med.stanford.edu/bugsanddrugs/dosing-protocols/_jcr_content/main/panel_builder/panel_0/download_2/file.res/Aminoglycoside%20Dosing%20Guide%202017-08-23.pdf. Date of access: 15 Dec. 2017.
- Namazi, S., Sagheb, M.M., Hashempour, M.M. & Sadatsharifi, A. 2016. Usage pattern and serum level measurement of amikacin in the internal medicine ward of the largest referral hospital in the South of Iran: A pharmacoepidemiological study. *Iran journal of medical science*, 41(3):191-199.
- Nezic, L., Derungs, A., Bruggisser, M., Tschudin-Sutter, S., Krähenbühl, S. & Haschke, M. 2014. Therapeutic drug monitoring of once daily aminoglycoside dosing: comparison of two

methods and investigation of the optimal blood sampling strategy. *European journal of clinical pharmacology*, 70(7):829-837.

Nicolau, D.P., Freeman, C.D., Belliveau, P.P., Nightingale, C.H., Ross, J.W. & Quintiliani, R. 1995. Experience with a once-daily aminoglycoside program administered to 2184 adult patients. *Antimicrobial agents and chemotherapy*, 39(3):650-655.

Nwobodo, N. 2014. Therapeutic drug monitoring in a developing nation: a clinical guide. *Journal of the Royal Society of Medicine*, 5(8):1-5.

Papp-Wallace, K.M., Endimiani, A., Taracila, M.A. & Bonomo, R.A. 2011. Carbapenems: Past, present, and future. *Antimicrobial agents and chemotherapy*, 55(11):4943-4960.

Pea, F. & Viale, P. 2006. The antimicrobial therapy puzzle: Could pharmacokinetic-pharmacodynamic relationships be helpful in addressing the issue of appropriate pneumonia treatment in critically ill patients? *Clinical infectious diseases*, 42(12):1764-1771.

Peloquin, C.A., Berning, S.E., Nitta, A.T., Simone, P.M., Gobble, M., Huitt, G.A., Iseman, M.D., Cook, J.L. & Curran-Everett, D. 2004. Aminoglycoside toxicity: daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clinical infectious diseases*, 38(11):1538-1544.

Petersen, L. & Rogers, C. 2015. Aminoglycoside-induced hearing deficits: a review of cochlear toxicity. *South African family practice*, 57(2):77-82.

Petri, W.A. 2011a. Sulphonamides, trimethoprim-sulfamethoxazole, quinolones, and agents for urinary tract infections. (In Brunton, L.L., Lazo, J.S. & Parker, K.L., eds. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York, NY: McGraw-Hill. p. 1463-1476).

Petri, W.A. 2011b. Penicillins, cephalosporins, and other beta-lactam antibiotics. (In Brunton, L.L., Lazo, J.S. & Parker, K.L., eds. Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York, NY: McGraw-Hill. p. 1477-1503).

Pietersen, J. & Maree, K. 2014. Standardisation of a questionnaire. (In Maree, K., ed. First steps in research. Pretoria: Van Schaik. p. 216-224).

Prayle, A., Watson, A., Fortnum, H. & Smyth, A. 2010. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax*, 65(7):654-658.

- Radigan, E.A., Gilchrist, N.A. & Miller, M.A. 2010. Management of aminoglycosides in the intensive care unit. *Journal of intensive care medicine*, 25(6):327-342.
- Roberts, J.A., Norris, R., Paterson, D.L. & Martin, J.H. 2012. Therapeutic drug monitoring of antimicrobials. *British journal of clinical pharmacology*, 73(1):27-36.
- Rossiter, D., Blockman, M., Barnes, K.I., Chughlay, M., Cohen, K., Decloedt, E., de Waal, R., Gounden, R., Irhuma, M., Maartens, G., McIlleron, H., Pandie, M. & Sinxadi, P.Z. 2014. Antibacterials for systemic use. (In Rossiter, D., ed. South African Medicines Formulary. Cape Town: Health and Medical Publishing Group. p. 281-374).
- Rougier, F., Claude, D., Maurin, M. & Maire, P. 2004. Aminoglycoside nephrotoxicity. *Current drug targets*, 4(2):153-162.
- Sawchuk, R.J. & Zaske, D.E. 1976. Pharmacokinetics of dosing regimens which utilize multiple intravenous infusions: gentamicin in burn patients. *Journal of pharmacokinetic biopharmaceutics*, 4(2):183-195.
- Sensi, P. 1983. History of the development of rifampin. *Reviews of infectious diseases*, 5(Suppl. 3):S402-S406.
- Shakil, S., Khan, R., Zarrilli, R. & Khan, A.U. 2008. Aminoglycosides versus bacteria – a description of the action, resistance mechanism, and nosocomial battleground. *Journal of biomedical science*, 15(1):5-14.
- Smith, B.S., Yogaratnam, D., Levasseur-Franklin, K.E., Forni, A. & Fong, J. 2012. Introduction to drug pharmacokinetics in the critically ill patient. *Chest*, 141(5):1327-1336.
- South Africa. Department of Health. 2015. Hospital level adults. Standard treatment guidelines and essential medicines list for South Africa. 4th ed. Pretoria.
- Stankowicz, M., Ibrahim, J. & Brown, D.L. 2015. Once-daily aminoglycoside dosing: An update on current literature. *American journal of health-system pharmacists*, 72(16):1357-1364.
- Stewardson, A.J., Gaïa, N., Francois, P., Malhotra-Kumar, S., Delémont, C., Martinez de Tejada, B., Schrenzel, J., Harbarth, S. & Lazarevic, V. 2014. Colateral damage from oral ciprofloxacin versus nitrofurantoin in outpatients with urinary tract infections: a culture-free analysis of gut microbiota. *Clinical microbiology and infection*.

[http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X\(14\)00102-5/fulltext](http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(14)00102-5/fulltext). Date of access: 18 Sep. 2017.

Surrey and Sussex Healthcare NHS Trust. 2015. Amikacin prescribing guidelines. <https://www.surreyandsussex.nhs.uk/wp-content/uploads/2013/04/Amikacin-Therapeutic-drug-monitoring.pdf>. Date of access: 1 Nov. 2018.

Sweileh, W.M. 2009. A prospective comparative study of gentamicin and amikacin induces nephrotoxicity in patients with normal baseline renal function. *Fundamental & clinical pharmacology*, 23(4):515-520.

Tabah, A., De Waele, J., Lipman, J., Zahar, J.R., Cotta, M.O., Barton, G., Timsit, J.F. & Roberts, J.A. 2015. The ADMIN-ICU survey: a survey on antimicrobial dosing and monitoring in ICUs. *Journal of antimicrobial chemotherapy*, 70(9):2671-2677.

Taber, S.S. & Mueller, B.A. 2006. Drug-associated renal dysfunction. *Critical care clinics*, 22(2):357-374.

Tamma, P.D., Cosgrove, S.E. & Maragakis, L.L. 2012. Combination therapy for treatment of infections with gram-negative bacteria. *Clinical microbiology reviews*, 25(3):450-470.

The Free Dictionary. 2017. Cross-sectional study. Medical-dictionary.thefreedictionary.com/cross-sectional+study Date of access: 27 Sep. 2017.

Touw, D.J., Westerman, E.M. & Sprij, A.J. 2009. Therapeutic drug monitoring of aminoglycosides in neonates. *Clinical pharmacokinetics*, 48(2):71-88.

Twycross, A. & Shields, L. 2004b. Validity and reliability – what's it all about? Part 2 Reliability in quantitative studies. *Pediatric nursing*, 16(10):36.

Vakulenko, S.B. & Mobashery, S. 2003. Versatility of aminoglycosides and prospects for their future. *Clinical microbiological reviews*, 16(3):430-450.

Watanabe, A., Nagai, J., Adachi, Y., Katsube, T., Kitahara, Y., Murakami, T. & Takanz, M. 2004. Targeted prevention of renal accumulation and toxicity of gentamicin by aminoglycoside binding receptor antagonists. *Journal of controlled release*, 95(3):423-433.

WHO (World Health Organization). 2017. WHO model lists of essential medicines. www.who.int/medicines/publications/essentialmedicines/en/. Date of access: 26 Jul. 2018.

Wong, G., Sime, F.B., Lipman, J. & Roberts, J.A. 2014. How do we use therapeutic drug monitoring to improve outcomes from severe infections in critically ill patients? *BioMedCentral infectious diseases*, 14(288).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4289211/pdf/12879_2014_Article_4062.pdf.

Date of access: 18 May 2017.

Xie, J., Talaska, A.E. & Schacht, J. 2011. New developments in aminoglycoside therapy and ototoxicity. *Hearing research*, 281(1-2):28-37.

Zaffiri, L., Gardner, J. & Toledo-Pereyra, L.H. 2012. History of antibiotics. From Salvarsan to cephalosporins. *Journal of investigative surgery*, 25(2):67-77.

Zarrilli, R., Tripodi, M., Di Popolo, A., Fortunato, R., Bagattini, M., Crispino, M., Florio, A., Triassi, M. & Utili, R. 2005. Molecular epidemiology of high-level aminoglycoside-resistant enterococci isolated from patients in a university hospital in southern Italy. *The journal of antimicrobial chemotherapy*, 56(5):827-835.

ANNEXURE A: PERMISSION FROM CORPORATE OFFICE

18 November 2016

Ms M du Toit

E-mail:

Cc:

Dear Mariëtte

THERAPEUTIC DRUG MONITORING OF GENTAMYCIN AND AMIKACIN IN HOSPITALISED PATIENT IN A PRIVATE HOSPITAL IN THE WESTERN CAPE

Please be advised that [REDACTED] hereby acknowledges the change in research site from [REDACTED] to [REDACTED] and confirms approval of the above-mentioned research.

Yours sincerely,

CHIEF CLINICAL OFFICER

ANNEXURE B: PERMISSION FROM LABORATORY MANAGER

17 March 2017

RE: Consent to use hospital data

Using hospital data to retrospectively determine whether therapeutic drug monitoring is being done in the hospital on patients receiving intravenous gentamicin or amikacin.

Investigator: M du Toit (B.Pharm)

The purpose of this study is to determine whether therapeutic drug monitoring is being done in our hospital. Gentamicin and amikacin can cause kidney damage and hearing loss if levels are not monitored correctly.

Data will be used from the hospital AS400, as well as information from the [redacted] laboratory user website.

The potential benefit from the study is that future policies and procedures may be changed to ensure that correct therapeutic drug monitoring will be done if it is found that currently this is not the case.

As only retrospective data will be used, there will not be any risk or discomfort to the patients. All data will be captured in a password protected excel spreadsheet and no patient details will be captured. The name of the prescribing doctor or hospital will not be mentioned in the research results.

If you have any questions about the study you are welcome to contact me at [redacted]

I [redacted] hereby give consent for the researcher to use data from [redacted] website for the research.

Signature

Date

20 March 2017

ANNEXURE C: PERMISSION FROM HOSPITAL MANAGER



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

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South Africa 2520

Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

Clinical Pharmacy
Tel: 018 2992364
Fax: 018
Email: dorcas.rekumakoe@nwu.ac.za

27 October 2016

Dear [REDACTED]

Permission to use data

As you are aware, I am currently enrolled for the MPharm in Advanced Clinical Pharmacy, and as part of the degree it will be required that I complete a research project.

I would appreciate it if you could give me permission to do my research in the hospital. The topic of my research project is *"Therapeutic drug monitoring of Gentamicin and Amikacin in hospitalised patients in a private hospital in the Western Cape."* Gentamicin and Amikacin are antibiotics used to treat infections and when administered in high doses, these drugs can cause renal toxicity and hearing loss. It is therefore of utmost importance that the levels of these antibiotics are monitored correctly. I plan to determine whether doctors measure these levels and adjust dosages accordingly. I will not have any direct contact with patients, as I will only use retrospective data from patient files.

All files of patients who received either one of these antibiotics will be collected from the archive facility. All research will be done outside of working hours and files will be sent back to the archive facility as soon as I am done collecting the data. I will give feedback to you and the Pharmacy Manager as soon as my research project is completed.

I will not use the name of the hospital in my research, so confidentiality of the facility and the patients is guaranteed.

I believe that future patients will indirectly benefit from this research, as protocols can be adjusted if found that the correct practices are not followed.

If you have any questions, please do not hesitate to contact me.

Regards

Mariette du Toit

Consent form for research to be done in the facility

I, C. [REDACTED] P.R., herewith give permission to the researcher, Mariëtte du Toit, to conduct research regarding therapeutic drug monitoring of Gentamicin and Amikacin in Mediclinic Durbanville, a 221-bed private hospital in the Western Cape Province.

Please note that this approval is subject to approval from the North West University Human Research Ethics Committee (NWU HREC).

[REDACTED]

31/1/17
Date

[Signature]
Mariëtte du Toit
Pharmacist

[REDACTED]

Dr DM Rakumakoe
Senior Lecturer

ANNEXURE D: PERMISSION FROM PHARMACY MANAGER



NORTH-WEST UNIVERSITY
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Web: <http://www.nwu.ac.za>

Clinical Pharmacy
Tel: 018 2992364
Fax: 018
Email: dorcas.raikumakoe@nwu.ac.za

28 December 2016



Permission to use data

As you are aware, I am currently enrolled for the MPharm in Advanced Clinical Pharmacy, and as part of the degree it will be required that I complete a research project.

I would appreciate it if you could give me permission to do my research in the hospital. The topic of my research project is *"Therapeutic drug monitoring of Gentamicin and Amikacin in hospitalised patients in a private hospital in the Western Cape."* Gentamicin and Amikacin are antibiotics used to treat infections and when administered in high doses, these drugs can cause renal toxicity and hearing loss. It is therefore of utmost importance that the levels of these antibiotics are monitored correctly. I plan to determine whether doctors measure these levels and adjust dosages accordingly. I will not have any direct contact with patients, as I will only use retrospective data from patient files.

All files of patients who received either one of these antibiotics will be collected from the archive facility. All research will be done outside of working hours and files will be sent back to the archive facility as soon as I am done collecting the data. I will give feedback to you and the Pharmacy Manager as soon as my research project is completed.

I will not use the name of the hospital in my research, so confidentiality of the facility and the patients is guaranteed.

I believe that future patients will indirectly benefit from this research, as protocols can be adjusted if found that the correct practices are not followed.

If you have any questions, please do not hesitate to contact me.

Regards

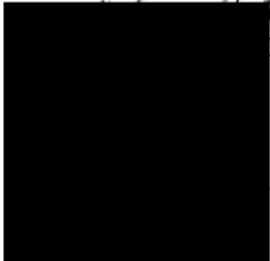
Marlétte du Toit

Consent form for research to be done in the facility

I,  S, herewith give permission to the researcher, Mariette du Toit, to conduct research regarding therapeutic drug monitoring of Gentamicin and Amikacin in Mediclinic Durbanville, a 221-bed private hospital in the Western Cape Province.

Please note that this approval is subject to approval from the North West University Human Research Ethics Committee (NWU HREC).

29 Dec 2016
Date


Yours sincerely

Dr DM Rakumakoe
Senior Lecturer

ANNEXURE E: ETHICS APPROVAL



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

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South Africa, 2520

Tel: (018) 299-4900

Faks: (018) 299-4910

Web: <http://www.nwu.ac.za>

Institutional Research Ethics Regulatory Committee

Tel: +27 18 299 4849

Email: Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by Health Research Ethics Committee (HREC) on 13/10/2016 after being reviewed at the meeting held on 19/11/2015, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Therapeutic drug monitoring of gentamicin and amikacin in hospitalised patients in a private hospital in the Western Cape.									
Study Leader/Supervisor: Dr DM Rakumakoe									
Student: M du Plessis									
Ethics number:									
N	W	U	-	0	0	3	6	3	-
Institution			Study Number				Year		Status
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation									
Application Type: Single study									
Commencement date: 2016-10-13									
Risk: Minimal									
Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.									

Special conditions of the approval (if applicable):

- Translation of the Informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
 - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviation from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
 - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via Ethics-HRECAppl@nwu.ac.za or 018 299 1206.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA
Du Plessis

Digitally signed by
Prof LA Du Plessis
Date: 2016.10.27
08:22:20 +02'00'

Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

ANNEXURE F: CONFIDENTIALITY AGREEMENT



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

CONFIDENTIALITY UNDERTAKING

entered into between:

I, the undersigned

Prof./Dr./Mr./Ms.

Identity Number:

Address:

hereby undertake in favor of the **NORTH-WEST UNIVERSITY**, a public higher education institution established in terms of the Higher Education Act No. 101 of 1997

Address: Office of the Institutional Registrar, Building Cf, 53 Borchard Street,
Potchefstroom, 2520

(hereinafter the "NWU")

1 Interpretation and definitions

1.1 In this undertaking, unless inconsistent with, or otherwise indicated by the context:

1.1.1 "Confidential Information" shall include all information that is confidential in its nature or marked as confidential and shall include any existing and new information obtained by me after the Commencement Date, including but not be limited in its interpretation to, research data, information concerning research participants, all secret knowledge, technical information and specifications, manufacturing techniques, designs, diagrams, instruction manuals, blueprints, electronic artwork, samples, devices, demonstrations, formulae, know-how, intellectual property, information concerning materials, marketing and business information generally, financial information that may include remuneration detail, pay slips, information relating to human capital and employment contract, employment conditions, ledgers, income and expenditures and other materials of whatever description in which the NWU has an interest in being kept confidential; and

1.1.2 "Commencement Date" means the date of signature of this undertaking by myself.

1.2 The headings of clauses are intended for convenience only and shall not affect the interpretation of this undertaking.

2 Preamble

2.1 In performing certain duties requested by the NWU, I will have access to certain Confidential Information provided by the NWU in order to perform the said duties and I agree that it must be kept confidential.

2.2 The NWU has agreed to disclose certain of this Confidential Information and other information to me subject to me agreeing to the terms of confidentiality set out herein.

3 Title to the Confidential Information

I hereby acknowledge that all right, title and interest in and to the Confidential Information vests in the NWU and that I will have no claim of any nature in and to the Confidential Information.

4 Period of confidentiality

The provisions of this undertaking shall begin on the Commencement Date and remain in force indefinitely.

5 Non-disclosure and undertakings

I undertake:

5.1 to maintain the confidentiality of any Confidential Information to which I shall be allowed access by the NWU, whether before or after the Commencement Date of this undertaking. I will not divulge or permit to be divulged to any person any aspect of such Confidential Information otherwise than may be allowed in terms of this undertaking;

5.2 to take all such steps as may be necessary to prevent the Confidential Information falling into the hands of an unauthorised third party;

5.3 not to make use of any of the Confidential Information in the development, manufacture, marketing and/or sale of any goods;

5.4 not to use any research data for publication purposes;

5.5 not to use or disclose or attempt to use or disclose the Confidential Information for any purpose other than performing research purposes only and includes questionnaires, interviews with participants, data gathering, data analysis and personal information of participants/research subjects;

5.6 not to use or attempt to use the Confidential Information in any manner which will cause or be likely to cause injury or loss to a research participant or the NWU; and

5.7 that all documentation furnished to me by the NWU pursuant to this undertaking will remain the property of the NWU and upon the request of the NWU will be returned to the NWU. I shall not make copies of any such documentation without the prior written consent of the NWU.

6 Exception

The above undertakings by myself shall not apply to Confidential Information which I am compelled to disclose in terms of a court order.

7 Jurisdiction

This undertaking shall be governed by South African law be subject to the jurisdiction of South African courts in respect of any dispute flowing from this undertaking.

8 Whole agreement

8.1 This document constitutes the whole of this undertaking to the exclusion of all else.

8.2 No amendment, alteration, addition, variation or consensual cancellation of this undertaking will be valid unless in writing and signed by me and the NWU.

Dated at Potchefstroom this 28th DECEMBER 2016

Witnesses:  

1 ...

2 ...



ANNEXURE G: GHANA MEDICAL JOURNAL GUIDELINES

Ghana Medical Journal

The Journal of the Ghana Medical Association

- [Home](#)
- [Current Issue](#)
- [Supplement](#)
- [Past Issues](#)
- [Author Guidelines](#)
- [Editorial Board](#)
- [Archives](#)
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GUIDELINES FOR AUTHORS

Contributions to the Ghana Medical Journal should be in English only

All submissions to the Ghana Medical Journal shall be through its online submission and review system at <https://mc.manuscriptcentral.com/gma-gmj>

1.0 CATEGORIES OF ARTICLES

The journal will consider manuscripts of the following categories for publication.

1.1 Original Research Article

Works publishable under this section include original work of suitable standard. Such work must be innovative or contribute further to well-established knowledge in a particular field. Manuscripts on all the medical specialties including the basic sciences, para-clinical and clinical sciences will be considered. Short or preliminary report on original works will be published under this section.

1.2 Special Article

Review articles, manuscripts on special medical events, clinical notes and clinical investigation will be accepted for publication under this section. Review articles should cite original works that lead to formulation of a concept, theory or hypothesis. Review articles that seek to draw attention to current medical practice must have ample support in the form of published observations by other authors as well as the author's own findings.

1.3 Case Report

Extremely rare clinical syndromes or presentations will be accepted for publication under this section. Also a collection of cases highlighting particular trends or problems in clinical practice are acceptable. In both instances, contributors are advised to give ample evidence in support of their claims.

1.4 Correspondence

Correspondence on articles published in the journal or letter to the editor shall be entertained. Such correspondence must reach the editor not more than 3 months after publication of an article. The correspondence may seek further clarification on a published article. In both cases the author(s) whose article has attracted correspondence from readers will be contacted for their comments and both comments and correspondence on published articles will be published together. Letters to the editor are welcome at all times.

Apart from correspondence and invited editorials, all submissions will be subjected to peer review.

2.0 LENGTH OF ARTICLES

The high cost of printing requires that manuscripts are not unduly lengthy. They must be concise.

2.1 Original Research Articles

Including text, figures, tables and references should occupy not more than the space for maximum of 6000 words including tables and illustrations. Short or preliminary reports should not exceed 1500 words.

2.2 Special Articles

Review articles should not exceed 7000 words including tables and illustrations. Manuscripts on special medical events should not exceed 1500 words including figures, tables and references.

2.3 Case Report

Case reports should not exceed 2500 words including figures tables and references.

3.0 PREPARATION OF MANUSCRIPT

Manuscripts should be prepared in accordance with the current edition of the *Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals* (ICMJE Recommendations) at <http://www.icmje.org/recommendations/>.

Guidelines on tables and figures, units and abbreviations, are also to be seen in the above references. All papers should be written using Microsoft Word with .doc or docx extension in A4 format with left right, top and bottom margins of 2.5cm.

3.1 Format

Original research articles, including short reports, must have the following parts: Title, Name(s) of Author(s), Address of author(s), Running title, Summary, Keywords, Source of funding for the work, Introduction, Materials/subjects and Methods, Data analysis/calculations, Results, Discussion, Conclusion, Acknowledgements and References.

Case reports and review articles or special articles on medical events need not comply with this format.

The title, name(s) of author(s), address (es) of author(s), address for correspondence, running title and conflict of interest statement of all types of manuscript should be submitted as a separate file.

3.2 Title

The title of each manuscript should not have more than 20 words, or 100 characters, and should express clearly, the aims of the work.

3.3 Names of Authors, Academic Qualifications, Title.

The name of the principal author should appear first, followed by other authors. The names of the authors should be stated in the format [First name] [Middle name initial] and [Surname]. The name and address of the author for correspondence must be indicated on the page for author name(s) and address (es). This information should be in a separate file and uploaded as Supplementary file during submission of the manuscript.

3.4 Summary

The summary should contain not more than 250 words and must be structured. The Summary should state: **Objectives:** This should provide a clear statement of the main aim of the study and the major hypothesis or research question asked

Design: Describe the study design (observational or analytical) indicating such features as pre-post, retrospective, randomization, placebo controlled, case control etc;

Setting: include the level of health of care, clinical department, community or groups; number of participating centres

Participants: Who, how selected, what entry and exclusion criteria, how many entering and completing the study

Interventions: What, how, for how long

Main outcome measures: Those planned in protocol, those finally measured (if different, explain why);

Results: Main results with levels of significance and 95% confidence intervals as appropriate; and

Conclusions: Primary conclusions and their implications, suggest areas for further research if appropriate.

3.5 Keywords

These should include words that emphasize the theme or central point of the research. Keywords should as far as possible be selected from the Medical Subject Heading (MeSH) list of Medline. A maximum of five (5) keywords should be listed.

3.6 Running Title

Where the title of the manuscript is lengthy, the running title may take a shortened form to reflect the main objectives of the paper. However, no running title is required if the title is short, for instance, not exceeding four words. The running title should not have more than 5 words or 30 characters arranged one after the other. A hyphen is counted as one character.

3.7 References

The number of references should be kept to a minimum. They should be numbered consecutively as they occur in the text. Identify references in the text, tables and legends by Arabic numerals placed in superscript. Where the citation is at the end of a sentence it should be placed after the 'full stop'.

The title of journals should be abbreviated according to the style used in Medline.

Reference should be based on the ICEMJ recommendations. The title of journals and books in the listed references should be in italics. The references should be listed in the order in which they appear in the text.

4.0 RESEARCH INTEGRITY

4.1 Ethical Clearance

Clinical studies are expected to conform to the Proposed International Guidelines for Biomedical Research involving Human Subjects issued by CIOMS, (Geneva 1982). Statements about ethical clearance (if appropriate) and obtaining participants' informed consent should be included in the manuscript. Experimental animals must be properly anaesthetized to avoid suffering and anaesthetic procedure fully explained in the text. Authors are required to provide evidence of ethical approval for their study.

Authors who do not comply with the said code of ethics both for humans and animals will have their manuscripts rejected.

4.2 Originality of Articles

Manuscripts submitted to the Ghana Medical Journal must not have been submitted for publication in another journal. The laboratory or institute of origin of research and the role of each author in the case of multiple authorship, must be indicated. Manuscripts must be accompanied by a written declaration that the materials have not been submitted for publication either in part or in full, in another journal. Such declaration may be included in the Submission letter.

The Ghana Medical Journal subscribes to the recommendations of the Committee on Publication Ethics (COPE) recommendations on publication misconduct including plagiarism.

4.3 Conflict of Interest

Authors and guest editors must disclose specific information relating to any financial relationship they may have with a sponsoring organization and any interest that organization presents, as well as with any for-profit product discussed or implied in the text of the manuscript. Helpful guidelines related to disclosure statements have been published by the International Committee of Medical Journal Editors (see <http://www.icmje.org/#conflicts>).

5.0 GENERAL INFORMATION

5.1 All correspondence should be addressed to the Editor-in-chief, Ghana Medical Journal, P. O. Box 1596, Accra, Ghana. Send e-mail correspondence to editor@ghanamedj.org

5.2 Peer Review

The journal uses blind peer-review and manuscripts should be anonymized for this purpose. Authors

should submit the full manuscript devoid of authors names, institutional affiliations and acknowledgement. There should also be no headers or footers. This document should be uploaded as the Main document/file.

A second file containing Title, author information and affiliation, running and conflict of interest statement title should be uploaded as Supplementary file.

Manuscripts that do not conform to the requirements of this journal shall be returned to the author(s) within two weeks from the date of submission. Returned manuscripts may be accepted for consideration if they are modified to an acceptable form.

Manuscripts are subject to peer-review and/or editorial revision to clarify them. As a general rule each submission is sent to two experts in the subject area of the manuscript for peer review. Additional reviewers may be used if the peer review decisions are not in agreement. Manuscripts may also be sent to statisticians for the review of that aspect of the study. The Editor-in-Chief makes the final decision on all manuscripts guided by the peer reviewers and the Editors. Where authors disagree with a review decision the Editor-in-Chief will have the decisions reviewed by another expert. The Editor-in-Chief makes the final decision.

5.3 Cover Letter or Submission Letter

The letter submitting the manuscript to the Editor-in-Chief should be on institutional letterhead; and signed by ALL authors.

5.4 Reprints

There shall be no reprints as published articles may be freely downloaded from the journal website.

5.5 Frequency of Publishing

The Ghana Medical Journal is published quarterly online and in print.

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
2. The submission file is in Microsoft Word document file format.
3. Where available, URLs for the references have been provided.
4. The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
5. The text adheres to the stylistic and bibliographic requirements outlined in the [Author Guidelines](#), which is found in About the Journal.
6. If submitting to a peer-reviewed section of the journal, the instructions in [Ensuring a Blind Review](#) have been followed.
7. **Author names** should be First Name Middle initial Surname (John B. Doe). Do not add titles and qualifications
8. Give full **institutional address** of each author
9. Provide information on **Funding** for the study
10. Make sure **Keywords** are consistent with Medline MeSH expressions
11. **Research Ethics Approval** All manuscript should indicate the name, institution and reference number of ethical approvals obtained on the study protocol. In the online submission process one of the checklist items is to indicate that ethical approval was obtained for the research.
12. Check **citations** in the text (Arabic numeral in superscript) 1 and make sure that citations at the end of a sentence are placed after the full stop NOT before it. They should NOT be in parenthesis (), [] or underlined. More than one citation should be separated by commas (,) like this^{1,2} If bibliographic software is used to generate the list of references make sure they are not placed as

footnotes or endnotes.

13. Check that the **list of references** is consistent with the citations and that the Journal names are in italics and as abbreviated as in Medline. The format is: Authors Title *Journal Name* Year, Volume (issue): pages
14. Internet references should indicate date of accession.
15. References to **unpublished communications** should be kept in the text and not listed in the list of references.
16. **Tables** should contain information relevant to findings and not a long list frequency data. Tables should be constructed using the Microsoft Word Table format in columns and rows. Data should not be separated with Tabs or spaces.
17. **Figures** should be clearly labeled and with appropriate caption. Pie charts containing less than 4 variables should be reported in words in the text. Avoid putting legend/title to Table in the first row of the Table. Give Figures separate legends. Do NOT include the legend or title in the figure.
18. Clinical and radiological images should be data size-minimized and presented in the JPEG format. They should be clear and sharp.

Open Access

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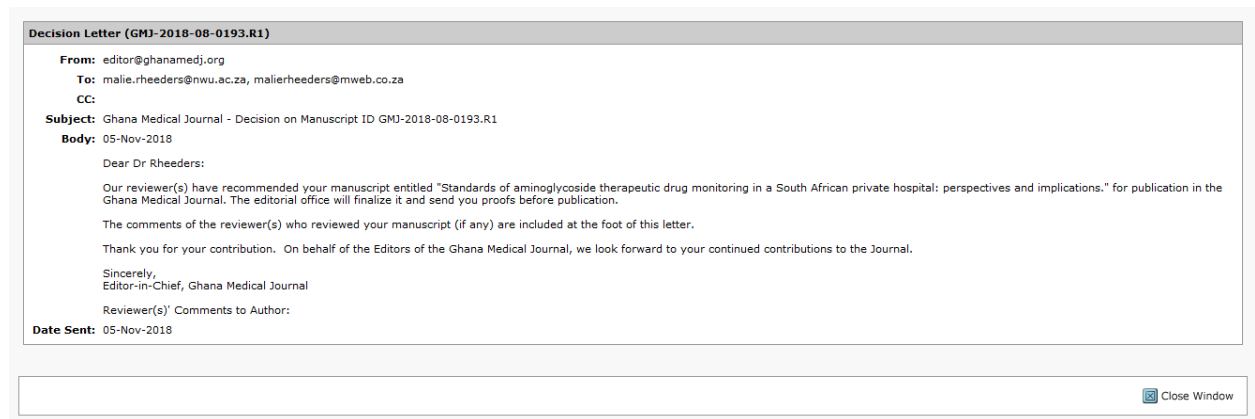
Publication Charges

Currently the journal does not charge manuscript processing or publication fees.

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ANNEXURE H: APPROVAL LETTER FROM GHANA MEDICAL JOURNAL



ANNEXURE I: DATA COLLECTION TOOL

Number	Weight	Age	Gender	Dosage/time	Time of day	Lab (TDM)	Results (Range)	New dose	eGFR	MDRD
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ANNEXURE J: LANGUAGE EDITING LETTER

Gill Smithies

Proofreading & Language Editing Services

59, Lewis Drive, Amanzimtoti, 4126, Kwazulu Natal

Cell: 071 352 5410 E-mail: moramist@vodamail.co.za

Work Certificate

To	Mariette du Toit
Address	Centre of Excellence for Pharmaceutical Studies, Division of Pharmaceutics, Potchefstroom Campus, North West University
Date	08/11/2018
Subject	Therapeutic drug monitoring of gentamicin and amikacin in hospitalised patients in a private hospital in Western Cape.
Ref	GS/MdT /01

I, Gill Smithies, certify that I have proofed the following,
Abstract, Preface and Chapters 1,2,3 & 4
to the standard as required by NWU, Potchefstroom Campus.

Gill Smithies

08/11/2018

ANNEXURE K: TECHNICAL EDITING LETTER

WHOM IT MAY CONCERN

I hereby declare that I have done the technical editing of the mini-dissertation with the title:

**Therapeutic drug monitoring of gentamicin and amikacin in hospitalised patients in a
private hospital, Western Cape**

by

M du Toit

20282095

Technical editing includes all tables, figures as well as the layout of the text.



E Oosthuizen

November 2018