Genetic and Pharmacokinetics Factors associated with Susceptibility to Kanamycin Induced Cochleotoxicity in a Cohort of Patients Undergoing MDR/RR-TB Treatment

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List of Abbreviations

Abbriviation	Definition
µg	Micrograms
µl	Microlitre
A	Adenine
ABR	Auditory Brainstem Response
ART	Acoustic reflexes threshold
ASHA	American Speech-Language-Hearing Association
ASSR	Auditory Steady State Response
ATP	Adenosine triphosphate
BCH	Brooklyn Chest Hospital
BDQ	Bedaquiline
Bp	Base pairs
C	Cytosine
CTCAE	Common Terminology Criteria for Adverse Events
CFZ	Clofazimine
CrCl	Creatinine Clearance
dB	Decibels
DCP	Department of Clinical Pharmacology
DPM	DP Marais Hospital
DPOAE	Distortion product atoacoustic emissions
DNA	Deoxyribonucleic acid
DoH	Department of Health
E.coli	Escherichia coli
f_1	Lower frequency of the pair of eliciting stimuli
f_2	Higher frequency primary
FDA	Food and Drug Administration
G	Guanine
H strand	Heavy strand
HFA	High Frequency Audiometry
HPCSA	Health Professions Council of South Africa
HREC	Human Research Ethics Committee
HIV/AIDS	Human Immune Deficiency Virus/Acquired Immune Deficiency Syndrome

Hz	Hertz
INH	Isoniazid
kHz	Kilohertz
L strand	Light strand
LC-MS	Liquid Chromatography-Mass Spectrometer
LFX	Levofloxacin
LZD	Linezolid
MAF	The minor allele frequency
MDR-TB	Multi-drug resistant tuberculosis
MET	Mechanoelectrical Transducer
Mg	Milligram
mRNA	Messenger Ribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
MT-RNR1	Mitochondrially encoded 12S RNA gene
MT-TS1	Mitochondrially encoded tRNA serine 1 (UCN) gene
NCR	Non-conding region
ROS	Reactive Oxygen Species
RR-TB	Rifampicin-resistant tuberculosis
rRNA	Ribosomal Ribonucleic acid
OAE	Otoacoustic emission
O_H	Non-coding control region
OHC	Outer hair cell
O_L	Origin of replication
OXPHOS	Oxidative phosphorylation
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics
PK	Pharmacokinetics
SNB	Single Nucleotide Polymorphism
SNHL	Sensorineural hearing loss
SRO	Sensitive range for ototoxicity
STS	Significant threshold shift
T	Thymine

TB	Tuberculosis
TDM	Therapeutic Drug Monitoring
TRD	Terizidone
TRP	Transient receptor potential
TEOAE	Transient Evoked Otoacoustic Emissions
tRNA	Transfer Ribonucleic Acid
UCT	University of Cape Town
UHF	Ultra-high Frequency
UHFA	Ultra-high Frequency Audiometry
Vd	Volume of distribution
WHO	World Health Organisation
XDR TB	Extremely Drug-Resistant Tuberculosis

Glossary of Terms

AUC: the **area under the curve** (**AUC**) is the area under a concentration versus time graph that describes the variation of a drug concentration in blood plasma as a function of time (Rowe, 2012).

Audiometry (pure tone): the evaluation and measurement (via testing) of hearing acuity across variations in sound intensity and pitch (American Speech-Language Hearing Association (ASHA, 2005).

ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/): a free available public archive of reports of the relationships among human variations and phenotypes hosted by the National Center for Biotechnology Information (NCBI) and funded by intramural National Institutes of Health (NIH) funding (Landrum et al., 2014).

Cochleotoxicity: hearing change from a baseline audiogram as a side effect of a drug (Ariano, Zelenitsky, & Kassum, 2008).

Idiosyncratic: idiosyncratic drug reactions are adverse effects that cannot be explained by the known mechanisms of action of the offending agent, are unrelated to the dose of the drug, and develop mostly unpredictably in only susceptible individuals (Uetrecht & Naisbitt, 2013).

The minor allele frequency (MAF): MAF is the frequency at which the second most common allele occurs in a given population. They play a surprising role in heritability since MAF variants, which occur only once, known as "singletons", drive an enormous amount of selection. MAF is widely used in population genetics studies because it provides information to differentiate between common and rare variants in the population (Hernandez et al., 2019; Sidore et al., 2015).

Mendelian pattern of inheritance: an inheritance pattern that follows the laws of segregation and independent assortment in which a gene inherited from either parent segregates into gametes at an equal frequency (Harel et al, 2015).

Multidrug resistance (MDR): resistance to at least both isoniazid and rifampicin (WHO, 2013).

MT-ND3: a gene of the mitochondrial genome coding for the NADH dehydrogenase 3 (ND3) protein (NIH, a2022)

MT-CYB*:* a gene of the mitochondrial genome coding for the cytochrome b protein (NIH, b2022)

Non-mendelian pattern of inheritance: any pattern of inheritance in which traits do not segregate in accordance with Mendel's laws. These laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus (Heyningen & Yeyati, 2004).

Pre–XDR TB: TB with resistance to isoniazid and rifampin and either a fluoroquinolone or second-line injectable agent but not both (WHO, 2021a).

Rate-limiting step: the slowest step in a metabolic pathway or series of chemical reactions, which determines the speed (rate) of chemical reactions in the pathway (Murdoch, 1981).

Rifampicin resistance (RR): resistance to rifampicin detected using phenotypic or genotypic methods, with of without resistance to other anti-TB drugs. It includes any resistance to rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR (WHO, 2013).

Single Nucleotide Polymorphism (http://www.ncbi.nlm.nih.gov/SNP): database (dbSNP): A free public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI) (Smigielski, Sirotkin, Ward, & Sherry, 2000).

Synonymous SNPs: synonymous SNPs are those SNPs that have different alleles that encode for the same amino acid (Srivastav, 2019).

Therapeutic Drug Monitoring (TDM): TDM is the clinical practice of measuring particular drugs at chosen periods to maintain a persistent concentration in a patient's bloodstream. TDM optimises individual dosage regimens (Kang & Lee, 2009).

Unipro UGENE: A multiplatform open-source software to manage, analyse and visualize the data (Okonechnikov et al., 2012).

Wild type gene: A gene when it is found in its natural, non-mutated form as it occurs in nature. Originally, the wild type was conceptualized as a product of the standard "normal" allele at a locus, in contrast to that produced by a non-standard, “mutant” allele (“Encyclopedia Britannica,” 2010).

XDR TB: TB with resistance to at least isoniazid, rifampin, a fluoroquinolone, and 1 of 3 injectable second-line drugs (amikacin, kanamycin, or capreomycin) (WHO, 2013).

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Declaration

I, Nazanin Ghafari, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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Abstract

South Africa is one of the countries with a high incidence of multidrug-resistant tuberculosis (MDR-TB) and rifampicin resistance tuberculosis (RR-TB). The standard MDR/RR-TB regimen prescribed in South Africa, at the time of the present study included Kanamycin, an aminoglycoside with a known cochleotoxic effect. Although kanamycin has recently been removed from the WHO MDR/RR-TB regimen, amikacin, another aminoglycoside derived from kanamycin, with similar structure and cochleotoxic side effects, has remained as part of the regimen for MDR/RR-TB patients with limited treatment options. In addition, some countries (e.g. India and Nigeria) have not completely removed kanamycin from their treatment regimen for MDR/RR-TB. Research has shown that genetic factors and factors affecting the pharmacokinetic of the drug could potentially be useful in identifying those who may be at a higher risk of aminoglycoside-induced cochleotoxicity. However, not much is known about the pharmacokinetics of Kanamycin and there is currently limited research available on the role of mutations involved in aminoglycoside-induced cochleotoxicity in South Africa. Therefore, this study aimed to determine: (1) the incidence of cochleotoxicity in MDR/RR-TB patients who are receiving kanamycin, (2) the pharmacokinetic properties of kanamycin that are associated with increased risk of cochleotoxicity, and (3) the association between participant's susceptibility to develop cochleotoxicity and two potentially pathogenic mitochondrial mutations (*T15312C* (I189T in MT-CYB) and *T10114C* (I19T in MT-ND3)).

The current study used a prospective cohort design. A total of 102 patients (median age was 34.9 years) on kanamycin-based MDR/RR-TB treatment participated in this study. The study site was the Metro Tuberculosis Hospital Centre, Cape Town. The majority of

the participants were males (*n* = 58, 56.9%,). Sixty five (63.7%) participants were HIV-positive, and 24 (23.5%) had been treated for MDR/RR-TB previously. Participants' hearing thresholds (0.25 to16kHz) were prospectively monitored for cochleotoxicity at the start of their treatment (baseline), and at 4, 8 and 12 weeks. The American Speech-Language- Hearing Association criteria (ASHA, 1994) were used to identify significant threshold shift (STS). Kanamycin concentrations were determined using liquid chromatography tandem mass spectrometry (LC-MS/MS), at steady-state in serial plasma samples over 10 hours. The *T15312C* (I189T in *MT-CYB*) and *T10114C* (I19T in *MT-ND3*) mutations was detected using PCR, ABI PRISM® 3130*xl* Genetic Analyser and UniPro UGene.

The results of the study revealed 82% (n = 84) of participants developed cochleotoxicity. The duration of treatment with kanamycin was associated with cochleotoxicity with a 120% and 220% increase in incidence of cochleotoxicity from week four of treatment to week eight and week 12 of treatment, respectively. Kanamycin exposure was significantly associated with cochleotoxicity with about 3% increased risk of hearing loss for every 10µg•hr/L increase in kanamycin $AUC_{0\text{-}10}$. The statistical analysis of the relationship between cochleotoxicity and two potentially pathogenic mutations, *T15312C* and *T10114C,* was not possible due to the low frequency of these mutations in the sample size. However, *T15312C* and *T10114C* were detected in 4.5% and 6%, respectively. Based on the MAF cut-off of 0.01 (1%), they are considered as common mutations. In addition, as *T15312C* and *T10114C* were just detected among participants who developed cochleotoxicity and not those who did not, they may be potentially pathogenic. However, since the presence of the known mutations associated with aminoglycoside-induced hearing loss in participants who carry *T15312C* and *T10114C* mutations had not been

investigated, it was not possible to draw a definite conclusion about the pathogenicity of *T15312C* and *T10114C*.

The results of the current study indicate that: (1) a high incidence of cochleotoxicity was detected among MDR/RR-TB patients receiving kanamycin, (2) the longer duration of treatment with kanamycin was associated with higher risk of cochleotoxicity, (3) higher Kanamycin $AUC_{0\text{-}10}$ was strongly associated with an increased incidence of cochleotoxicity, and (4) the *T15312C* and *T10114C* were common mutations in South African MDR/RR-TB patients who participated in this study and they may be potentially pathogenic for cochleotoxicity, and that should be assessed in future studies.

This study recommends that aminoglycoside-sparing regimens should be used for MDR/RR-TB patients. A routine ototoxic monitoring programme (at least once a month) including ultra-high frequency audiometry should be implemented for MDR/RR-TB patients who receive aminoglycosides, from the time of ototoxic drug exposure until six months post treatment. Therapeutic drug monitoring should be implemented for all the MDR/RR-TB patients on aminoglycosides and AUC value should be used for clinical decision making to reduce the risk of cochleotoxicity. Screening for the known mutations that contribute to the risk of cochleotoxicity, prior to the start of aminoglycoside therapy is recommended to lower the incidence of aminoglycoside induced hearing loss, especially in countries such as South Africa with a high incidence of MDR/RR-TB.

Chapter 1: Background and Rationale

This chapter presents the background and rational to this study. The chapter will set the scene by presenting the burden of TB and MDR/RR-TB both globally and in South Africa. The chapter also highlights the link between MDR/RR-TB treatment and cochleotoxicity.

1.1. Background

Tuberculosis (TB) still is one of the world's top infectious killers. In 2020, it is estimated that about 10 million people contracted TB and officially 1.3 million died from this disease globally (WHO, 2021a). According to estimates about 2 billion people, a quarter of world's population have a latent TB infection and hence are at risk of developing this disease (WHO, 2021a). South Africa is one of the countries with high number of new cases of TB after India, China, Indonesia the Philippines, Pakistan, Nigeria and Bangladesh (WHO, 2021a). In 2019, 58 000 people died of TB in South Africa and about 360 000 people fell ill with this disease which shows a 20% increase over WHO report in 2018 (WHO, 2020).

Individuals who are HIV positive have a much higher risk of developing TB (15-22 times higher than HIV negative individuals) and TB remains the main cause of death among HIV positive individuals (WHO, 2020). It was estimated that in 2020, TB was the cause of death of 214 000 HIV-positive patients and an additional 1.3 million HIV-negative patients globally (WHO, 2021a). About 25% of (10.4 million) people with TB and about 70% of (25.4 million) people with HIV live in Africa (WHO, 2021a, 2021b) . In some parts of Africa, including South Africa, over 50% of people infected with TB are co-infected with HIV, speeding the progress of both infections (WHO, 2021a). It has also been reported that TB and HIV co-infection may increase the risk of developing more

dangerous type of TB known as multi-drug resistant tuberculosis (MDR-TB) (Mesfin, Hailemariam, Biadglign, & Kibret, 2014).

Emergence of MDR-TB, defined as a form of TB that is resistant to at least two of the most powerful first-line anti-TB drugs, isoniazid and rifampin, is a formidable obstacle to TB control (WHO, 2013). It is estimated that in 2019, 465 000 people developed MDR-TB or rifampicin-resistant tuberculosis (RR-TB) globally (WHO, 2020). In South Africa, the incidence of MDR/RR-TB was estimated to be about 23 per 100 000 population in 2019. The recorded number of detected MDR/RR-TB cases in South Africa was about 10000, in 2009, which increased to almost 19000 in 2015 and then declined to about 14000 in 2019 (WHO, 2020). The emergence of MDR/RR-TB as a global epidemic is of great concern to TB control because this type of TB is usually more difficult and expensive to treat. Of particular concern to hearing health care professions is the fact that its treatment may result in irreversible cochleotoxic hearing loss (Knight, 2008; Xing, Chen, & Cao, 2007).

Until 2018, the second-line injectable agents (aminoglycosides and capreomycin), despite their frequent adverse events including cochleotoxicity, were part of the drug regimen recommended by WHO for treatment of patients who are diagnosed with MDR/RR-TB (WHO, 2016). The standard MDR-TB regimen prescribed in South Africa, at the time of the present study (Kanamycin, Pyrazinamide, Ethionamide, Moxifloxacin, Terizidone) also included intensive use of Kanamycin, an aminoglycoside with known cochleotoxic effect (WHO, 2016). Although, kanamycin (the subject of the present study) has recently been removed from the WHO MDR-TB regimen, due to its increased risk of treatment failure and relapse, amikacin, another aminoglycoside derived from kanamycin, with

similar structure and cochleotoxic side effects has remained as part of the regimen for the treatment of MDR/RR-TB patients who cannot use agents from groups A and B medicines (Table 2.1.) (WHO, 2018b).

Cochleotoxicity is the toxic damage to the cochlea that typically manifests as a permanent and bilateral hearing loss (Kaland & Salvatore, 2002; Petersen & Rogers, 2015). Cochleotoxicity initially occurs to the outer hair cells at the base of the cochlea that are responsible for the high frequency sound detection. Therefore, cochleotoxicity firstly damages the ability to hear high frequency sound. With prolonged exposure to the cochleotoxic agent, hair cell damage progresses to the apex of the cochlea that are responsible for the low frequency sounds detection (Petersen & Rogers, 2015). Individuals with only high frequency hearing loss usually experience difficulties in sound localization and hearing certain kinds of environmental sounds. They may have difficulty in hearing the consonant sounds, which lead to problems in understanding the speech when it is fast or in the presence of background noise (Moore et al., 2008; Knight, 2008). In younger children, loss of ability to detect higher frequencies impedes speech acquisition and discrimination (Stelmachowicz et al., 2004). Once the cochleotoxic hearing loss extends into the speech frequency regions (0.5-4 kHz), their ability in speech recognition becomes even worse (De Andrade et al., 2016; B. Li et al., 2017).

The severity of the impact of cochleotoxic hearing loss on one's life depends on its grade and the age of the patient (Brock et al., 1991; Ramma, 2016; Theunissen et al., 2014). Specific to young children cochleotoxic hearing loss can interfere with the speech and language development. It may adversely influence the child's academic achievement; children with hearing loss have poor vocabulary and difficulty understanding grammar,

word order and idioms. In addition, children with hearing impairment have difficulty in reading, writing and mathematical reasoning (ASHA, 2015; Johnson & Seaton, 2020; Khairi Md Daud et al., 2010; Northern & Downs, 2002). Cochleotoxic hearing loss may also negatively influence a child's cognitive, social and emotional development; children with hearing loss are often unsuccessful in social activities and unhappy in the school environment or other social gatherings (ASHA, 2015; Olusanya et al., 2000; Rall, 2007; Smith, 2001).

In adults, cochleotoxic hearing loss may adversly influence their work life, social participation and mental health. Hearing impaired persons may face various problems in obtaining, performing and keeping a job (Copley & Friderichs, 2010). As such, unemployment rate, low wages and fewer full-time job opportunities are higher among this population (Punch et al., 2004).

Hearing loss has also been linked to feelings of depression, anxiety, frustration, social isolation, and fatigue in adults (Kochkin & Rogin, 2000). Adults who lose their hearing often show changes in their personality. They may be confused or apprehensive about their inability to communicate as clearly as they once did. The fear of losing one’s income, relationships or social standing can have a huge emotional impact, causing high levels of stress, which in turn may affect other areas of health such as mental health.

There is also a strong link between risk of developing dementia and degree of hearing loss. Individuals with mild hearing loss were twice as likely to develop dementia as those with typical hearing, those with moderate hearing loss were three times more likely, and those with severe hearing loss had five times the risk (Copley & Friderichs, 2010; Lin et al.,

2011a). Furthermore, it has been demonstrated that hearing impairment is associated with accelerated brain atrophy (Alfandari et al., 2018; Lin et al., 2014). However, the usage of hearing restorative devices may decrease the hazard of long-term cognitive decline (Yeo et al., 2022).

Given the negative impact that cochleotoxicity-induced hearing loss may have on patients, it is important to have a method to prevent or minimize the occurrence of cochleotoxic hearing loss. Research has shown that there are certain treatment factors (i.e. dose and treatment duration) and patient factors (i.e. genetic), which could potentially be useful in identifying those who may be at a higher risk of aminoglycoside-induced cochleotoxicity (Human, 2009; Lu et al., 2010; Modongo et al., 2015). Therefore, identification of these factors and considering them when prescribing the treatment regimen could potentially be a method for preventing cochleotoxic hearing loss (Guan, 2005; Konrad-Martin et al., 2005). The main purpose of the present study was to identify factors that could be used to develop an alternative approach to prevent or minimze cochleotoxicity among MDR/RR-TB patients by: (1) determination of the pharmacokinetic properties of kanamycin that are associated with increased risk of cochleotoxicity and (2) identification of genetic markers in individuals with susceptibility to cochleotoxicity.

1.2. Rationale for the Study

Until recently (2018), a significant proportion of South African patients undergoing MDR/RR-TB treatment were treated with kanamycin and that exposed them to the risk of developing cochleotoxicity-induced hearing loss (Ghafari et al., 2015; Harris et al., 2012). There are a number of risk factors that predispose MDR/RR-TB patients to drug-induced cochleotoxicity. Higher aminoglycoside plasma concentration is one of these risk factors. However, not much is known about the pharmacokinetics of Kanamycin that is an

aminoglycoside with known cochleotoxic effects and was also a key component of WHO-recommended regimen at the time of this study. Genetic factors are also known to be another predisposing factor to cochleotoxicity. There is currently limited research available on the role of mutations involved in aminoglycoside induced-cochleotoxicity in South Africa. Given the increasing number of MDR/RR-TB infections there is a need to identify genetic markers that predispose patients to aminoglycoside cochleotoxcity. Human (2009) conducted a study on the frequencies of six known mitochondrial genetic mutations in a group of South African MDR-TB patients. However as the conventionally defined racial groups differ in genetic factors display significant differences in vulnerability to specific diseases or sensitivity to therapeutic drugs (Exner et al., 2001; Karter et al., 2002), the data on the role of mutations involved in aminoglycoside induced-cochleotoxicity among South Africans with high genetic diversity is so limited. Therefore, the main purposes of this study are to determine (1) the pharmacokinetic properties of kanamycin associated with increased risk of cochleotoxicity and (2) the genetic susceptibility factors for aminoglycoside-induced cochleotoxicity in South African MDR/RR-TB patients. This information can help to better predict patient's prognosis and guide treatment provided by the clinician to the patient (Huang & Ratain, 2009).

Currently cochleotoxicity monitoring is the main strategy used to prevent treatment-induced ototoxicity (Konrad-Martin et al., 2005). However, it has several limitations e.g. some patients may experience cochleotoxicity after completion of their treatment only. Moreover, in a subset of patients carrying certain mitochondrial mutations e.g. m.1555A>G, hearing loss can occur following a single dose (O'Sullivan et al., 2017; Usami et al., 1998). Therefore, cochleotoxcity monitoring is not useful for these patients. It is necessary to develop a more effective index that helps with individualisation of drug

therapy and reliable identification of patients not suitable for aminoglycoside pharmacotherapy to prevent cochleotoxicity. This index can constitute genetic factors and pharmacokinetic properties of drug information that the present study aims to investigate.

It is important to note at this point that although WHO has recently removed kanamycin from MDR-TB regimen, due to its increased risk of treatment failure and relapse, another aminoglycoside, amikacin, with similar structure and cochleotoxic side effects, has remained as part of the regimen for the treatment of some of the MDR/RR-TB patients (Kumana & Yuen, 2012; WHO, 2018). In addition, some countries e.g. India and Nigeria have not completely removed kanamycin from their treatment regimen for MDR/RR-TB (Bada et al., 2020; Shelar, 2022), which makes the present study on kanamycin-induced cochleotoxicity relevant in the current clinical management of MDR-TB patients.

1.3. Research Questions

1. What is the incidence of significant hearing threshold shift due to cochleotoxicity in MDR/RR-TB patients treated with kanamycin?
2. What is the association between pharmacokinetics of Kanamycin and the risk of developing cochleotoxicity?
3. What is the association between T10114C (I19T in MT-ND3) and T15312C (I189T in MT-CYB) mutations and patient's susceptibility to aminoglycoside induced cochleotoxicity among South African MDR/RR-TB patients
4. What patient factors (other than genetic markers) and/or treatment factors are associated with aminoglycoside-induced cochelotoxicity.

1.4. Chapter Outlines

This thesis comprises seven chapters.

Chapter 1. Background and rational, includes a review of the area being researched and justifies the need for conducting the study.

Chapter 2. MDR/RR-TB treatment and cochleotoxicity, provides a review of the WHO regimens for MDR/RR-TB. This chapter also discusses the aminoglycoside-induced cochleotoxicity, cochleotoxicity monitoring and the cochleotoxicity criteria/grading scales.

Chapter 3. Pharmacology of kanamycin and cochleotoxicity, introduces the pharmacology and its main subsets followed by the therapeutic drug monitoring with aminoglycosides. Then, pharmacokinetics properties of kanamycin predictive of cochleotoxicity are discussed.

Chapter 4. Pharmacogenomics of kanamycin and cochleotoxocity, commences with introduction of the effect of the mitochondrial RNA mutations on aminoglycoside induced-hearing loss. Then, this chapter introduces the mitochondrial disorders due to mutations as well as the known mitochondrial mutations associated with non-syndromic hearing loss. Subsequently, the mitochondrial 12S rRNA mutations and aminoglycoside cochleotoxicity is discussed.

Chapter 5. The methodology commences with a description of the aims, sub-aims, research design, participants, study site, equipment and pilot study. It then continues to discuss the data collection process, data management and analysis as well as the ethical considerations, which expanded upon in the Appendices.

Chapter 6. Results, presents the findings of the study according to its aims and sub-aims.

Chapter 7. Discussion and Conclusion, discusses the findings of the study in relation to existing literature and finally concludes.

Chapter 2: MDR/RR-TB treatment and Cochleotoxicity

This chapter will present a review of the WHO regimens for MDR/RR-TB. A critical review of existing literature on aminoglycoside-induced cochleotoxicity will also be presented in this chapter. Last, cochleotoxicity monitoring and the cochleotoxicity criteria/grading scales will be introduced.

2.1. WHO regimens for MDR/RR-TB

Over the previous decades, treatment of drug resistant TB (DR-TB) especially MDR and XDR tuberculosis has been difficult due to its long duration (up to two years), toxic side effects, high costs and unsatisfactory outcomes (Pontali et al., 2018; World Health Organization (WHO, 2018b). The first attempt for treating DR-TB commenced when it emerged as a major challenge to TB control, in the late 1990s (Pablos-Mendez & Laszlo, 1998). To address this, in 1999, WHO and their international partners launched an approach known as DOTS-plus, which recommended the use of second-line agents (amikacin, kanamycin, capreomycin, viomycin, ciprofloxacin, moxifloxacin, ofloxacin, ethionamide, prothioamide, cycloserine and *p*-aminosalicylic acid) in resource-limited settings with high burden of MDR-TB (Gupta et al., 2002; WHO, 2000). In 2006, after the approval of the favourable results of DOTS-plus, the WHO published the "Guidelines for the programmatic management of DR-TB" which classified the MDR-TB drugs into five groups and recommended the minimum of 18 months of treatment following culture conversion (WHO, 2011). In 2011, another version of the WHO guidelines published that updated the composition of second-line regimen for MDR-TB (Falzon et al., 2011). Despite the low evidence, the recommended MDR-TB regimen included at least four second-line anti-TB (including an injectable) agents likely to be effective for a

recommended duration of 20 months, as well as pyrazinamide, during 8-month intensive phase of treatment (Falzon et al., 2011).

The first addition to MDR-TB regimen's recommendations was issued in 2016, after observation of the successful outcomes of the shorter standardised regimen (9-12 months) in Bangladesh and other countries. This addition included the WHO recommendation on the use of shorter MDR-TB regimen in national TB programmes when no exclusion criteria applicable to the patient (Falzon et al., 2017; Van Deun et al., 2010). The shorter MDR-TB regimen includes a 4–6 month intensive phase with kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high-dose isoniazid and ethambutol followed by a 5-month course with moxifloxacin, clofazimine, pyrazinamide and ethambutol. Shorter MDR-TB regimen was later tested in an international, randomised controlled trial and its good results proved that it is an alternative option to the longer WHO regimen under specific conditions (Aung et al., 2014; Nunn et al., 2019). Unfortunately, shorter MDR-TB regimen still contains an injectable drug, kanamycin, which may result in cochleotoxic hearing loss in patients.

Over the past a few years, it was suggested by several studies and reports that DR-TB treatment success could be improved by using new anti TB agents or new combination of drugs (Dalcolmo et al., 2017; Norbert Ndjeka et al., 2018; Nunn et al., 2019). A recent meta-analysis on MDR-TB patients' data revealed that there is positive-correlation between treatment success and the use of linezolid, levofloxacin, carbapenems, moxifloxacin, bedaquiline and clofazimine (Ahmad et al., 2018). Using of linezolid, levofloxacin, moxifloxacin or bedaquiline, significantly decreased the death rate. The comparison of regimens with and without injectable agents showed that amikacin

provided only modest benefits for the patients, while there was association between worst outcomes and using kanamycin and capreomycin (Ahmad et al., 2018). However, the negative impact of kanamycin and capreomycin on outcomes could be attributed to using them for the worst clinical cases, changing the drugs during patient's treatment and misclassification of treatment outcomes (Ahmad et al., 2018). The meta-analysis study, therefore, rather than recommending removing the injectables, emphasised on the use of later generation fluoroquinolones, bedaquiline, linezolid and clofazimine for MDR-TB treatment. The study also revealed that the clinical outcomes could be improved by using bedaquiline as a replacement for second-line injectables (Ahmad et al., 2018).

As a result of all the accumulated evidence from the new studies, WHO significantly changed the MDR/XDR-TB regimens via a rapid communication in 2018 and the updated consolidated guidelines in 2019 (WHO, 2019; WHO, 2018b). In the updated guidelines, WHO regrouped the drugs used to compose the longer regimen (18–20 month) into 3 categories (Table 2.1) and recommended the use of shorter regimen whenever possible. WHO also emphasised the need to exclude kanamycin and capreomycin from short and long regimens and using amikacin if an injectable agent is required (WHO, 2019).

In South Africa, usage of bedaquiline for treatment of RR-TB started after U.S. FDA approval in 2013, before WHO recommendation in 2018 for the use of this drug in the MDR/XDR-TB regimens (Guglielmetti et al., 2017; Mahajan, 2013; Ndjeka et al., 2015). Department of Health (DoH), in 2015 issued a policy framework for introduction of new drugs and drug regimens, which made provision for substitution of the injectable agent with BDQ for long and short treatment regimens (DoH, 2015). A retrospective analysis of MDR-TB patients in the Western Cape revealed improved treatment results when second-

line injectable agents were substituted with bedaquiline (Zhao et al., 2019). Another retrospective analysis of MDR/RR-TB and XDR-TB South African patients showed that using bedaquiline was associated with an almost 4 times reduction in mortality (Schnippel et al., 2018). Therefore, the DoH in June 2018 made bedaquiline routinely available within injectable-free regimens for all RR-TB patients. In October 2018, in response to the WHO Rapid Communication for key changes to treatment of MDR/RR-TB (WHO, 2018b), the DoH released interim clinical guidance on implementation of new injectable-free regimens in South Africa (see Table 2.1) (DoH, 2018). This guidance recommended a new long regimen (lZD-BDQ-LFX-CFZ-TRD) and short regimen (lZD, BDQ, LFX, CFZ-INH high dose-Z-E) for MDR/RR-TB, and excluded the Kanamycin and Capreomycin from the treatment of MDR/RR-TB due to their poor TB treatment outcomes. However this document suggested that in exceptional cases where treatment options are severely limited, amikacin, should be considered the injectable agent of choice (DoH, 2018).

Evidence presented in the preceding paragraphs seems to suggest that, despite all the efforts that have been made in developing more effective and safer regimen for MDR/RR-TB, for some patients, its treatment still includes aminoglycosides (amikacin or streptomycin) with well-known ototoxic side effects that can expose patients to high risk of developing hearing loss.

Table 2.1: Grouping of medicines recommended for use in longer MDR-TB regimens

Groups & steps	Medicine	
Group A: Include all three medicines	levofloxacin *OR* moxifloxacin	Lfx Mfx
	bedaquiline	Bdq
	linezolid	Lzd
Group B: Add one or both medicines	clofazimine	Cfz
	cycloserine *OR* terizidone	Cs Trd
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	ethambutol	E
	delamanid	Dlm
	pyrazinamide	Z
	imipenem–cilastatin *OR* meropenem	Ipm–Cln Mpm
	amikacin (*OR* streptomycin)	Am (S)
	ethionamide *OR* prothionamide	Eto Pto
	p-aminosalicylic acid	PAS

Note. Adapted from "WHO consolidated guidelines on drug-resistant tuberculosis treatment" by WHO, 2019.

2.2. Aminoglycoside Induced Cochleotoxicity

Aminoglycosides can cause toxicity of the auditory system (cochleotoxicity) and/or vestibular system (vestibulotoxicity) (Ariano et al., 2008). Some of the aminoglycoside agents are preferentially vestibulotoxic e.g. Gentamicin, tobramycin and streptomycin, while some others are mainly cochleotoxic e.g. amikacin and kanamycin (Ariano et al., 2008; Black, Pesznecker, & Stallings, 2004). Cochleotoxicity due to aminoglycoside typically manifests as hearing loss and/or tinnitus. Tinnitus can be transient or permanent and has been reported to precede measurable changes in hearing (Arora et al., 2009; Dille et al., 2010). Hearing loss is usually bilateral, permanent and proceeds from high to low frequency (Einarsson et al., 2010; Kaland & Salvatore, 2002).

2.3. Incidence of Aminoglycoside Cochleotoxicity among TB patients

One of the main adverse effects of aminoglycoside treatment for TB patients is cochelotoxicity. The reported incidence of aminoglycoside cochleotoxicity is variable and it has been reported to range from 18% to 93% in previous studies (see Table 2.2). In South Africa, aminoglycoside induced cochleotoxicity has been reported to affect 57% of the adult, and up to 48% of the paediatric MDR-TB patients (Ghafari et al., 2015; Harris et al., 2012). The reported incidence rates indicate that a high percentage of the South African population who are being treated for MDR/RR-TB is at risk of developing hearing loss. Table 2.2 shows a summary of studies reporting the incidence of aminoglycoside-induced cochleotoxicity among patients who are on treatment for TB.

Table 2.2. Incidence of Cochleotoxicity

Author	Year	Drug	Method	Participant	Incidence	Treatment duration
De Jager & Van Altena	2002	AMK, KAM, STM	Conventional PTA	110 adults with MDR-TB	18%	> 14 days
Peloquin et al	2004	STM, KAM or AMK	Conventional PTA	87 adults with TB	37%	1–139 weeks
De Lima et al	2006	STM	UHF & Conventional PTA	36 adults with TB	64%	> 14 days
Duggal & Sarkar	2007	MAK, KAM, CAP	Conventional PTA	64 adults with MDR-TB	25%	18–24 months
Sturdy et al	2011	AMK, CAP, STM	Conventional PTA	50 adults with MDR-TB	28%	> Two weeks
Harris et al	2012	KAM, STM, CAP	Conventional PTA	153 adults with MDR-TB	57%	Monitored for three months
Ramma & Ibekwe	2012	KAM, AMK	Conventional PTA	53 adults with MDR/XDR-TB	47%	1–18 months
Ghafari et al	2015	AMK, CAP, STM	DPOAE, AABR, Conventional PTA	25 children with MDR-TB	48%	> 14 days
Van Altena et al	2017	AMK, KAM	Conventional PTA	80 adult with MDR-TB	31%	> 3 days
Heysell et al	2018	KAM	Conventional PTA	40 adults with MDR-TB	78%	1–8 months
Hollander	2018	KAM, CAP	UHF DPOAE, UHF & Conventional PTA	15 adults with DR-TB	93%	Monitored for three months
Ghafari et al	2019	KAM	UHF PTA	102 adults with MDR/RR-TB	82%	Monitored for three months
Hong et al	2020	KAM, AMK	Conventional PTA	238 DR-TB patients ≤13 years old	63%	Monitored for six months
Lodiong et al	2021	AMK, KAM, CAP	Conventional PTA	70 DR-TB patients >15 years old	53%	> six months

AMK= Amikacin, Kanamycin= KAM, Streptomycin = STM, Capreomycin = CAP, PTA = pure tone audiometry, TEOAE= Transient evoked otoacoustic emissions, DPOAE = Distortion product otoacoustic emissions, AABR= Automated Auditory Brainstem Response, UHF= ultra-high frequency, AG = Aminoglycoside,
Note. Adapted from "Aminoglycoside-induced hearing deficits – a review of cochlear ototoxicity" by L Petersen and C Rogers, 2012, *South African Family Practice 57*:2, 77-82.

A review of the studies summarised in Table 2.2 indicated a wide variability in incidences of aminoglycoside-induced hearing loss reported. According to ASHA (1994), the actual incidence of aminoglycoside induced hearing loss is unclear due to the inconsistent reporting of results. Variability in incidence rates reported are due to many variables such as; differences in the criteria used to define cochleotoxicity, protocols used to assess hearing loss, population groups studied, sample size and treatment parameters (Edson & Terrell, 1999; Petersen & Rogers, 2015). In general studies that include monitoring of shift in hearing threshold at ultra-high frequencies (>8kHz) tend to report higher incidence rates when compared to those that monitor shift in conventional frequencies (Konrad-Martin et al., 2005). Regarding the population groups studied, it should be noted that patients' variables might have impact on occurrence of cochleotoxicity. For instance, patients with renal dysfunction, pre-existing hearing loss and susceptibility to aminoglycoside cochleotoxicity are more likely to develop cochleotoxicity (Human, 2009; Rybak & Ramkumar, 2007).

2.4. Aminoglycoside Cochleotoxicity and mechanism of hair cell loss

In the cochlea, the outer hair cells (OHC) are the most susceptible components to the toxicity of aminoglycosides. It is suggested that aminoglycosides can also injure spiral ganglion cells (Hinojosa et al, 2001). Aminoglycoside rapidly enter the cochlea following systemic administration of the drug (Huth, Ricci, & Cheng, 2011). The mechanism by which aminoglycoside enters the cochlea and target hair cells is not fully understood (Kim & Ricci, 2021). Aminoglycosides appear to primarily enter endolymph via the stria vascularis (Li & Steyger 2011; Kim & Ricci, 2022). A research on mice identified megalin, which is an endocytic receptor as the prime transporter of aminoglycoside into the

endolymph and the mechanoelectrical transducer (MET) channels located at the top of hair cell stereocilia (Kim & Ricci, 2021). Evidences suggest that aminoglycosides mainly enter to the hair cell via MET channels and block these channels (Hashino & Shero, 1995; Marcotti, van Netten, & Kros, 2005). Aminoglycosides block the MET channel in a way that makes it function like a one-way valve, resulting in intracellular accumulation of aminoglycosides which might explain the increased vulnerability of hair cells to this drug compared to other cell types (Ricci, 2002; Waguespack & Ricci, 2005). Accumulation of aminoglycosides within the hair cell results in increased production of reactive oxygen species (ROS) or free radicals (Priuska & Schacht, 1995; Sha & Schacht, 1999a). ROS are electrophilic molecules generated by the partial reduction of oxygen to form superoxide, hydrogen peroxide, and hydroxyl radicals. The odd number of electron(s) in a free radical makes it unstable, short-lived and highly reactive (Forge & Schacht, 2000; Huth et al., 2011; Phaniendra et al., 2015). A common mechanism for the formation of ROS needs Iron salts (Thomas, Mackey, Diaz, & Cox, 2009). The combination of aminoglycoside with iron salts increases iron-catalyzed oxidation and, hence directly promotes the formation of ROS (Priuska & Schacht, 1995). This reaction needs electron for which unsaturated fatty acids, mostly arachidonic acid act as electron donors and oxidized to lipid peroxides (Sha & Schacht, 1999a; Sha & Schacht, 1999b). As arachidonic acid is present in the phospholipids of cell membrane; ROS can affect cellular membrane permeability. ROS via lipid peroxidation can also affect proteins and nucleic acids and therewith interfere with activity of enzymes, receptors and ion channels. (Cheng et al., 2005; Halliwell & Gutteridge, 1990). ROS are formed in the cell as a regular byproduct of cellular metabolism (Gutteridge & Halliwell, 2000; Halliwell & Gutteridge, 1990). Normally, the cell protects itself from harmful accumulation of ROS with neutrilising intrinsic antioxidants (Gutteridge & Halliwell, 2000; Yamasoba, Harris, et al., 1998;

Yamasoba et al.,1998). However, when ROS formation exceeds the neutrilising capacity of the protective intrinsic antioxidants, the cell undergoes apoptotic cell death (Cheng et al., 2005; Jeong et al., 2011).

Exposure to aminoglycoside also inhibit the synthesis of mitochondrial protein which leasd to damaging the RNA translation and ATP (Guan, 2011; Hobbie et al., 2008; Prezant et al., 1993). Decreasing the energy production may compromise the mitochondrial integrity and result in the leakage of cytochrome c from mitochondria which in turn activates apoptotic cascades (Inoue et al., 1996). Furthermore, it is thought that in the presence of aminoglycosides, mitochondrial RNA mutations cause increased formation of ROS, which encourages apoptotic cell death (Guan, 2011).

Hair cell loss progresses from the base of the cochlea (an area for high frequency sound detection) to the apex (an area for low frequency sound detection) (Chen et al., 2007). A possible explanation for this mechanism of hair cell loss is that hair cells operating at higher frequencies have higher metabolic demand and are more susceptible to reduced ATP due to the mitochondrial malfunction than lower frequency cells, that is, basal versus apical (Huth et al., 2011). The degree of hair cell damage and hence hearing loss is directly linked to the duration and dose of the aminoglycoside to which the hair cells are exposed (Modongo et al., 2015; Rybak & Ramkumar, 2007).

2.5. Predisposing Factors to Cochleotoxicity

There are a number of risk factors that predispose DR-TB patients to drug-induced cochleotoxicity. These factors are important to consider when patients are introduced to treatment that includes cochleotoxic drugs (e.g. MDR-TB regimen) as they can alert the clinicians to the patients' risk profile with respect to cochleotoxic hearing loss. In general,

the risk factors for cochleotoxicity can be categorized into “Patient-related factors” and “Treatment-related factors.” Some of the known patient and clinical risk factors will be discussed in this chapter, however may not account for all known factors.

2.5.1. Patient-related factors

Age. Older adults are at increased risk of cochleotoxic hearing loss since they may have fewer hair cells due to age-related hearing loss (Lin et al., 2011). Furthermore, there are also various factors that can contribute to drug accumulation with increased risk of hearing loss among elderly populations, including decreased hepatic and renal function, polypharmacy, drug interactions and sensitivity to adverse drug reactions (Coggins, 2014; Roland & Rutka, 2004). Young children are also at higher risk of cochleotoxicity as their auditory system is still developing and, therefore, more susceptible to damage (Knight et al, 2005; Bass et al., 2016; Stelmachowicz et al., 2004). Patients older than 60 years of age and younger than six years were found to be most susceptible to aminoglycoside induced hearing loss (Henry, 1983; Li et al., 2004b).

Body mass index (BMI). Underweight patients (low BMI, <18.5 kg/m2) are more prone to aminoglycoside induced hearing loss (Hong et al., 2020). Aminoglycoside molecular concentration is influenced by body size of the patient and in critically ill patients (e.g. MDR/RR-TB patients) larger loading doses of aminoglycosides are needed to achieve target concentrations (Blot et al., 2014). Therefore, the higher risk of cochleotoxicity among patients with low BMI may be due to the pharmacokinetic vulnerability of underweight patients to high serum doses of aminoglycoside (Pai et al., 2011; Sandri et al., 2013). Overweight patients (high BMI, ≥ 25 kg/m2) are also at higher risk of aminoglycoside induced hearing loss (Lodiong et al., 2021). It has been shown that excess weight and its related cardiovascular disease (CVD) can change the

pharmacokinetics of the drug (Blot et al., 2014; Sandri et al., 2013). CVD by reducing the volume of distribution (vd) of aminoglycoside as well as diminishing its clearance in the kidneys, due to decreased blood flow to this organ, may increase the risk of cochleotoxicity (Sandri et al., 2013; Shammas & Dickstein, 1988; Woosley et al., 1986; Yang et al., 2020).

Renal dysfunction. Aminoglycosides are excreted by the kidney. Higher aminoglycoside doses, in patients with renal failure, may result in accumulation of the aminoglycosides in the inner ear fluids for longer periods of time, and subsequently cochleotoxic hearing loss (Human, 2009; Rybak & Ramkumar, 2007). In addition, nephrotoxicity is a common side effect of aminoglycosides, which can increase the risk of cochleotoxicity (Gonzalez & Spencer, 1998; Range et al., 2007).

Genetic factors. Genetic factors contribute to aminoglycoside-induced cochleotoxicity (Guan, 2011; Human, 2009). Several mitochondrial mutations in the 12S rRNA gene have been found to predispose carriers to aminoglycoside-induced hearing loss. An in-depth discussion of mutations that associated with susceptibility to aminoglycoside-induced cochelotoxicity will be discussed in chapter 4. Also, one of the aims of this study is to investigate specific mutations that are thought to potentially contribute to susceptibility to aminoglycoside-induced cochleotoxicity in South African patients undergoing treatment for MDR/RR-TB.

Exposure to excessive noise. During aminoglycosides treatment, exposure to noise extents the open state of the MET channel (at the top of hair cell stereocilia) leading to an increased concentration of aminoglycosides inside the hair cell, which may increase

the risk of cochleotoxicity (Ricci et al., 2005). Following treatment with aminoglycosides, because of the residual drug levels within the inner ear fluids, exposure to excessive noise may also have a synergistic cochleotoxic effect (Li & Steyger, 2009). A study on infants, treated with aminoglycosides, revealed that exposure to excessive noise increased the failure rate of hearing screening by 14% (Rees, 2007).

2.5.2. Treatment-related factors

History of cochleotoxic treatment. In patients with previous history of cochleotoxictherapy, cochleotoxic medications may still be present in their cochlea, and exposure to further cochleotoxic drugs causes an extra effect, which may lead to cochleotoxic hearing loss (Schellack & Naude, 2013).

Simultaneous administration of cochleotoxic agents. Concurrent administration of aminoglycosides and other known cochleotoxic drugs such as loop diuretics, cisplatin and vancomycin may increase the risk of aminoglycoside-induced cochleotoxicity (Schellack & Naude, 2013).

HIV. The risk of aminoglycoside-induced hearing loss can be influenced by HIV coinfection as a result of severe immunosuppression along with antiretroviral therapy (Hong et al., 2018). It has been shown that HIV-positive MDR-TB patients on highly active antiretroviral therapy (HAART), are more likely to develop cochleotoxicity than HIV-negative MDR-TB patients (Harris et al., 2012). A recent meta-analysis revealed that the risk of aminoglycoside induced hearing loss among patients with MDR-TB and HIV coinfection is 22% higher than non-HIV infected patients (Hong et al., 2018).

Aminoglycoside blood concentrations. When aminoglycosides are prescribed for treating acute infection, peak and trough serum concentrations should be monitored to

confirm that serum levels are within the therapeutic and nontoxic ranges, respectively. However, for DR-TB patients measuring serum peak/trough levels may not be practical as most of them are treated in the community and for prolonged periods (Peloquin et al., 2004). In addition, even when their aminoglycoside blood concentrations are within the recommended therapeutic range, cochleotoxicity can still occur. In such cases as patients are exposed to aminoglycosides for a prolonged period of time, the risk of cochleotoxicity may be more strongly associated with other pharmacokinetic properties of aminoglycosides such as cumulative duration of therapy (Beaubien et al., 1989; Modongo et al., 2015). For further information in this regard please see section 3.4. One of the aims of the present study was to find the pharmacokinetic properties of kanamycin, which may be associated with increased risk of chochleotoxicity in MDR-TB patients.

2.6. Cochleotoxicity monitoring

Monitoring of cochlear and auditory function during aminoglycoside therapy or other potentially cochleotoxic treatments can help to identify the toxic effect of the medication to the auditory system (Schellack & Naude, 2013). Monitoring for cochleotoxicity allows for the early detection of changes in patients' hearing thresholds which alerts the treating physician to explore modifications to patients' treatment regimen (e.g. drug type, dosage level) to prevent further deterioration of hearing thresholds. Early detection of changes in patients' thresholds also allows for early intervention once hearing handicap has occurred (ASHA, 1994; Durrant et al., 2009; Schellack & Naude, 2013).

2.6.1. Audiologic monitoring guidelines

Prospective monitoring of patients' hearing thresholds when they are being treated with potentially cochleotoxic drugs (i.e. cochleotoxicity monitoring) is a common approach for detecting treatment-induced changes in patients's hearing thresholds. There are currently various guidelines available that are used for ototoxicity/cochleotoxicity monitoring. The most widely used guidelines are those issued by ASHA in 1994, and the American Academy of Audiology in 2009 (Durrant et al., 2009). Locally, the DoH released its ototoxicity/cochleotoxicity monitoring guidelines in 2015, followed by the HPCSA in 2018. However, both the HPCSA and DoH guidelines are essentially an adaptation of ASHA, 1994 guidelines (Lord, 2019).

There are also various challenges with implementation of cochleotoxic monitoring in South Africa. Shortage of audiological equipment and personnel are some of the key challenges. Therefore, investigation of alternate ways of monitoring patients' hearing thresholds when they are treated with potentially cochleotoxic treatment is essential. One approach is patient's self-report hearing loss via a questionnaire (DoH, 2015). However, Ramma and Ibekwe (2012) found this approach to be poor at detecting changes in auditory function for mild to moderate hearing loss and hence recommend that it is essential for audiometric equipment to be available for patients on MDR/RR-TB treatment (Ramma & Ibekwe, 2012). Given resource constraints in South Africa and the above mentioned challenges of current guidelines, it is essential to develop a specific protocol for MDR/RR-TB patients within South Africa that include fewer tests as well as an intervention method to improve the shortcomings of current guidelines (Hollander, 2018).

2.6.2. Assessment of cochleotoxiciy

There are several guidelines in existence that are recommended for cochleotoxicity monitoring. However, all of the existing guidelines in South Africa (e.g. ASHA, 1994; DoH, 2015; HPCSA, 2018) recommend the following as key components of a cochleotoxicity monitoring protocol: Pre-treatment counseling, and audiological testing prior to, during and after receiving cochleotoxic treatment. Ideally, the baseline assessment must be administered before the commencement of the first treatment. However, when it is not possible, it is suggested that for patients on cochleotoxic medication, the baseline should be obtained within 72 hours of the first dose administration (ASHA, 1994). Periodic monitoring of patients' hearing thresholds is recommended once or twice per week, and post-treatment evaluations one month, three months and six months following final treatment (Konrad-Martin et al., 2005). The South African guidelines, considering the challenges with cochleotoxicity monitoring in South Africa, recommend that the baseline assessment can be obtained even within the first seven days of treatment, and recommend subsequently weekly testing, and if this is not possible, monthly testing (DoH, 2015; HPCSA, 2018). They also recommend three monthly testing for a total of six months for the continuation phase of DR-TB treatment, (DoH, 2015). An outline of assessment and monitoring procedures, including the pre-treatment counselling, baseline measures and monitoring evaluations are detailed below.

2.6.2.1. Pre-treatment counselling

Prior to the commencement of ototoxic treatment, the patients should get informed of the risks and benefits of the drugs. All patients should be counselled regarding the cochleotoxic side effects of the drugs on the auditory system as well as the signs and symptoms of cochlear damage. The audiologist should make the patients aware of the characteristic of cochleotoxic hearing loss, tinnitus, dizziness, and predisposing factors e.g.

noise exposure (HPCSA, 2018; Konrad-Martin et al., 2005). Following informing the patients regarding the side effects of the cochleotoxic medications the patient's consent to treatment with these drugs should be obtained (HPCSA, 2018).

2.6.2.2. Baseline assessment

Baseline assessment is a pre-treatment record to which monitoring measures during and after treatment will be compared, for identification of changes in hearing sensitivity. Ideally, baseline measures should be conducted before the administration of the first dose of the cochleotoxic regimen (ASHA, 1994; Durrant et al., 2009). Baseline assessment preferably should include all tests that may be required in future testing, although only a few of them are used for the follow-up monitoring. Therefore, baseline assessment needs to be as comprehensive as possible and by most accounts must include: case history, otoscopic examination, tympanometry, acoustic reflexes (ipsi and contralateral), conventional and UHF pure tone audiometry, speech audiometry, otoacoustic emissions (OAEs), and auditory brainstem response (ABR) (Ganesan et al., 2018; Konrad-Martin et al., 2005; Reavis et al., 2011). However, as administration of all these tests take long time and may not be practical at every follow-up, the DoH (2015) recommends only otoscopy, tympanometry, PTA (If resources allow, UHF) and OAEs.

For some patients, especially very young children and those who are ill and cannot cope with a complete assessment, the testing procedures should be adapted. In these cases, modifications to the testing procedure are necessary and the most crucial information should be obtained for baseline audiogram purposes. Efficient objective testing protocol includes a full immittance test battery, distortion product otoacoustic emissions (DPOAE) testing, and a diagnostic ABR threshold estimation assessment (Fischel-Ghodsian, 2005; Knight et al., 2007; Lord, 2019; Theunissen et al., 2015). For patient that subjective testing

can be conducted, reduced number of pure tone thresholds selected for measurement (e.g. 4, 6, 8, 10, 12.5 kHz), should be prioritised, and more comprehensive measurements can be implemented after improvement in the patient's condition (HPCSA, 2018).

2.6.2.3. Monitoring assessment

Monitoring assessment include follow-up questionnaires reporting tinnitus, dizziness, self-reported hearing loss as well as the recent addition of any synergistic components such as noise exposure and other ototoxic treatments (DoH, 2015). Audiological tests implemented during monitoring evaluation are otoscopy and pure-tone air conduction threshold testing including UHF or DPOAEs for patients in whom behavioral measure cannot be conducted (HPCSA, 2018). When a significant change relative to baseline audiogram/DPOAEs are detected, a comprehensive audiological assessment should be implemented within 24 hours or before the next administration of ototoxic medication to confirm that hearing loss is due to the medication. The HPCSA (2018) recommends that a comprehensive assessment (at the minimum) must include: case history, otoscopic examination, immittance testing, pure-tone audiometry (air and bone conduction) for adults, or DPOAE for children younger than five years and non-responsive patients. For patients that DPOAEs are absent, Auditory Brainstem Response (ABR) and Auditory Steady State Response (ASSR) should be conducted. An outline of the tests and rational for using them in baseline and or monitoring assessment of cochleotoxicity are detailed below.

Case history. A comprehensive case history considering predisposing factors to cochleotoxicity (family history, HIV medication, noise exposure, history of ear disease, renal dysfunction etc.) should be obtained during the baseline assessment to identify patients who are at higher risk of developing hearing loss. Patient's medical record should

be reviewed to gather information about the treatment plan including type, dosage and number of treatment cycles (Lord, 2019).

Otoscopic examination. Otoscopy should be part of the baseline and monitoring assessments. It is used to evaluate the state of the external auditory meatus and tympanic membrane for any abnormalities such as obstructions and infection that can affect patients' hearing thresholds and/or could influence additional testing procedures (Rappaport & Provencal, 2002).

Tympanometry and acoustic reflexes. Tympanometry and acoustic reflex testing should be part of the baseline assessment for identification of the middle ear pathology. Tympanometry evaluates middle ear status and Ipsi and contra acoustic reflexes thresholds (ART) in conjunction with tympanometry can provide corroborating evidence for presence of middle ear abnormality (Clark et al., 2007; Lord, 2019). Implementation of ART and tympanometry may also be needed during the treatment phase. During the treatment phase, tracking changes in the ARTs can provide valuable information regarding the hearing of the patients who cannot be tested subjectively. The patients' ART will be absent or elevated beyond the normal range, when they develop severe to profound hearing loss (Lord, 2019; Margolis & Shanks, 1990). In addition, as otitis media with effusion is so common among children, it is recommended that tympanometry should be routinely performed as part of the monitoring process for this group (Brooks & Knight, 2018). This provides the ability to exclude middle ear pathology when the pure tone audiometry and otoacoustic emission (OAE) tests show changes in hearing in comparison to the baseline (Lord, 2019). In South Africa, middle ear infections are prevalent even among the adults due to high rates of HIV and TB co-infection. Therefore, conducting tympanometry is essential during the treatment phase among TB patients (Lord, 2019; Seddon et al., 2012).

Pure tone audiometry. The pure tone air conduction audiogram should be obtained as part of the baseline assessment to document hearing loss prior to treatment and to be compared with future serial testing (ASHA, 1994; Studebaker, 1962). As cochleotoxicity usually starts from ultra-high frequency (UHF) (9-16kHz) and extends to lower frequency, it is recommended that the frequencies from 0.25 to 16 kHz including both 3 and 6 kHz should be tested (Reavis et al., 2011). A significant change in pure tone air conduction levels is defined by ASHA; a ≥ 20 dB pure tone threshold shift at a single frequency, ≥ 10 dB shift at 2 consecutive frequencies or threshold response shifting to "no response" at three consecutive frequencies (ASHA, 1994).

Pure tone bone conduction threshold should also be conducted as part of the baseline assessment at octave frequencies from 0.5 to 4 kHz. This is especially necessary at frequencies that the air conduction thresholds are greater than 10 dB HL. Measurement of bone conduction thresholds is not imperative during the treatment phase, however, it should be retested when significant change in air conduction thresholds is detected. This helps to determine if the change in hearing is conductive or sensorineural in nature (Lord, 2019).

Speech audiometry. Speech reception threshold (SRT) and word recognition score (WRS) tests are included in the baseline assessment. Administration of speech audiometry is not necessary during the treatment phase, however, it should be measured if a significant change in pure tone air conduction thresholds is detected. When this change is in speech frequency range, WRS should be retested to determine if it also affected. If hearing loss involves the WRS, the patient should get informed not only about the hearing loss but also the effect it is having on their ability to understand speech, especially in

adverse listening conditions. Sharing this information with the patient can increases the probability of pursuing hearing loss treatment by the patient (Bass & Bhagat, 2014; Reavis et al., 2011).

Otoacoustic emissions (OAEs). OAEs are ideally suited to assess minor changes of cochlear function at the level of the OHCs for patients who cannot be tested subjectively. Therefore, both TEOAEs and DPOAEs can be used as an ototoxic monitoring test. However, although TEOAEs are more sensitive to marginal and milder sensory hearing loss compared to DPOAE, its upper frequency (up to 5 kHz) is more limited than DPOAE's (up to 8 to10 kHz) and hence cannot be used for early cochleotoxic hearing identification. Therefore, DPOAE is the test of choice for use in the audiologic ototoxic monitoring programme (Lord, 2019).

The DPOAE can detect subclinical ototoxic damage to the cochlear, before significant changes to pure tone thresholds from 0.5 kHz to 8 kHz. It is a fast test and takes less than 1 minute for each ear (Clark et al., 2007; S A Fausti et al., 1994; Leigh-Paffenroth et al., 2005). The DPOAE levels should be obtained as part of the baseline assessment to which measurements taken during treatment can be compared. It is recommended to test the frequencies from 1.5 kHz to 10 kHz with primary tone intensity levels set to f1 = 65 dB SPL and f2 = 55 dB SPL at a f2/f1 frequency ratio of 1.22. It is suggested to use the frequency resolution of 1/6 octave or six points per octave (Lord, 2019).

Currently, there is no accepted protocols or criteria for a significant change in DPOAE levels compared with baseline (Brooks & Knight, 2018; Durrant et al., 2009;

Konrad-Martin et al., 2020). Cunningham (2011) suggested that a change of 3-6 dB SPL is accepted as a significant change, indicating damage in cochlear function. Dhar and Hall (2011) recommended a change of 4 or 5-dB for a limited number of the highest frequencies (Dhar & Hall, 2011). Other authors suggested a change of at least 6 dB from baseline at sensitive frequency range for cochleotoxicity (Fischel-Ghodsian, 2005; Ganesan et al., 2018).

Auditory brainstem response (ABR). Although DPOAE is an excellent test for objective assessment of cochlear function, it cannot estimate behavioural hearing threshold (Abdala & Visser-Dumont, 2001). For patients who cannot be tested behaviourally, ABR is an ideal objective test at the level of auditory nerve and brainstem that can estimate frequency-specific thresholds. The frequency range for clinical ABR is 0.25 to 8 kHz using broadband and frequency-specific tone burst stimuli, which is not ideal for early ototoxic hearing identification. However, research has shown that frequency specific tone burst at higher frequency (8 to 14 kHz), and a high-frequency filtered click can also be measured by ABR (Fausti et al., 2003).

ABR is not included in the baseline assessment for the patients who can be tested by conventional means. Although ABR is an objective test, it requires subjective interpretation. It also needs a quiet, cooperative patient; therefore, it is difficult or impossible to be conducted in infants and young children without sedation. It is also a time-consuming test when the aim is to estimate threshold at multiple frequencies. Fausti et al. (2003) recommended that threshold searching for cochleotoxic monitoring is not feasible due to the time issue and questionable reliability of the responses near threshold

(Fausti et al., 2003). They suggested that for confirmation of cochleotoxic hearing changes, confirming the absence or presence of a response is the most reliable ABR measurement.

ASSR. Another objective test for estimating behavioral threshold is the auditory steady-state response (ASSR). For ASSR, unlike ABR, subjective interpretation of the test result is not required. ASSR equipment can utilize modulated pure tone or narrow band octave band chirp at multiple frequencies binaurally. Using the chirp stimuli increases amplitude of the response and also reduces test time (Lord, 2019). In a study conducted by Sininger et al (2018), the test time and accuracy of octave band CE chirp stimuli presented binaurally at 0.5, 1, 2 and 4 kHz was compared with single tone burst ABR at the same frequencies. They found that the intensity of ASSR thresholds were lower than tone burst ABR and also the average test time of ASSR (19.93 minutes) was less than ABR (32.15 minutes). Sininger et al concluded that for behevioral estimation, ASSR has advantage over ABR both in time and in accuracy in threshold estimation.

2.6.3. Sensitive region for ototoxicity (SRO)

The American Speech-Language Hearing Association (ASHA, 1994) recommends that those patients with limited responsiveness should be tested using a shortened monitoring protocol which only include those measures that significantly contribute to the ototoxicity monitoring programme's goal of detecting threshold changes e.g. the sensitive range for ototoxicity (SRO). Fausti et al. (1999) introduced SRO to reduce audiometric testing time. The SRO method is defined as the highest frequency with a threshold of ≤100 dB SPL followed by the next six frequencies below (which have thresholds better than 100 dB SPL) in 1/6th-octave steps, or the one octave range near the highest audible frequency (Durrant et al., 2009). The SRO is determined at the baseline assessment prior to ototoxic treatment and at future serial monitoring for comparison. When a hearing change is

observed, a more complete evaluation is necessary. The test's results in the follow-up assessment will allow verifying hearing changes and ruling out threshold shifts due to middle ear dysfunction. Thresholds actually can be tested in one-sixth octave interval steps within the SRO, whether the SRO is located above or below 8 kHz. It is reported that almost 90% of all initial ototoxic hearing thresholds shifts were detected within the seven frequency SRO (Fausti et al., 2003). Therefore, SRO approach by decreasing test time makes the monitoring potentially more efficient and cost effective.

Equipment plays a role in including the SRO into the test protocol. In developing countries, such as South Africa, there are financial constraints on healthcare system due to the competing budgetary demands from life-threatening and/or communicable diseases (A. Harris et al., 2012). Govender (2015) conducted a research in South Africa and reported that 19% of audiologists conduct HFA, which indicated that there is a lack of high frequency audiometers due is to financial constraints. Therefore, the lack of appropriate equipment in many South African audiology departments (Koekemoer & Ndjeka, 2013), limit the conduction of HFA audiometry and consequently SRO approach in ototoxicity monitoring.

2.6.4. Intervention and management

The DoH (2015) suggested that upon the detection of cochleotoxicity, the physician should stop the drug or reduce the dosage and/or increase the length of the dosing interval. However, if no changes in the patient's regimen for decreasing the drug side effects is possible and the current therapy has to be retained, increasing the frequency of monitoring for early identification of further deterioration is suggested. Regarding the management and treatment of cochleotoxicity, it is recommended by some authors including DoH

(2015) that referral for aural rehabilitation should be done when ototoxicity occurs (Konrad-Martin et al., 2005; Vasquez & Mattucci, 2003). It means that counselling and communication strategies may need to be conducted as part of the management of cochleotoxic hearing loss while monitoring is taking place. The time for considering the hearing aid fitting is also not clear; upon the detection of hearing loss or post treatment. In addition, in South Africa, because of budget limitation, on one hand, hearing aids are not often available in the public sector and on the other hand, the benefit of hearing aids is minimal to moderate (DoH, 2015). Cochlear implantation is also limited in South Africa due to the unavailability of facilities (SACIG, 2017).

2.7. Cochleotoxicity Grading Scales

Grading the severity of cochleotoxicity following the aminoglycosides treatment is essential for evaluating the impact of treatment, and for considering alternative treatment protocol (Jacob et al., 2006). There are varieties of grading scales for cochleotoxicity. Scales that use serial audiogram for detecting changes in hearing thresholds including ultra-high frequency (UHF) thresholds are the most effective indicator of cochleotoxicity (Crundwell, Gomersall, & Baguley, 2015; Konrad-Martin et al., 2005). Hearing loss at UHF is considered as an alert signal for cochleotoxicity before the lower (speech) frequencies are affected. However, most of existing cochleotoxiciy grading scales such as Common Terminology Criteria for Adverse Events version 5 (CTCAEv5), Brock pediatric grading system and International Society of Paediatric Oncology Boston Ototoxicity Scale (SIOP) do not use UHF information (Konrad-Martin et al., 2005). American Speech-Language- Hearing Association (ASHA, 1994) criteria, which is very well known and was developed to identify ototoxicity at the earliest opportunity by monitoring threshold shifts from baseline testing, is not a scale. It is a binary (yes/no) classification that does not

determine the differentiation between affected frequencies, which is essential for determination the clinical impact of hearing loss (Crundwell et al., 2015; King & Brewer, 2018). The commonly used scales, which recommend the use of UHF are Chang and Tune that have other limitations; Chang grading system developed specifically for evaluation of cisplatin cochleotoxicity in children and only consider hearing loss up to 12kHz. This grading system needs no baseline measurement, therefore, is not able to determine that hearing loss is due to chemotherapy specifically (Chang & Chinosornvatana, 2010). Tune is a grading system for cochleotoxicity in adults that only use UHF hearing loss up to 12.5kHz (Theunissen et al., 2014). This grading system has not been validated yet and is not clear about grading the patients with a pre-existing PTA of 35 dB HL that changes to 50 dB HL on post treatment test (King & Brewer, 2018). In 2016, the University of Cape Town (UCT) developed a cochleotoxicity grading scale for adults with suggestions to include UHF thresholds up to 16kHz and to improve the potential shortcomings of the current criteria and grading systems (Table 5.8). UCT scale is a combination of ASHA criteria and pure tone average (Ramma, 2016). However, this scale has not been validated yet and does not incorporate subjective reports of hearing loss or tinnitus. In addition, UCT scale is not a functional scale, which means that grades of hearing loss do not necessarily correspond to specific functional limitations. From the explanations in the preceding paragraphs it can be concluded that still there is no standard and validated grading scale for cochleotoxicity which is a challenge for understanding the severity of cochlcleotoxicity and adjusting the treatment based on that.

Chapter 3: Pharmacology of Kanamycin and Cochleotoxicity

This chapter will introduce the basic concepts of pharmacology with emphasis on the pharmacokinetics and pharmacodynamics. Therapeutic drug monitoring with aminoglycosides will also be presented in this chapter. The chapter will conclude with pharmacokinetics properties of kanamycin predictive of cochleotoxicity.

3.1. Pharmacology

Pharmacology is the study of drugs, their chemical composition, their biological action, and their beneficial and adverse effect to living organisms (Hacker et al., 2009). The two broad divisions of pharmacology are pharmacokinetics and pharmacodynamics. Pharmacokinetics (PK) is the study of the effects of the living organisms on the drugs including absorption, distribution, metabolism, and elimination. Pharmacodynamics (PD) is the study of the effects of drugs and the mechanism of these effects on living organisms (Hacker et al., 2009).

3.2. Pharmacokinetic

Pharmacokinetic considerations allow the clinician to determine the route of administration of drug, drug dosage, drug concentration, duration of action and frequency of administration. Understanding and applying the pharmacokinetics information can enhance the prospect of therapeutic success and diminish the occurrence of adverse drug effects in the body (Lucer & Penzak, 2016). The key processes involved in pharmacokinetics (absorption, distribution, metabolism and elimination) are elaborated below with the focus on kanamycin and its toxicity.

3.2.1 Absorption

Absorption refers to the movement of a drug from its site of administration into the plasma (Chillistone & Hardman, 2017). Absorption of drug generally affected by route of administration (Chillistone & Hardman, 2017). Aminoglycosides including kanamycin are poorly absorbed from the gastrointestinal tract and mostly administered parenterally (Huth et al., 2011). The peak serum concentrations of kanamycin are generally achieved within one hour and the serum concentration of intravenous administration of kanamycin over a period of one hour is similar to those obtained by intramuscular administration (WHO, 2018a). Therefore, kanamycin for MDRRR-TB patients is usually administered by intramuscular route, which let about 40%-80% of the dose to be absorbed (WHO, 2018a). However, the fastest route of absorption is inhalation (Trevor et al., 2002). Inhaled drugs can be given for either local or systemic effects. Drugs given for their effect on the respiratory tree (e.g. bronchodilators) are given by aerosol or nebulizer (Chilistone & Hardman, 2017). Recently aerosolized administration of aminoglycoside in the treatment of MDR-TB has been described. This method has the potential to reduce systemic exposure, and hence hearing loss, with enhanced drugs concentrations at the bronchi (Mohammad et al., 2017). However, further research is required to determine the safety and efficacy of aerosolized administration of aminoglycoside in the treatment of patients with MDR/RR-TB.

3.2.2. Distribution

Distribution is post-absorptive transfer of drug from systemic circulation to their effect sites (Tray, 2004a). Aminoglycosides are large polar molecules so their distribution is largely limited to the extracellular fluid compartment. The volume of distribution (Vd) of the aminoglycosides including kanamycin is 25% of lean body weight, which is almost

equal to extracellular fluid volume (Fisher et al., 2000; Schentag et al., 2006). Aminoglycosides cannot enter most cells, so have a poor penetration into lung and bronchial secretions, which makes the MDR/RR-TB treatment long and hard (Radominska-Pandya et al., 1999; Schentag et al., 2006). However, inner ear and renal proximal tubule have active transport systems for aminoglycosides, so this drug enters exceptionally into these organs, which may explain the nephrotoxicity and ototoxicity seen with this drug (Fisher et al., 2000; Schentag et al., 2006).

3.2.3. Metabolism

Drug metabolism changes drugs into compounds, which are easier to eliminate, and called metabolites (Tray, 2004b). Enzymes mostly in the liver carry out drug metabolism reactions. However, aminoglycosides are not metabolized in the body and are rapidly excreted in the urine, therefore, they may increase the risk of nephrotoxicity in the patient (Gonzalez & Spencer, 1998).

3.2.4. Elimination

Drug elimination is the process of eliminating of an administered drug from the body (Garza et al., 2020). A drug may be excreted in its intact form or may undergo metabolic biotransformation and be eliminated as biologically active, or inactive, metabolites (Garza et al., 2020). The kidneys or the liver eliminates most of drugs. Aminoglycosides are rapidly excreted unchanged by glomerular filtration of kidney. The plasma half-life of aminoglycosides is about 2-3 hours (Huth et al., 2011). Approximately one-half of the administered dose of kanamycin is cleared within 4 hours and excretion is complete within 24 to 48 hours (WHO, 2018a). Since aminoglycosides including kanamycin are not metabolized in the body and are excreted in their active form, they can cause renal

toxicity, which is a risk factor for cochleotoxicity (Human, 2009; Rybak & Ramkumar, 2007).

3.3. Pharmacodynamics

During the journey of drug in the body, after its absorption and distribution, the drug reaches its sites of action where it interacts with its receptors, and produces its biological effects, which is known as "Pharmacodynamic" (Malangu, 2018). In other words, pharmacodynamics is the relationship between drug concentration at the site of action and any resulting effects, including the intensity and time course of the effect and adverse effects (DiPiro et al., 2010). Pharmacodynamics is affected by drug's binding with a receptor. Receptors, usually proteins and enzymes, may be present on the cell surface or with in the cell of patient or bacteria. The intensity of a drug's effect for most drugs depends on the concentration of the drug at the receptor site (DiPiro et al., 2010). However, other factors such as disease, aging, other drugs or genetic mutations can influence on a drug's affect and hence a drug's pharmacodynamics. These factors can change PD response, by their ability to change receptor binding/sensitivity (Campbell & Cohall, 2017).

In essence both pharmacodynamics and pharmacokinetics of aminoglycosides such as kanamycin explain the relationship between the dose and response of the drug. Therefore, clinicians should consider both factors to assess and control the effects of kanamycin/aminoglycosides through therapeutic drug monitoring (DiPiro et al., 2010; Niward, 2019).

3.4. Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is a clinical practice that involves measuring drug concentration and individual dose adjustment to provide the optimum treatment for the patient and, in particular, to avoid concentration-dependent toxicity (Niward, 2019). TDM is critical for some drugs including those that have a narrow therapeutic window, severe adverse effect, marked pharmacokinetic inter-individual variability (Hallworth, 2014; Niward, 2019). Aminoglycosides unlike most antimicrobial drugs require TDM to avoid toxicity and ensuring efficacy. They exhibit simple pharmacokinetics and must be administered parenterally for DR-TB patients. Aminoglycosides are not metabolized in body and are excreted through kidneys. The plasma half-life of this drug is two to three hours, but the drug may accumulate in tissues and exhibit significant toxicity. The main toxic effects are nephrotoxicity, which is often reversible, and ototoxicity, which causes irreversible hearing loss (Hallworth, 2014; Huth et al., 2011).

The drug concentration at which therapeutic effects are achieved is not easy to determine due to the differing conditions at the site of infection and variable penetrance of the drug to the infection site. Aminoglycosides also have several pharmacodynamic characteristics such as post-antibiotic effect that makes difficult the definition of target plasma concentrations. This difficulty has recently been compounded by changes in the dosing interval of this drug (Hallworth, 2014; Maglio et al., 2002). Aminoglycosides have been traditionally administered every eight to 12 hours. However, it has been proven in recent years, that less frequent dosing (every 24 h or more) produces higher peak concentrations, which enhance bacterial eradication, and lower trough concentrations, which reduce the risk of toxicity. Such regimens that are known as “once daily dosing” are more convenient, less toxic, and reduce adaptive resistance. In once daily dosing or more accurately ‘extended dosing interval’, a plasma concentration measurement is used to design an

individual dosing interval, which reflects the patient's needs and renal function (Hallworth, 2014).

In South Africa, the administration of the aminoglycosides for treatment of DR-TB, at the time of this study, was daily at a minimum of six times a week (for minimum 750mg, maximum 1000mg and average 15mg/kg per dose) or 5 to 3 times a week for patients who developed ototoxicity or nephrotoxicity (SADH, 2013). This approach for administration of aminoglycosides is not based on individual pharmacokinetic parameters while, TDM by using the knowledge of pharmacology is able to adjust the dose of aminoglycosides according to the characteristics of an individual patient, in the decreasing of toxicity and in the increasing of treatment effectiveness. However, before considering TDM as the standard of care for DR-TB patients, further understanding of the pharmacokinetics of kanamycin among these patients with regards to relationships to cochleotoxicity is necessary.

3.5. Pharmacokinetic properties of kanamycin predictive of cochleotoxicity in MDR/RR-TB patients

When a dose of aminoglycoside is taken, its amount in the blood ascends for a period of time, peaks level, and then descends, reaching its lowest level, or trough level, just before the next dose. The next dose is timed to coincide with the trough level of aminoglycoside in the blood. The trough level is widely used for drug safety (Anaizi, 1997; Weimann, 2003). It is recommended that for monitoring purposes, in patients with adequate renal function a trough level of the drug should be measured. If the trough level is >1 mg/L, extending the dosing interval is suggested (Anaizi, 1997). It is suggested that for gentamicin, tobramycin and netilmicin, the risk of cochleotoxicity is increased if peak levels are consistently maintained above 12 to 14 mcg/ml or trough levels consistently

exceed 2 mcg/ml. For amikacin, peak levels above 32 to 34 mcg/ml or trough levels greater than 10 mcg/ml have been associated with a higher risk of cochleotoxicity (Pelton, 2014). To the best of our knowledge, the association between cochleotoxicity and peak/trough levels of Kanamycin has not yet been conclusively demonstrated.

Although peak and trough concentrations are used to adjust dosing, in order to minimize the risk of cochleotoxicity under the hypothesis that cochleotoxicity is concentration dependent (Black et al., 1976), some studies have shown otherwise. Setiabudy et al. (2013) found that there was no relationship between cochleotoxicity and serum trough concentration of gentamicin and amikacin in neonates. In South Africa a study on pharmacokinetics and pharmacodynamics of kanamycin and capreomycin in DR-TB patients found no conclusive positive or negative relationship between dosage and trough levels with the progression of hearing loss (Hollander, 2018). In Netherlands, Van Altena et al. (2017) conducted a study on the therapeutic drug monitoring (TDM) of kanamycin and amikacin in the treatment of MDR-TB and found that weighted C_{max} was not related to cochleotoxic hearing loss and cochleotoxicity correlates with the cumulative drug dose per kg of body weight during daily administration (Van Altena et al., 2017). In Botswana, Modongo et al. (2015) investigated the effects of amikacin concentrations on cochleotoxicity in MDR-TB patients, and found that cochleotoxicity best correlated with both plasma cumulative AUC (area under the concentration-time curve) and duration of therapy, but not with peak or trough concentrations. The finding about the lack of significant relationship between cochleotoxicity and peak and trough concentrations of aminoglycosides reported by the above mentioned studies might be explained by speculating on how PK concepts might relate to cochleotoxicity. Presumably cochlear toxicity is related to the concentration at the site of action together with the carry-over

effects/irreversible damage caused by concentrations at an earlier time point. Higher plasma peaks might imply greater tissue penetration, and higher plasma troughs might indicate greater accumulation. However, if the rate-limiting step for efflux is at the cochlear level, plasma troughs may not reflect cochlear accumulation, and duration of treatment/cumulative exposure might reflect this best (Pelton, 2014; Salt, 2005).

The above-mentioned studies suggest that: (1) the evidence linking cochleotoxicity to peak/trough levels of the aminoglycosides is inconclusive and (2) the pharmacokinetics (PK) of Kanamycin with regard to relationship to cochleotoxicity has not yet been conclusively demonstrated. One of the main aims of the present study is to determine the pharmacokinetic properties of kanamycin that are predictive of cochleotoxicity among MDR/RR-TB patients. This information may help to prevent hearing loss in MDR/RR-TB patients who are being treated with this drug.

Chapter 4: Pharmacogenomics of Kanamycin and Cochleotoxicity

This chapter will introduce pharmacogenomics briefly. Mitochondrial function, structure and genetics will also be discussed in this chapter. This chapter will present the mitochondrial disorders due to mutations as well as the known mitochondrial mutations associated with non-syndromic hearing loss. Lastly the mitochondrial 12S rRNA mutations and aminoglycoside cochleotoxicity will be discussed.

4.1. Pharmacogenomics

Pharmacogenomics is the study of the role of the genetic factors in drug response or toxicities in treated patients (Shukla, 2020). Pharmacogenomics may influence both the pharmacokinetic pathways (drug absorption, distribution, metabolism, and elimination) and pharmacodynamic interactions (effects mediated through a drug's biological targets) (Bishop, 2018). Utilizing pharmacogenomic information allows a physician to choose the right drug and dose for each individual patient (precision medicine) to have the best therapeutic effect and avoid the drug's side effect.

One of the main side effects of aminoglycosides is cochleotoxicity. Aminoglycoside induced cochleotoxicity occurs both in a dose-dependent and idiosyncratic fashion. The idiosyncratic pathway is presumably due to the some of the mutations in the mitochondrial 12S ribosomal RNA gene (Fischel-Ghodsian, 2005). The dose-dependent pathway has been discussed in the previous chapter (chapter 3) and the idiosyncratic pathway will be discussed in this chapter.

4.2. Mitochondrial Function and Structure

For better understanding the impact of mitochondrial dysfunctional on organism due to mitochondrial mutations, it is necessary to get familiar with its function and structure. It is suggested that mitochondria are derived from bacteria that were assimilated early in the evolution of eukaryotic cells. Mitochondria produce energy by converting oxygen and nutrients into adenosine triphosphate (ATP). The process of producing ATP known as oxidative phosphorylation (OXPHOS) is the source of more than 80% of the required energy by the cell. Mitochondria have also other essential functions, such as signaling between cells, cellular differentiation, cell growth, and cell death (McBride et al., 2006).

The elaborate structure of a mitochondrion plays an important role in the functioning of the organelle. Mitochondria are characterized by an outer membrane and an inner membrane that divide the organelle into an intermembrane space and an internal matrix. Mitochondrial intermembrane space is the location of cytochrome C that is responsible for the initiation of apoptosis. Matrix and inner membrane play an important role in the production of ATP. The matrix contains a high concentration of enzymes, including those required for the Krebs cycle. Matrix also contains special mitochondrial ribosomes, transfer RNAs (tRNA) and the mitochondrial DNA genome (Lewin, 1998).

4.3. Mitochondrial Genetics

The human mitochondrial genome is circular, double-stranded DNA molecules (mtDNA) containing 16,569 DNA base pairs (Goodman, 2008). In total, human mtDNA encodes 37 genes, with 22 coding for tRNA and 13 coding for protein subunits. The remaining two genes, *MT-RNR1* and *MT-RNR2*, code for ribosomal RNAs (rRNA) namely, 12S rRNA (small ribosomal subunit) and 16S rRNA (large ribosomal subunit), respectively

(Barbarino et al., 2016).

In humans, mitochondrial DNA inheritance is exclusively maternal. However, it is suggested that under certain circumstances paternal mtDNA transmission is also possible (Bhagavan & Ha, 2011). Mitochondrial DNA is, therefore, inherited in the non-Mendelian pattern. The segregation process of mitochondrion is random and much less organized than nuclear chromosome during mitosis (Mendelian pattern), so the mutation rate of mitochondrial genome is higher (about 10-fold higher) than the nuclear genome. This can cause a mixture of wild type and mutant mtDNA at a given nucleotide position present in the same cell which is known as heteroplasmy and may result in mitochondrial disease (Naviaux, 2000).

4.4. Mitochondrial Disorders

As mitochondria are vital components of all nucleated cells, mitochondrial disease can have great impact in human health. A mitochondrial disease can be due to mutations in one of the 37 mitochondrial genes or in one of the 1000 nuclear genes that code for mitochondrial components. Mitochondrial mutations can be inherited or acquired in either mitochondrial or nuclear DNA. However, most of mitochondrial diseases are due to inherited rather than acquired mutations of mitochondrial DNA. These mutations can also be caused by some extrinsic factors such as drugs, toxins and infections (Finsterer, 2004).

Most pathogenic mutations are heteroplasmic with the exception of a few such as the homoplasmic A1555G mutation associated with non-syndromic deafness (Prezant et al., 1993). Heteroplasmic mutation can result in mitochondrial disease only when the proportion of mutant versus wild type DNA is above a certain threshold level. However,

for some mitochondrial diseases the phenotype is not related to the levels of mutant DNA. The phenotypic manifestations of these mitochondrial diseases are influenced by other factors such as age, environment, nuclear genes and other mitochondrial genes (Leonard & Schapira, 2000).

The severity of the mitochondrial disease ranges from asymptomatic to fatal and depends on the problem in the mitochondria and the tissue the affected mitochondria are in. The postmitotic (nondividing) and more metabolically active cells such as myocytes, neurons, pancreatic cells and cochlear hair cells are more affected by mitochondrial disease, therefore, cardiomyopathy, loss of vision, diabetes and sensorineural hearing loss are the most common diseases resulting from mitochondrial mutations (Hutchin & Cortopassi, 2000).

4.5. Mitochondrial mutations and non-syndromic hearing loss

It has been estimated that 67% of patients with mtDNA disease, develop hearing loss (Guaran et al., 2013). Non-syndromic hearing loss occurs as the only clinical anomaly of a mitochondrial disease without any other signs and symptoms in other parts of the body. (Bravo et al., 2006; Young et al., 2006). Mitochondrial inherited forms of hearing loss usually arise due to mutations in the genes involved in the protein synthesis machinery: rRNAs and tRNAs (Guan, 2004). Some mtDNA mutations associated with non-syndromic hearing loss, have been identified in the *MT-RNR1* (12S rRNA) and *MT-TS1* (tRNA(Ser)(UCN)) genes (Usami & Nishio, 2018). Pathogenic variants in *MT-TS1* (tRNA(Ser)(UCN)) are usually related to the childhood onset of hearing loss while pathogenic variants in *MT-RNR1* (12S rRNA) can be associated with late-onset hearing loss and/or susceptibility to aminoglycoside induced hearing loss (Usami & Nishio, 2018).

4.6. Mitochondrial 12S rRNA mutations and aminoglycoside cochleotoxicity

Aminoglycosides interfere with protein synthesis and cause cellular death by binding to highly conserved sequences of bacterial 16S ribosomal ribonucleic acid (rRNA). Human mitochondrial ribosomes share similarities with bacterial ribosomes. It has been suggested that hair cell mitochondria may be an early target of aminoglycosides. A higher incidence of aminoglycoside-induced hearing loss is likely in individuals with an inherited mitochondrial RNA defect. In patients carrying certain mitochondrial mutations (12S rRNA mutations) (i.e., m.1555A>G; m.1494C>T; m.1095T>C), the structure of the mitochondrial rRNA has an even greater resemblance to that of the bacterial ribosome. This close resemblance increases the potential for aminoglycosides to bind to rRNA; this in turn increases the potential for cochleotoxic effects on the hair cells following exposure to aminoglycosides (Roland & Pawlowski, 2009).

The first mutation found to be linked with non-syndromic and aminoglycosides hearing loss was 12S rRNA A1555G (Prezant et al., 1993). In human mitochondria, the A nucleotide at the 1555 position in the 12S rRNA gene is equivalent to positions 1491 of *E. coli* 16S rRNA (Bottger, 2010). When the1555 A is mutated to G, the new structure of 12S rRNA more closely resembles the bacteria 16S rRNA subunit. It is suggested that the newly formed G-C pair generates an altered binding site for aminoglycoside, which causes a hypersensitivity to this drug in those who harbor this mutation (Moazed & Noller, 1987; Noller, 1991; Purohit & Stern, 1994). 1555A>G has been reported as the most frequent mutation associated with aminoglycoside-induced hearing loss worldwide (Guan, 2011; Lu et al., 2010). However, this mutation can cause hearing loss even in the absence of aminoglycosides (Hutchin, 1999; Inoue et al., 1996). Following discovery of 1555A>G, several other mutations in the 12S rRNA gene have been identified to be associated with

both aminoglycoside and non-syndromic hearing loss. The C1494T mutation, similar to 1555A>G, also found in the highly conserved A site of 12S rRNA. The frequency of this mutation is much lower than 1555A>G and, to date, has been found in three countries; China, Spain and USA (Foster & Tekin, 2016; Johnson et al., 2010; Rodríguez-Ballesteros et al., 2006; Zhao et al., 2004). The T1095C and A827G mutations are also involved in the pathogenesis of both non-syndromic and aminoglycoside induced hearing loss (Chaig et al., 2008; Xing et al., 2006a, 2006b). The T1095C has been reported in Chinese and Italian descent (Wang et al., 2005) and the A827G in Chinese and Argentinian descent (Chaig et al., 2008; Xing et al., 2006a, 2006b). In 2006, the T1291C variant was discovered in a Cuban family with non-syndromic hearing loss (Ballana et al., 2006). It is suggested that this variant may also be pathogenic but further investigation will be required to prove or disprove it (Ballana, 2006). Moreover, some mutations at position 961 in MT-RNR1 such as 961delT+insC(n) and T961G have been identified that are possibly associated with aminoglycoside induced hearing loss (Bacino et al., 1995; Casano et al., 1999; Konings et al., 2008; Tang et al., 2002). However, position 961 in MT-RNR1 is not evolutionarily conserved and its functions are unknown. Therefore, it is possible that what described as the mutations at this position may be polymorphisms and not be involved in aminoglycoside induced hearing loss (Gao et al., 2017; Human, 2009). In addition, after screening of 12S rRNA gene sequences of hearing impaired Chinese children, more mitochondrial mutations were suggested to may be related to aminoglycoside ototoxicity or non-syndromic hearing loss; m.745A>G, m.792C>T, m.801A>G, m.839A>G, m.856A>G, m.1027A>G, m.1192C>T, m.1192C>A, m.1310C>T, m.1331A>G, m.A374A>G, m.1452T>C and m.1537C>T (Gao et al., 2017; Konings et al., 2008; Lévêque et al., 2007; R. Li et al., 2004a; Lu et al., 2010). All of these thirteen mutations were also in highly conserved nucleotides in the 12S rRNA, but had a very low frequency

(Gao et al., 2017). In 2016, a comprehensive meta-analysis of mitochondrial variations that increase sensitivity to aminoglycosides, found a significant relationship between aminoglycoside induced hearing loss and five of previously reported mitochondrial mutations; 839A>G, 1095T>C, 1107T>C, 1494C>T, 1555A>G (Foster & Tekin, 2016).

The explanations in the preceding paragraphs seem to suggest that in different population, different mitochondrial mutations may be associated with aminoglycoside induced hearing loss. Moreover, the conventionally defined racial groups differ in genetic factors show significant differences in vulnerability to specific diseases or sensitivity to therapeutic drugs (Exner et al., 2001; Karter et al., 2002). Therefore, in South Africa with high genetic diversity, vast genetic investigation is needed to identify the different mitochondrial mutations associated with aminoglycoside-induced hearing loss. Currently, only two studies investigated genetic factors associated with aminoglycoside-induced cochletotoxicity among South Africans. The first study reported that mitochondrial mutation 1555A>G was responsible for sensorineural deafness in a South African family under treatment with streptomycin (Gardner et al., 1997). The second study, conducted by Human (2009), used SNaPshot screening method to assess the frequencies of six known genetic mutations (A1555G, C1494T, T1095C, 961delT+insC$_{(n)}$, T961G and A827G), in a group of South African MDR-TB patients and in control samples. The pathogenic A1555G and A827G mutations were found in 0.9% of the Black control samples and 1.1% of the Afrikaner control samples. Human concluded that a considerable proportion of the South African population is genetically predisposed to develop aminoglycoside-induced hearing loss (Human, 2009). They also sequenced the entire mitochondrial genomes of eight patients with ototoxicity but with no known aminoglycoside-induced hearing loss mutations and found two potentially pathogenic variants, T10114C (I19T in MT-ND3)

and T15312C (I189T in MT-CYB). They, therefore, recommended that the possible role of T10114C (I19T in MT-ND3) and T15312C (I189T in MT-CYB) in ototoxicity should be elucidated in future studies (Human, 2009). Human also suggested that 961delT+insC$_{(n)}$ and T961G variants are probably non-pathogenic polymorphisms in South Africans, while they have been reported to be pathogenic in some other countries (Bacino et al., 1995; Chen & Guo, 2015; Rydzanicz et al., 2010). The limited data available on the role of mutations involved in aminoglycoside induced-cochleotoxicity in South Africa, with increasing number of MDR-TB infections, emphasizes the need for further research in this arena. Identification of additional genetic variations that contribute to the risk of cochleotoxicity will allow for the development of effective screening panels and provide a way to tailor the therapy optimally. Therefore, one of the aims of this study was to investigate the role of two potentially pathogenic variations, T10114C (I19T in MT-ND3) and T15312C (I189T in MT-CYB), in cochleotoxicity, as recommended in the study conducted by Human (2009) among the South African populations.

Chapter 5: Methodology

This chapter presents the methodological aspects of this study. Aims and sub-aims as well as the study design, sampling and their justification are presented. Furthermore, threats to reliability and validity as well as ethical considerations in this study are discussed.

This study was part of a larger study ("Pharmacometric optimization of second line drugs for MDR/RR tuberculosis treatment") conducted by the Division of Clinical Pharmacology (DCP), Faculty of Health Sciences, University of Cape Town (HREC/REF:065/2015). The scope of the data required for this study required input from the Divisions of Clinical Pharmacology and Human Genetics. Technical work on Pharmacokinetics (for aim 2) was led by the Division of Pharmacology while technical on genetic analysis (aim 3) was led by the Division of Human genetics. Therefore, the current study is a collaborative research project between the Division of Communication Sciences & Disorders [Audiology], Division of Human Genetics and Division of Clinical Pharmacology [DCP]) and it has the following as its aims:

5.1. Aims and sub-aims

In MDR/RR-TB participants (>18 years) at Metro Hospital Tuberculosis Centre, who are receiving cochleotoxic medication (kanamycin) as part of their treatment:

Aim 1: To determine the incidence of cochleotoxicity during MDR/RR-TB treatment

1.1. To determine significant thresholds shift (STS) in hearing based on ASHA criteria

1.2. To grade the severity of cochleotoxicity based on CTCAE, Tune, UCT criteria/scales

1.3. To determine the variation in incidence of cochleotoxicity as a function of the

following factors:

a. Gender

b. Age

c. Comorbid presentation of HIV and MDR/RR-TB

d. Previous MDR/RR-TB treatment

b. BMI (body mass index) at enrolment to the study

c. Renal dysfunction

d. Exposure to excessive noise

Aim 2: To determine the association between risk of developing cochleotoxicity during MDR/RR-TB treatment and the following pharmacokinetics (PK) factors of Kanamycin:

a. Dose & cumulative dose

b. Peak, trough

c. Half-lives

d. Area under the curve (AUC)

Aim 3: To determine the association between participant's susceptibility to develop cochleotoxicity and two potentially pathogenic mitochondrial mutations:

a. T15312C (I189T in MT-CYB)

b. T10114C (I19T in MT-ND3)

5.2. Research Design

The current study used a prospective cohort design. In this study design, baseline data are collected from the participants at the start of the study, and data are repeatedly collected for a period of time (Jekel et al., 2007). Therefore, audiometric data was collected at the time of enrolment into the study (baseline) and up to 12 weeks after starting the MDR-TB treatment (the average duration of hospitalization of patients). This data was then used to determine the relationship between cochleotoxicity and its predisposing factors.

The advantage of this study design is that the investigator can control and standardize data collection, as the study progresses and can check the outcome events carefully when they occur, ensuring that they are correctly classified (Jekel et al., 2007). The major limitation of this design, however, is the possible loss of study participants to follow-up (attrition). To minimize this limitation, the study recruited slightly more participants than the calculated sample size (i.e. over enrolled participants into study). According to Schulz and Grimes (2002), attrition in prospective studies can be as high as 5%; therefore, this was factored in during recruitment of participants to cater for possible attrition.

5.3. Participants

Participants in this study were all patients who were accessing treatment for MDR/RR-TB and hospitalized within the Metro Tuberculosis Centre in Cape Town. This comprised predominantly patients from the Cape Town metropolitan area as well as patients from neighbouring health districts. However, because this facility is a Centre of Excellence in the province for management of DR-TB, there also patients from other parts of the province.

5.3.1. Recruitment

Participants for this study were recruited from a pool of participants in the primary study ("Pharmacometric optimization of second line drugs for MDR/RR-TB tuberculosis treatment) that was conducted by DCP. They were identified by the researcher in collaboration with the research team of the DCP study. Participants were given information sheets regarding the study and were allowed adequate time to think and discuss participation with their significant others if necessary (Appendix A). Patients who were willing to participate in this study were interviewed (for recruitment) and fully informed of the current study [by the researcher and a trained translator (if necessary)], as

well as given opportunity to ask any questions and had them answered to their satisfaction. Written informed consent to participate were obtained in English, Afrikaans, or Xhosa, from all participants (Appendix A1, A2 & A3).

5.3.2. Sampling method

Non-probability purposive sampling (Maxwell & Loomis, 2003) was used to select participants. Purposive sampling is the deliberate selection of individuals on the basis of predefined criteria (Maxwell & Loomis, 2003). The logic and power of purposive sampling lies in intentionally selecting specific cases that will provide the most information for the research questions (Maxwell & Loomis, 2003). When using this method, one of the first things to do is to verify that the respondent does in fact meet the criteria for being in the sample (Trochim & Donnelly, 2006). For this study MDR/RR-TB inpatients at Metro Tuberculosis Centre in Cape Town who had met the inclusion criteria were included in the study. As a result, a non-probability purposive sampling method was best suited for this study.

5.3.3. Sample size

The sample size required for this study was determined using a power calculation formula. This calculation was based on ASHA criteria for significant shift of hearing threshold in cochleotoxicity (Konrad-Martin et al., 2005), with a standard deviation of ≤10dB HL for pure tone audiometric measurement (baseline vs follow up). The result of the calculation showed that with a sample size of 60 participants a two-sided two-tailed t test at the 5% level of significance, as a standard value (Lavrakas, 2008), would have 99% power to detect a mean difference of 10db with the pure tones assuming a common standard deviation of 10dB.

5.3.4. Inclusion/Exclusion Criteria

Inclusion criteria. Participants were recruited and included in this study if they met the following criteria:

1. ≥ 18 years old
2. Had confirmed pulmonary MDR/RR-TB
3. Were eligible for standard MDR/RR-TB regimen (Kanamycin, Pyrazinamide, Ethionamide, Moxifloxacin, Terizidone) as determined by medical personnel at the hospital
4. Had not started with MDR/RR-TB treatment
5. Had passed Mini-Cog test (cognitive screening test)

Exclusion criteria. Patients were excluded from the study if:

1. They were too ill or medically unstable (as determined by a physician/nurse) to have hearing tests done during the course of this study
2. They had Pre-XDR/XDR TB
3. They had bilateral middle ear pathology (in patients with unilateral middle ear pathology the ear which was involved was excluded from the study)

The number of MDR/RR-TB patients hospitalized at Metro Tuberculosis Centre in Cape Town, who might be eligible to be recruited for this study was estimated to be 100 per annum. However, the actual number was much lower due to participants attrition as a result of: death, withdrawal, and discharge from the hospitals. Therefore, the data collection period was extended to 28 months to maximize the chance of obtaining the required sample size. From August 2015 to December 2017, 147 patients were enrolled for the present study. Of these 147 participants, 45 participants were excluded from the

study, thereby, leaving 102 participants to form the final study sample. For more detail see Table 5.1.

Table 5.1. Detailed participant enrolment description

Patients enrolment from August 2015 to December 2017	BCH *n*	DPMH *n*
No. of MDR/RR-TB patients who were eligible for participation in this study at research site	59	88
No. of participants who were enrolled	59	88
No. of participants who excluded from the study	15	30
Deceased	3	2
Discharged from the hospital	2	8
Withdrew from the study	2	3
Too ill to undergo hearing tests	2	3
Left the hospital against medical advice	1	1
Transferred to another hospital	0	1
Had middle ear pathology	0	6
Had less than 2 audiograms	5	6
Total remaining participants	44	58

5.4. Study Sites/Context

The study was conducted in Cape Town at the Metro Tuberculosis Hospital Centre in the Western Cape, a province with one of the highest incidence of TB in the country (Western Cape Provincial AIDS Council, 2016). Metro Tuberculosis Hospital Centre consists of two facilities: the BCH, Brooklyn (Northern part of the metro) and DPMH, Retreat (Southern part of the Metro). Both hospitals are the main TB referral centers in the Western Cape. The BCH is the only hospital in the province that provides services for XDR-TB patients. It is a 330 beds hospital: With 270 beds (and 7 wards) for adult and 60 beds (and 2 wards) for paediatric patients. DPMH offers in-patient services to drug

sensitive and MDR/RR-TB patients. It has 260 beds and 6 wards. The total number of MDR/RR-TB patients admitted within the Metro Tuberculosis Centre in 2018 was 365; 133 patients at BCH and 232 patients at DPM hospitals.

5.5. Equipment/Study Tools

The equipment/tools that were used in this study as well as their respective application and rational for their use are shown in Table 5.2. All equipment was calibrated as per manufacturer specification and South African National Standards (SANS) requirements and in accordance with the hospital regulations.

Table 5.2. Equipment/tools, their application and rational for use

Equipment	Application	Rationale for use
Mini-Cog test	For screening the cognitive issues	To make sure that participants make truly informed decision about participating in this study
Heine Minilux 2000-otoscope	For otoscopy (visual examination of outer ear & TM)	To detect outer ear & TM problems (e.g. wax)
Audiometic Sound Booth	For examination the pure tones with AC40-audiometer	Control the ambient noise that may interfere with the accuracy of the PTA
Welch Allyn TM 262-immittance instrument*	For tympanometry	Assessment of TM & middle ear to detect middle ear problems
Interacoustic AC40 - audiometer	To conduct pure tone AC & BC audiometry	Determination the STS and grade of HL up to 16 kHz for early detection of loss
Tandem Mass spectrometer (MS/MS)**	Therapeutic drug monitoring	Determination the concentration of kanamycin
SimpliAmp Thermal Cycler***	Polymerase chain reaction (PCR), Amplification of genome	Genetic analysis

AC= air conduction, BC= bone conduction, HL= hearing loss, PTA=pure tone audiometry, STS=significant threshold shift.
* TM 262-immittance instrument is only equipped with ipsilateral stimulus
** MS/MS was used by DCP
*** Thermal Cycler was used by DHG

5.6. Pilot study

Pilot study is conducted to assess the feasibility of the study and to address any possible problems (Van Teijlingen & Hundley, 2002). A pilot study was, therefore, conducted to establish any possible issues that could emerge, since this data collection process had never been applied in practice. The intention was to testing adequacy of study tools and to ensure that the data collection process was proper and contained all the aims that the

researcher wanted to address in the study. The pilot study was conducted after ethical clearance had been obtained to proceed with the study. It included 10% of the total required sample size (6 MDR/RR-TB patients at BCH & DPM) (Connelly, 2008). Written informed consent was obtained from the participants who participated in the pilot study. Pilot study was part of the data collection process and involved the first six participants. After the first six participants, it was decided that there is no need to change the protocol and these six participants were included in the main study.

5.7. Data Collection

Prior data collection, necessary ethical approval and permission for conducting the study were acquired as follow: Ethical approval for the main study ("Pharmacometric optimization of second line drugs for MDR/RR tuberculosis treatment") conducted by DCP was obtained from both the National Health Research Ethics Council (DOH No: 27-0416-5057) and, the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 065/2015) (Appendix B1 & B2). A separate ethical approval to conduct the present study (as a sub-study linked to the main one) was sought from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 595/2018) (Appendix B3). Permission to have access to the research sites (BCH and DPMH) was obtained from the Western Cape Department of Health (Ref: WC-2015RP40-269) (Appendix C).

Data collection included a number of procedures and involved a research team. Firstly, the research team is described, followed by a description of the data collection procedures, including the audiological, pharmacological and genetic aspects. Then, the validity and

reliability of the procedure and test is discussed. Lastly the data management and analysis are described.

5.7.1. Research team

The present study was conducted by a multidisciplinary team including professionals from the following disciplines: audiology, pharmacology, genetics as well as a statistician for data analysis:

Audiologist and researcher: The primary researcher for this study is an audiologist and currently holds an MSc in audiology and over 13 years of clinical experience in a hospital setting and over nine years work experience as a lecturer and clinical educator at UCT. The primary researcher was responsible for the development of the study protocol, obtaining relevant permissions and ethical clearances, and conducting the audiological assessment (ototoxicity monitoring) and analyzing the data. The primary researcher was also trained by a specialist physician from the Division of Clinical Pharmacology to assist with pharmacokinetic calculations and analysis under his supervision.

Specialist physician: A specialist physician from the Division of Clinical Pharmacology was responsible for the development and implementation of the pharmacology clinical study protocol as well as conducting the pharmacokinetic calculations of the kanamycin.

Pharmacology laboratory technicians: A laboratory technician from the Division of Clinical Pharmacology was responsible for the pharmacokinetics measurement of kanamycin in blood samples of participants.

Geneticist: A geneticist from the Division of Human Genetics assisted in the development of the protocols and procedures for the genetic data collection as well as conducting the genetic analysis on the biological samples collected.

Statistician: An independent, experienced statistician from UCT, Department of Statistical Sciences was responsible for the final statistical analysis of the results and assisting with interpretation of them.

Translator: An audiology undergraduate student who was familiar with test procedures and fluent in the English, Afrikaans and IsiXhosa languages was recruited to explain the instructions in the language appropriate to the participant. However, there was no need for the translator, since all of the participants could speak either English or Afrikaans and the resident audiologists at BCH and DPMH, who could speak both languages, assisted the researcher in this regard.

Nurse: An experienced research nurse was appointed by DCP. She was responsible for the phlebotomy procedures and collection of relevant biometric data (e.g height body weight etc), as well as collating the relevant data from the patient files, case history and follow-up records.

5.7.2. Data collection procedure

Data collection procedures in this study involved several procedures that included screening for cognitive problems and collecting audiological, pharmacological and genetic data from the participants. The following is a description of the data collection procedures for each aspect of the study:

5.7.2.1. Cognitive screening

Mini-Cog test (for 3 minutes) (Appendix D) was done on all patients who agreed to participate in the study. These screening tests was conducted on participants to ensure that they did not have cognitive issues that may impair their decision-making with respect to participating in this study, such as dementia (which is linked to HIV and AIDs), and were able to make a truly informed decision (Carnero-Pardo et al., 2013; Watkins & Treisman, 2015). No patients failed Mini-Cog screening and, therefore, none of the participants had to be excluded from the study or be referred to the attending medical doctor for management.

5.7.2.2. Collection of audiological data

Collection of audiological data was done by the primary researcher of this study. Audiological assessments were carried out by the researcher once a month for up to three months after starting the MDR-TB regimen. Because the average duration of hospitalization of patients at BCH and DPMH was about three months, the hearing assessment of most of the participants post three months period was not possible and the resident audiologist at the hospital followed up participants who stayed at the hospital over three months. Audiological evaluation was performed at enrolment into the study (baseline) and at 4, 8 and 12 weeks after starting the MDR/RR-TB treatment. Audiology assessment was timed to ensure that it coincides with the monthly serum creatinine tests for checking the kidney failure, which can increase the risk of hearing damage. Monthly hearing assessments were part of the standard care at BCH and DPMH so the study did not add any extra burden to participants. For this study, pure tone audiometry, tympanometry and ipsilateral acoustic reflexes were chosen. Considering the physical weakness of participants due to their MDR/RR-TB infection, contra lateral acoustic reflexes and speech audiometry were not assessed for this study to keep the time of testing shorter (Durrant et

al., 2009). In addition, as South Africa is a multilingual country and speech test materials are usually available only in English, speech audiometry was not included for this study (Ramkissoon & Khan, 2003).

Each participants underwent the following audiological measures at enrolment to the study (baseline), 4, 8 and 12 weeks post treatment initiation: Comprehensive case history, otoscopic examination, tympanometry, pure tone audiometry (0.25-16 kHz).

5.7.2.2.1. Comprehensive case history

A comprehensive case history interview with the participant was conducted by the researcher at the baseline to record the information relevant to cochleotoxicity. A short case history was also taken at the follow-ups at 4, 8 and 12 weeks post treatment initiation to determine any changes in hearing status (See Appendix E, for case history form). When required a trained translator, translated the information for those who speak Afrikaans or isiXhoza.

5.7.2.2.2. Otoscopic examination

A visual inspection of the outer ear and tympanic membrane (TM) was performed to identify visible abnormalities e.g. discharge, perforation and excessive wax (Swart, 2006). Participants with excessive wax, after waxing removal by the medical officer (doctor), were retested. All participants with visible ear abnormalities that may result in conductive hearing loss (e.g. discharge) were excluded from the study and referred to a medical officer (doctor) at the hospital for medical management.

5.7.2.2.3. Tympanometric examination

Tympanometric examination was conducted to confirm the result of otoscopy and rule out any middle ear abnormality. This occurred at the baseline measure and

subsequently, at the follow-ups at 4, 8 and 12 weeks post treatment initiation. Participants with middle ear abnormalities were excluded from the study and referred to a medical officer (doctor) at the hospital for medical management.

5.7.2.2.4. Audiometric assessment

Pure tone audiometry hearing thresholds were conducted to determine STS based on ASHA criteria (1994), type of hearing loss (sensorineural, conductive or mixed) and grade of cochleotoxicity. Extended UHFA was implemented for this study to increases the test sensitivity for detecting cochleotoxicity (Konrad-Martin et al., 2005). Air conduction thresholds were obtained from 250 Hz until and including 16000 Hz at baseline, 4, 8 and 12 weeks post treatment initiation. Bone conduction was tested at 250-4000 Hz to rule out conductive hearing loss at baseline and when it was necessary. The hearing thresholds were established using Modified Hughson-Westlake procedure (Carhart & Jerger, 1959), as this procedure is not long and the task is not difficult for the participant (Lecluyse & Meddis, 2009).

There is no universal cochleotoxicity grading system and a variety of scales are used to grade the cochleotoxicity worldwide. The existing grading systems for adults are CTCAE, Tune and UCT. Considering the specific shortcomings and strengths of these scales (see section 2.7), conclusion was drawn that all these three scales to be used to grade the cochletoxicity of MDR/RR-TB participants in this study.

5.7.2.2.5. Management of patients with cochleotoxicity

All participants received immediate feedback about their hearing test results. Participants with cochleotoxicity were referred to the resident audiologist at the hospital

for diagnostic assessment. Participants who showed significant threshold changes were assessed every 2 weeks for earlier detection of further changes in their hearing (as part of the routine care at BCH and DPMH). Furthermore, participants with cochleotoxicity were also referred to the attending medical doctor. The medical doctor was then altered the treatment regimen to a non-cochleotoxic drug (Bedaquiline), or reduced the dose and/or frequency of kanamycin (from 6 to 3 days a week), if it was feasible (as part of the routine care at BCH and DPMH). Changing the drug or dose and/or frequency of kanamycin could decrease plasma concentrations of kanamycin, which could effect on hearing loss; this effect was assessed by follow up audiometry (Konrad-Martin et al., 2005; Pelton, 2014).

All participants were followed up monthly for audiometric monitoring, up to three months after starting the MDR/RR-TB medication or until dropout or losing eligibility requirements. As mentioned above, the results were shared with the patient, resident audiologist and the medical doctor for appropriate management/treatment modification. Participants who stayed at the hospital over three months and/or needed hearing amplification were assessed and followed up by the resident audiologist.

5.7.2.3. Collection of pharmacokinetic data

The pharmacological tests included the creatinine testing and the pharmacokinetics of kanamycin, which was managed by DCP. During the study period, the standard regimen for MDR-TB consisted of pyrazinamide, moxifloxacin, kanamycin, terizidone and either ethionamide or isoniazid (depending on the presence of *katG* and *inhA* mutations identified by line-probe assay in the pre-treatment sputum culture, indicating high-level resistance to isoniazid or low-level resistance to isoniazid and resistance to ethionamide, respectively) (Caminero et al., 2010). Ethambutol was added if the risk of ethambutol resistance was considered to be low. Kanamycin was dosed intramuscularly daily, 6 times

per week at 15mg/kg per dose according to the South African Department of Health guidelines during the study period, (DoH, 2013) and adjusted for renal dysfunction at the discretion of the treating clinician. The renal function was assessed at 4, 8 and 12 weeks post treatment initiation.

The pharmacokinetic sampling was performed once patients were established on treatment between two and six weeks. Five serial blood samples were drawn at the following time points: pre-dose and at two, four, six, eight and ten hours post-dose. Dosing was strictly observed and performed under fasting conditions. Blood samples were immediately centrifuged and the plasma was stored at -70°C.

Kanamycin concentrations were measured via liquid chromatography tandem mass spectrometry (LC-MS/MS) using methods validated according to US Food and Drug Administration (FDA, 2018) and European Medicines Agency guidelines (European Medicines Agency, 2011). The samples were processed with a solid phase extraction method using 50µl plasma. Five microliters of the extracted sample were injected onto the HPLC column. Isocratic chromatographic separation was achieved on a Discovery C18, 5µm, 50 mm x 4.6 mm analytical column using four mM HFBA in 0.1% formic acid in water / acetonitrile (80:20, v/v) at a flow-rate of 500 µl/min. The mobile phase flow was split (1:1) at the source of the mass spectrometer. An AB Sciex API 3000 mass spectrometer was operated at unit resolution in the multiple-reaction monitoring mode, monitoring the transition of the protonated molecular ions at m/z 485.2 to the product ions at m/z 163.2 for kanamycin A and the protonated molecular ions at m/z 494.3 to the product ions at m/z 165.3 for the Kanamycin-d9 internal standard. Electrospray ionization was used for ion production. The assay was validated over the concentration range of

0.625 to 40 µg/ml. The combined accuracy (%Nom) and precision (%CV) statistics of the lower limit of quantification (LLQ), low, medium, and high-quality controls (3 validation batches, n=18) were between 101.3% and 107.0%, and 3.0% and 14.3%, respectively. These data were then used to determine the following PK measurements for each patient: dose, cumulative dose, peak, trough, half-lives and area under the curve (AUC).

5.7.2.4. Collection of genetic data

The blood samples for genetic analysis were collected at the same time as the blood samples for PK analysis. Blood samples were immediately centrifuged and the buffy coat was stored at -70°C. Genomic DNA was extracted from frozen buffy coat preparations containing leukocytes with the Chemagic™ 360 automated nucleic acid extraction system (PerkinElmer). For extraction, the DNA blood 5k kit was used according to the manufacturer's instructions. Extracted DNA was suspended in 300µL elution buffer. The concentrations and purity of DNA (260/280 and 260/230 ratios) were assessed with a Nanodrop ND1000 spectrophotometer (Thermo Fisher Scientific) and DNA was diluted accordingly. DNA was amplified by PCR, using the primers which were designed for two known mutations in the Mitochondrial genes *mtCYB* and *mtND3* (Table 5.3). PCR reactions consisted of 100ng DNA, 1X Colourless GoTaq® PCR buffer (Promega, Madison, WI USA), 200 µM of each dNTP (Bioline, London, UK), 0.8 µM of each primer, and one unit GoTaq® DNA polymerase (Promega) in a total reaction volume of 25 µl. Run condition consisted of five steps; an initial denaturation at 95°C for 5 minutes, 35 amplifications cycles through denaturation at 95°C for one minute, primer annealing at 58.5°C (for *mtCYB*) or 54°C (for *mtND3*) for 30 s, and elongation at 72°C for one minute, which was followed by a final extension step at 72°C for 10 minutes. Samples were run in batches of 20 as the samples would not amplify properly at higher batches.

PCR clean-up was performed in 10 µl reactions containing 8.9µl PCR products, 1 µl *FastAP*™ thermosensitive alkaline phosphatase (Thermo Scientific, Waltham, MA USA) and 0.1µl exonuclease I (Thermo Scientific). These reactions were incubated at 37°C for one hour, followed by 15 minutes at 72°C. Direct cycle sequencing was carried out using 5µl cleaned PCR product, 4µl dilution buffer, 2µl Terminator mix, 0.5µl primer and 8.5µl dH20 to make up a 20µl reaction mix. Direct cycle sequencing was carried out in 4 steps; an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 96°C for 30 seconds, primer annealing at 50°C for 15 seconds, and elongation at 60°C for 4 minutes.

The sequencing clean-up was implemented by mixing 5µl of 0.125 ethylene-diamine-tetraacetic acid (EDTA) with the 20µl of the sequencing reaction, in a 96 well plate. Then 60µl 100% ethanol was mixed into the samples and they were sealed and transferred onto an ice block and placed in the freezer for 15minutes. The samples were then centrifuged for 45 minutes at 1870g; the seal was removed, and the plate was inverted prior to the samples being centrifuged at 180g for one minute. Afterward, 60µl of 70% ethanol was added to the samples and the plate was once again sealed and centrifuged at 1870g for 15 minutes. The seal was then removed, and the plate was inverted prior to the samples being centrifuged for one minute at 180g. The samples were air-dried in the dark for 10 minutes at room temperature and subsequently, 10µl of HiDi Formamide was added to the samples. Then the samples were denatured at 95°C for five minutes before flash-freezing on an ice block for one minute. Finally, the sequences were run on the ABI PRISM® 3130*xl* Genetic Analyser (Applied Biosystems, Austin, TX USA) and analysed on UniPro UGene.

Table 5.3. Primers used for the amplification of target regions of *mt-ND3* and *mt-CYB*

Target region	Sense/antisense	Primer sequence (5`-3`)	Expected product size
mt-ND3	Sense	AGT ACT TCG AGT CTC CCT TC	881 bp
	Antisense	GTG GGT GTT GAG GGT TAT G	
mt-CYB	Sense	CTT ACT ATC CGC CAT CCC ATA C	1141 bp
	Antisense	CTG CGG CTA GGA GTC AAT AAA	

Bp= base pairs

5.7.3. Reliability & Validity

Reliability and validity are important concepts used to assess the quality of research. This section discusses relevant threats to reliability and validity as far as this study is concerned. An explanation of how those threats were mitigated was also presented.

5.7.3.1. Reliability

Reliability refers to the consistency of an assessment tool or research study (Gravetter & Forzano, 2011). Evaluation of the stability of the measures (test-retest reliability) is one of the best ways to estimate the reliability of any measure (LoBiondo-Wood & Haber, 2014). Stability is tested using test-retest and parallel or alternate-form reliability testing (LoBiondo-Wood & Haber, 2014). Test-retest reliability is assessed when the same test/instrument is given to the same participants more than once under similar circumstances to see if the scores are the same. This provides an indication of the reliability of the test/instrument (LoBiondo-Wood & Haber, 2014).

Test-retest reliability was obtained for subjective hearing test, pure tone audiometry (PTA). For participants who showed significant changes (≥ 10dB) in their hearing thresholds in comparison to their baseline audiogram, measurements of thresholds were obtained twice for the same ear during the same test session to confirm the threshold. Testing of all participants could not repeatedly be done as they tired quickly.

Furthermore, test-retest reliability of Ultra-high Frequency Audiometry (UHFA) that has been used in this study for early detection of cochleotoxicity have also been shown by several studies. Sinks and Goebelt (1994) investigated the test-retest reliability of serial monitoring of UHF (8-18 kHz) audiometry at bedside with critically ill patients and concluded that it is 99% reliable. Later in 2001, Ahmed et al. also confirmed the test-retest reliability of UHFA and showed that it is as reliable as the conventional audiometry.

With regards to the pharmacokinetics and genetic, test-retest reliability was established prior to the collection of participant samples with the mass spectrometer and thermal cycler, respectively. Plasma levels with kanamycin and genetic tests for target variations were repeatedly tested to confer consistent results. This is explained further in 5.7.2.3 for PK data and 5.7.2.4 for genetic data.

5.7.3.2. Validity

Validity refers to how well the results of a study were able to scientifically answer the questions that it was supposed to answer (Gravetter & Forzano, 2011). Validity needs a reliable assessment tools. However, the assessment tools can be reliable without being valid. Validity is evaluated by examining the internal and external validity (Kimberlin & Winterstein, 2008).

5.7.3.2.1. Internal validity

Internal validity is the extent to which a study establishes a reliable causal relationship between experimental treatment/condition and outcome (Patino & Ferreira, 2018). As such, in this study, the internal validity would refer to whether the genetic and/or pharmacokinetic factors were responsible for the change of hearing thresholds of participants receiving kanamycin for MDR/RR-TB. The internal validity of a study can be affected by many factors and researchers should think about and avoid these errors (Patino & Ferreira, 2018).

Internal validity can be affected by demand characteristics, in research that individuals are involved, which is when the participants who are supposed to perform a task try to figure out what is expected of them and perform accordingly. Other factors that may influence the internal validity of the current study are participant predisposition effects (such as HIV and previous ototoxic treatments) as well as experimenter bias (Kirk, 2009). As almost all of the measures of this study were objective (such as pharmacological and genetic testing as well as tympanograms and acoustic reflexes), the participants and experimenter had no control over the outcome of these measures, which reinforced the internal validity. With regards to PTA, the effects of the demand characteristics could affect the validity, however, as tones were presented inconsistently, the participant could not 'learn' the response.

5.7.3.2.2. External validity

External validity refers to the generalisability of the treatment/condition outcomes (Rothwell, 2005). As there are two main TB referral centers in the Western Cape, BCH and DPMH, and both of them were included in this study, it can be assumed that the accessible population is representative of the target population. Data collection was

conducted on all participants who met the inclusion and exclusion criteria, which were determined to exclude variables that could contribute to hearing loss unrelated to the study aims.

5.7.4. Test validity

Test validity determines whether a test accurately measures what it is supposed to measure (Patino & Ferreira, 2018). The validity of the audiological tests have been used in this study are well established in every day practice (Karzon, 1991; Tyler & Wood, 1980). In this study, the hearing thresholds of participants were established using conventional PTA and High-frequency PTA. Using High-frequency measures allowed for the detection of early changes with hearing thresholds as testing included up to 16 kHz with pure tones. In addition, ASHA (1994) recommends the use of UHFA to detect changes in hearing of patients receiving ototoxic medications which emphasis on the validity of this measure. Measures such as otoscopy, tympanometry, and acoustic reflexes ensured that the PTA revealed valid data, and outer/middle ear status were not affecting the results.

The validity of using mass spectrometer for detection of the kanamycin plasma levels has been approved by various studies (Cuyckens, 2019; Stead, 2000; Yi, Lokesh, & Akowuah, 2020). The geneticist prior testing the samples confirmed the validity of using thermal cycler for detection of target variations. The validity of the tests has been used in the current study and is further enhanced by sensitivity and specificity of the data presented in Table 5.4.

Table 5.4. Demonstrates the sensitivity and specificity of each test

Tests	Sensitivity	Specificity	Application	Limitation	Solution	Reference
Tympanometry	90%	75%	Identification of TM problems	Can't be done when wax/discharge present	Refer + repeat once each clear	Onusko, 2004
Pure tone audiometry	74.4%	92.1%	Identification of hearing loss	Diagnosis of MEE	Use of test battery	Forster & Kumar, 1997
Polymerase chain reaction (PCR)	96%	99.4%	Amplification of genome			Sharaan, Wu, Petersen, & Zhang, 2008
Liquid chromatography tandem mass spectrometry (LC-MS/MS)	Highest	Very high	Therapeutic drug monitoring			Wu & Lynch, 2012

TM= tympanic membrane, MEE= middle ear effusion

5.8. Data Management

All data was captured and managed as per UCT Research Data Management Policy (2018). All participants were given a study number generated by a specified database. As a result, all data could be synchronised.

In the data management a well-designed database can help to maximise the quality of the data (Needham et al., 2009). Therefore, an online secure database was designed by the database design IT employee at UCT to store the study's data. The researchers captured the data weekly on the database. This database made it easy for the authorised members of the research team to have access to the data of the study. In addition, this online database will preserve and keep the data of the study accessible and useable for future research.

Quality assurance reviews of both collection and entry and system-based controls reduce the likelihood of error (Needham et al., 2009). In order to minimise the missing data and errors, the researcher reviewed the paper-based data/spreadsheets and two trained undergraduate students reviewed the captured data onto the database to identify and correct the missing data and errors with system-based controls.

The data quality was ensured via various aspects; the validity and reliability of the data was assured by choosing suitable equipment as well as methods of collection and analysis of data, the integrity of the data was maintained by prevention of any unauthorised changes to the data and disabling data changes on database after confirmation of the correct data entry.

5.9. Data Analysis

A statistical software programme (Stata version 15) was used to analyse this study's data (StataCorp, 2017). Stata enabled importing data from Microsoft Excel spreadsheet into the database. Stata also provided summarise command for performing descriptive statistical analysis: Measures of central tendency (mean & median), variance (standard deviation, range), interquartile range (IQR) and frequency statistics (proportions).

Inferential statistical analysis of data was also conducted using Chi-square/Fisher's exact test, cox regression and Wilcoxon Mann–Whitney rank sum test. The Chi-Square test is used to determine if there is a significant relationship between two nominal variables and Fisher's exact tests is used when the samples are small for Chi-Square test (cochleotoxicity and presence of T10114C and T15312C mutations) (Balakrishnan et al., 2013). Wilcoxon Mann–Whitney rank sum test is used to test the hypothesis of a zero-median difference between two independently sampled populations (Harris & Hardin,

2013). Cox regression (or proportional hazards regression) is a method for investigating the effects of several risk factors or exposures simultaneously (predisposing factors of cochleotoxicity) to a particular event happening (cochleotoxicity) at a particular point in time (up to 12 weeks post MDR-TB treatment) (Nikulin & Wu, 2016). P-values < 0.05 were considered statistically significant. Usually, at the end of a study, some of the participants may not have experienced the event (cochleotoxicity), however, if the study continued, they may have experienced it. Moreover, the outcome is unknown for participants who have opted to leave the study or were lost to follow up. Therefore, Kaplan-Meier analysis was also used in this study, which allowed estimation of cochleotoxicity over time, even for participants who were studied for different lengths of time (Kishore et al., 2010).

The minor allele frequency (MAF) was calculated for *T10114C and T15312C* to determine the frequency of these mutations among the participants. The MAF was considered as low frequency (rare variant) when MAF$\leq$ 0.01 (Xiong et al., 2009). A summary of data analysis methods has been provided in Table 5.5.

Table 5.5. Summary of data analysis methods

Aims/objectives		Type of data	Data collection tools &/or sources	Analysis methods
Incidence of cochleotoxicity		Nominal	Audiometry, ASHA criteria	Frequency counts & Kaplan-Meier
Grade of cochleotoxicity		Ordinal	CTCAE, TUNE and UCT criteria/scales	Frequency Counts & Kaplan-Meier
Association between with cochleotoxicity & participant/treatment-related factors	Comorbid presentation of MDR/RR-TB & HIV	Nominal	Participant's hospital records/case history & audiometry	Descriptive statistics & Cox regression
	Previous MDR/RR-TB treatment	Nominal		
	Age & BMI	Ratio		
	Gender	Nominal		
	Renal dysfunction	Nominal		
Association between pharmacokinetics of Kanamycin & cochleotoxicity		Ratio	Measurement of kanamycin plasma concentrations & audiometry	Descriptive statistics, Cox regression & Wilcoxon rank-sum
Association between two mtRNA mutations (T10114C and T15312C) & cochleotoxicity		Nominal	Genetic analysing & audiometry	MAF, Chi-Square/Fisher's exact

5.9.1. Audiological data analysis

The incidence of cochleotoxicity was determined based on ASHA criteria for STS for cochleotoxicity (1994) (Table 5.6). The data obtained from the baseline audiogram was compared with the last audiogram to determine the STS and analysed via frequency counts. The frequency range of STS was determined according to ASHA criteria for STS in three different frequency ranges; low (0.25 to 2kHz), high (3 to 8 kHz) and ultra-high (9 to 16 kHz) and analysed via frequency counts. The incidence of cochleotoxicity over time was estimated using Kaplan-Meier failure analyses (Friis & Sellers, 2014; Kishore et al., 2010).

Table 5.6. Detection of significant threshold shift

Pure Tone Audiometry (.25-16kHz)	At one test frequency	At two adjacent test frequencies	At three adjacent test frequencies
Comparison between baseline & the follow-up audiograms	20 dB decrease	10 dB decrease	Loss of response where they were previously obtained

Note. Adapted from "Guidelines for the audiologic management of individuals receiving cochleotoxic drug therapy" by American Speech-Language-Hearing Association, 1994.

The degree of hearing loss was determined based on the last audiogram that was obtained from each ear and analysed via frequency counts. The World Health Organization grades of hearing impairment (WHO, 2008) were used for determination of degree of hearing impairment at conventional frequency range (0.25 to 8 kHz) (Table 5.7).

Table 5.7. Degree of hearing impairment for adults (0.25 to 8kHz)

Degree	Level of hearing loss (dBHL)
No impairment	≤ 25
Slight	26-40
Moderate	41-60
Severe	60-80
Profound	≥ 81

The STS was investigated based on the frequency range; low (0.25-2kHz), High (3-8kHz) and ultra-high (9-16kHz) and analysed via frequency counts (Seddon et al., 2012). The frequency range of STS was determined for each ear based on the audiograms, which were obtained at 4, 8 and 12 weeks after starting the MDR-TB medication.

The grade of cochleotoxicity was ascertained according to the three different scales, CTCAE, TUNE and UCT (Table 5.8). The cochleotoxicity was graded per ear and analysed via frequency counts.

Table 5.8. TUNE, UCT & CTCAE cochleotoxicity grading scales for adults

CTCAEv5	**TUNE**	**UCT** * [PTA: 0.5, 1, 2 & 4 kHz] (SANS 10154-1)
Grade 0: Not defined	Grade 0: No hearing loss	0 (No impairment): No significant change in hearing thresholds
Grade 1: Adult (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of 15 to 25 dB averaged at 2 contiguous test frequencies in at least 1 ear	Grade 1a: Threshold shift ≥ 10dB at [8 10-12.5]	Grade 1a (UHF threshold shift): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 9-16kHz, PTA: 10-15 dB HL
	Grade 1b: Threshold shift ≥ 10dB at [1-2-4]	Grade 1b (Slight impairment): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz, PTA: 16-25 dB HL
Grade 2: Adult (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least 1 ear	Grade 2a: Threshold shift ≥ 20dB at [8-10-12.5]	Grade 2a (Mild Impairment): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz, PTA: 26-40 dB HL
	Grade 2b: Threshold shift ≥ 20dB at [1-2 4]	Grade 2b (Moderate Impairment): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz, PTA: 41-60 dB HL
Grade 3: Adult (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least 1 Ear	Grade 3: Hearing level ≥ 35 dB at [1-2-4] de novo	Grade 3 (severe Impairment): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz, PTA: 61-80 dB HL
Grade 4: Adult: Profound bilateral hearing loss (> 80 dB at 2 kHz and above)	Grade 4: Hearing level ≥70 dB at [1-2-4] de novo	Grade 4 (Profound Impairment): ≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz, PTA ≥ 81 dB HL

Abbreviations: [8-10-12.5], pure tone average 8-10-12.5 kHz; [1-2-4], pure tone average 1-2-4kHz, PTA=pure tone average, UHF=ultra-high frequency
* Appendix F

In order to determine the association between cochleotoxicity and participant/treatment-related factors, the data obtained from the participant's hospital records regarding HIV status, history of previous MDR/RR-TB treatment, gender, BMI (healthy range: 18.5 to

<25 kg/m2) and age of participant at enrolment to the study were analysed using descriptive statistics & Cox regression. It was not possible to assess the relationship between exposure to excessive noise and cochleotoxicity, as none of the participants exposed to excessive noise during their MDR/RR-TB treatment.

The creatinine clearance (CrCl) was calculated using Cockroft-Gault method. The creatinine clearance over 90 mL/min/1.73m2 was considered as normal for kidney function (NIAID, 2017). The severity of renal dysfunction was graded as moderate (< 90 to 60 ml/min or ml/min/1.73 m2), severe (< 60 to 30 ml/min or ml/min/1.73 m2) and potentially life-threatening (< 30 ml/min or ml/min/1.73 m2) based on the DAIDS grading system (2017).

5.9.2. Pharmacological data analysis

The pre-dose kanamycin plasma concentrations below Lower limit of quantification (LLQ) (0.625 µg/mL) was imputed as half the LLQ value and STATA version 15.0 was used to perform the non-compartmental analyses. Area under the concentration-time curve (AUC) from 0 to 10 hours after the dose (AUC_{0-10}), AUC to infinity (AUC_{∞}), half-life, peak concentration, and time to peak concentration were assessed. The trapezoidal rule was applied for computation of the AUC_{0-10} and the exponential extrapolation option was used to calculate AUC_{∞}. The cumulative dose of kanamycin was calculated by multiplying the dose by the number of days a particular dose was administered before hearing loss developed. The average daily dose was calculated by dividing the cumulative dose of kanamycin by the number of days recorded from treatment initiation to first detection of hearing loss. Cumulative AUC was measured by multiplying the AUC_{0-10} on the PK sampling day by the number of days the same dose was administered before first

detection of hearing loss. If the dose was changed during the treatment period, the change in AUC was predicted by increasing or decreasing the exposure proportionally to the change in dose, since AUC after parental administration equals dose divided by clearance. For example, if the dose of kanamycin was halved by the treating clinician, assuming linear kanamycin pharmacokinetics, the AUC was 50% lower for the time period that the lower dose was administered. The average daily AUC of kanamycin was calculated by dividing the cumulative $AUC_{0\text{-}10}$ of kanamycin by the number of days from treatment initiation to first detection of cochleotoxicity.

The PK factors and cumulative kanamycin exposure measures associated with cochleotoxicity were analysed using univariate Cox proportional hazards regression. The covariates with a p value of <0.2 were included in the multivariate model. The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare cumulative and average daily dose and AUC between participants with and without cochleotoxicity.

5.9.3. Genetic data analysis

The associations between *T10114C and T15312C* mutations and the development of cochleotoxicity were analysed using Chi-square/Fisher's exact test. The minor allele frequency (MAF) was calculated for T10114C and T15312C. MAF is the frequency of minor or recessive allele in a given population. MAF is used to differentiate between common and rare variants in the population (Hernandez et al., 2019; Xiong et al., 2009). The MAF was considered as low frequency (rare variant) when MAF≤ 0.01 (Xiong et al., 2009).

5.10. Ethical Considerations

The following ethical principles have been compiled in accordance with the World Medical Association (WMA) Declaration of Helsinki, (WMA, 2013).

5.10.1. Autonomy

Participants of the bigger study conducting at the DCP were informed [by the researcher and a trained translator (if necessary)] both verbally and in writing about the aims and nature of the present study and what was expected of them (Appendix A: Information letter & consent form). They were given the opportunity to discuss their participation with their loved ones, ask questions and seek clarification prior to signing the informed consent form. Patients who signed the consent form for participation in the study (Appendix A) had a Mini-Cog screening test (a three-minute test for detection of cognitive impairment). Participants who passed the Mini-Cog test remained in the study.

All participants were verbally and in writing informed of their rights to voluntary participation in the study and to withdraw their participation at any time once the study has begun, without their treatment (by the hospital) being affected in any way (Appendix A).

5.10.2. Confidentiality

All participants were assigned a research number and data were recorded and analysed using this number and not their names. Participant' name was only used for identification in repeated hearing assessments. The participant's hospital records and the results of the hearing, blood and genetic tests were handled with caution, and were not disclosed to anyone other than the health care professionals working with the patient (for appropriate management/treatment). The research team from the Division of Clinical Pharmacology (DCP) had access to the hearing tests results, for which informed patient consent and

ethical approval had been granted (HREC/REF:065/2015). Participants' data, saved on the researcher's laptop were password protected and only were accessible to the researcher. Participants' data with assigned research number (no name) will be kept for five years from the commencement of the study and thereafter will be removed from the researcher's laptop. All the participants were informed in the information letter that they would not be identified in any way in any publications arising from the study.

5.10.3. Non-Maleficence

The hearing tests of this study (at BCH and DPMH) were non-invasive, non-painful and part of routine care and should not cause any harm. The present study only added pure tone audiometry of four extra frequencies for determination of UHF hearing loss, which added up to five minutes to the length of the test. The study was designed in such a way that there were none/minimal risks involved in participation.

Information needed for the current study that pertains to blood sampling was obtained from a parallel study undertaken by the DCP. The ethical approval for the parallel study had been granted by DCP (HREC/REF:065/2015).

5.10.4. Beneficence

At the time of this study regular hearing monitoring was not possible at DPMH, due to lack of a resident audiologist and limited access to kuduwave audiometer (one day a week). At BCH, at the time the present study the hearing assessment was restricted from 250 Hz to 8kHz, not including higher frequencies, which are affected firstly by cochleotoxicity. Participants of the present study received regular monthly hearing monitoring (up to three months after starting the MDR/RR-TB treatment) including the pure tone audiometry at

UHF (up to 16kHz). The UHFA allows for earlier detection of cochleotoxicity before there is damage to speech frequency range. Information about the abnormality of the auditory system were shared immediately with the attending physician, who could alter the medication/drug dosage to decrease the hearing loss side effect of the medication, therefore, preserving the hearing loss at speech frequency.

All participants got immediate feedback about their hearing status. Participants who needed further audiological assessment/management were referred to the resident audiologist at BCH. The results of the study were presented to the superintendent, which may be used for the development of new protocols/strategies.

Travel and other costs incurred as a result of participation in the study were reimbursed from the funds of a parallel study conducted at the DCP.

5.10.5. Justice

The principal of justice considers that the selection of participants is fair, as well as the risks and benefits of participation are distributed equally (Kornblau & Burkhardt, 2012). Participants of this study were selected as they were at risk for developing cochleotoxicity, which was under investigation by this study and their ethnic origin, gender, socio-economic or linguistic background did not influence the selection. Participants of the study were likely to gain from the findings of this study. The present study was designed in such a way that there were none/minimal risks involved in participation and the participants were able to benefit from the outcomes of the research as, by early identification of their hearing loss they had the opportunity to preserve their hearing before there was damage to their speech frequency range.

Participants of this study were considered vulnerable since they were in-patients and also

had the risk of having dementia linked to HIV/AIDS (Watkins & Treisman, 2015), so Mini-Cog test was conducted to ensure that patients could make a truly informed decision. They also had the opportunity to read the patient information sheet, think about it and discuss their participation with their loved ones prior to signing the informed consent form. For patients who spoke in Afrikaans and isiXhosa a trained translator and informed consent form in Afrikaans and isiXhosa were available. Informed consent in this study was obtained in such a way to reassure ethics of patient autonomy.

5.10.6. Professional Competence

The researcher is a senior Audiologist, with 13 years of work experience (familiar with test procedures and equipment to be used in this study), and an MSc in Audiology.

5.10.7. Dissemination

The results of the study were made available to all relevant stakeholders such as the BCH, DPMH and all other interested parties. It was also sheared with chief director of TB control and management at national Department of Health. The results of the study will also be published in reputable academic journals. Parts of the results of study has already been published in International Journal of Audiology in 2019 “Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi drug resistant tuberculosis” (Appendix G).

Chapter 6: Results

This chapter will present the findings of this study according to its aims and sub-aims. Firstly, the incidence and grade of cochleotoxicity as well as covariates associated with it are presented. Subsequently, the pharmacokinetic (PK) of kanamycin in relation to cochleotoxicity is illustrated. Finally, findings regarding two mitochondrial mutations, T10114C (I19T in MT-ND3) and T15312C (I189T in MT-CYB), and their association with kanamycin-induced cochleotoxicity are presented.

6.1. Participants Description

A total of 147 participants were initially recruited to take part in this study. Forty-five were excluded from the study due to various reasons (see Table 3.3). In the end, there were 102 participants who had analysable audiological data. The median age of these 102 participants at baseline was 34.9 years and there were slightly more males (57%) than females. While the majority of participants (n=65, 64%) had a normal renal function (CrCL> 90 ml/min/1.73m2), 13 (13%) had severe renal impairment (CrCL< 60 ml/min/1.73m2). Participant characteristics are shown in Table 6.1.

Table 6.1. Baseline characteristics of study (n=102)

Variable	Value
Number. (%) male	58 (56.9%)
Median age, year (Range)	34.9 (27.2-42.2)
Median BMI, kg/m^2 (IQR)	17.3 (15.6-18.9)
Median duration of treatment to first detection of hearing loss, day (IQR)	61 (43 to 81)
Number. (%) HIV infected	65 (63.7%)
Number. (%) Antiretroviral therapy (ART)	65 (63.7%)
Number. (%) with previous MDR-TB treatment	24 (23.5%)
Creatinine clearance, mL/min (IQR)	79.7 (58.8 to 98.8)

Where appropriate, the percentage/ interquartile range (IQR) is shown in brackets

6.2. Incidence of Cochleotoxicity

6.2.1. Significant threshold shift (STS) in hearing

Of the 102 participants with analysable hearing data, 84 (82.4%) showed STS in their last audiogram based on ASHA criteria for cochleotoxicity (1994). Of these 84 participants, 61 (73%) had STS bilaterally and 17(20%) had unilateral STS. The remaining 6 (7%) participants had unilateral STS and unilateral middle ear infection (which excluded one of their ears from the study).

The results of analysis of the frequency range of STS during the 12 weeks of treatment showed that in all of the participants the STS started from UHF. The STS was detected at UHF before affecting other frequency ranges in 43 (42%), 39 (38%) and 36 (35%) participants at week 4, 8 and 12 after starting their treatment, respectively. The STS extended from UHF to conventional frequency range (0.25 to 8kHz) in 15 (15%) participants during the 4 weeks of treatment, which increased by 120% and 220% to 33 (33%) and 48 (47%) participants during 8 and 12 weeks of treatment, respectively (see Table 6.2).

Table 6.2. Frequency range of STS as a function of duration of treatment (n=102)

Frequency Range	UH	Conventional *		Total number of participants with STS
	9-16	0.25-8		
		High	Low	
kHz		3-8	0.25-2	0.25-16
Number of participants with STS during 0-4 weeks	43 (42%)	6 (6%)	9 (9%)	58 (57%)
Number of participants with STS during 0-8 weeks	39 (38%)	15 (15%)	18 (18%)	72 (71%)
Number of participants with STS during 0-12 weeks	36 (35%)	27 (26%)	21 (21%)	84 (82%)

UH=ultra high, Frequency range of STS for participant is based on the most severely affected ear.
*STS started at ultra-high frequency and extended to High frequency and then Low frequency, so overtime, the number of participants who had just UHF hearing loss decreased as they developed high and/or low frequency hearing loss.

The results of analysis of the degree of hearing loss based on conventional frequency range (0.25 to 8 kHz) showed that out of 84 participants with STS in their last audiogram, 37 (44%) had slight to severe (26-80dB) degree of loss (see Table 6.3). The type of hearing loss was sensorineural for all these 37 participants who had hearing loss at conventional frequency range.

Table 6.3. Degree of hearing impairment in participants with STS based on their last audiogram (n=84)

Degree	No impairment	Slight	Moderate	Severe	Profound
	≤ 25 dB	26-40 dB	41-60 dB	61-80 dB	≥ 81dB
Number of participants	47	17	10	10	0
%	56	20	12	12	0

Degree of hearing impairment is based on WHO grading system (2008). Degree of impairment for participant is based on the most severely affected ear.

6.2.2. Grade of cochleotoxicity

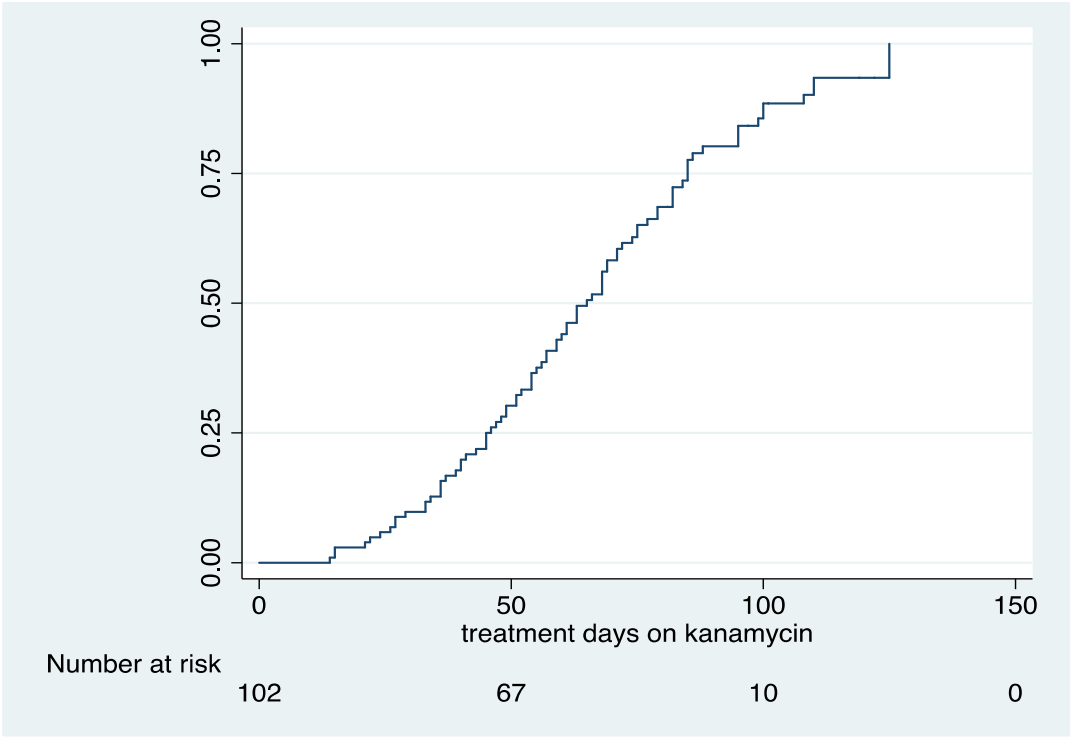
For surveillance of hearing loss, cochleotoxicity was graded in 84 participants who had STS based on ASHA criteria (1994), using three different cochleotoxic grading scales; CTCAEv5, TUNE and the UCT scale (Table 5.8). The result of grading of cochleotoxicity showed that 45 (53%), 65 (77%) and 84 (100%) of these participants, had cochleotoxicity above grade 0 (grade 1/1a to 3) based on CTCAE, TUNE and UCT grading scale, respectively (see Table 6.4).

Table 6.4. Grade of cochleotoxicity in participants with STS (n=84)

Grade	**Cochleotoxicity Scales**		
	CTCAEv5	**TUNE**	**UCT**
0	39 (46.5%)	19 (23%)	0 (0%)
1/1a	16 (19%)	16 (19%)	10 (12%)
1b		13 (15.5%)	37 (44%)
2/2a	13 (15.5%)	22 (26%)	17 (20%)
2b		7 (8.3%)	10 (12%)
3	16 (19%)	7 (8.3%)	10 (12%)
4		0 (0%)	0 (0%)
Total No of participants with grade > 0	45 (53.5%)	65 (77%)	84 (100%)

UCT criteria for cochleotoxicity in adults (Ramma, 2016)
To determine the grade of impairment for each participant, CTCAEv5, TUNE and UCT criteria were applied based on the most severely affected ear.

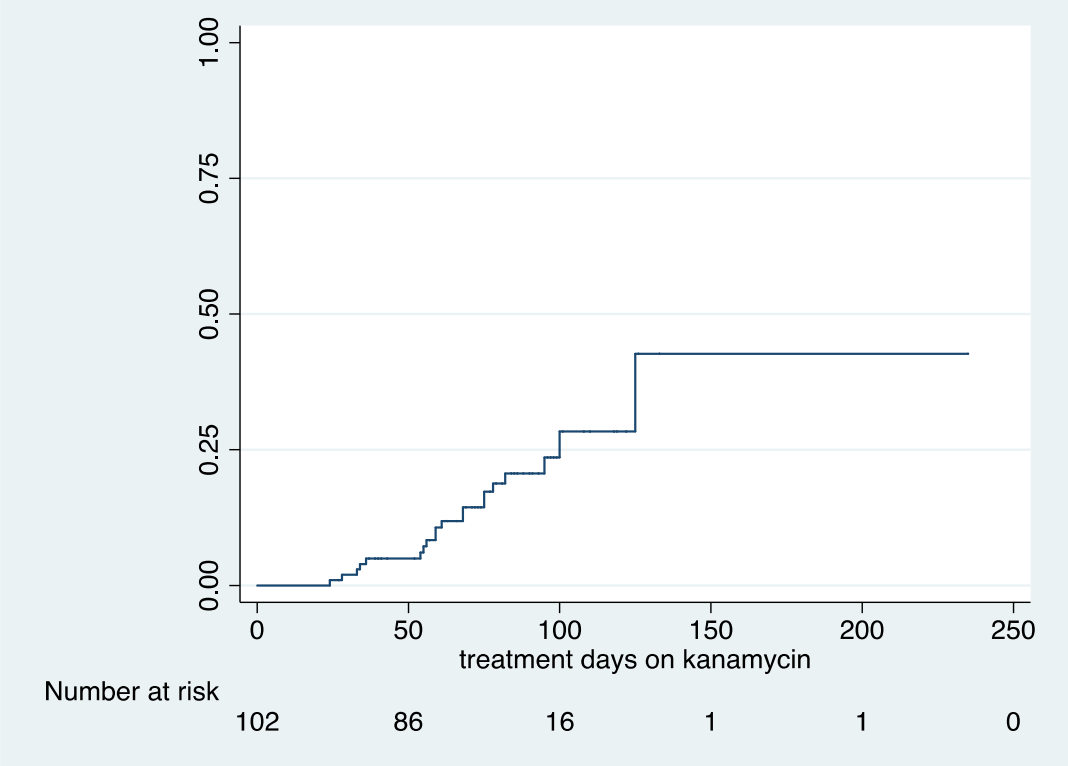
Kaplan-Meier failure analysis was used to estimate the incidence of cochleotoxicity based on ASHA criteria (1994) amongst participants over time (see Figure 6.1). Figure 6.1 showed that if follow up would be continued up to 125 days, all of the participants (100%) would develop cochleotoxicity.



Cochleotoxicity was determined based on ASHA criteria (1994)

Figure 6.1. Time to (> grade 0) cochleotoxicity amongst MDR/RR-TB participants (n=102)

Kaplan-Meier failure analysis was also used to estimate the incidence of moderate-severe grade of cochleotoxicity (grade 2b or 3) based on UCT grading scale amongst participants over time (see Figure 6.2). Figure 6.2 showed that if follow up would be continued up to 125 days 86 (84%) participants would develop moderate-severe grade of cochleotoxicity.



Moderate and severe grade of cochleotoxicity based on UCT scale (Ramma, 2016)

Figure 6.2. Time to moderate-severe grade of cochleotoxicity (grade 2b & 3) amongst MDR/RR-TB participants (n=102)

The follow up time period for Figures 6.1 and 6.2 extends beyond the 90 days study period as the final hearing tests for some participants were either delayed or postponed for logistical reasons. According to Figures 6.1 and 6.2, it took slightly longer to develop moderate-severe grade of cochleotoxicity than any grade of cochleotoxicity and there were relatively fewer participants who developed moderate–severe grade of cochleotoxicity (86 vs 100).

6.2.3. Association between cochleotoxicity and participant/treatment-related factors

The variation in incidence of cochleotoxicity was analysed amongst participants as a function of the following factors: Sex, age, HIV infection, previous MDR/RR-TB treatment and BMI. The result of the statistical analysis revealed that there was no statistically significant relationship between cochleotoxicity and these variables. See

Tables 6.5 and 6.6 for more details.

Table 6.5. Participant/treatment-related factors associated with cochleotoxicity amongst MDR/RR-TB participants (n=102)

Variable	Univariate	
	HR (95%CI)	P value
Sex	0.99 (0.64 to 1.53)	0.968
Age	0.98 (0.96 to 1.00)	0.058
HIV	1.06 (0.67 to 1.67)	0.813
Previous MDR-TB treatment	0.90 (0.56 to 1.45)	0.670
BMI	0.98 (0.92 to 1.05)	0.761
Creatinine clearance	1.04 (0.62 to 1.65)	0.819

aHR: adjusted Hazard Ratio
HR: Hazard ratio
CI: Confidence Interval

Table 6.6. Participant/treatment-related factors associated with cochleotoxicity amongst MDR/RR-TB participants with moderate-severe grade of cochleotoxicity (n=20)

Variable	Univariate	
	HR (95% CI)	P value
Sex	0.98 (0.40 to 2.36)	0.959
Age	1.02 (0.98 to 1.06)	0.302
HIV	1.71 (0.67 to 1.67)	0.300
Previous MDR-TB treatment	0.61 (0.62 to 4.75)	0.409
BMI	0.96 (0.90 to 1.03)	0.712
Creatinine clearance	1.60 (0.62 to 1.51)	0.578

HR: Hazard ratio
CI: Confidence Interval
Moderate and severe grade of cochleotoxicity based on UCT scale (Ramma, 2016)

6.3. Association between Pharmacokinetic (PK) of Kanamycin and the risk of cochleotoxicity

The key pharmacokinetics factors for kanamycin were compared between participants with and without cochleotoxicity. These measures included doses (mg/kg), peaks (µg/ml), troughs (µg/ml), AUC_{∞} (µg•hr/L), $AUC_{0\text{-}10}$ time (µg•hr/L), half-lives (hours). The result

of the statistical analysis showed no statistically significant difference with respect to key PK measures of kanamycin between the two groups of participants (see Table 6.7).

Table 6.7. Comparison of the key PK measures of kanamycin in MDR/RR-TB participants (n=102)

PK measure	Cochleotoxicity (n=84)	No cochleotoxicity (n=18)	P value
Dose (mg/kg/day)	15.9 (15.0 to 17.5)	16.0 (14.5 to 17.2)	0.443
Peak concentration (µg/mL)	36.4 (29.4 to 42.7)	34.1 (29.5 to 38)	0.512
*Trough concentration (µg/mL)	0.3125	0.3125	0.420
AUC_{∞} (µg•hr/L)	168.8 (134.6 to 244.0)	160.1 (128.9 to 199.3)	0.432
AUC_{0-10}	155.6 (127.3 to 212.1)	152.5 (121.2 to 168.2)	0.425
Half- life (hours)	2.5 (2.2 to 3.4)	2.5 (2.2 to 2.9)	0.345

Median is shown with interquartile range in brackets
*Most of Participants had the same trough concentration so there was no interquartile range

The cumulative kanamycin exposure was compared between participants who developed cochleotoxicity and those who did not. The result of the statistical analysis revealed that there was no statistically significant difference with respect to cumulative kanamycin exposure between the two groups (see Table 6.8).

Table 6.8. Comparison of cumulative kanamycin exposure in MDR/TB participants (n=102)

Variable	Cochleotoxicity (n=84)	No cochleotoxicity (n=18)	P value
AUC_{0-10}	155.6 (127.3 to 212.1)	152.5 (121.2 to 168.2)	0.425
Cumulative AUC_{0-10}	9450.9 (6541.3 to 12615.2)	7226.8 (4794.7 to 9885.0)	0.103
Average daily AUC_{0-10}	639.2 (450.9 to 689)	644.1 (477.6 to 666.7)	0.567
Cumulative dose (mg)	46625 (33687.5 to 59375)	41678 (27750 to 62500)	0.390
Average daily dose (mg)	639.2 (450.9 to 689.0)	644.1 (477.6 to 666.7)	0568

Median is shown with interquartile range in brackets

The association between cochleotoxicity and the key kanamycin pharmacokinetic measures and cumulative kanamycin exposure measures were analysed using both univariate and multivariate models. The results of univariate analysis showed no statistically significant association between cochleotoxicity and any of the kanamycin PK measures and cumulative kanamycin exposure measures. Then, variables for PK of kanamycin, cumulative kanamycin exposure as well as participant/treatment related factors (Table 6.5) with p values <0.2 in univariate analysis (age and AUC-time [AUC_{0-10}]) were analysed in the multivariate model. The analysis showed that age was marginally significant (p=0.050), however, kanamycin exposure was significantly associated with cochleotoxicity with about 3% increased risk of cochleotoxicity for every 10µg•hr/L increase in kanamycin AUC_{0-10} (p=0.028) (Table 6.9). The AUC_{0-10} was also analysed in a subgroup of 20 participants with moderate-severe grade of cochleotoxicity and revealed a stronger association between kanamycin AUC_{0-10} and cochleotoxicity. The results showed that for every 10µg•hr/L increase in average daily AUC_{0-10}, the risk of moderate and severe grade of cochleotoxicity increases by about 6% (HR: 1.05, 95% CI: 1.01 to 1.10; p=0.017).

Table 6.9. PK and participant/treatment related factors associated with cochleotoxicity in MDR/RR-TB participants (n=102)

Variable	Univariate		Multivariate	
	HR (95%CI)	P value	aHR (95%CI)	P value
Age	0.98 (0.96 to 1.00)	0.058	0.97 (0.95 to 1.00)	0.050
AUC_{0-10} (per 10 µg•hr/L increase)	1.03 (1.00 to 1.05)	0.051	1.03 (1.00 to 1.06)	0.028

aHR: adjusted Hazard Ratio
HR: Hazard ratio
CI: Confidence Interval

6.4. Association between cochleotoxicity and two potentially pathogenic mitochondrial mutations, T15312C (I189T in MT-CYB) and T10114C (I19T in MT-ND3)

The statistical analysis of the association between cochleotoxicity and two potentially pathogenic mitochondrial mutations, T15312C and T10114C, was not possible due to the low frequency of these mutations in the study's sample size. Out of 102 MDR/RR-TB participants with reliable hearing test results, the DNA samples of 95 participants were available to be tested. Of these 95 participants, the DNA sample of 17 participants failed to be sequenced for T15312C (I189T in *mt-CYB*) variation. Of the remaining 78 participants 66 participants had cochleotoxicity and the rest had no sign of cochleotoxicity. The T15312C variation was detected in 3 (4.5%) of these 66 participants with cochleotoxicity and it was not detected in participants who had no sign of cochleotoxicity (n=12). The T15312C was detected in cohort at minor allele frequency (MAF) of 0.045 (see Table 6.10). A representative chromatogram of the T15312C (I189T in *mt-CYB*) sequencing results is shown in Figure 6.3.

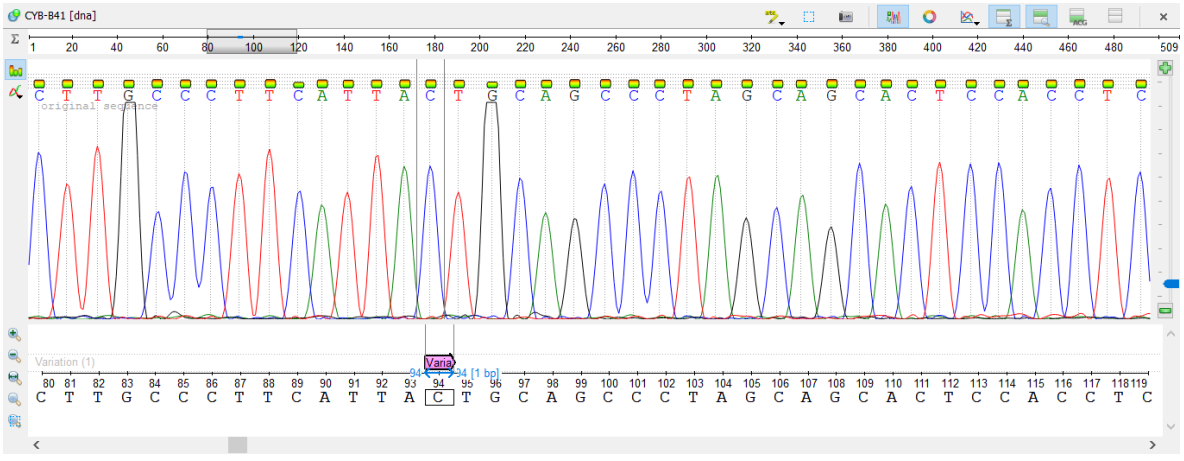
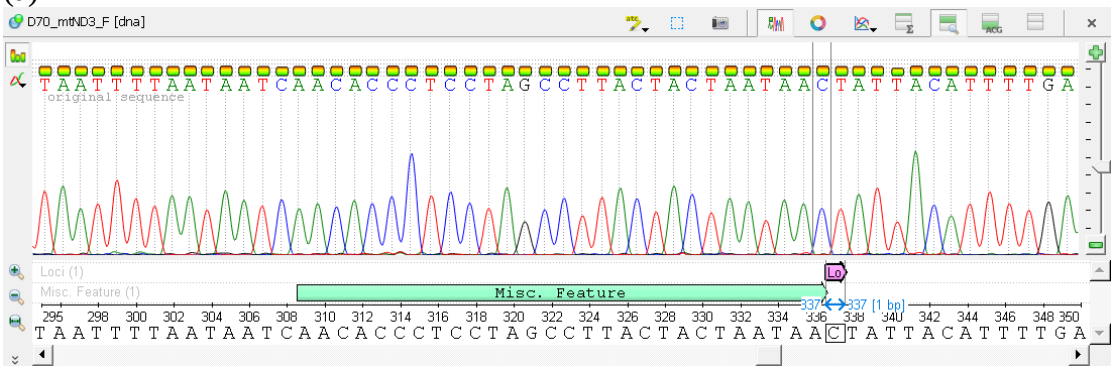


Figure 6.3. Chromatogram of the T15312C (I189T in *mt-CYB*) sequencing results (Homozygous Target Variation). The position of the mutation is indicated with the purple arrow.

For the T10114C (I19T in *MT-ND3*) variation, out of 95 participants, the DNA samples of

15 participants failed to be sequenced. In the remaining 80 participants, 65 had cochleotoxicity and the rest had no sign of cochleotoxicity. The T10114C was detected in 4 (6% [three patients were homozygous for the variation and one patient was heterozygous]) of these 65 participants with cochleotoxicity and it was not detected in participants who had no sign of cochleotoxiciy (n=16). The MAF for T10114C is 0.061 (see Table 6.10). A representative chromatogram of the T10114C (I19T in *MT-ND3*) sequencing results is shown in Figure 6.4.

(a)



(b)

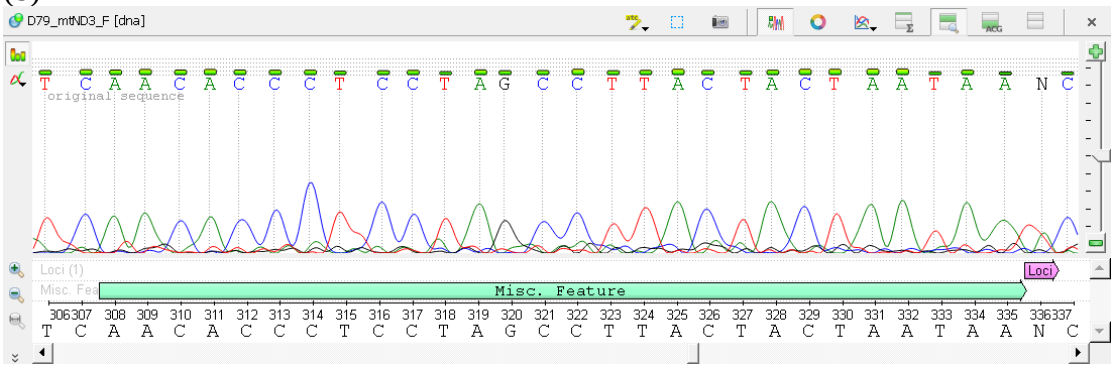


Figure 6.4. Chromatogram of the T10114C (I19T in *mt-ND3*) sequencing results. The position of the mutation is indicated with the purple arrow. (a) Homozygous and (b) Heterozygous Target Variation.

Table 6.10. Prevalence of T15312C (I189T in *MT-CYB*) and T10114C (I19T in *MT-ND3*) variations in participants with sequenced DNA samples (n=78 for *mt-CYB* & N=80 for *mt-ND3*)

Gene	Genotypes	SNP	Variant	**Hearing loss** n=66 for *mt-CYB* & n=65 for *mt-ND3*	**No hearing loss** n=12 for *mt-CYB* & n=15 for *mt-ND3*	MAF
mt-CYB	Homozygous	rs1603335215	m.15312T>C	3 (4.54%)	0 (0%)	0.045
mt-ND3	Homozygous	rs779734442	m.10114T>C	3 (5.35%)	0 (0%)	0.046
	Heterozygous	rs779734442	m.10114T>C	1 (1.53%)	0 (0%)	0.015

SNP = single nucleotide polymorphisms

Out of the 66 samples that were analysed for T15312C (I189T in *mt-CYB*) variation, two samples (3.03%) contained the non-target variant (G15301A) that was homozygous for different variations around the same site as the T15312C variant (see Table 6.11). Out of the 65 samples that were analysed for the T10114C (I19T in *MT-ND3*) variation, five samples (9.23%) contained the non-target variation (T10115C), which were homozygous for different variations around the same site as the T10114C variant (see Table 6.11).

Table 6.11. Prevalence of non-target variations in participants with sequenced DNA samples (n=78 for *mt-CYB* & n=80 for *mt-ND3*)

Gene	Genotypes	SNP	Variant	**Hearing loss** n=66 for *mt-CYB* & n=65 for *mt-ND3*	**No hearing loss** n=12 for *mt-CYB* & n=15 for *mt-ND3*	MAF
mt-CYB	Different homozygous variation	rs193302991	mt.15301G>A	2 (3.03%)	0 (0%)	0.030
mt-ND3	Different homozygous variation	rs38999188	Mt.10115T>C	5 (7.69%)	2 (13.33%)	0.092

SNP = single nucleotide polymorphisms

Chapter 7: Discussion

In this chapter, the results of this study will be discussed in relation to the existing literature. Recommendation for policy and future research will be presented, and lastly, the chapter will conclude with a discussion of implications for clinical practice; specifically, ototoxicity monitoring of patients who are on treatment that includes ototoxic aminoglycosides.

Summary of the aims and key findings of the study

The present study aimed to determine the incidence of cochleotoxicity among MDR/RR-TB patients receiving kanamycin as well as determine the pharmacokinetic properties of kanamycin that are associated with increased risk of cochleotoxicity among these patients. The study also sought to determine the association between participant's susceptibility to develop cochleotoxicity and two mitochondrial mutations, T15312C (I189T in MT-CYB) and T10114C (I19T in MT-ND3). Overall this study found that there is a high incidence of cochleotoxicity (82%) amongst MDR/RR-TB patients who were receiving kanamycin as part of their treatment. The longer duration of treatment was associated with an increased incidence of cochleotoxicity. Higher Kanamycin $AUC_{0\text{-}10}$ was also found to be strongly associated with an increased risk of cochleotoxicity amongst participants in this study. Lastly, the *T15312C* and *T10114C* were common mutations amongst South African MDR/RR-TB patients who participated in this study and may play a role in patients' susceptibility to aminoglycoside-induced cochleotoxicity which should be assessed in future studies.

7.1. Incidence of cochleotoxicity during MDR/RR-TB treatment

7.1.1. STS based on ASHA criteria

About 82% of MDR/RR-TB patients in this study who were receiving kanamycin developed cochleotoxicity. This was higher than the incidence rate of cochleotoxicity reported in most of the previous studies that investigated the incidence of aminoglycoside-induced cochleotoxicity amongst their participants who were also MDR/RR-TB patients; De Jager and Van Altena (2002) in the Netherlands (18%), Peloquin et al. (2004) in the USA (37%), De Lima et al. (2006) in Brazil (64%), Duggal and Sarkar (2007) in India (25%), Sturdy et al. (2011) in the UK (28%), Harris et al. (2012) in South Africa (57%), Ramma and Ibekwe (2012) in South Africa (47%), Ghafari et al. (2015) in South Africa (48%), Van Altena et al. (2017) in the Netherlands (31%), Heysell et al. (2018) in Bangladesh (78%), Hong et al. (2020) in South Africa (63%) and Lodiong et al. (2021) in Uganda (53%). A possible explanation for the high incidence of cochcleotoxicity reported in this study when compared to other studies is the fact that the present study determined the STS at UHF up to 16 kHz which is more sensitive than conventional audiometry at detecting STS (Konrad-Martin et al., 2005), while majority of the previous studies did not.

The higher incidence rate of cochlotoxicity (82%) reported in this study was, however, consistent with a study by Hollander (2018). In their study, which also involved South African MDR/RR-TB patients, and using UHFs (up to 16kHz) to monitor cochelotoxicity, Hollander (2018) reported an incidence rate of 93% amongst their study participants. The slightly higher incidence rate of cochelotoxicity in Hollander's study compared to the current study might be due to using DPOAEs measures in addition to UHFA to monitor patients. The DPOAEs are generally more sensitive to changes in hearing than pure tone audiometry (Guthrie, 2008), which can also be a reason for the higher incidence rate in

their study in comparison to the present study.

The current study identified a STS at UHF before affecting other frequency ranges in 43 (42%), 39 (38%) and 36 (35%) participants at week 4, 8 and 12 after starting their treatment, respectively (Table 6.2). This finding indicates that UHF allows for early detection of hearing threshold changes before it becomes evident in the high frequencies which are important for speech intelligibility, specifically in a noisy environment (Knight, 2008; Moore et al., 2008). The finding of the present study is in agreement with the findings by Hollander (2018) and Fausti et al. (1993). Hollander (2018) found a significant change in 77% of ears with UHF PTA at week 2, while no changes at high frequencies was detected at the same time. Fausti et al. (1993) reported that in their study, UHF identified 95% of ears in comparison to 67% with high frequencies. Therefore, the present study emphasises on the importance of using UHF for cochelotoxicity monitoring that has been recommended for early detection of cochleotoxicity (Konrad-Martin et al., 2005).

In the present study, 44% (n=37) of participants developed slight to severe degree (26 to 80 dB) of hearing loss in the conventional frequency range (0.25 to 8 kHz) based on their last audiogram (Table 6.3). This indicates that in about half of the participants, the speech frequency range is impaired from a mild to severe degree. Even a mild degree of hearing loss has an adverse effect on patient's communication and quality of life (Lin et al., 2011b; Northern & Downs, 2002).

In the current study, the number of participants who developed hearing loss in conventional frequency range (0.25 to 8kHz) during 4 weeks of treatment, increased by 120% and 220% during 8 and 12 weeks of treatment, respectively (Table 6.2). Moreover,

the Kaplan-Meier failure analysis showed that the longer the patient is on treatment with kanamycin, the risk of cochleotoxicity is higher (see Figures 6.1 and 6.2). These findings indicates that cochleotoxicity is associated with the duration of treatment and the incidence of cochleotoxicity in the second month of treatment is almost doubled in the first month and in the third month, it is tripled from the first month of treatment. However, it should be noted that in a subset of patients carrying certain mitochondrial mutations (e.g. m.1555A>G), cochleotoxicity can occur following a single dose (O'Sullivan et al., 2017; Usami et al., 1998).

Majority of participants (73%) developed bilateral cochleotoxicity. Previous studies have also reported that a high proportion of patients who were treated for MDR/RR-TB developed bilateral hearing loss. For instance, Sagwa et al. (2015) reported that 83% of their participants who were receiving kanamycin and amikacin in Namibia developed bilateral hearing loss. Hearing loss resulting from aminoglycosides is usually bilateral (Black et al., 1976; Harris & Heinze, 2013) (Black et al., 1976; Harris & Heinze, 2013). Bilateral hearing loss (depending on its severity) may lead to verbal communication problems and delays in speech and language acquisition (Probst, 2006). The impact of unilateral hearing loss is not as severe as that of bilateral loss, however, individuals with unilateral hearing loss may have problem in understanding the speech in the presence of environmental noise. In addition, unilateral hearing loss compromise the auditory localization in patients (Mondelli et al., 2010).

Cochleotoxicity is usually associated with bilateral high-frequency sensorineural hearing loss (Einarsson et al., 2010; Kaland & Salvatore, 2002). In the present study, out of 84 participants with STS, 73% had bilateral STS. In all of these 84 participants, the STS

started from UHF. All of participants with hearing impairment at conventional frequency range (0.25 to 8 kHz) had sensorineural hearing loss. The findings of the current study are consistent with those of previous studies regarding the features of cochleotoxicity in the MDR/RR-TB population (Harris et al., 2012; Harris & Heinze, 2013).

In the current study, 18% of participants did not develop cochleotoxicity during the 3 months of monitoring. However there is a risk of developing cochleotoxicity up to six months post-treatment (Konrad-Martin et al., 2005). In addition Kaplan-Meier failure analysis showed that if the follow up would be continued up to 125 days, all of the participants (100%) would develop cochleotoxicity. Therefore these participants with no sign of cochleotoxicity also are at risk of developing cochlotoxicity and should be followed up till six months after conclusion of their treatment.

7.1.2. Grade of Cochleotoxicity

Severity of cochleotoxicity was determined using various cochleotoxicity grading scales. Cochleotoxic grading scales are used to categorise the severity of cochleotoxicity and to determine its likely impact on patient's daily life and need for therapeutic and or audiological referral (King & Brewer, 2018). Three scales for grading the cochleotoxicity in adults, namely CTCAE, TUNE and UCT were used to grade the severity of cochleotoxicity in this study. The result of grading of cochleotoxicity based on CTCAE, TUNE and UCT grading systems in 84 participants with cochleotoxicity revealed that 53%, 77% and 100% of them developed cochleotoxicity above grade 0 (grade 1/1a to 3), respectively. The wide difference in the percentage of cochleotoxicity above grade 0 based on the different grading scales (53% to 100%) was reported in similar studies. For instance, Hollander (2018) found that out of 15 participants, 93% showed cochleotoxicity above

grade 0 with TUNE and 7% with CTCAE. A study among cancer children revealed that the prevalence of cochleotoxicity above grade 0 was 59% according to Muenster, 48% according to SIOP, 40% according to Brock, 40% according to Chang, and 57% according to CTCAEv4.03 (Clemens et al., 2019). This variation in the grade of cochleotoxicity, based on the different grading scales in the same study limits the ability to prognosticate risk for patients and indicates the need work towards a universal grading scale for cochleotoxicity.

7.1.3. Association between cochleotoxicity and patient/treatment-related factors

The findings of this study showed no statistically significant gender differences in cochleotoxicity between males (57%) and females (43%) (p = .968). Similar findings were reported by De Jager and Van Altena (2002), Harris et al. (2012), Peloquin et al. (2004), Ramma and Ibekwe (2012) and Van Altena et al. (2017). This is in contrast to a Brazilian study conducted by De Lima et al. (2006) which found that cochleotoxicity was more prevalent in females than males while in Uganda Lodiong et al. (2021) reported that it is more prevalent in males than females. However, most studies on cochleotoxicity seem to suggest that patient's gender plays no role as a risk factor for cochleotoxicity. The results of the present study and most of studies indicate that both male and female are equally at risk of cochleotoxicity and should receive equal access to audiological and health care services.

The present study also found no statistically significant relationship between cochleotoxicity and comorbid presentation of HIV and MDR/RR-TB (p = .813). However, it has been reported by some studies that the risk of cochleotoxicity among patients with

MDR-TB and HIV co-infection is significantly higher than non-HIV infected patients (Harris et al., 2012; Hong et al., 2018). The fact that all the HIV infected participants of this study were on antiretroviral drugs and the reports that early initiation of antiretroviral therapy may prevent hearing loss (Fansula, Ogunkeyede, & Afolabi, 2019) could be an explanation for why there was no association between HIV infection and cochelotoxicity. However, there is no consensus on the effect of antiretroviral medications on hearing (Assuiti, 2013), therefore, the present study suggests that the effect of antiretroviral drugs on hearing especially in MDR/RR-TB population should be assessed in future studies.

Assessment of the relationship between cochleotoxicity and age using multivariate model revealed marginally significant association between the frequency of cochleotoxicity and decreased age (p = .050). De Jager and Van Altena (2002), Harris et al. (2012), Ramma and Ibekwe (2012) and Van Altena et al. (2017) found no significant correlation between age and cochleotoxicity. In the United Kingdom, Sturdy et al. (2011) and in Uganda Lodiong et al. (2021) showed that increased age was significantly associated with ototoxicity. Peloquin et al. (2004) in USA were in agreement with the findings of Sturdy et al. (2011) and Lodiong et al. (2021), and reported that among the patients with a median age of 56 years, for every 5-year increase in age, the odds of hearing loss increased by 24% (Pp.04; OR, 1.24; 95% CI, 1.01–1.51). De Lima et al. (2006) in Brazil also found that older patients are more at risk of ototoxicity, they reported that patients between 40 to 59 years-old exhibited higher percentages of hearing loss (81.3%) than those between the ages of 20 to 39 (70%) ($p > 0.05$). The finding of a trend towards younger participants being at higher risk of cochleotoxicity was an unexpected finding in the present study. A possible explanation for this unexpected trend may be due to a higher incidence of HIV in younger patients, which has previously been described as a risk factor for hearing loss

(Harris et al., 2012; Hong et al., 2018). However, the current study did not find HIV to be associated with cochleotoxicity. Therefore, the present study suggests that although in this study there is a trend toward younger participants being at higher risk of cochleotoxicity, MDR/RR-TB patients of all age groups are in need of appropriate audiological services.

In this study, there was also no significant relationship between previous MDR/RR-TB treatment (23%) and cochleotoxicity (p =.409) while, history of cochleotoxic treatment has been reported as a risk factor for cochleotoxicity (Schellack & Naude, 2013; Vasquez & Mattucci, 2003). However, in the present study, there was limited information on previous aminoglycoside use, which may be the reason for the lack of relationship between previous MDR/RR-TB treatment and cochleotoxicity in this study.

Assessment of the relationship between BMI and cochleotoxicity revealed no statistically significant relationship between the two variables. This was consistent with the findings of a study by Van Altena et al. (2017), which also concluded that BMI (P _ 0.432) did not correlate with the occurrence of cochelotoxicity. However, Hong et al. (2020) in their study using multivariable logistic regression model predicting hearing loss showed that low BMI (<18.5 kg/m2) is associated with aminoglycoside induced hearing loss. Conversely, Lodiong et al. (2021) reported that high BMI (≥ 25 kg/m2) is associated with cochleotoxicity. These conflicting results emphasise on the need for further research to ascertain the relationship between BMI and cochleotoxicity especially in MDR/RR-TB patients.

In this study, there was also no statistically significant relationship between renal dysfunction and cochleotoxicity. However, it has been reported by some studies that the

decreased renal function is significantly associated with cochleotoxicity (De Jager & Van Altena, 2002; Sturdy et al., 2011). This association can be explained by the pharmacokinetic of aminoglycosides in the body; as aminoglycosides are not metabolised in the body and are excreted in their active form by kidney, they can cause renal toxicity, which is a risk factor for cochleotoxicity (Human, 2009; Rybak & Ramkumar, 2007). In the present study, the prevalence of renal failure among the participants was low which may explain the lack of association between renal dysfunction and cochleotoxicity in this study.

7.2. Association between Pharmacokinetic (PK) of Kanamycin and the risk of cochleotoxicity

The current study found a statistically significant association between kanamycin exposure and cochleotoxicity, with about 3% increased risk of hearing loss for every 10µg•hr/L increase in kanamycin $AUC_{0\text{-}10}$ (see Table 6.11). When the kanamycin exposure was assessed in a subgroup of participants who developed moderate-severe grade of cochleotoxicity, the effect of kanamycin exposure on cochleotoxicity was enhanced (HR: 1.057, 95% CI: 1.01 to 1.10; p=0.017). The results showed that for every 10µg•hr/L increase in average daily $AUC_{0\text{-}10}$, the risk of moderate and severe grade of cochleotoxicity increases by about 6%.

These findings of the present study are consistant with the literature and can be illustrated by the absorption, distribution and elimination process of kanamycin from the body; Kanamycin is administered by intramuscular route for MDR/RR-TB patients for having a high absorption level, however, kanamycin has a poor penetration into most cells including lungs' cells which make MDR/RR-TB patients to need the high dosage of the drug for a long duration (4–6 month) to get cured (WHO, 2016). Nevertheless, inner ear,

unlike lung, has active transport system for kanamycin, so this drug enters exceptionally into this organ and concentrates at high level in the endolymph and perilymph (Fisher et al., 2000; Schentag et al., 2006). Therefore, the patients who are exposed to kanamycin at high plasma concentration, as well as those who receive this drug for a long period of time may be more susceptible to cochleotoxicity.

Van Altena et al. (2017) study findings were similar to those of the current study since they also found that AUC and the duration of amikacin treatment were predictors of cochleotoxicity. Similarly, Peloquin et al. (2004) in their study showed that duration of treatment and the related total dose received were both associated with cochleotoxicity. In Botswana, Modongo et al. (2015) also found that cochleootoxicity best correlated with plasma cumulative AUC and duration of therapy, which is consistent with findings of the current study. However, in the present study, although the $AUC_{0\text{-}10}$ was correlated with cochleotoxicity, the cumulative assessments of kanamycin exposure including AUC and dose as well as the average daily dose and AUC were not significantly higher in those participants who developed hearing loss compared with those who did not (see Table 6.10). A possible reason for the lack of association between cumulative exposure and cochleotoxicity, which has been described by Modongo et al. (2015), is that the treating clinicians responded to the hearing test results in real time by either stopping or decreasing the dose of kanamycin, which may have attenuated the effect of cumulative exposure in those patients who developed cochleotoxicity. A second possible reason could be statistical: the relationship between cumulative AUC and cochleotoxicity described previously is non-linear while the regression method we used in our study follows a linear analytical approach.

The findings of this study indicated that there was no correlation observed between peak and/or trough concentrations of kanamycin and cochleotoxicity. This is consistent with the findings by Modongo et al. (2015), which also found that peak and trough levels were not good predictors of cochleotoxicity. Setiabudy et al. (2013) in Indonesia and Hollander (2018) in South Africa also reported that there was no correlation between trough levels and cochleotoxicity. Therefore, based on the findings of the present study, along with those of previous studies (Modongo et al, 2015; Setiabudy et al, 2013; Hollander, 2018) it can be concluded that peak and/or trough concentrations are not the most suitable PK properties of kanamycin for predicting cochleotoxicity (Black et al., 1976).

7.3. Association between cochleotoxicity and T15312C (I189T in *MT-CYB*) and T10114C (I19T in *MT-ND3*) mutations

Multiple variations within the mitochondrial *MT-RNR1* (12s rRNA) gene have been associated with the development of aminoglycoside-induced hearing loss (Barbarino et al., 2016). The current study aimed to assess the relationship between cochleotoxicity and two potentially pathogenic variations, *T10114C* and *T15312C*, in mitochondrial *MT-ND3* and *MT-CYB* genes, respectively. The statistical analysis of the association between cochleotoxicity and the T15312C and the T10114C mutations was not possible due to the low frequency of these mutations in the study's sample size. However, as these two mutations are just detected among participants who developed cochleotoxicity and not those who did not, they may be potentially pathogenic. This is consistent with the finding of Human (2009) study, which also suggested, that these two potential pathogenic variants: *T15312C* and *T10114C* may play a role in increasing susceptibility for cochleotoxicity. However, in the current study, the presence of the known mutations associated with aminoglycoside-induced hearing loss in participants who carry *T15312C*

and *T10114C* mutations had not been investigated. Therefore, it was not possible to draw a definite conclusion about the pathogenicity of *T15312C* and *T10114C*. It is, therefore, recommended that future studies should screen the presence of the known mutations associated with aminoglycoside-induced hearing loss in patients with cochleotoxicity who carry *T15312C* and *T10114C* variations to determine the true pathogenicity of these variations.

The MAF for *T15312C* and *T10114C* variants, which is used to differentiate between common and rare variants in the population (Xiong et al., 2009), is not available on publicly available databases such as gnomAD and the mitochondrial database. In the current study, based on the MAF cut-off of 0.01 (1%), both the *T15312C* (MAF= 0.045) and the *T10114C* (MAF= 0.061) are considered as common mutations in South Africans. In addition, according on the results of the present study and Human (2009), the *T15312C* and *T10114C* may be potentially pathogenic and play a role in increasing susceptibility for cochleotoxicity. If proven to be pathogenic the presence of *T15312C* and *T10114C* as common mutations in the MDR/RR-TB population is alarming since these individuals are exposed to very high concentrations of aminoglycosides. The lack of frequency data as well as accurate pathogenicity data on these variants are shortcomings that indicate the need for larger studies in South Africa to understand the mitochondrial implication in cochleotoxicity. Larger studies on *T15312C* and *T10114C* that screen other variations associated with aminoglycoside-induced hearing loss could also allow developing the interactions between mitochondrial variants in patients with cochleotoxicity. A further analysis could be targeted genome sequencing looking specifically at these mitochondrial variants and variants that have been associated with cochleotoxicity in other populations.

The present study also found that 9.23% (n=5) of the participants carry the non-target variant of *T10115C* that was homozygous for different variation around the same site as the *T10114C* variant (see Table 6.12). The *T10115C* variant is tagged as synonymous in Ensembl genome browser and dbSNP. Synonymous mutations are usually considered to be silent and the change is often assumed to be neutral (Cuevas, Domingo-Calap & Sanjuán, 2012). This study further found that, 3.03% (n=2) of the participants harbour the non-target variation of *G15301A*, which is homozygous for different variation around the same site as the *T15312C* variant. The *G15301A* variant is tagged as synonymous in Ensembl genome browser but in the Single Nucleotide Polymorphism Database (dbSNP), it is tagged as likely pathogenic. The likely pathogenic result for *G15301A* is corroborated by clinVar database; however, the functional consequence of *G15301A* is unknown which emphases on the need for further functional research on this variant.

7.4. Limitations of the study

The findings of the present study must be interpreted whilst considering its methodological limitations. First, participants hearing thresholds were only followed up to three months post MDR/RR-TB treatment initiation. This may have led to under-reporting the proportion of participants who developed cochleotoxicity following treatment initiation. For instance, previous studies (Konrad-Martin et al., 2005) showed that there is a risk of developing cochleotoxicity up to six months post-treatment. Furthermore, the Kaplan-Meier failure analysis revealed that if hearing follow-up would have been continued up to 125 days, 100% of participants would develop some grade of cochleotoxicity (> grade 0) and about 84% would develop a moderate-severe grade of cochleotoxicity (grade 2b and 3). This supports the need for following up with patients up to six months after conclusion of aminoglycoside therapy (Konrad-Martin et al., 2005).

Second, for participants who developed cochleotoxicity, MDR/RR-TB treatment was modified (i.e. kanamycin stopped or its dosage reduced), which may have attenuated the effects of cumulative exposure in these participants. Third, there was no information on the presence of known mutations associated with aminoglycoside-induced hearing loss, which preclude drawing a definite conclusion about the pathogenicity of *T15312C* and *T10114C*. Forth, 24% of the participants had no DNA sample or their DNA samples failed to be sequenced. As a result, this study sample size was small for genetic analysis which could have influenced the reliability of the results.

7.5. Strengths of the study

Despite its limitations, this study also had some strengths. First, due to its multidisciplinary nature, i.e. collaboration between the disciplines of audiology, pharmacology and human genetic fields, this study enabled the researchers to analyse the risk factors associated with kanamycin-induced cochleotoxicity from multiple perspectives. Second, inclusion of UHFA up to 16kHz which is more sensitive than conventional audiometry for cochleotoxicity as part of the protocol for ototoxicity monitoring, enabled the researchers to identify a high proportion of individuals who developed STS. Third, the participants of the present study were inpatient at BCH or DPMH with the 12 weeks average duration of hospitalisation, which facilitated the follow-up for a hearing test.

7.6. Conclusion

The current study set out to determine the incidence of cochleotoxicity amongst MDR/RR-TB patients who receive kanamycin as well as variation in incidence of cochleotoxicity as a function of different patient factors. This study also aimed to determine the association between the risk of developing cochleotoxicity during MDR/RR-TB treatment and

different kanamycin pharmacokinetic (PK) factors. Lastly, the study also aimed to determine the association between participant's susceptibility to develop cochleotoxicity and two potentially pathogenic mitochondrial mutations. Based on the findings of this study, the following conclusions can be drawn: (1) there was a high proportion of participants who developed cochleotoxicity (82%) following MDR/RR-TB treatment that included kanamycin; (2) there was no statistically significant association between developing cochleotoxicity (i.e. STS) and a number of patient factors (sex, age, HIV infection, previous MDR/RR-TB treatment, renal failure and BMI); (3) the longer duration of treatment with kanamycin was associated with an increased incidence of cochleotoxicity; (4) higher kanamycin $AUC_{0\text{-}10}$ (area under the concentration-time curve) was strongly associated with an increased incidence of cochleotoxicity; (5) *T10114C* (I19T in *MT-ND3*) and *T15312C* (I189T in *MT-CYB*) were common mutations amongst South African MDR/RR-TB patients who participated in this study and may be involved in the pathogenesis of aminoglycoside induced hearing loss. However, further studies are required to confirm their pathogenicity.

Following the WHO recommendation in 2018 for the use of the injectable-free regimen for MDR/RR-TB patients, South Africa also removed the kanamycin and capreomycin from the treatment of MDR/RR-TB. However, in exceptional cases where treatment options are severely limited, amikacin, is considered the injectable agent of choice (DoH, 2018). Amikacin has similar structure and cochleotoxic side effects to kanamycin. Therefore, the results of the current study on kanamycin-induced cochleotoxicity still can be useful for MDR/RR-TB patients who receive amikacin. In addition, some developing countries (e.g. India and Nigeria) have not completely removed kanamycin from their treatment regimen for MDR/RR-TB (Bada et al., 2020; Shelar, 2022), which makes the

present study relevant in the current clinical management of MDR/RR-TB patients.

7.6.1. Clinical Implications

The findings of this study have a number of important clinical implications for future practice: (1) kanamycin should not be used for treatment of MDR/RR-TB patients due to its high level of cochleotoxicity and, therefore, the present study supports the WHO's recommendation to remove kanamycin from MDR-TB treatment regimens (WHO, 2018b). (2) A routine ototoxic monitoring programme that includes UHFA should be implemented for MDR/RR-TB patients who receive aminoglycosides, from the time of ototoxic drug exposure until six months post treatment. (3) Cochleotoxicity grading scales that apply UHF thresholds should be used to grade the cochleotoxicity. (4) Therapeutic drug monitoring should be implemented for all the MDR/RR-TB patients on aminoglycosides and the AUC value should be used for clinical decision making to reduce the risk of cochleotoxicity. (5) There is also a need to develop rapid clinical tests that can screen for the known mutations that contribute to the risk of cochleotoxicity, prior to the start of aminoglycoside therapy to lower the incidence of aminoglycoside induced hearing loss, especially in countries such as South Africa with a high incidence of MDR/RR-TB.

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Appendixes

Appendix A: Information letter & consent form

Appendix A1: Information letter & consent form in English

Dear patient

I am Nazanin Ghafari a PhD student in Audiology at the University of Cape Town. I am conducting research on the hearing of patients receiving MDR-TB medication. For the purpose of this study I need to access your medical records to obtain medical history and other relevant information. This study has been given ethical approval by the Research Ethics Committee (REC) of the Faculty of Health Sciences, University of Cape Town. (REC Reference Number: 065/2015 & 595/2018).

Purpose of the study:

You may receive MDR-TB medication that places you at risk for developing a hearing loss. The purpose is to identify whether you have got hearing difficulties after receiving MDR-TB treatment. The earlier your hearing loss is detected, the better for you. If you are found to have a hearing loss, I will refer you for further assessments/management of your hearing. I will send the details of your hearing test results to your doctor at Hospital and she/he will consider changing your drug dosage or use alternative medicines that are not/less harmful to your hearing.

What does taking part in the study mean?

I will first ask you a few questions about your hearing and then I will examine your outer ear. It is important to look in your ear, in order to make sure that there is nothing in the ear and that the eardrum is normal. If your ear is full of wax, I will refer you to your doctor for waxing removal and will recheck your outer ear after that. If you have ear infection and

perforated eardrum, I will refer you to the doctor for further management/treatment and you will exit from the study.

I will test your middle ear to see how well it is functioning. During this test (Tympanometry) I will place a probe in your ears. If you have a middle ear problem, that needs treatment/management, I will refer you to the doctor at the hospital and you will exit from the study.

I will assess your inner ear and auditory nerve function by placing headphones on your ears. I will ask you to indicate when you hear the tones. With this test, I will record your hearing threshold and determine if you have hearing loss or not.

If you have hearing loss, I will inform your doctor and she/he will change your drug or drug dosage and I will also refer you to the audiologist at the hospital. The audiologist will test your hearing every two weeks to check if your hearing gets worse or not, and will keep your doctor updated.

The hearing tests that I will do is the same as the hospital's routine hearing tests, other than I will test your hearing at four extra frequencies to determine if you have UHF hearing loss, which may add five minutes to the length of the tests.

For these tests, you will be seated in a sound proof room or laid down comfortably on your bed (if you cannot move); I will only carry out the test once you have agreed to the tests. I will do the hearing tests at enrolment (before the beginning of MDR-TB treatment) and at 4, 8 and 12 weeks after starting the MDR-TB medication. The tests will take about 15 (if your hearing is normal) to 30 minutes (if you have hearing problem).

I will use the results of your blood tests performed by the Division of Clinical Pharmacology to find the effect of the dosage of your drug on your hearing. I will also use

your blood samples collected by the division of clinical pharmacology for genetic testing to find the relationship between your genes and hearing loss.

Risk and Discomfort:

- These tests are routinely utilised in hearing assessment of TB patients.
- There are no risks for you, all tests are non-invasive and are not painful to you.

Benefits:

If you have a hearing loss because of using TB drugs, I will identify it early and refer you for further hearing tests. When your doctor know about the effect of medication on your hearing, he/she will be able to change the medication/dosage that may protect you from further hearing loss. The hospital may use the results of this study for making better treatment strategies for MDR-TB patients.

Voluntary Participation:

You don't have to say yes to join the study. If you don't want to, that is fine. You will then be treated just like all the other patients in the hospital. You can also say yes now, but can change your mind at any time later. I will ask for your permission at each follow up visit.

Confidentiality:

I will be looking in your folder to find out about the medicines you are getting, your age and any health issue that you have. I will be writing down all your information. I will not take your name when I do this. Only I will know who you are. I will give you a number when I put your results into my laptop. I only give your hearing results to the doctor and

the audiologist at the hospital who work with you to be able to give you appropriate treatment/management. The research group from the Division of Clinical Phamacology who have your consent will also have access to your hearing tests results but without your name. I will publish a few papers using your hearing data but no one is able identify you in these papers.

For any further information please feel free to contact me and my supervisors. My name is Nazanin Ghafari, my contact number is 0788335350 and my email address is nazanin_gh59@yahoo.com.

My supervisors are:

1. Prof LebogangRamma

Phone: 021-4066954; email: Lebogang.remma@uct.ac.za

2. Mrs Lucretia Petersen

Phone: 021-4066993; email: Lucretia.Petersen@uct.ac.za

If you have any ethical questions or issues about this study please contact the Chairperson of the Research Ethics Committee.

Chairperson of the Research Ethics Committee, Prof Marc Blockman

Phone: 021-4066993

Thank you for your assistance.

University of Cape Town

Division of Communication Sciences and Disorders

I _____ have read (or _____ read to me) the Information Letter. I understand what taking part in this study means and you answered all my questions. I know that I do not have to join the study. I freely say "yes" to join the study. I know that I can change my mind and say "no" at any time later. It is fine if I say "no" and I will be treated just like all the other patients.

Signed:

_____ _____

Participant Date and place

_____ _____

Researcher Date and place

_____ _____

Witness (if necessary) Date and place

Appendix A2: Information letter & consent form in Afrikaans

Ingeligte toestemmingsvorm

Geagte Meneer/Mevrou,

Ek is Nazanin Ghafari , ‘n nagraadse student in Oudiologie aan die Universiteit van Kaapstad. Ek doen navorsing oor die gehoor van pasiënte wat MDR-TB medikasie ontvang. Vir die doel van hierdie studie moet ek toegang tot u mediese rekords verkry om mediese geskiedenis en ander relevante inligting te bekom. Hierdie navorsing is eties goedgekeur deur die Navorsingetiekkommitee, staan in engels bekend as die *Research Ethics Committee* (REC),van die Fakulteit van Gesondheidswetenskappe by die Universiteit van Kaapstad. (REC Verwysings nommer: 065/2015 & 595/2018).

Doel van die studie:

Pasiënte kan soms middleweerstandige tuberkulose ,wat in engels bekend staan as *multidrug-resistant TB* (MDR-TB), medikasie ontvang wat hulle in gevaar stel om ‘n gehoorverlies te ontwikkel. Die doel van die studie is om te identifiseer of die pasiente wat MDR-TB het en wat MDR-TB middels ontvang, gehoorprobleme het. Hoe vroeër ‘n gehoorverlies opgespoor word , hoe beter is dit vir die pasiënt. As daar bevind word dat die pasiënt ‘n gehoorverlies het, kan hy/sy verwys word vir verdere assessering/behandeling (vir sy gehoor) en kan vroegtydig behandel word. Dit is ‘n waarskuwing vir die dokters ook, sodat hulle alternatiewe medisyne kan oorweeg, wat moontlik nie skadelik vir die gehoor is nie.

Wat beteken u deelname aan die studie?

Die navorser sal eerste jou buiteoor ondersoek. Dit is belangrik om in die oor van die pasiënt te kyk om seker te maak dat daar niks in die oor is nie en dat die oordrom normal is. Daarna sal jou middeloor getoets word om te sien hoe goed dit funksioneer. Gedurende hierdie toetse (tympanometrie en akoetiese refleks) gaan ‘n opname-toestel (‘n klein mikrofoon) in jou oor ingeplaas word. As jy ‘n middeloorprobleem het, sal jy na ‘n dokter in Brooklyn Chest Hospitaal/DP Marais Hospitaal verwys word.

Jou binne-oor en gehoorsenuweefunksies sal ondersoek word deur oorfone op jou ore te plaas. Jy sal dan gevra word om aan te dui waneer jy die klanke hoor.

As jy ‘n probleem in jou binne-oor of gehoorsenuwee het, sal jy na ‘n oudioloog verwys word vir gereelde gehoormonitering/verdere diagnostiese assesserings.

Vir hierdie toetse sal jy gemaklik sit of lê; ons sal die toets eers uitvoer sodra jy vir die toetse ingestem het. Die gehoortoets sal uitgevoer word tydens inskrywing (basislyn) en na 4, 8 en 12 weke na die aanvang van die MDR-TB medikasie. Die toetse duur ongeveer 15 tot 30 minute, afhangende van die gehoorstatus van die pasiënt.

Risiko en ongemak:

- Hierdie toeste word gereeld gebruik om die gehoor van TB-pasiënte te toets.
- Daar is geen risiko aan verbonde nie, alle toetse is nie -indringend en is nie pynlik nie.

Voordele:

As jy ‘n gehoorverlies het, kan ons dit vroegtydig identifiseer en jou vir verdere toetse of ‘n ondersoek verwys. As die dokter gewaarsku word oor die effek van die medikasie op u gehoor, kan hy/sy die medikasie verander en op hierdie manier word u beskerm teen

verdere dwelmverwante gehoorverlies. Die resultate van hierdie studie kan ook daartoe bydra dat ‘n verskeidenheid dienste aan pasiënte met MDR-TB, wat medisyne ontvang wat hulle gehoor kan beïnvloed, gelewer word.

Vrywillige deelname:

Deelname aan hierdie studie is vrywillig.As u besluit dat u nie aan hierdie studie wil deelneem nie, sal u voortgaan met standaardversorging sonder benadeling of ‘n boete. As u instem om deel te neem, maar later besluit om die gehoortoetse te staak, is u welkom om dit te enige tyd te doen.

Ek wil u aanmoedig om u gehoor te laat toets sodat u die nodige sorg kan kry. As u wil, kan die oudioloog van die Brooklyn Chest Hospitaal / DP Marais-hospitaal u gehoor toets.

Vertroulikheid/Konfidensialiteit:

Alle inligting wat tydens u verblyf in die Brooklyn Chest-hospitaal / DP Marais-hospitaal en na u ontslag versamel word, sal met vertroulikheid hanteer word. U hospitaalrekords en die uitslae van die toetse sal met omsigtigheid hanteer word en aan niemand anders as die gesondheidswerkers wat saam met u werk, getoon word nie. U sal in geen publikasies van hierdie studie geïdentifiseer word nie.

Vir enige verdere navrae kan u my of my toesighouers skakel. My naam is Nazanin Ghafari, my kontak nommer is 0788335350 en my e-pos adres is nazanin_gh59@yahoo.com.

My toesighouers is:

1. Professor Lebogang Ramma

   Kontak nommer: 021-4066954; e-pos: Lebogang.remma@uct.ac.za

2. Mev. Lucretia Petersen

Kontak nommer: 021-4066993; e-pos: Lucretia.Petersen@uct.ac.za

Voorsitter van die Navorsingsetiekkomitee , Professor Marc Blockman

Kontak nommer :021-4066993

Dankie vir u samewerking.

Appendix A3: Information letter & consent form in IsiXhosa

Ifomu yemvume enolwazi

Mnu / Nkszk Ebekekileyo

Igama lam ndinguNazanin Ghafari umfundi ophumelele isidanga kwi-Audiology kwiDyunivesithi yaseKapa. Ndenza uphando malunga nokuva kwezigulane ezifumana amayeza e-MDR-TB. Ngenjongo yolu phononongo kufuneka ndifikelele kwiirekhodi zakho zonyango ukuze ndifumane imbali yezonyango kunye nolunye ulwazi olufanelekileyo. Olu phononongo lunikezwe imvume yokuba luqhubeke yiKomiti yeenqobo ezisesikweni yoPhando (REC) yeFakhalthi yeSayensi yezeMpilo, kwiDyunivesithi yaseKapa , kuba luyilandela imigaqo yokuziphatha. (Inombolo yokubhekisa ye-REC: 065/2015 & 595/2018).

Injongo yophando:

Izigulane ngamanye amaxesha zinokufumana amayeza e-MDR-TB azibeka emngciphekweni wokuphulukana nokuva. Injongo yoluphando kukufumanisa ukuba ingaba izigulane ezine-MDR-TB kwaye ezifumana amayeza e-MDR-TB zinengxaki yokuva na. Ukufunyaniswa kokuphulukana nokuva kwangoko kufunyaniswe kuluncedo kwisigulane. Ukuba isigulana sifunyenwe singeva kakuhle, singathunyelwa kuvavanyo lweendlebe kwaye sinokunikwa unyango kwangoko. Ikwazisa oogqirha, ukuze bakukhangelele amanye amayeza angenabungozi ekuveni kwakho.

Kuthetha ntoni ukuthatha inxaxheba kolu phando?

Umphandi uya kuqala ahlole indlebe yakho yangaphandle. Kubalulekile ukujongwa kwendlebe yesigulana, ukuze uqiniseke ukuba akukho nto ingaqhelekanga kwindlebe.

Indlebe yakho ephakathi iya kuvavanywa ukubona ukuba isebenza njani. Ngexesha lovavanyo (iTympanometry kunye neAcoustic Reflex) kuya kufakwa iprobhu kwiindlebe zakho. Ukuba unengxaki ngaphakathi endlebeni, uya kuthunyelwa kugqirha kwisibhedlele iBrooklyn Chest okanye iDP Marais.

Indlebe yakho yangaphakathi kunye nokusebenza komthambo-luvo uya kuvavanywa ngokubeka ii-headphones kwiindlebe zakho. Uya kucelwa ukuba ubonise xa usiva isandi.

Ukuba unengxaki kwindlebe yangaphakathi okanye ingxaki yemithambo-luvo, uya kuthunyelwa kwiinkonzo ze-Audiology zokubeka iliso rhoqo kwindlebe okanye kuvavanyo olongezelelweyo lokuqonda isigulo.

Kwezi mvavanyo, uza kuhlala phantsi okanye ubekwe phantsi kakuhle; siza kwenza uvavanyo kuphela wakuba uvumile ukuvavanywa. Uvavanyo lokuva luya kwenziwa kubhaliso (isiseko) nakwiiveki ezi-4, 8 nezili-12 emva kokuqala amayeza e-MDR-TB. Olu vavanyo luya kuthatha malunga nemizuzu eli-15 ukuya kwengama-30 kuxhomekeke kwimeko yokuva kwesigulane.

Umngcipheko kunye nokungahlali kakuhle:

• Olu vavanyo lusetyenziswa qho kuvavanyo lwezigulana ezine-TB.

• Akukho bungozi kuwe kwaye lonke uvavanyo alunabuhlungu.

Inzuzo:

Ukuba unengxaki yokungeva siya kuba nakho ukuyifumanisa kwangoko kwaye sikuthumele kuvavanyo olungaphaya. Ukuba ugqirha uyaziswa ngesiphumo seyeza kwindlebe yakho, anganakho ukutshintsha amayeza kwaye ngale ndlela ukhuselekile ekulahlekelweni kokuva okunxulumene namachiza. Iziphumo zophononongo zinokuba

negalelo kuluhlu lweenkonzo ezinikezelwa kwizigulana ezine-MDR-TB, ezifumana amayeza anokuchaphazela ukuva kwazo.

Ukuthatha inxaxheba ngokuzithandela:

Ukuthatha inxaxheba kolu phando kungokuzithandela. Ukuba uthatha isigqibo sokuba awufuni ukuthatha inxaxheba kolu phononongo, uya kuqhubeka ufumana ukhathalelo oluqhelekileyo ngaphandle kokukhethwa okanye ukohlwaywa. Ukuba uyavuma ukuthatha inxaxheba kodwa kamva uthathe isigqibo sokuyeka uvavanyo lokuva, wamkelekile ukuba wenze njalo nangaliphi na ixesha.

Ndingathanda ukukhuthaza ukuba kuvavanywe iindlebe zakho ukuze ufumane ukhathalelo olufunekayo. Ukuba unqwenela, ugqirha wezandi ongumhlali kwisibhedlele iBrooklyn Chest / iDP Marais, unokuvavanya ukuva kwakho.

Imfihlo:

Lonke ulwazi oluqokelelweyo ngexesha lokuhlala kwakho kwisibhedlele sase eBrooklyn Chest/ DP Marais, nasemva kokuphuma kwakho luya kuphathwa luyimfihlo. Iirekhodi zakho zesibhedlele kunye neziphumo zeemvavanyo ziya kuphathwa ngononophelo, kwaye azizukuboniswa namnye umntu ngaphandle koochwepheshe abasebenza nawe. Awuyi kuvezwa kulo naluphi na upapasho lolu phononongo.

Ngalo naluphi na ulwazi oluthe vetshe nceda ukhululeke ukunxibelelana nam nabaphathi bam. Igama lam ndinguNazanin Ghafari, inombolo yam yoqhagamshelwano ithi 078833535, idilesi yam ye-imeyile ithi nazanin_gh59@yahoo.com.

Abaphathi bam ngaba:

1. UNjingalwazi Lebogang Ramma

Inombolo yomnxeba: 021-4066954; imeyile: Lebogang.remma@uct.ac.za

2. UNksk Lucretia Petersen

Inombolo yomnxeba: 021-4066993; imeyile: Lucretia.Petersen@uct.ac.za

USihlalo weKomiti yeeNqobo eziseSikweni yoPhando, nguNjingalwazi Marc Blockman

Inombolo yomnxeba: 021-4066993

Enkosi ngoncedo lwakho.

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Appendix B: Ethical Approvals

Appendix B1: Ethical approval from National Health Research Ethics Council

Application ID:	4057	DOH Number	DOH-27-0416-5057	Page:	1/3
Applicant Details					

Organisation :	University of Cape Town
Applicant Type	Academic Investigator
: Contact Name	Marilyn Solomons
: Address :	Division of Clinical Pharmavcology, K45 Old Main Building, Groote Schuur Hospital, Observatory, 7925
Telephone :	0214066779
Fax :	0214481989
E-mail :	marilyn.solomons@uct.ac.
Responsible Contact person (for public)	za Marilyn Solomons
Telephone :	021 4066779
Research contact person	Helen McIlleron
Telephone :	0214066292

Trial Application Details	
Issue Date :	2015/04/14
Sponsors :	NIH National Institutes of Health
Primary Sponsor :	
FundingType :	Grant Funded
Research Site Names :	DP Marias Hospital, Retreat Cape Town Brooklyn Chest Hospital, Milnerton, Cape Town
Primary Research Site Name :	

Total National Budget for Trial : R 20634915

Protocol / Grant Reference Number	R01 AI116155-01

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Study Descriptive Information

Brief Tiltle of Study	MDR
: Full Title of Study :	Pharmacometric optimization of second line drugs for MDR tuberculosis treatment
Anticipated Start Date :	2015/06/01
Anticipated End Date	2020/05/31
: Target Sample Size	240
: Study Phase :	Phase 3
Study Scope :	Multiple Site, Multi-National
Study Type :	Observational
Disease Type Heading :	Respiratory Tract
Disease Type Condition :	Tuberculosis, Pulmonary
Intervention Name (Generic) :	
Intervention Duration :	No. Type 0 Months

Observational

Purpose :	Screening
Duration :	Longitudinal
Sample Selection :	Defined Population Prospective
Timing :	

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Application ID:	4057	DOH Number	DOH-27-0416-5057	Page:	2/3
Study Descriptive Information					

Recruitment Status as at Date: Recruitment Status :	2015/07/01 Not Yet Recruiting
Gender :	Both
Ethnicity :	All
Age :	From 18 Years To Years
Qualifying Disease Condition for Inclusion :	Age > 18 years Current diagnosis of pulmonary MDR-TB: Baseline sputum sample with positive Gene Xpert MTB/RIF test, or confirmed positive Mycobacterium tuberculosis culture displaying resistance to rifampicin and isoniazid on standard DST. Eligible for standard MDR-TB treatment regimen (see Table 1), or, started on standard MDR-TB regimen within the past 1 month.
Major Exclusion Criteria :	Written confirmation of informed consent to participate. Pregnant women satisfying all other eligibility criteria may be enrolled. Critically ill or medically unstable* e.g. organ failure - on ventilator, receiving dialysis for acute renal failure, fulminant hepatitis (*can be recruited once stabilized if still eligible), or severe haemoptysis. Unwilling to participate, or unable to understand the Participant information and provide full informed consent

TRIAL APPLICATION

Key Primary Outcomes :

1.To describe the population PK of moxifloxacin, terizidone,

ethionamide, pyrazinamide and kanamycin in a cohort of 142 South African patients diagnosed with MDR-TB.

•Develop LC-MS/MS assays to accurately quantify moxifloxacin, terizidone, ethionamide, pyrazinamide and kanamycin in plasma.

•Determine plasma concentrations of the 5 drugs in serial samples (5 samples drawn during a dosing interval) in each patient.

•Develop population nonlinear mixed effects models to describe the plasma PK of the 5 drugs in patients with MDR-TB.

•Estimate individual PK measures of exposure for each drug.

•In those patients who consent to pharmacogenetic evaluation, collect and store a suitable blood sample.

2. To describe the individual susceptibility and MIC distributions of the infecting strains of Mtb in the study population.

•Determine moxifloxacin, kanamycin, ethionamide, isoniazid, cycloserine and pyrazinamide MICs in baseline culture isolates in each patient and in positive 8-week cultures.

3. Develop a treatment response model using time to positivity (TTP) in serial MGIT sputum cultures as a surrogate marker to quantify viable mycobacterial burden by time and hence response to treatment during the initial phase of treatment.

•Using TTP data from serial MGIT cultures taken weekly during the first 12 weeks of treatment, to develop a nonlinear mixed effects model describing the population response to standard MDR-TB treatment.

•Individual model parameter estimates will be obtained from the model.

4. To describe the key drivers of treatment response in the standard multi-drug regimen for MDR-TB

•Quantify the effects of PK exposure and MIC on key treatment response parameters

•Identify key PK thresholds for treatment response.

5. Describe the safety and tolerability of standard MDR-TB treatment through serial standardized collection of laboratory results and AE data, and describe PK associations with such toxicity.

Key Secondary Outcomes : N/A

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Committees

Ethics Committee :	Approval Status	Ethics Number	Ethics Date
University of Cape Town	Approved	065/2015	2015/02/2

Appendix B2: Ethical approval from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences Human
Research Ethics Committee

Room E52-24 Old ggain Building Groote
Schuur Hospital Observatory 7925

Telephone [021] 406 6492 • Facsimile [021] 406 6411

Email: Sumayah.ariefdien @uct.ac.za Website: www.
health.uct.ac.za/fhs/research/humanethics/forms

03 February 2015

HREC/REF: 065/201B

Prof H F4cIIIeron
Clinical Pharmacology K-
45

OMB

Dear Prof McIIIeron

Project Title: PHAR IACOMETRIC OPTIt INATION OF SECOND LINE DRUGS FOR UDR TUBERCULOSIS TREATMENT.

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 28 February 2016.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN CHAIRPERSON, CISF HUIAN ETHICS

Federal Wide Assurance Number: FWA00001637. Institutional

Review Board (IRB) number: IRB00001938

Hrec/ref:065/2O15

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (ARC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPNP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix B3: Ethical approval from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

24 October 2018

HREC REF: 595/2018

A/Prof Lebogang Ramma
Communication Science and Disorders
Health and Rehab
F-floor, OMB

Dear A/Prof Ramma

PROJECT TITLE: GENETIC AND PHARMACOKINETICS FACTORS ASSOCIATED WITH SUSCEPTIBILITY TO KANAMYCIN INDUCED COCHLEOTOXICITY IN A COHORT OF PATIENTS UNDERGOING MDR-TB TREATMENT (SUB-STUDY LINKED TO 065/2015) (PhD Candidate - Mrs N. Ghafari)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 October 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Mrs Nazanin Ghafari will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	30-7-23
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	18/7/2022

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown

Comments to PI from the HREC
Thank you for the deviation received JR

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	10/6/2022		
HREC REF Number	595/2018	Current Ethics Approval was granted until	30/10/2019
Protocol title	Genetic and Pharmacokinetics Factors associated with Susceptibility to Kanamycin Induced Cochleotoxicity in a Cohort of Patients Undergoing MDR/RR-TB Treatment		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ☐X No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Lebogang Ramma		
Department / Office Internal Mail Address	Department of Health and Rehabilitation Sciences Lebogang.ramma@uct.ac.za		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ X No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes	
1.3 Annual Approval for **full committee** review	- R 3450 (inclusive of vat)
For invoicing purposes, please provide:	
Sponsor's name	
Contact person	
Address	
Telephone number	
Email Address	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
✓☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	147
Number of participants enrolled, since last HREC Progress report (continuing review)	147

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

✓☐	No prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
NA

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
✓☐ Yes	☐ No	☐ Not applicable
If yes, please describe:		
Participants with middle ear infection and those who developed hearing loss were refered to the doctor in the hospital		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	✓☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	✓☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	✓☐ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
✓☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓☐ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

14. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	7-7-2022

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix C: Permission to have access to the research sites (BCH and DPMH)

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 6857; fax: +27 21 483 9895
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_2015RP40_269
ENQUIRIES: Ms Charlene Roderick

University of Cape Town
Anzio Road
Observatory
Cape Town
7935

For attention: **Dr Helen McIlleron**

Re: PHARMACOMETRIC OPTIMIZATION OF SECOND LINE DRUGS FOR MDR TUBERCULOSIS TREATMENT

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact **PAUL SPILLER** ON **(021 508 7401)** to assist you with any further enquiries in accessing the following sites:

Brooklyn Chest Hospital
DP Marais TB Hospital

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final feedback (annexure 9) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za)

3. The reference number above should be quoted in all future correspondence.

Yours sincerely

A HAWKRIDGE.

DR A HAWKRIDGE
DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 24/6/2015.
CC **K GRAMMER** **DIRECTOR: SOUTHERN/ WESTERN**

Appendix D: Instructions for the Mini-Cog Test

Step 1. Ask the patient to repeat three unrelated words, such as “apple,” “watch ”, and “penny”.
Step 2. Ask the patient to draw a simple clock set to 10 minutes after eleven o’clock (11:10), A correct response is a drawing of a circle with all of the numbers placed in approximately the correct positions, with the hands pointing to the 11 and 2.
Step 3. Ask the patient to recall the three words from Step 1. One point is given for each item that is recalled correctly.

Interpretation

Number of items correctly recalled	Clock drawing test result	Interpretation of screen for dementia
0	Normal	Positive
0	Abnormal	Positive
1	Normal	Negative
1	Abnormal	Positive
2	Normal	Negative
2	Abnormal	Positive
3	Normal	Negative
3	Abnormal	Negative

Note. Adapted from “Instructions for the Mini-Cog Test ” retrieved from http://geriatrics.uthscsa.edu/tools/MINICog.pdf

Appendix E: Case history form

Case History Form			
			Comments
Date of birth	DD/MM/YY		
Kanamycin-start date	DD/MM/YY		
History of cochleotoxic treatment	DD/MM/YY		
Other Medication			
HIV	Y	N	
Dizziness (Ringing or noises in the ear)	Y	N	
Aural fullness			
Hearing loss noted	Y	N	
Noise exposure	Y	N	
Ear infection history	Y	N	
Renal dysfunction	Y	N	
Family history of hearing loss	Y	N	
Ear pain (Otalgia)	Y	N	

Appendix F: UCT Scale for Cochleotoxicity

UCT Criteria for Ototoxicity in Adults (Adapted from World Health Organization's (2015) Grades of Hearing Impairment)

Grade of Impairment	Audiometric Value [PTA: 0.5, 1, 2 & 4 kHz] (SANS 10154-1)	Description of activity limitation	Recommended Intervention
0 (No impairment)	**No significant change in hearing thresholds**	None	None
Grade 1a (UHF **impairment)**	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 9-16kHz **PTA: 10-15 dB HL**	None; able to hear a whisper	None
Grade 1b (Slight impairment)	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz **PTA: 16-25 dB HL**	Slight hearing problems especially in the presence of background noise	Counselling. Adaptive listening strategies
Grade 2a (Mild Impairment)	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz **PTA: 26-40 dB HL**	Able to hear and repeat words spoken in normal voice at 1 meter. Likely to experience difficulties listening in noisy environments	Counselling. Aural Rehabilitation (mainly adaptive listening strategies). Amplification considered based on patient's listening needs/ demands
Grade 2b (Moderate Impairment)	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz **PTA: 41-60 dB HL**	Able to hear some words when shouted into better ear	Aural Rehabilitation (including hearing amplification) indicated
Grade 3 (severe Impairment)	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz **PTA: 61-80 dB HL**	Able to hear some speech when shouted into better ear; more likely to have poor word discrimination scores	Aural Rehabilitation (including hearing amplification) indicated
Grade 4 (Profound Impairment)	≥10 dB threshold shift relative to baseline at ≥2 frequencies OR ≥ 20 dB threshold shift at ≥ 1 frequency; 2-16kHz **PTA ≥ 81 dB HL**	Unable to hear speech even at a shouted voice. Less likely to benefit from conventional hearing aids	Aural Rehabilitation (including hearing amplification other than conventional hearing aids) indicated

[*Ears to be graded separately]

Note. How to use this criteria: 1. Establish that a criteria for hearing thresholds shift has been met; 2. Calculate PTA (0.5-4kHz), 3. Assign the hearing loss to the appropriate grade

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Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi-drug resistant tuberculosis

Nazanin Ghafari, Richard Court, Maxwell Tawanda Chirehwa, Lubbe Wiesner, Lucretia Petersen, Gary Maartens, Tawanda Gumbo, Helen McIlleron & Lebogang Ramma

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ORIGINAL ARTICLE

Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi-drug resistant tuberculosis

Nazanin Ghafari[a], Richard Court[b], Maxwell Tawanda Chirehwa[b], Lubbe Wiesner[b], Lucretia Petersen[a], Gary Maartens[b], Tawanda Gumbo[c], Helen McIlleron[b] and Lebogang Ramma[a]

[a]Department of Health & Rehabilitation Sciences, University of Cape Town, Cape Town, South Africa; [b]Division of Clinical Pharmacology, University of Cape Town, Cape Town, South Africa; [c]Baylor Research Institute, Baylor University Medical Center, Dallas, TX, USA

ABSTRACT

Objective: The toxicity associated with the use of kanamycin includes irreversible hearing loss. There are limited data describing the relationship between hearing loss and kanamycin pharmacokinetics (PK). We explored the association of kanamycin PK with hearing loss in patients on MDR-TB treatment.

Design: We prospectively recruited patients on kanamycin-based MDR-TB treatment in Cape Town. Hearing thresholds from 0.25 to 16 kHz were tested at baseline and at 4, 8 and 12 weeks. We determined kanamycin concentrations at steady-state in serial plasma samples over 10 h, and explored factors associ- ated with hearing loss.

Study sample: One hundred and two participants including 58 (56.9%) men had analysable audiometric data; median age was 34.9 years, 65 (63.7%) were HIV-positive, and 24 (23.5%) had been treated for MDR- TB previously.

Results: Eighty-four participants (82.4%) developed hearing loss. We found a 3% (95% CI: p ¼ 0.028) increased risk of cochleotoxicity for each 10 mg h/L increase in 0–10

Conclusion: We describe a high incidence of hearing loss in MDR-TB patients treated with kanamycin, with higher $AUC_{0–10}$ significantly associated with hearing loss.

ARTICLE HISTORY
Received 3 April 2019
Revised 1 November 2019
Accepted 2 November 2019

KEYWORDS
Cochleotoxicity; AUC; aminoglycosides; kanamy-cin; hearing loss; pharmacokinetics

Introduction

Risk factors for aminoglycoside-induced hearing loss, which ranges from 18% to 90% (Harris et al. 2012; Ghafari et al. 2015; Ramma and Ibekwe 2012; Sturdy et al. 2011; Brits et al. 2012; De Jager and Van Altena 2002; Heysell et al. 2018) include: older age, HIV, excessive noise, the presence of identi- fied mitochondrial mutations, prior use of a drug known to cause hearing loss and high aminoglycoside plasma concentra- tions (Brits et al. 2012; Human et al. 2010; Kokotas, Petersen, and Willems 2007). The WHO currently recommends that an aminoglycoside be included in the shortened treatment regimen for MDR-TB, and be considered in longer regimens where there is toxicity or intolerability of one of the group A or group B drugs (WHO 2018). Although kanamycin-induced hearing loss is well described (Harris et al. 2012; Ramma and Ibekwe 2012; De Jager and Van Altena 2002), there are limited data describing the relationship between kanamycin pharmaco- kinetics (PK) including area under the concentration-time curve, and hearing loss (Van Altena et al. 2017; Modongo et al. 2015). Furthermore, few studies have included ultra-high- frequency audiometry. One recent study showed a cumulative dose-response relationship between amikacin exposure and hearing loss in patients on treatment for MDR-TB (Modongo et al. 2015). We measured ototoxicity prospectively including ultra-high-frequency audiometry in a cohort of patients treated for MDR-TB, and determined the PK parameters of kanamycin.

We then explored some of the risk factors for kanamycin- induced hearing loss including kanamycin PK.

Materials and methods

We performed a prospective observational cohort study in adult patients on treatment for pulmonary MDR-TB at two TB hospi- tals in Cape Town: Brooklyn Chest Hospital and DP Marais Hospital. We enrolled patients 18 years of age or older initiated on therapy for MDR-TB within the previous month and explored the relationship between covariates including kanamy- cin exposure with hearing loss. Patients with middle ear path- ology were excluded from the hearing analysis. During the study period, the standard regimen for MDR-TB consisted of pyrazina- mide, moxifloxacin, kanamycin, terizidone and either ethiona- mide or isoniazid (depending on the presence of *katG* and *inhA* mutations identified by line-probe assay in the pre-treatment sputum culture, indicating high-level resistance to isoniazid or low-level resistance to isoniazid and resistance to ethionamide, respectively) (Caminero et al. 2010). Ethambutol was added if the risk of ethambutol resistance was considered to be low. Kanamycin was dosed intramuscularly daily, 6 times per week at 15 mg/kg per dose according to the South African Department of Health guidelines during the study period (South African Department of Health 2013), and adjusted for renal dysfunction at the discretion of the treating clinician. We assessed renal

CONTACT Nazanin Ghafari ghfnaz001@myuct.ac.za Department of Health and Rehabilitation Sciences (DHRS), University of Cape Town, F45 Old Main Building, Groote Schuur Hospital Observatory, Cape Town, South Africa

function at 4, 8 and 12 weeks post-treatment initiation, using the Cockroft-Gault method to calculate creatinine clearance.

Audiological assessment

We performed pure tone audiometry including ultra-high fre- quencies (12.5–16 kHz), using an Interacoustic AC40-audiometer. Hearing assessments were performed at baseline and at 4, 8 and 12 weeks after starting treatment. American Speech-Language- Hearing Association (1994) criteria were used to define cochleo- toxic hearing loss, comparing the baseline with follow-up audiograms as follows: a shift of 10 dB at any two contiguous frequencies with reference to the baseline audiogram, a 20 dB shift at any one test frequency, or loss of response at three con- tiguous frequencies where responses were previously obtained (American Speech-Language-Hearing Association 1994). A min- imum of two audiograms were required to include participants in the hearing analysis. We used the University of Cape Town (UCT) cochleotoxic criteria (Ramma 2016) to classify the grade of hearing loss as the currently used cochleotoxicity scales for adults (CTCAEV & TUNE) (Theunissen et al. 2014; Crundwell, Gomersall, and Baguley 2016; King and Brewer 2018) use con- ventional frequency ranges and do not include ultra-high-fre- quency testing (12.5–16 kHz), which we performed in our study.

Pharmacokinetic sampling

We performed PK sampling once patients were established on treatment between 2 and 6 weeks. Blood was drawn at the fol- lowing time points: predose and at 2, 4, 6, 8 and 10 h postdose. Dosing was strictly observed and performed under fasting condi- tions. Blood samples were immediately centrifuged and the plasma was stored at 70 ^{0}C. Kanamycin concentrations were measured using liquid chromatography-tandem mass spectrom- etry (LC-MS/MS) using methods validated according to US Food and Drug Administration Center for Drug Evaluation and Research (2018) and European Medicines Agency (2011) guide- lines. The samples were processed with a solid phase extraction method using 50 mL plasma. Five microliters of the extracted sample were injected onto the HPLC column. Isocratic chroma- tographic separation was achieved on a Discovery C18, 5 mm, 50 mm 4.6 mm analytical column using 4 mM HFBA in 0.1% formic acid in water/acetonitrile (80:20, v/v) at a flow-rate of 500 mL/min. The mobile phase flow was split (1:1) at the source of the mass spectrometer. An AB Sciex API 3000 mass spectrom- eter was operated at unit resolution in the multiple reaction monitoring mode, monitoring the transition of the protonated molecular ions at *m/z* 485.2 to the product ions at *m/z* 163.2 for kanamycin A and the protonated molecular ions at *m/z* 494.3 to the product ions at *m/z* 165.3 for the Kanamycin-d9 internal standard. Electrospray ionisation was used for ion production. The assay was validated over the concentration range of 0.625–40 mg/mL. The combined accuracy (%Nom) and precision (%CV) statistics of the lower limit of quantification (LLQ), low-, medium-, and high-quality controls (3 validation batches, *n* 18) were between 101.3% and 107.0%, and 3.0% and 14.3%, respectively.

Statistical analysis

We imputed predose kanamycin plasma concentrations below LLQ (0.625 mg/mL) as half the LLQ value and used STATA

version 15.0 (Stata Corp, College Station, TX, USA) to perform the non-compartmental and statistical analyses. Area under the concentration-time curve (AUC) from 0 to 10 h after the dose ($AUC_{0–10}$), AUC to infinity (AUC_{∞}), half-life, peak concentra- tion and time to peak concentration were assessed. The trapez- oidal rule was applied for computation of the $AUC_{0–10}$ and the exponential extrapolation option was used to calculate AUC_{∞}. We calculated the cumulative dose of kanamycin by multiplying the dose by the number of days a particular dose was adminis- tered before hearing loss developed. The average daily dose was calculated by dividing the cumulative dose of kanamycin by the number of days recorded from treatment initiation to first detec- tion of hearing loss. Cumulative AUC was measured by multi- plying the $AUC_{0–10}$ on the PK sampling day by the number of days the same dose was administered before first detection of hearing loss. If the dose was changed during the treatment period, we predicted the change in AUC by increasing or decreasing the exposure proportionally to the change in dose, since AUC after parental administration equals dose divided by clearance. For example, if the dose of kanamycin was halved by the treating clinician, assuming linear kanamycin PK, we consid- ered the AUC to be 50% lower for the time period that the lower dose was administered. We calculated the average daily AUC of kanamycin by dividing the cumulative $AUC_{0–10}$ of kanamycin by the number of days from treatment initiation to first detection of hearing loss.

We explored factors associated with hearing loss using Cox proportional hazards regression, including the following factors in the univariate model: sex, age, previous exposure to second-line anti-TB drugs, HIV status and $AUC_{0–10}$. We included cova- riates with a p value of <0.2 in the multivariate model, and used Kaplan–Meier failure analyses to estimate the incidence of coch- leotoxicity over time. We used the two-sample Wilcoxon rank- sum (Mann–Whitney U) test to compare cumulative and average daily dose and AUC between participants with and without hear- ing loss.

Ethics approval

The study protocol was reviewed and approved by the Human Research Ethics Committee at the UCT (HREC 065/2015). Written informed consent was taken from each participant in a language of their choice (either English, Afrikaans or isiXhosa).

Results

Participant characteristics are shown in Table 1. Of the 147 par- ticipants initially recruited into the study, 102 (69.4%) had ana- lysable hearing data. The reasons why 45 participants (30.6%) were unable to complete the two valid hearing tests required for the analysis were as follows: 10 were discharged from hospital prior to study completion, five withdrew from the study, five died, five were too sick for the hearing tests to be completed

Table 1. Participant characteristics of 102 patients with analysable hearing data on treatment with kanamycin for multidrug-resistant tuberculosis.

Variable	Value
No. (%) male	58 (56.9%)
No. (%) HIV infected	65 (63.7%)
No. (%) with previous MDR-TB treatment	24 (23.5%)
Median age, years	34.9 (27.2–42.2)
Median BMI, kg/m^2	17.3 (15.6–18.9)
Creatinine clearance, mL/min (n ¼ 95)	79.7 (58.8–98.8)

Where appropriate, the percentage/interquartile range is shown in brackets.

Table 2. Grade of cochleotoxicity in 84 participants who developed hearing loss during the first 12 weeks of treatment with kanamycin for multidrug-resistant tuberculosis.

Grade of cochleotoxicity*			
Grade of Impairment	Change in hearing thresholds [PTA: 0.5, 1, 2 and 4 kHz] (SANS 10154-1)	Description of activity limitation	*n* ¼ 84
0 (no impairment)	No significant change in hearing thresholds	None	0
Grade 1a (UHF impairment)	2:10 dB threshold shift relative to baseline at 2 frequencies OR 2: 20 dB threshold shift at 1 frequency; 9–16 kHz PTA: 10–15 dB HL	None; able to hear a whisper	10
Grade 1b (slight impairment)	2:10 dB threshold shift relative to baseline at 2 frequencies OR 2: 20 dB threshold shift at 1 frequency; 2–16 kHz PTA: 16–25 dB HL	Slight hearing problems especially in the presence of background noise Able to hear and repeat words spoken in normal voice at 1 m. Likely to experience difficulties listening in noisy environments	37 17
Grade 2a (mild impairment)	2:10 dB threshold shift relative to baseline at 2 frequencies OR 2: 20 dB threshold shift at 1 frequency; 2–16 kHz PTA: 26–40 dB HL	Able to hear some words when shouted into better ear	10
Grade 2b (moderate impairment)	2:10 dB threshold shift relative to baseline at 2 frequencies OR 2: 20 dB threshold shift at 1 frequency; 2–16 kHz PTA: 41–60 dB HL	Able to hear some speech when shouted into better ear; more likely to have poor word discrimination scores	10
Grade 3 (severe impairment)	2:10 dB threshold shift relative to baseline at 2 frequencies OR 2: 20 dB threshold shift at 1 frequency; 2–16 kHz PTA: 61–80 dB HL	Unable to hear speech even at a shouted voice. Less likely to benefit from conventional hearing aid	0
Grade 4 (profound impairment)	2:10 dB threshold shift relative to baseline at 2:2 frequencies OR 2:20 dB threshold shift at 2:1 frequency; 2–16 kHz PTA 2: 81 dB HL		

*UCT criteria for cochleotoxicity in adults (Ramma 2016).
Grade of impairment is based on the most severely affected ear. PTA: pure tone average; UHF: ultra-high frequency.

Table 3. Pharmacokinetic measures of kanamycin exposure in participants on treatment for multidrug-resistant tuberculosis.

Pharmacokinetic measure	Hearing loss (*n* ¼ 69)	No hearing loss (*n* ¼ 16)
Dose (mg/kg/day)	15.9 (15.0–17.5)	16.0 (14.5–17.2)
Peak concentration (mg/mL)	36.4 (29.4–42.7)	34.1 (29.5–38)
Trough concentration (mg/mL)	0.3125 (0.3125–0.84)	0.3125 (0.3125–0.3125)
$AUC_{0–10}$ (mg h/L)	155.6 (127.3–212.1)	152.5 (121.2–168.2)
AUC_{1} (mg h/L)	168.8 (134.6–244.0)	160.1 (128.9–199.3)
Half-life (hours)	2.5 (2.2–3.4)	2.5 (2.2–2.9)

Median is shown with interquartile range in brackets.

Table 4. Covariates associated with hearing loss in 102 participants on treat-ment with kanamycin for multidrug-resistant tuberculosis.

	Univariate		Multivariate	
Variable	HR (95%CI)	*p* value	aHR (95%CI)	*p* value
Sex	0.99 (0.64–1.53)	0.968		
Age	0.98 (0.96–1.00)	0.058	0.97 (0.95–1.00)	0.050
HIV	1.06 (0.67–1.67)	0.813		
Previous MDR-TB treatment	0.90 (0.56–1.45)	0.670		
$AUC_{0–10}$ (per 10 mg h/L increase)	1.03 (1.00–1.05)	0.051	1.03 (1.00–1.06)	0.028

Table 5. Covariates associated with moderate-severe hearing loss in participants on treatment with kanamycin for multidrug-resistant tuberculosis.

	Univariate	
Variable (*n* ¼ 28)	HR (95% CI)	*p* value
Sex	0.98 (0.40–2.36)	0.959
Age	1.02 (0.98–1.06)	0.302
HIV	1.71 (0.67–1.67)	0.300
Previous MDR-TB treatment	0.61 (0.62–4.75)	0.409
AUC (per 10 mg h/L increase)	1.05 (1.01–1.10)	0.017

$_{0–10}$

HR: hazard ratio; CI: confidence interval.

Table 6. Comparison of key pharmacokinetic measures in participants with and without hearing loss, treated with kanamycin for multidrug-resistant tuberculosis.

Variable	Hearing loss (*n* ¼ 84)	No hearing loss (*n* ¼ 18)	*p* value
$AUC_{0–10}$	155.6 (127.3–212.1)	152.5 (121.2–168.2)	0.425
Cumulative $AUC_{0–10}$	9450.9 (6541.3–12,615.2)	7226.8 (4794.7–9885.0)	0.103
Average daily	639.2 (450.9–689)	644.1 (477.6	0.56

aHR: adjusted hazard ratio; HR: hazard ratio; CI: confidence interval.

dose (mg)

Median is shown with interquartile range in brackets.

timeously, two left hospital against medical advice, one partici- pant was transferred out to another facility, six had comorbid medical conditions that made the hearing tests uninterpretable, and 11 participants had hearing test results, which were deter- mined by the study audiologist to be unreliable.

The median (IQR) duration of kanamycin therapy to first detection of hearing loss was 61 (43–81) days. Of the 102 partici- pants with analysable hearing data, 84 (82.4%) developed hearing loss on treatment with kanamycin including 20 participants (23.8%) who developed moderate-severe hearing loss. The grade of cochleotoxicity in those participants who developed hearing loss is shown in Table 2 (Ramma 2016). The key PK measures of kanamycin are shown in Table 3. Covariates associated with any degree of hearing loss and moderate to severe hearing loss are shown in Tables 4 and 5, respectively. On multivariate analysis in those participants who developed any degree of cochleotoxicity, hearing loss was significantly associated with kanamycin $AUC_{0–10}$ (aHR: 1.03, 95% CI: 1.00–1.06; p 0.028) – see Table 4. We observed a stronger association between kana- mycin $AUC_{0–10}$ and moderate-severe hearing loss in a subgroup of 20 participants (HR: 1.05, 95% CI: 1.01–1.10; p 0.017) – see Table 5. Table 6 compares cumulative kanamycin exposure between those who developed hearing loss and those who did not. Figure 1(A,B) show time to any grade of hearing loss and time to moderate-severe hearing loss respectively in the 102 par- ticipants with analysable hearing data. The follow-up time period for Figure 1(A,B) extends beyond the 12-week study period as the final hearing tests for some patients were either delayed or postponed for logistical reasons.

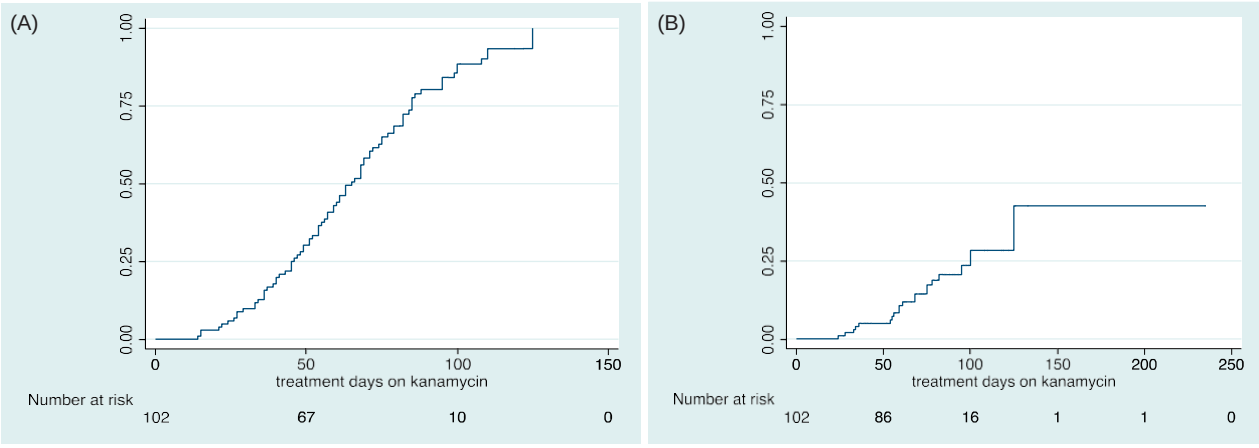

Figure 1. (A) Time to any grade of hearing loss in 102 participants on treatment with kanamycin for multidrug-resistant tuberculosis. (B) Time to moderate-severe grade of hearing loss in 102 participants on treatment with kanamycin for multidrug-resistant tuberculosis.

Discussion

We describe a nearly universal (>8 in 10 patients) incidence of hearing loss in patients treated with kanamycin for MDR-TB. Given that kanamycin-containing regimens have been associated with worse outcomes compared with regimens without it, this high-level toxicity is not balanced by efficacy benefits (Ahmad et al. 2018). Our findings therefore support the WHO's recent recommendation to remove kanamycin from MDR-TB treatment regimens (WHO 2018). There are several possible reasons for the high incidence of hearing loss we observed. First, considering aminoglycosides initially affect the highest hearing frequencies, we tested ultra-high frequencies (up to 16 kHz) in all partici- pants, which is higher than the maximum conventional fre- quency (8 kHz) tested in most studies (Harris et al. 2012; Ghafari et al. 2015; De Jager and Van Altena 2002). Thus, we diagnosed patients with hearing loss who would otherwise not be identified at conventional frequency thresholds. Second, HIV infection is a risk factor for hearing loss (Harris et al. 2012) and there was a large proportion of HIV-infected patients in our cohort (63.7%). However, HIV infection was not a significant risk factor in the univariate analysis in our study. Third, 24/102 participants (23.5%) had documented evidence of prior treatment for MDR- TB with aminoglycosides, which may have predisposed some participants to developing hearing loss. We did not find prior aminoglycoside use to be significantly associated with hearing loss, although we had limited information on previous MDR-TB treatment exposure at the time of recruitment.

We found kanamycin exposure to be significantly associated with hearing loss with a 3% increased risk of hearing loss for every 10 mg h/L increase in kanamycin $AUC_{0–10}$ (see Table 3). When we explored the association of kanamycin exposure in a subgroup of 20 participants who developed moderate-severe hearing loss, the effect of kanamycin exposure on hearing loss was enhanced (see Table 5). Cumulative assessments of kanamycin exposure including AUC and dose as well as the average daily dose and AUC were not significantly higher in those partic- ipants who developed hearing loss compared with those who did not (see Table 6). A possible reason for the lack of association between cumulative exposure and hearing loss, which has been described previously (Modongo et al. 2015), is that the treating clinicians responded to the hearing test results in real time by either stopping or decreasing the dose of kanamycin, which may have attenuated the effect of cumulative exposure in those patients who developed hearing loss. A second possible reason

could be statistical: the relationship between cumulative AUC and hearing loss described previously is non-linear while the regression method we used in our study follows a linear analyt- ical approach. There was an unexpected trend toward younger patients being at higher risk of hearing loss, possibly due to patients with age-related hearing loss at baseline being excluded from the analysis. We also considered this may be due to a higher incidence of HIV in younger patients, which has previ- ously been described as risk factor for hearing loss (Harris et al. 2012), although we did not find HIV to be associated with hear-ing loss in this study.

Aerosolised administration of kanamycin in the treatment of MDR-TB has been described as having the potential to reduce systemic exposure, and hence hearing loss, with enhanced kana- mycin concentrations at the bronchi (Momin et al. 2017). Further research is required to determine the safety and efficacy of aerosolised kanamycin in the treatment of patients with MDR-TB. As aminoglycoside-induced hearing loss is progressive from high to low frequencies, we used ultra-high-frequency audi- ometry (up to 16 kHz) to detect early cochleotoxic hearing loss before damage to the speech frequency range occurs. In clinical practice, testing ultra-high frequencies may allow earlier detec- tion of hearing loss thereby prompting clinicians to stop amino- glycosides before speech range hearing loss develops.

Our study has some limitations. First, a baseline audiogram could not be obtained before commencement of MDR-TB treat- ment in all participants, as some participants initiated treatment at local clinics prior to referral to the study sites. This may have led to under-reporting of hearing loss if only one audiogram was able to be performed at the TB hospitals. Second, because we measured the AUC to a maximum of 10 h post-dose, the cumula- tive $AUC_{0–10}$ is likely an underestimation of the true cumulative AUC until the first detection of hearing loss. Third, we had lim- ited information on previous aminoglycoside use and other risk factors such as genetic factors which are known to influence patients' susceptibility to aminoglycoside-induced ototoxicity (Kokotas, Petersen, and Willems 2007). Fourth, we were unable to include patients with only one hearing test or those with pre-exist- ing hearing loss, which had a negative effect on our sample size.

Conclusion

Using ultra-high-frequency audiometry, we report a high inci- dence of hearing loss in patients on treatment with kanamycin for MDR-TB, with approximately a quarter of patients with ana- lysable data developing moderate to severe hearing loss. Higher kanamycin $AUC_{0–10}$ is strongly associated with an increased inci- dence of hearing loss, which adds to the growing body of evi- dence in support of the rollout of injectable-sparing treatment regimens for MDR-TB.

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Declaration of interest

There is no conflict of interest regarding the publication of this article.

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