RISK OF AMINOGLYCOSIDE-INDUCED HEARING LOSS AMONG PATIENTS WITH DRUG-RESISTANT TUBERCULOSIS IN SOUTH AFRICA

by

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ABSTRACT

Problem Statement

Individuals treated for multidrug-resistant tuberculosis (MDR-TB) with aminoglycosides (AGs) in resource-limited settings often experience permanent hearing loss, but there is no practical, cost-effective means to identify those at higher risk. This dissertation aimed to estimate the risk of AG-induced hearing loss for MDR-TB-infected individuals in South Africa.

Methods

We nested this analysis within a cluster randomized trial of nurse-led case management in 10 South African TB hospitals. All participants ≥13 years old received kanamycin or amikacin. Hearing loss was defined as a poorer hearing threshold compared to baseline clinical and audiometric evaluation. We developed the prediction model using data from 265 patients at hearing frequencies from 250 to 8,000Hz and validated the model using data from 114 separate patients at both normal (250-8,000Hz) and ultrahigh frequencies (9,000-16,000Hz). We estimated standardized weekly AG exposure as:

{prescribed daily AG dose (mg) x frequency of dosing per week} ÷ weight (kg)

Cox proportional hazard and logistic regression were used for multivariable adjustment.

Results

Of 936 participants, 54% were male; mean age was 36 years; 75% were HIV coinfected at baseline. Comparing patients with high (≥75mg/kg/week) versus low (<75mg/kg/week) AG exposure, the adjusted hazard (aHR) of regimen cessation due to ototoxicity was 1.33 (p=0.006); aHR for audiometric hearing loss was 1.34 (p=.038). Pre-

existing hearing loss (aHR=1.71, p<.001) and age (aHR=1.02, p=.031) were also associated with increased hazard of hearing loss. Predictors of ototoxicity in the final prediction model included: standardized weekly AG exposure, HIV status, CD4 count, age, serum albumin, BMI, and pre-existing hearing loss. This model demonstrated moderate discrimination (AUC=0.72) and good calibration (χ^2[8]=6.10, p=.64) at normal frequencies and better discrimination (AUC=0.81) at ultrahigh frequencies that might represent early manifestations of AG ototoxicity. Discrimination for AG regimen cessation due to ototoxicity (among 671 patients without baseline audiometric data) was weaker (AUC=0.60). Using a cutoff of 85% predicted probability of hearing loss, the positive predictive value was 100% and the negative predictive value was 41%.

Conclusions

This model identifies patients at high risk for AG-induced hearing loss and may inform clinical guidelines regarding which patients to prioritize for injectable-free regimens.

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PREFACE

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CHAPTER 1: INTRODUCTION

In many low- and middle-income countries, tuberculosis (TB) remains the leading cause of death. Drug-resistant tuberculosis (DR-TB) has the potential to increase the length and intensity of treatment, reduce patients' quality of life, and increase the likelihood of mortality. DR-TB is known as multidrug-resistant (MDR-TB) TB and is resistant to both first-line anti-TB drugs (isoniazid and rifampicin)—and extensively drug-resistant (XDR-TB)—TB resistant to core second-line anti-TB drugs (i.e., fluoroquinolones and injectable agents).[1] In particular, the World Health Organization (WHO) recommendations for rifampicin-resistant (RR-TB) or MDR-TB treatment currently include second-line anti-TB drugs and is divided into two phases: (1) the intensive phase is at least 4 months (up to 6 months) and includes an injectable agent either aminoglycoside (AG) (e.g., kanamycin or amikacin) or capreomycin along with 6-7 oral drugs, (2) the continuation phase includes daily oral drugs for at least 5 months.[1] MDR-TB treatment regimens in South Africa have evolved based on WHO guidelines; however, newer short-course regimens requiring only 9 months of treatment, while traditional longer regimens for 18 to 20 months, are still used for some patients.[2] During the first 4-6 months of the intensive phase, a large proportion of MDR-TB patients develop permanent hearing loss due to ototoxic effects from AG.[3,4] AG ototoxicity may cause early AG regimen modification (i.e., reduced or discontinued), leading to failed or delayed TB culture conversion due to attenuated bactericidal efficacy of AG, particularly in resource-limited settings without a substitute for AG. AG-induced hearing loss typically presents with high-frequency hearing loss, which may be accompanied by tinnitus, prior to presentation of hearing loss in audible lower frequencies. It is often

under-detected if audiological evaluation is not available or inconsistently performed.[5,6] Myriad therapeutic efforts have been proposed to target various steps of the complex cascade of AG ototoxicity; however, clinical application is impractical in many resource-limited settings. The exact mechanism of AG ototoxicity is unknown, but it has been hypothesized that AG accumulation in the inner ear catalyzes the formation of free-radicals.[3,4,7-9] When free-radical formation overwhelms the capacity of the intrinsic protective and repair systems, hair cells in the inner ear along with ancillary sensory cells and neurons undergo apoptotic cell death, resulting in irreversible hearing loss.[6,9-13] Chapter 2 explains this pathway in greater detail.

There are several risk factors that appear to aggravate AG ototoxicity. High AG plasma concentrations and frequent or prolonged dosing may increase risk, yet monitoring of drug concentrations is not possible in most resource-limited settings. Further, the amount of AG that maintains therapeutic levels (to contribute meaningfully to multidrug therapy) but not supra-therapeutic (leading to hearing loss) has not been defined. The risk of hearing loss is impacted by human immunodeficiency virus (HIV) coinfection—up to 70% of South African MDR-TB patients are living with HIV—as a result of severe immunosuppression and the adverse effects of antiretroviral therapy (ART).[14,15] Thus MDR-TB/HIV coinfected patients have a 22% greater risk of developing AG-induced hearing loss than non-HIV-infected MDR-TB patients.[16] Both ART and anti-TB drugs may cause renal impairment, which hastens ototoxicity due to decreased renal excretion of AGs.[17-21] Clinical manifestations of TB, such as malnutrition and severe, disseminated inflammation may be associated with increased incidence of hearing loss.[22-28] Pre-existing hearing loss, prior use of ototoxic drugs for MDR-TB

treatment, comorbidities, advanced age, female gender, poverty, and substance use may increase the risk for subsequent hearing loss.[29,30] Despite these known risks, there is no practical, cost-effective means to identify those at highest risk for developing hearing loss. Thus, practical tools to estimate the risk of AG-induced hearing loss are desperately needed to avoid this unnecessary adverse event and to guide clinical decision-making.

PURPOSE AND STUDY AIMS

The goal of this dissertation was to estimate the risk of AG-induced hearing loss for MDR-TB patients in South Africa. An ongoing cluster randomized clinical trial offered a unique opportunity to explore hearing loss and its determinants prospectively via a secondary data analysis.

The specific aims of this study were:

Aim 1: To explore the prognostic impact of cumulative AG exposure on AG-induced hearing loss in MDR-TB patients following initiation of injectable-containing multidrug therapy for MDR-TB

Hypothesis: Patients within high cumulative AG exposure would have a shorter time to development of hearing loss than those with lower cumulative exposure.

Aim 2: To develop a prediction model of AG-induced hearing loss in MDR-TB treatment

Hypothesis: A model with potential predictors including cumulative (or weekly) AG exposure (daily dose x weekly frequency), HIV/ART status, CD4 count, presence of lung cavities, renal impairment, weight/BMI, serum albumin, pre-existing hearing loss, previous TB history, comorbidities, age, sex, poverty, and substance use can help categorize patients into high- or low-risk of AG-induced hearing loss.

PARENT STUDY

Study Setting

The parent study—A Nurse Case Management Intervention to Improve MDR-TB/HIV Coinfection Outcomes with and without HIV Coinfection—is a NIAID-funded cluster randomized trial [R01 AI104488-01A1, PI: J. Farley]. The primary aim of the parent study is to determine the impact and cost effectiveness of a nurse case management (NCM) model on MDR-TB outcomes (i.e., cure, death, or default) in patients with MDR-TB with and without HIV coinfection in the Eastern Cape and KwaZulu-Natal provinces of South Africa.[31,32] These two provinces have the highest MDR-TB incidence in the country.[2] The trial includes 10 study sites (5 intervention and 5 control sites) in which nurse case managers (NCMs) facilitate and coordinate treatment plans initiated by other clinicians for MDR-TB treatment. Participants included in this dissertation research were recruited into the parent study on initiation of MDR-TB treatment at the 10 study sites between November 2014 and June 2017 and were followed throughout the MDR-TB treatment course (~2 years), although the parent study is ongoing and actively recruiting patients. Participants in the parent study included those ≥13 years of age with microbiologically confirmed MDR-TB receiving standard of care for MDR-TB at a participating center. All eligible participants with known or suspected MDR-TB with rifampicin with/without isoniazid resistance from GeneXpert (cartridge-based Xpert® MTB/RIF; Cepheid, Sunnyvale, CA, USA) should be started on standardized MDR-TB treatment within 5 days.[2] Also, GeneXpert, LPA, and culture phenotype drug sensitivity tests should be followed to confirm either MDR-TB or XDR-TB and to modify the MDR/XDR-TB treatment regimen depending upon the

susceptibility.[2] If participants had been diagnosed with XDR-TB from drug sensitivity tests during the 6-months intensive phase, they were excluded from the study. All participating TB centers are public facilities and patients were generally poor or had exhausted medical aid available in the private sector. The centers do not collect racial statistics; however, the majority of individuals receiving care in these settings were black South Africans. All racial groups were screened for eligibility and had equal access to recruitment, and those who signed informed consent within seven days of treatment initiation were finally included.

Standard of Care MDR-TB and Monthly Follow Up

According to the South African National Department of Health guidelines, the standard MDR-TB regimen consists of at least 6 months of intensive phase treatment (= injectable phase) with one intramuscular injectable AG (e.g., kanamycin or amikacin) and four oral antimycobacterials (e.g., moxifloxacin, ethionamide, terizidone, and pyrazinamide).[2] Dosing of AG is weight(kg)-based.[2] Frequency of AG dosing varies from once-weekly to five times per week and is determined by physicians' clinical judgement, based on patients' pre-existing conditions in real settings. The clinical and laboratory evaluations are conducted at baseline and every month during the intensive phase, which includes microbiological assessment (i.e., sputum culture and microscopy), weight, BMI, vital signs, chest x-ray, albumin, full blood count, urea, electrolytes, vision testing, adverse drug reactions, and adherence.[2] Audiometry is conducted at baseline and repeated monthly during the intensive phase or as symptoms warrant by an audiologist or providers.[2] Although the WHO recommends therapeutic drug monitoring (TDM) for AG

treatment, it is not the standard of care according to the South African MDR-TB guidelines, and no clinical sites perform TDM due to limited MDR-TB treatment budgets.[33]

Data Collection

Data for the parent study were collected by NCMs at intervention sites or by research assistants (RA) at control sites. On the day of admission to the MDR-TB treatment program, patients were interviewed for sociodemographic data, medical history, and self-reported symptoms. Data were also collected through medical chart review and the National Health Laboratory System (NHLS) online laboratory portal. NCM intervention sites conducted additional patient level assessments through one or more interviews. All sites recorded weekly data from baseline to the end of the intensive phase of MDR-TB treatment, including patient vital signs, symptoms, medication changes, lab results, and treatment outcomes based on chart review and patient interviews. RAs at control sites collected baseline data from the medical records, NHLS online portal, and baseline patient interviews. All other follow-up data were abstracted from chart reviews and the NHLS portal.

CONCEPTUAL FRAMEWORK

The conceptual framework development process was guided by the OECD's Guidance on Developing and Assessing the Completeness of Adverse Outcomes Pathways.[34,35] The *Adverse Outcome Pathway* (AOP) is a model often used to predict the pharmacokinetic and pharmacodynamic responses of a drug and the relationship of these

to an adverse drug effect.[36] The model consists of conceptual constructs and depicts existing knowledge of linkages between drug initiation, physiologic and molecular responses, followed by organ, then finally organism-level responses.[36] Applying such an approach is helpful in hypothesizing relationships between different covariates when developing a prediction model associated with drug-induced adverse effects. Since AG-induced hearing loss is influenced by pre-treatment and treatment-related conditions, relationships between risk factors were depicted through this model. See Chapter 2 for detailed description and development process of an AOP for AG ototoxicity in MDR-TB treatment.

INNOVATION

In 2016 WHO released new treatment guidelines offering for the first time a shortened MDR-TB treatment of 9-12 months.[1] The regimen includes 7 drugs; AGs are given for at least 4 months.[1] An AG-sparing regimen is reserved for those with substantial risk of hearing loss.[1] Today that risk is based solely on clinical expertise without a tested and validated measure to support those decisions. This is the first study to develop and validate a hearing loss prediction model. The prediction model was developed by utilizing existing clinical data collected based upon South African national guidelines for MDR-TB management. Thus, additional lab tests or clinical evaluations would not be required to use the developed model. We expect that predicting hearing loss risk would reduce ototoxic drug use for those at highest risk and thereby reduce hearing loss. This is also one of the few studies to explore the impact of cumulative AG exposure using a reasonable surrogate measure of AG concentration on time to developing hearing

loss, adding richness to the understanding of risk of ototoxicity. The study addressed a critical need to estimate the risk for developing hearing loss in a low-resource setting where advanced screening for ototoxicity is not feasible.

DISSERTATION ORGANIZATION

This dissertation is organized into seven chapters. Chapter 1 includes introductory and background materials, the purpose and specific aims, and the introduction of conceptual framework. Chapter 2 is a manuscript on the development of AOP conceptual model. Chapter 3 is a data-driven manuscript on the prevalence of pre-existing hearing loss and ototoxicity risk factors in MDR-TB patients. Chapter 4 is a published manuscript on increased risk of AG-induced hearing loss in MDR-TB patients with HIV coinfection through meta-analysis. Chapters 5 and 6 are data-driven manuscripts on the risk of AG-induced hearing loss. Chapter 5 specifically explores the impact of cumulative AG exposure on AG-induced hearing loss outcome. Chapter 6 specifically develops the AG-induced hearing loss prediction model. Finally, Chapter 7 provides a summary of findings, discusses further limitations of this study, and suggests implications for research, practice and policy.

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CHAPTER 2

Adverse Outcome Pathway for Aminoglycoside Ototoxicity in Drug-Resistant Tuberculosis Treatment

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ABSTRACT

Up to 69% of individuals with multidrug-resistant tuberculosis (MDR-TB) in sub-Saharan Africa experience permanent hearing loss due to ototoxic effects of aminoglycosides (AGs)—injectable antibiotics for MDR-TB treatment. Despite our knowledge of the cellular mechanisms of ototoxicity and the global clinical experience among providers, AG-induced hearing loss has never been conceptually integrated or causally linked to MDR-TB patients' pre-treatment health condition. Therefore, this study aimed to develop a framework that examines the relationships between pre-treatment conditions and AG ototoxicity among MDR-TB-infected individuals in sub-Saharan Africa.

The adverse outcome pathway (AOP) approach was used to develop a framework linking key events (KEs) within a biological pathway that result in adverse outcomes (AO), which are associated with chemical perturbation of a well-defined molecular initiating event (MIE). This AOP describes pathways initiating from AG accumulation in hair cells, sound transducers of the inner ear immediately after intramuscular or systemic administration of AG. After administration, the drug catalyzes cellular oxidative stress due to overproduction of reactive oxygen species. Since oxidative stress inhibits mitochondrial protein synthesis, inner ear hair cells undergo apoptotic cell death—resulting in irreversible hearing loss (AO). We identified the following pre-treatment conditions that worsen the causal linkage between MIE and AO: HIV, malnutrition, smoking, alcohol use, aging, and noise. The KEs are: (1) pre-existing hearing loss, nephrotoxicity, and hypoalbuminemia that catalyzes AG accumulation; (2) antioxidant deficiency and immunodeficiency that trigger oxidative stress pathways; and (3) co-

administration of mitochondrial toxic drugs that hinder mitochondrial protein synthesis, causing apoptosis.

This AOP clearly warrants the development of personalized interventions for patients undergoing MDR-TB treatment. Such interventions (i.e., choosing less ototoxic drugs, scheduling frequent monitoring, modifying nutritional status, avoiding poly-pharmacy) will be required to limit the burden of AG hearing loss for those at highest risk.

INTRODUCTION

Despite decades of effort to eradicate *Mycobacterium tuberculosis* (M.tb.), ~2.7 million people have been diagnosed with tuberculosis (TB) in sub-Saharan Africa—26% of the total global incidence of TB in 2015.[1] Particularly, multidrug-resistant TB (MDR-TB) has emerged as a global epidemic and results in significant mortality.[1] Because MDR-TB is resistant to the powerful first-line regimens (i.e., rifampicin and isoniazid), second-line antimicrobials are used to treat this infection. Up to now, second-line regimens for MDR-TB have consisted of one injectable drug along with four or more oral anti-TB drugs. The most widely used injectable drug is an aminoglycoside (AG) given during the first phase of treatment (at least 4 months).[2] The U.S. Food and Drug Administration-approved AGs are gentamicin, tobramycin, amikacin, kanamycin, capreomycin, streptomycin, neomycin, and paromomycin for the treatment of serious infections caused by aerobic gram-negative bacilli; however, amikacin and kanamycin are the most frequently prescribed AGs globally for MDR-TB treatment recommended by the World Health Organization (WHO).[2]

One of the most debilitating adverse outcomes from long-term use of AGs is ototoxicity. Up to 69% of individuals with MDR-TB infection in sub-Saharan Africa experience hearing loss.[3] This incidence is almost 2-3 times higher than in high-resource countries, such as the U.S. (13%),[4] the Netherlands (18%),[5] and the U.K. (28%).[6] AG ototoxicity appears to significantly contribute to hair cell injury, damaging both the cochlear and vestibular apparatus of the inner ear (Figure 1).[7] Typical manifestations of cochleotoxicity consist of tinnitus and/or hearing loss, which begins with high-frequency hearing loss, which may or may not be clinically apparent, and often progresses to more

severe hearing loss even after discontinuation of AGs; while those of vestibulotoxicity include disequilibrium and dizziness with occasional nausea and vomiting.[7-9] Vestibulo-cochlear impairment, moreover, can be permanent. While hearing loss is one of the most common and debilitating adverse outcomes of AGs from MDR-TB treatment, strategies to reduce the risk such as selection of less ototoxic antibiotics or systematic monitoring of hearing loss are limited in many TB programs or clinical settings in sub-Saharan Africa due to cost and constraints on human resources for health care. Compared to less-toxic antibiotics, AGs are extremely inexpensive relative to the high potency they offer.[7,10,11] Financial considerations may, in part, explain the higher incidence of AG-induced hearing loss in resource-limited countries compared to high-resource countries. However, HIV coinfection, which is substantially more common in many low resource settings may also play a major role.[3] Presently, there are no practical and cost-effective tools to identify those at highest risk for developing hearing loss from AG treatment. To avoid the unnecessary occurrence of this adverse outcome and to guide clinical decision-making, it is critical to assess individuals' potential risk for ototoxicity before initiation of MDR-TB regimen.

The Adverse Outcome Pathway (AOP) is a conceptual framework representing a set of plausible connections from an initiating event to an adverse outcome considered relevant in risk assessment in predictive toxicology.[12,13] The framework consists of conceptual constructs and depicts existing knowledge concerning the predictive and/or causal linkages between drug initiation, physiological and molecular responses, and organ and organism-level responses.[12] Since the AOP includes integrated sequential pathways, it is often used to develop integrated tools for predictive toxicology, regulatory

toxicity testing, and risk assessment.[12,14] Despite examination of the mechanisms of AG-induced hearing loss at a cellular level, AG-induced hearing loss has never been conceptually integrated or causally linked to MDR-TB patients' pre-treatment health condition, which may play a pivotal role in aggravating the ototoxicity pathway. Therefore, this study aimed to develop a framework that examines the relationships between pre-treatment conditions and AG ototoxicity among MDR-TB-infected individuals in sub-Saharan Africa.

MATERIAL AND METHODS

AOP Development

The conceptual framework development process was guided by the Organization for Economic Co-operation and Development's (OECD) Guidance on Developing and Assessing the Completeness of Adverse Outcomes Pathways.[12,15] AOP methodology shares a common schematic representation consisting of a molecular initiating event (MIE), intermediate key events (KE), and an adverse outcome.[12] MIE is defined as a chemical interaction with a biological target.[12] In this study, the MIE refers to AG molecular accumulation in the interstitium of hair cells in the inner ear that initiates the toxicity pathway. The MIE is associated with a set of potential apical hazard endpoints,[12] but the AG-induced sensori-neural hearing loss (SNHL) is the apical adverse outcome of interest in this pathway. The MIE and adverse outcomes are causally linked with a series of KEs that are direct chemical effects or responses initiated from or prior to the target sites through the cellular or higher levels of biological organization, scientifically proven by *in vitro* and/or *ex vivo* studies.[12] In this pathway, three key events were identified: (1)

KE1—prerequisite events that directly impact MIE highlighted in orange arrows, (2) KE 2—prerequisite events that impact initial cellular responses highlighted in green arrows, and (3) KE 3—prerequisite events that impact the latter cellular responses highlighted in a purple arrow in the proposed AOP framework (Figure 3). To evaluate whether scientific qualitative and quantitative data precisely support a causal relationship between the observed outcomes and a given chemical, Weight-of-Evidence supporting the AOP was assessed by modified Bradford Hill Criteria per OECD guidelines.[12,16] Institutional Review Board approval was not required for this study as human subjects were not involved in the research.

RESULTS

3.1. MDR-TB treatment

Second-line injectable AGs recommended by the WHO for MDR-TB treatment include amikacin, kanamycin and streptomycin;[2] however, streptomycin is no longer considered a second-line agent because it was previously widely used for TB retreatment, and MDR-TB strains are more likely to be resistant to streptomycin than the other aminoglycosides.[2] The selection of amikacin versus kanamycin for providers and organizations is determined by the likelihood of effectiveness, availability, and cost.[2]

3.2. AG – Mechanism of Action

AGs are highly potent and broad-spectrum bactericidal agents used for the treatment of serious gram-negative bacteria or mycobacteria including *M.tb.*[17] Their primary site of action is the 30S ribosomal subunit.[17,18] To reach the site, molecules cross

the bacterial cell wall through active transport into the cell cytosol; thereby, they inhibit bacterial protein synthesis that results from misreading of the genetic code.[17-20] AGs have very poor oral bioavailability because they are highly polar cations. Only 0.3–1.5% of an orally or rectally administered dose of aminoglycoside reaches the systemic circulation and then appears in the urine.[19] Thus the route of AG administration is intravenous (IV), intramuscular (IM), intraosseous (IH), topical (cream/ointment), and ophthalmic. AGs are water-soluble and freely filtered across the glomerulus; almost all of the drug is then excreted.[18,21]

3.3. MIE – Molecular interactions

Although AGs preferentially target the bacterial ribosome, the inner ear and kidney are known to receive collateral damage.[7] The mechanisms of AG uptake into sensory hair cells and renal epithelial cells increase the susceptibility to both ototoxicity and nephrotoxicity, which can be explained by the physiological similarities between the cochlea and kidneys in terms of active transport of fluid and electrolytes to achieve iso-osmotic balance.[8] The accumulation of AGs appears to be dose- and duration-dependent; uptake into the inner ear occurs rapidly and exposures persist for longer than other organs. In animal studies, AGs enter the cochlea within a few minutes and hair cells within 3 hours after systemic administration.[22-24] AG concentrations in the inner ear are higher than plasma concentrations because the half-life of AGs in perilymph fluid are 10 to 15 times longer than in serum.[25] The receptor-mediated endocytosis at the apical surface of hair cells in the cochlea plays a role in AG uptake—AG molecules are found in vesicles beneath the hair cells.[26] AGs are also taken up into the renal epithelial cell line

via an endocytotic process, which explains the nephrotoxicity after glomerular filtration of the agent.[26] Along with endocytosis, the presence of several ion channels at the hair cells, such as the mechanoelectrical transducer (MET) cation channel quickens AG accumulation. The MET channel increases the potential differences between extracellular fluid and cytoplasm and functions like a one-way valve, promoting the likelihood of cellular uptake and accumulation of cationic AGs in the cytoplasm in the hair cells and renal cells.[27-29] Consequently, AG molecules accumulate rapidly and are eliminated slowly from the inner ear; thus, hair cells are more susceptible to AG-related processes than other cell types.

3.4. Cellular and Organ Responses.

Reactive oxygen species (ROS) are byproducts of normal mitochondrial metabolism; ROS contribute to organ homeostasis by controlling normal cell growth, differentiation, development, and death.[30,31] TB infection induces ROS production through activation of phagocytes—a part of host defense mechanism against *M.tb.*[32] Further, the AG molecules that enter hair cells readily bind to cytosolic proteins, specifically calreticulin, which plays a role in Ca^{2+} homeostasis.[33] AG binding to calreticulin dysregulates cytosolic Ca^{2+} concentration,[34] which in turn induces mitochondrial Ca^{2+} overload, producing cytoplasmic ROS and causing mitochondrial oxidation.[35] In addition, since AGs act as iron chelators, the formation of redox-active iron-AG complexes catalyzes oxygen-derived free radicals.[36,37] Thus, ROS over-production with exhaustion of the capacity of the intrinsic protective and repair system results in oxidative stress.[31,38] Moreover, the Transient Receptor Potential (TRP) cation

channels—particularly the subfamily TRPA1-containing pore helices—are located in the outer hair cells. The TRPA1 channels function as inflammatory, irritant, and oxidative stress sensors.[39,40] Activation of TRPA1 channels resulting from oxidative stress or noise-exposure, enlarges the pore diameter to dimensions larger than AG molecules, thereby facilitating AG uptake into the hair cell[41]. Oxidative stress contributes to mitochondrial depolarization and dysfunction, and mitochondrial protein synthesis inhibition, which in turn activates programmed cell death-signaling pathways, such as mitogen-activated protein kinases (MAPK).[38,42-45] Consequently, hair cells along with ancillary sensory cells and neurons—mainly the cochlear portion of the auditory nerve—undergo apoptotic cell death, resulting in irreversible SNHL.[36,37,46,47]

3.5. Key event 1: Prerequisite events that directly impact MIE

3.5.1. Nephrotoxicity

Individuals with renal impairment may experience decreased AG clearance and increased AG accumulation, as AGs are mostly eliminated by glomerular filtration. As a result, sustained and excessive peak serum concentrations are considered risk factors for hearing loss. AGs are also nephrotoxic; renal function at treatment initiation directly influences the level of AG accumulation in hair cells. Thus ototoxicity can be caused by AG toxic levels or renal impairment, which leads to reduced AG clearance and more drug accumulation.[7] Comorbid conditions that influence renal function directly or indirectly through chronic use of nephrotoxic drugs would induce AG ototoxicity. A common example in sub-Saharan Africa is HIV coinfection. Renal complications of HIV infection are common and include proteinuria, interstitial nephritis, renal tubular damage, and

nephrolithiasis; HIV-associated nephropathy—coupled with the use of nephrotoxic antiretroviral drugs such as Nucleoside Reverse Transcriptase Inhibitors (NRTIs) in particular—lead to excessive AG accumulation.[48-52]

3.5.2. Pre-existing hearing loss.

Pre-existing hearing loss at MDR-TB diagnosis commonly originates from previous exposure to ototoxic drugs, noise exposure, advanced age, or idiopathic SNHL.[53] Particularly, because acoustic stimuli increase permeability of cation channels such as MET and TRP, the noise exposure also increases the AG uptake and directly accelerates intracellular accumulation of AGs within hair cells.[54-56] Age-related hearing loss, or presbycusis, caused by the degeneration of cochlear cells is also a major cause of pre-existing hearing loss.[57] As tissue ages, the hair cells also undergo progressive oxidative mitochondrial DNA damage modified by excessive ROS generation and chronic inflammatory damage due to immunosenescence.[58-62] This results in auditory sensory cell degeneration. Further, HIV can cause pre-existing hearing loss directly and indirectly. A primary HIV infection in either the central nervous system or peripheral auditory nerve causes SNHL, although the exact mechanism of nervous destruction is still unclear.[63] A human study did not find histopathologic changes using electron microscopy supporting that HIV directly damages the cochlear end organs.[64] However, a recent observational study found that HIV-infected adults had significantly poorer hearing threshold in both low and high frequencies than HIV-uninfected adults.[65] Opportunistic infections are one of the common indirect causes of pre-existing hearing loss. The most frequent otologic opportunistic infections found in HIV-infected

individuals include seborrheic dermatitis of the external ear, otitis externa with otomycosis, and serous otitis media.[66,67] Because these infections are mostly caused by community-acquired organisms, such as *Pseudomonas aeruginosa, Aspergillus, fumigatus, Candida albicans* in outer ear and *Streptococcus pneumoniae*, *Hemophilus influenzae* and *Moraxella catarrhalis* in middle ear,[66,67] frequently recurrent acute or chronic ear infections lead to conductive hearing loss before or during AG treatment.[68]

Due to lack of trained healthcare providers or devices, it is difficult to confirm SNHL by differentiating it from conductive hearing loss by comprehensive audiological assessment, including otoscopy, tympanometry, and air-bone conduction audiometry in most countries in sub-Saharan Africa.[3] Thus, underdiagnosed ear infections may masquerade as AG-induced hearing loss, and undertreated ear infections aggravate oxidative stress from altered metabolic pathways.[68] While otosyphilis is a rare complication of syphilis, it is not an uncommon cause of inner ear infection in people living with HIV. A clinical manifestation of acute syphilis with cochleovestibular involvement includes sudden SNHL;[69] otosyphilis amplifies oxidative stress but thereby may magnify symptoms of AG-induced hearing loss. Otosyphilis seems to lead to endolymphatic hydrops in the cochlea or atrophy of the organ of Corti, spiral ganglion, and *stria vascularis* (Figure 2),[70] which may reduce endocochlear potential, resulting in cochlear sensitivity to sound. Also, because HIV drugs, particularly NRTIs—including zidovudine, didanosine, stavudine, and lamivudine—have ototoxic potential via their effect of reducing mitochondrial DNA content, the use of NRTIs prior to MDR-TB treatment may potentiate the ototoxic effect of AGs.[71,72] This association has been specified in key event 3 (Figure 3).

3.5.3. Hypoalbuminemia.

Malnutrition—an insufficiency or unbalance of nutrition—is a significant health issue in people living with MDR-TB with or without HIV and is more prominent in resource-limited environments due to food insecurity.[73-77] Malnutrition is a result of a deficiency of both macronutrients (nutrients that provide calories or energy, including carbohydrates, proteins, and fat) and micronutrients (i.e., vitamins and minerals), vital dietary components necessary for physical and mental development, disease prevention, and well-being.[78,79] Most individuals with active TB are in a catabolic state and experience weight loss and signs of vitamin and mineral deficiencies.[80] Protein-energy malnutrition (PEM) caused by insufficient intake of protein and calories is more prominent among TB and HIV coinfected patients and is worsened by TB-induced muscle wasting.[78,81-84] In the case of PEM, albumin synthesis is impaired, leading to low serum albumin concentration (hypoalbuminemia).[84,85] Since albumin plays a pivotal role in maintaining colloid oncotic pressure, hypoalbuminemia results in an abnormal increase of inner ear fluid volume by diminishing the osmotic gradient,[86,87] accelerating AG accumulation because AGs are water-soluble.[21]

3.6. Key event 2: Prerequisite events that impact initial cellular responses

3.6.1. Immunodeficiency

HIV infection weakens the human immune system by killing T-helper cells, macrophages, and dendritic cells, thus causing immunodeficiency.[88] HIV infection leads to chronic activation of nuclear factor (NF)-κB—a master regulator of pro-inflammatory genes, which produces pro-inflammatory cytokines, such as interleukin 1 (IL-1), IL-6,

and tumor necrosis factor-alpha (TNF-α).[89] Soon after the secretion of pro-inflammatory cytokines, neutrophils and other immune cells migrate to the infection site in various cell types depending on opportunistic infections including TB, where they ingest bacteria and kill them by releasing ROS, which causes oxidative stress and mitochondrial DNA damage.[32] Further, HIV-infected individuals with advanced disease have increased levels of oxidative DNA damage biomarkers (i.e., 7,8-dihydro-8-oxoguanine) in CD4+ T cells and show declines in DNA glycosylase activity for the repair of oxidative base lesions in these cells.[90] In addition, the number of CD4+ cells is positively associated with the levels of intracellular concentration of antioxidants, especially glutathione.[91,92] In particular, people living with HIV who are not taking ART may have increased risk of AG ototoxicity since ART restores the numbers of CD4+ T-cells while it augments the imbalanced redox status.[93]

3.6.2. Antioxidant deficiency.

Antioxidant deficiency causes hair cells to be more vulnerable to oxidative stress, which contributes to apoptotic hair cell death. For example, the presence of glutathione—an endogenous antioxidant resulting in detoxification of xenobiotics and protection against ROS[94]—protects the hair cells against oxidative stress.[95,96] Dietary nutrient-based antioxidant supplementation, including vitamin A, β-carotene (one of the provitamin A carotenoids), vitamin C, and vitamin E, significantly attenuated outer hair cell damage, as they have anti-inflammatory properties.[46,97,98] Albumin also has antioxidant properties through its multiple binding sites and capacity to trap free radicals.[99] Thus, hypoalbuminemia also worsens antioxidant deficiency. Antioxidant deficiency is caused

not only by poor intake of dietary sources but also by smoking, alcohol consumption, and aging, which inhibit synthesis of antioxidant enzymes and reduce antioxidant concentrations.[100-106]

3.7. Key Event 3: Prerequisite events that impact latter cellular responses

3.7.1. Mitochondrial toxicity

Use of mitochondrial toxic drugs may potentially worsen AG ototoxicity. NRTIs can inhibit human DNA polymerases, including gamma polymerase—important to mtDNA replication—that may damage mtDNA; key event 3 may also directly activate the MAPK pathway.[107-109] In particular, Tenofovir Disoproxil Fumarate (TDF) is one of the most common choices of NRTI; however, it targets the mitochondria of both hair cells and renal proximal tubules, increasing the risk of ototoxicity, as does key event 1. Since the combination of two NRTIs constitutes the backbone of ART regimens,[110] individuals on both NRTIs and AGs are at higher risk of apoptotic hair cell death.

3.8. Assessment and Confidence Testing

Bradford Hill Criteria consist of 6 items: evaluating the concordance, strength, consistency, and specificity of associations between conceptual constructs within AOP, as well as the biological plausibility and coherence of experimental evidence.[12] To achieve confidence in the proposed AOP, 5 items were addressed to evaluate the mechanistic understanding of biological systems.[12]

3.8.1. Assessment of the AOP According to the Bradford Hill Criteria [16]

3.8.1.1. Concordance of dose-response relationships.

AG-induced ototoxicity occurs in basal outer hair cells and then extends to inner hair cells and further apical outer hair cells with an increasingly cumulative AG dose.[8] Many classic laboratory animal studies have revealed that AG-induced vestibulocochlear toxicity ranges over duration and levels of exposure.[111-114] Cochleotoxicity was tested in response to a range of amikacin doses in adult rats for 5 consecutive days.[113] Hair-cell stereocilia degeneration occurred in the high-dose group (i.e., 600-1000 mg/day): the low-dose group did not develop cochlear abnormalities (i.e., 200 mg/day).[113] In addition, the pattern of hair cell degeneration—most severe in the basal regions of the cochlea with decreasing gradient towards the apex—was dependent on the administered dose of amikacin.[113] Streptomycin also causes vestibulotoxicity in a dose-response manner. Vestibular disturbance was observed in cats 12-19 days after receiving a low-dose of streptomycin (100-200 mg/kg daily); however, the cats that received 400 mg/kg became ataxic shortly after administration of the first dose, which persisted for almost 24 hours.[114]

3.8.1.2. Temporal concordance among the key events and adverse outcome

The temporal relationship among the three key events are dependent on the pre-treatment conditions that are present. Each pre-treatment factor may influence multiple key events that occur in sequential order. Prerequisite events are mediated by the presence of pre-treatment factors with or without exposure to toxicants, which precede AG accumulation in the interstitium of hair cells (MIE). Since AG accumulation is an

essential prerequisite for apoptosis of hair cells, the temporal sequence from pre-treatment conditions through AG-induced SNHL is well supported.

3.8.1.3. Strength, consistency, and specificity of association of adverse outcome and initiating event

We explained that AG-induced SNHL is caused by the oxidative stress that results from excessive AG accumulation in the hair cells (MIE). The causality of this pathway can be inversely proven by the following experimental and clinical studies that tested protective effects by targeting various steps of the ototoxic cascades:

(1) Reducing AG uptake: Evidence for the molecular identity of the MET channel strongly supports the potential modification of MET channel permeability, reducing AG uptake.[27,115,116]

(2) Iron chelators and antioxidants as ROS scavengers: Attenuated hair cell apoptosis capacity have been confirmed by administration of iron chelators or antioxidative agents, such as salicylates,[117,118] deferoxamine,[119] N-Acetylcysteine,[97,120] D-Methionine,[121,122] α-lipoic acid,[123] ascorbic acid (vitamin C),[98,124] α-tocopherol (vitamin E),[98,125] magnesium,[98] and misoprostol.[126]

(3) Inhibiting the MAPK pathway: Inhibition of the MAPK pathway by application of D-JNKI-1,[127] CEP 11004,[128] and estradiol[129] prior to AG administration result in significant protection from hair cell death in vitro and hearing loss in vivo.

3.8.1.4. Biological plausibility, coherence, and consistency of the experimental evidence

The biological plausibility, coherence, consistency, and strength of the experimental evidence that supports the proposed AOP is detailed in Table 1.

3.8.1.5. Alternative mechanism(s) that logically present themselves and the extent to which they may distract from the postulated AOP.

The mechanism of AG-induced ototoxicity with hearing loss is less understood. However, one potential alternative hypothesis is the presence of N-methyl-D-aspartate (NMDA) at the synapse between cochlear hair cells and spiral ganglion neural afferents.[130,131] At NMDA receptors, AG mimics the positive modulation of polyamines, potentially leading to excitotoxic damage at the hair cell-afferent nerve synapses.[132] Since hair cell apoptosis resulting from ROS overproduction is a significant modifiable pathogenesis of AG ototoxicity, our AOP did not include mechanisms of NMDA receptors; thereby, separate AOP could depict such alternative mechanism.

3.8.1.6. Uncertainties, inconsistencies and data gaps

Assessments of human tissue from patients with MDR-TB, with or without HIV coinfection, for evidence of AG-related pathophysiology have not been conducted for obvious ethical reasons. Although AG ototoxicity has been comprehensively studied, the major events within the proposed AOP have been causally explained by healthy preclinical animal models, while AG has mostly been administered to those with severe infections in clinical settings. However, a recent animal study induced systemic host-

mediated inflammatory conditions by injecting lipopolysaccharide (LPS), an important component of bacterial endotoxin, to experimental mice.[133,134] While LPS alone did not affect hearing, mice that received LPS prior to ototoxic agents had worse hearing loss than those that did not receive LPS pretreatment resulting from accelerated AG uptake.[133,134] Such animal studies are unable to fill the gap entirely, but evidence from preclinical work supports the hypothesis that persistent inflammation contributes to AG ototoxicity.

3.8.2. Confidence in the AOP

3.8.2.1. How well-characterized is the AOP?

AG-induced ototoxicity is a well-understood phenomenon. We adapted the *Mitochondrial Free Radical Theory of Aging* to explain the relationship between AG molecules and active free radicals, which are generally produced in the organism at the cellular level.[106] Such relationship is supported by experimental data, as specified in Table 1.

3.8.2.2. How well are the initiating and other key events causally linked to the outcome?

Multiple experiments have demonstrated that AGs are causally linked to SNHL in a dose-dependent way in both animals and humans. Evidence is strong to support a causal relationship between each key event and SNHL.

3.8.2.3. What are the limitations in the evidence in support of the AOP?

There are unmeasurable variables that may confound the relationship outlined in the AOP, such as known and unknown genetic mutations or additional confounders we may not have thought of. Specifically, mtDNA mutation is a risk factor that may be considered as one of the pre-treatment conditions as several genetic mutations also increase the susceptibility to ototoxicity. The mitochondrial rRNA mutation, particularly in the 12S rRNA gene, such as A1555G (most common), C1494T, T1095C, T1291C, 961delT+C(n), and A827G, among others, increase the structural similarity of human mitochondrial ribosomal RNA (rRNA) to bacterial 16S rRNA.[135-137] As a result, mutated mitochondrial ribosomes in the cochlea become target-binding sites for AGs,[138,139] and AGs lead to misreading of the genetic code along with perturbation of ribosomal translation.[136,137] This causes mitochondrial ribosomal damage and further cytotoxicity as it directly activates the MAPK pathway with apoptosis.[127,140-142] The most common type of mitochondrial A1555G gene mutation is most prevalent in Europeans (0.19%)[143,144] but not in sub-Saharan Africans, where the prevalence of the mutation is extremely low (0% to 0.09%).[145-148] As a result, mtDNA mutation was not addressed and generalizability is limited because this model targets evidence obtained within the sub-Saharan African MDR-TB populations and in resource-limited settings. To date, numerous experimental studies in this area are ongoing, so new evidence may change this AOP.

3.8.2.4. Is the AOP specific to certain tissues, life stages/age classes?

Advanced age may increase the risk for AG ototoxicity. Presbycusis is difficult to characterize because of genetic and environmental influences, and because of its complexity of structural changes confounded by various medical, psychological, and pharmacologic factors.[149] However, presbycusis is also caused by apoptotic hair cell death resulting from excessive oxidative cellular stress, which in turn stimulates the MAPK pathway. Age-dependent renal function is also closely related to this pathway because AG elimination is mostly completed through renal clearance. Age-related reduction in creatinine clearance among elderly populations increase the risk of ototoxicity.[150,151] The glomerular filtration rate is low at birth, reaches about adult levels by the end of the second year of life, and declines after the fourth decade.[151] Thus, infants, young children, and the elderly are more susceptible to AG-induced SNHL, but this AOP is developed targeting adult populations.

3.8.2.5. Are the initiating and key events expected to be conserved across taxa?

Experimental studies in multiple types of animals across species, including zebrafishes,[22,152] bullfrogs,[24] chicks,[24,26] mice,[24,115,133,134,153,154] rats,[113,120,155] turtles,[27] cats,[114] and guinea pigs[23,24,54,95,111,122,156,157]—all show evidence in support of this pathway. Human autopsies have also shown this relationship.[20,158]

DISCUSSION

Overall, AG ototoxicity caused by apoptotic hair cell death is a complex process, although our understanding of it has increased in recent years. Based on the modified

Bradford Hill Criteria, we believe this AOP provides critical, evidence-based insights into AG-induced hearing loss. AG-induced hearing loss prevention in TB programs is a real challenge due to complicated clinical conditions, and the causal relationship between treatment and adverse outcomes is often difficult or impossible to determine definitively. Although maintaining therapeutic levels, but not supra-therapeutic, AG concentration aids in hearing loss prevention and cure of MDR-TB, frequent therapeutic drug monitoring (TDM) is impractical in most resource-limited settings. While the causative genomic variants have been studied to determine the phenotype-genotype correlations with AG-induced hearing loss,[159] genetic services are not available in many clinical settings as a screening tool. As there are no practical screening tools to aid in the prevention of ototoxicity, knowing the mechanism of AG ototoxicity and its linkage with pre-treatment physical conditions associated with MDR-TB is critical for designing strategies to prevent AG-induced irreversible SNHL.

This is the first attempt to develop an AOP framework that outlines the apoptotic cascade in AG toxicity. This AOP framework will broaden our understanding of the complexity of AG-induced hearing loss and interactive health conditions in individuals before and after AG exposure. Such schematic representations can be used as a tool for healthcare providers to make clinical decisions, particularly in developing personalized interventions, such as choosing less ototoxic drugs or scheduling more frequent toxicity monitoring. The proposed AOP can be favorably applied not only in clinical practice but also widely in public health research as it is helpful in hypothesizing the relationships between different covariates associated with drug-induced adverse outcomes. Examples

of clinical implications and recommendations based on the key elements and contributors to hearing loss are summarized in Table 2.

Since AG ototoxicity is concentration-dependent, AG dose and use should be tightly regulated in inpatient settings, with serial measurement of creatinine and estimation of creatinine clearance coupled with TDM, which is a measurement of aminoglycoside peaks and troughs, and adjustment of dosing to remain in the targeted therapeutic ranges.[18] In outpatient settings or home-visiting programs, however, optimizing AG dosing is considerably challenging because TDM is unavailable in real time. As a result, detection of ototoxicity could be delayed because cochlear damage is initially asymptomatic. Thus, future research should consider the development of a surrogate measure of AG concentration without laboratory testing and examine its practical feasibility in resource-limited environments.

Although the proposed AOP is theoretically and practically useful, application is limited to MDR-TB treatment in resource-limited settings particularly in sub-Saharan Africa because this study does not account for genetic variance. Furthermore, we acknowledge that the proposed AOP oversimplifies the complex pharmacopathological and pharmacotoxicological process, which did not capture all potential mechanisms. Since this AOP was developed based on currently available scientific evidence, it must be considered an open and flexible framework that requires continuous refinement. There is a need for well-designed and adequately powered observational studies to identify the risk factors for AG ototoxicity that are present at MDR-TB treatment initiation and during treatment, through thorough history-taking and frequent hearing screening. Since polypharmacy is common among people with MDR-TB and HIV,[160] future studies may

be helpful in elucidating drug-drug interactions and drug-gene interactions with AG and would be a good scientific addition to understanding and prevention of AG-induced hearing loss. Continuous attention to the prevention of AG-induced hearing loss during MDR-TB treatment is critical not only in resource-limited settings but also as a global policy.

Table 1. Summary of Information on the Key Events of the AOP

Key Events	Description of Events	Experimental Support and References
MIE: AG accumulation in the inner ear hair cell	The receptor-mediated endocytosis and presence of MET cation channel lead to rapid accumulation and slow elimination of AG in the inner ear	(1) KM was taken up into sensory hair cells via receptor-mediated endocytosis at their apical surfaces because AG molecules are found in vesicles beneath the hair cells from White Leghorn chicks, confirmed by immuno-gold electron microscopy.[26,161]
		(2) MET channels on hair cell functions as open transducer channels that is the main route for aminoglycoside entry. AGs functioned as voltage-dependent MET channel blockers that also rapidly permeate through MET channels into hair cells, which was found in bullfrog model,[162] turtle model,[27] and mouse model.[29] The AG molecules enter the channel and block the ion-conducting pathway, thus such blockage increases voltage. Increased AG entry through the channel pore into the hair cell due to the large electrical driving force also increases the affinity for the blocker. This boosts both the entry of AG into the channel and the channel's affinity for the drug.[27,29,162]
KE 1: Prerequisite events impacting MIE	Nephrotoxicity (KE1-1), hypoalbuminemia (KE1-2), and pre-existing hearing loss (KE1-3) accelerate AG accumulation in the interstitium of hair cell	(1) TDF is mitochondrial toxic, increasing number of abnormal mitochondria including irregular mitochondrial shape, and sparse, fragmented cristae. Abnormal proximal tubule functioning and decreased GFR occurred in patients who had been taking TDF in multiple studies.[48-52]
		(2) The albumin-like proteins, including albumin, IgG, IgA, transferrin, antitrypsin, and haptoglobin were the major protein compositions of luminal fluid in inner ear.[86,87] Among patients (n=11) with enlarged vestibular aqueducts, patients with recent hearing loss and increased volume of luminal fluid showed a significantly decreased proportion of the albumin-like proteins in the interstitial space.[86]
		(3) Pre-existing hearing loss includes mainly noise-induced and age-related hearing loss. Histological evaluation using mice (n=22), received repetitive exposure the acoustic stimuli (~4.5 kHz with 120.5 dB sound pressure level), showed significant structural hair cell damage.[56] It has been found that atrophy of the stria vascularis of cochlear duct was observed in older mice (older than 12 months at least), and that lipofuscin (aging associated pigment granules) accumulation in inner and outer hair cells of the mice were also found from the same age.[62] This structural change is accelerated by the age-related dysfunctions of the systemic immune system accelerated, worsening presbycusis.[58-62]

KE 2: Prerequisite events impacting initial cellular responses	Immunodeficiency (KE2-1) and antioxidant deficiency (KE2-2) trigger cellular oxidative stress	(1) In comparison between 8 HIV-infected patients (mean CD4+ T-cells count = 280×10^6/L), 7 AIDS patients (mean CD4+ T-cells count = 45×10^6/L), AIDS patients had increased levels of 7,8-dihydro-8-oxoguanine in CD4+ T cells and marked declines in DNA glycosylase activity for the repair of oxidative base lesions in these cells.[90] People living with HIV showed elevated pro-inflammatory cytokines, including interleukin 2 (IL-2), IL-6, and tumor necrosis factor-alpha (TNF-α) and biomarkers associated with inflammation and coagulation, including C-reactive protein (CRP) and D-dimer due to chronic inflammation and immune activation.[163-166] In cross-sectional human study, the level of lipid peroxidation (LPO) and glutathione were used as a means of determining oxidative stress. The mean LPO levels were significantly higher in HIV-infected patients (n=100; mean= 0.7 ± 0.1 μmol/ml) as compared to healthy controls (n=30; mean= 0.3 ± 0.1 μmol/ml). The mean glutathione level in HIV-infected patients (0.06 ± 0.01 μmol/ml) was significantly lower in compared to healthy controls (0.09 ± 0.01 μmol/ml).[91,92]
		(2) A human study found that compare to younger control subjects, elderly subjects had significantly lower level of glutathione (2.08 ± 0.12 vs. 1.12 ± 0.18 mmol/L RBCs; $P < 0.05$); glutathione synthesis rates (1.73 ± 0.16 vs. 0.55 ± 0.12 mmol/L RBCs per day; $P < 0.01$); and higher plasma oxidative stress (304 ± 16 vs. 346 ± 20 Carratelli units; $P < 0.05$) simultaneously.[167] This indicates that glutathione deficiency in elderly humans resulted from a marked reduction in synthesis, related to oxidative stress.
KE 3: Prerequisite events impacting latter cellular responses	Mitochondrial toxicity (KE3) worsens inhibition of mitochondrial protein synthesis of hair cells	Since reductions in mitochondrial DNA content induced by NRTIs, significantly more HIV-infected patients had or developed persistent hearing loss with/without tinnitus during follow-up.[107-109] After short-term exposure to AZT, d4T, ddC, ddI, and FLT (6-72 hours), mtDNA copy numbers were markedly decreased because the NRTIs inhibit mtDNA replication.[168] Upregulation of glutathione S-transferase 4 expression were significantly increased, which suggests that ROS defense mechanisms likely to be induced by NRTI administration due to mtDNA intoxication.[168]
AO: SNHL	When programmed cell death-signaling pathways has been activated, hair cells, ancillary sensory cells, and neurons undergo apoptotic cell death, resulting in irreversible SNHL	ROS formation through ototoxicants, including gentamicin and kanamycin, in cochlear tissues of was directly observed in guinea pig by electron paramagnetic resonance spectrometry[46] and in chick by using dichlorofluorescin.[47] When chicks and mouse cochlear and vestibular hair cells were exposed to gentamicin, the incorporation of methionine-free medium over 24 hours was reduced by 30–60% compared to control conditions observed by fluorescence microscopy.[169] This indicates gentamicin inhibited the medium uptake into hair cells by

		inhibiting protein synthesis in hair cells and activate a c-Jun N-terminal kinase (JNK) pathway as JNKs activate apoptotic signaling.[169]

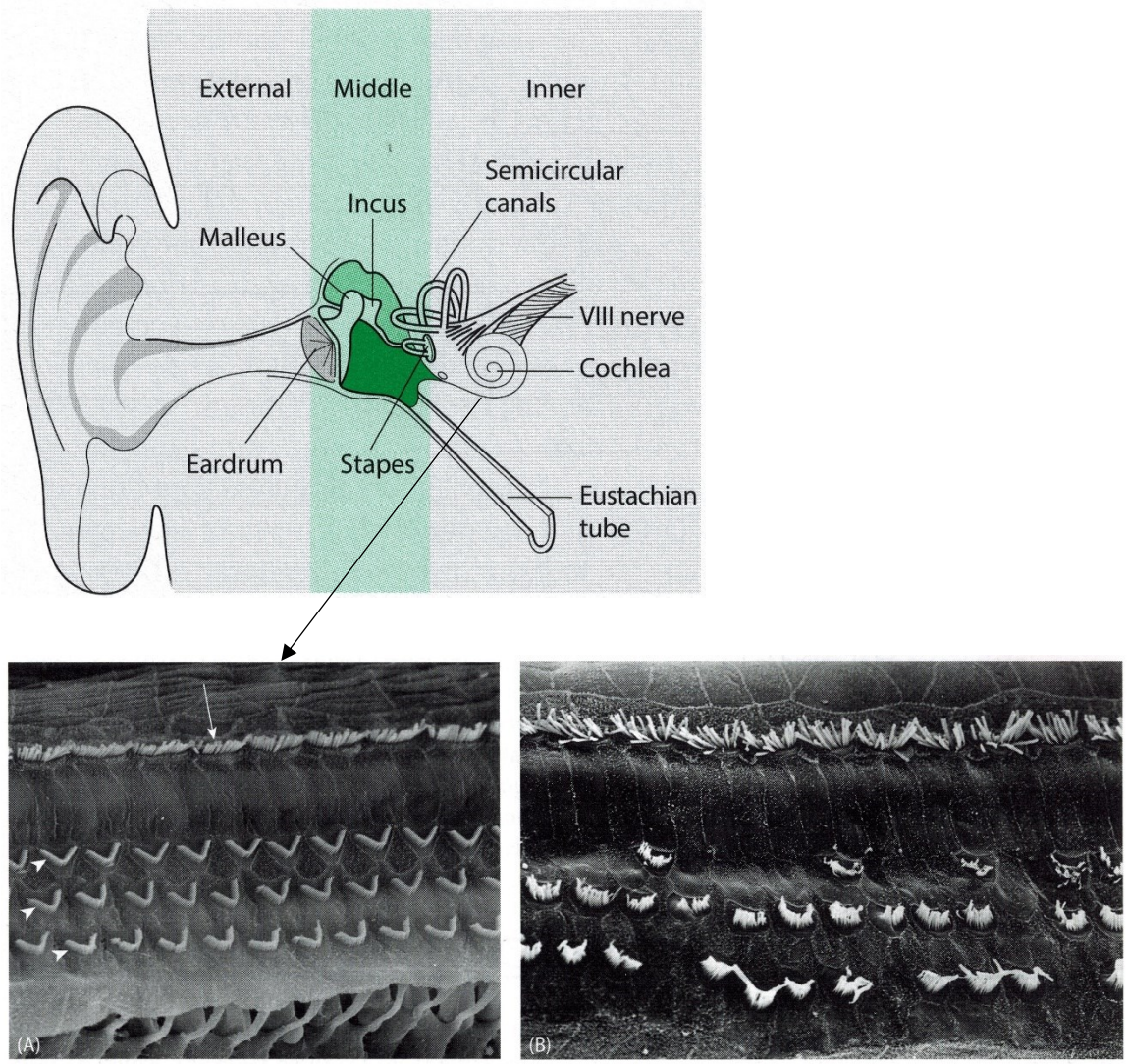
Abbreviations: MIE= molecular initiating event; KE= key event; AO= adverse outcome; HIV= human immunodeficiency virus; CD4= cluster of differentiation 4; SNHL= sensorineural hearing loss; MET= mechanoelectrical transducer; NRTI= Nucleoside Reverse Transcriptase Inhibitor; KM= kanamycin; TDF= Tenofovir disoproxil Fumarate; ROS= reactive oxygen species; AZT= zidovudine (3'-Azido-3'-deoxythymidine); d4T= stavudine (2',3'-didehydro-2',3'-deoxythymidine); ddC= zalcitabine (2',3'-dideoxycytidine); ddI= didanosine (2',3'-dideoxyinosine); FLT= alovudine (3'-deoxy-3'-fluorothymidine)

Table 2. AOP implications and recommendations in MDR-TB treatment

Pre-treatment conditions or prerequisite events	Recommendations
Untreated HIV and/or NRTI use	• Monitor CD4+ T-cell count and viral load. • Consider NRTI-sparing antiretroviral regimen. • Monitor oto- and nephro-toxicity more closely.
Renal insufficiency	• Monitor renal function more closely including BUN, creatinine (serum or urine), and creatinine clearance, etc. • If HIV-infected, consider NRTI-sparing antiretroviral regimen and avoid other nephrotoxic agents
Antioxidant deficiency Hypoalbuminemia	• Provide dietary counseling. • Consider macro- and micronutrient supplementation. • Monitor serum albumin level more closely.
Pre-existing SNHL	• Conduct comprehensive audiological evaluations including occupational/recreational noise exposure, family history of ototoxicity or hearing loss, audiometry, tympanometry, and otoscopy prior to AG initiation. • If moderate to severe hearing loss screened consider AG-sparing MDR-TB regimen or more frequent systematic audiological evaluations should be followed.
Substance abuse	• Provide alcoholism and smoking cessation counseling and rehabilitation

Abbreviations: AG= aminoglycoside; CD4= cluster of differentiation 4; HIV= human immunodeficiency virus; SNHL= sensorineural hearing loss; BUN= blood urea nitrogen; MDR-TB= multidrug-resistant tuberculosis; NRTI= Nucleoside Reverse Transcriptase Inhibitor

Figure 1. Anatomy of inner ear and hair cells



(A) Electron micrograph of normal outer (arrowheads) and inner (arrow) cochlear hair cells; (B) Electron micrograph of damaged cochlear hair cells.

This illustration and image were adapted with permission from Taylor & Francis Group, LLC.[8]

Figure 2. Cross-sectional view of the cochlear duct

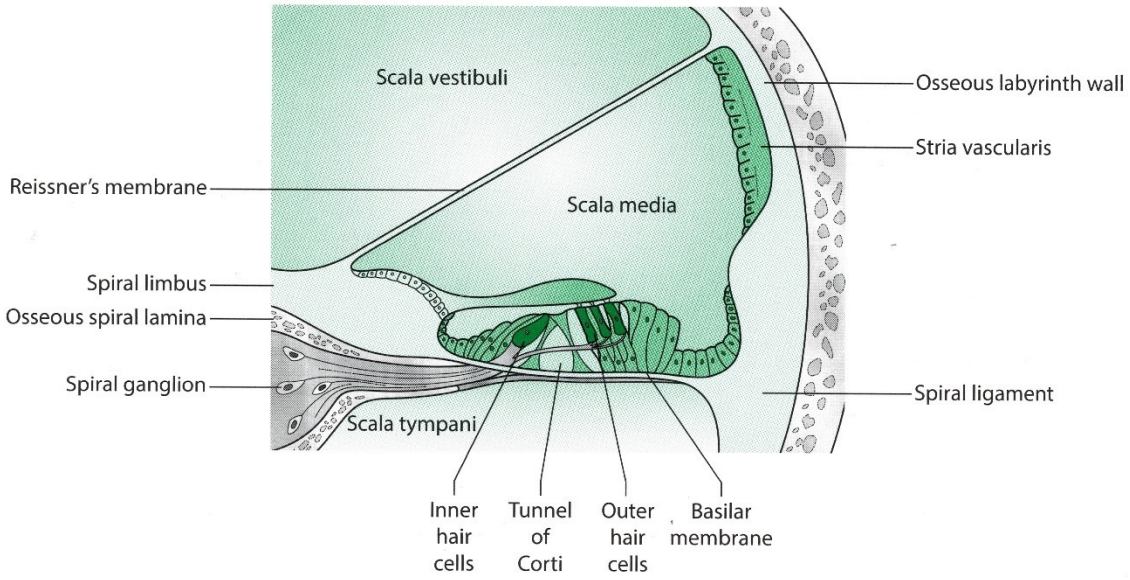
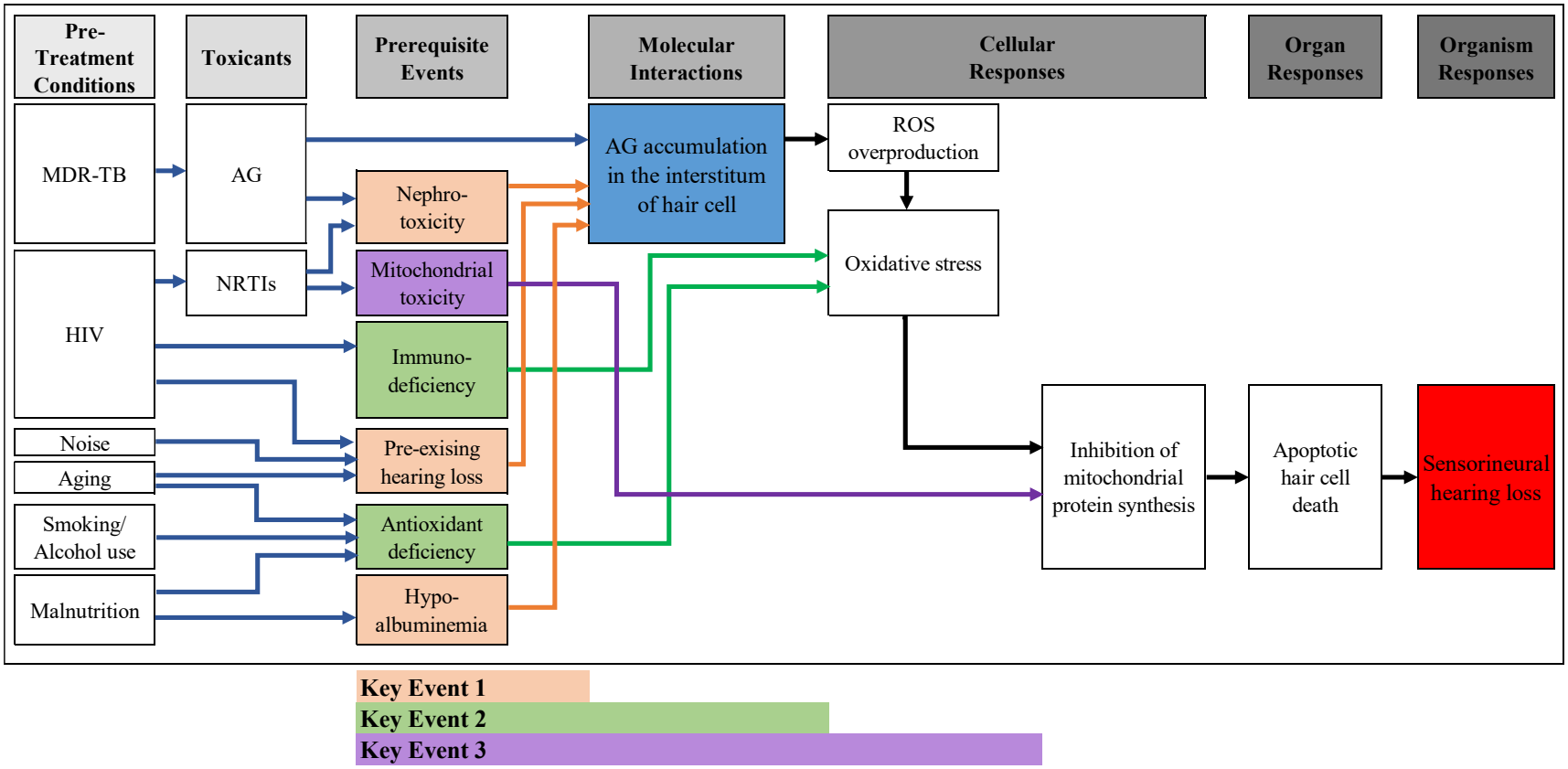


Diagram not to relative scale. This illustration was adapted with permission from Taylor & Francis Group, LLC.[8]

Figure 3. Conceptual framework of Adverse Outcome Pathway on AG ototoxicity in MDR-TB treatment



Abbreviations: AG= aminoglycoside; AO= adverse outcome; HIV= human immunodeficiency virus; KE= key event; MDR-TB= multidrug-resistant tuberculosis; MIE= molecular initiating event; mt= mitochondrial; NRTI= Nucleoside Reverse Transcriptase Inhibitor; ROS= reactive oxygen species; SNHL= sensorineural hearing loss

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CHAPTER 3

Prevalence of Pre-Existing Hearing Loss

among Drug-Resistant Tuberculosis Patients in South Africa

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Running head: Prevalence of hearing loss among South African DR-TB patients

Keywords: ototoxicity, auditory symptom, audiometry

ABSTRACT

Setting: Among drug-resistant tuberculosis (DR-TB) patients, permanent hearing loss from the ototoxic effects of injectable aminoglycosides (AG) is common. Pre-existing hearing loss prior to DR-TB treatment may accelerate further AG ototoxicity compared to those with normal hearing at baseline.

Objective: To determine the risk factors associated with pre-existing hearing loss for DR-TB patients in South Africa prior to the initiation of treatment for DR-TB.

Methods: A cross-sectional study across 10 hospitals in the Eastern Cape and KwaZulu-Natal provinces of South Africa as part of an ongoing cluster-randomized trial. All patients older than 13 years with confirmed DR-TB were included. The clinical, audiological and laboratory evaluations were conducted before DR-TB treatment initiation.

Results: Of 936 patients, 54% were male, mean age 36.2 (SD=11.04), 75% HIV coinfected, and 11% had a prior history of second-line DR-TB treatment. The prevalence of pre-existing auditory symptoms was 15.2% (n=142). Of 482 patients (51.5%) tested at baseline by audiometry, 60.2% (n=290) had pre-existing audiometric hearing loss. Prevalence of pre-existing auditory symptoms was 5.53 times higher ($p<.001$) and that of pre-existing audiometric hearing loss was 1.63 times higher ($p<.001$) among patients ≥ 50 years of age than among teenagers. The prevalence of pre-existing auditory symptoms was 1.73 times higher ($p <.001$) and the unadjusted prevalence of pre-existing audiometric hearing loss was 1.33 higher ($p=.031$) among those who had a prior TB history with second-line treatment than among those who never had TB.

Conclusions: We found a high prevalence of pre-existing hearing loss in this study setting in South Africa. Advanced age and prior second-line TB treatment history were significantly associated with higher prevalence of pre-existing hearing loss, particularly for DR-TB patients. DR-TB providers should adhere to DR-TB treatment guidelines to screen those at higher risk for developing AG-induced hearing loss.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of infectious disease-related deaths worldwide, and is particularly common and lethal in HIV/AIDS-endemic areas such as South Africa.[1] A growing concern in South Africa is drug-resistant TB (DR-TB)—TB that is resistant to first-line anti-TB drugs rifampicin and/or isoniazid.[1,2] DR-TB is treated with second-line injectable anti-TB drugs (for at least 4 months), including aminoglycosides (AGs) (e.g., amikacin and kanamycin) and polypeptides (e.g., capreomycin).[2,3] Among DR-TB patients, permanent hearing loss may result from long-term use of AG due to its ototoxic adverse reaction. Although the exact mechanism of AG ototoxicity is not fully understood, one of the hypotheses is that AGs generate free radicals within the inner ear, which causes apoptotic death of hair cells and ancillary sensory cells within the cochlea.[4,5] Such irreversible sensorineural damage leads to permanent hearing loss, starting from high frequencies with or without tinnitus.[4,5] The cumulative incidence of AG-induced hearing loss varies between 24% and 69% for DR-TB-infected individuals in South Africa,[6] and hearing loss is the most common cause of AG discontinuation, increasing the risk for treatment failure along with further transmission of DR-TB in the household and community.[3,7]

Although it is still unclear who are at higher risk for AG ototoxicity, it has been studied that pre-existing hearing loss prior to DR-TB treatment is associated with AG ototoxicity compared to patients with normal hearing at baseline.[5] Pre-existing sensorineural hearing loss at DR-TB diagnosis commonly originates from previous exposure to ototoxic drugs, noise exposure, advanced age (presbycusis), or idiopathic sensorineural hearing loss.[8,9] These conditions also result in irreversible hair cell loss that

causes the remaining hair cells to be more vulnerable to apoptosis after AG administration. For this reason, the South African Department of Health DR-TB treatment guidelines recommend that an AG-sparing regimen should be considered for those who have pre-existing hearing loss at baseline evaluation.[2] However, due to the lack of trained audiologists or testing facilities, pre-existing hearing loss is often underdiagnosed at baseline.

While there is plausible evidence that several pre-treatment health conditions may be associated with the presence of pre-existing hearing loss prior to AG treatment that intensifies further AG-induced hearing loss, no evidence exists at the population level. Aging leads to auditory sensory cell degeneration due to excessive oxidative stress, which in turn damages hair cells before, during, and even after AG treatment.[10] HIV coinfection impacts hearing loss because the virus directly demyelinates the central nervous system (CNS) and peripheral auditory nerves or causes opportunistic infections, such as cytomegalovirus (CMV) infection that involves CNS or vestibulocochlear nerves.[11-13] Frequent use of ototoxic drugs to treat opportunistic infection and to manage the associated symptoms may increase the risk of AG ototoxicity. Malnutrition may also damage the hair cells in the inner ear. Acute malnutrition causes risk of infection of the auditory system, and thus untreated or recurring infection leads to hearing loss.[14] Chronic malnutrition in childhood and young adulthood results in stunted auditory nerve systems, causing sensorineural hearing loss.[14] In South Africa, a history of DR-TB treatment may indicate previous AG exposure.[2] As a result, those with a history of DR-TB treatment may experience more substantial hearing loss from repeated AG treatment than those who never had DR-TB.[15-18]

Since pre-existing hearing loss is directly and indirectly associated with the risk of further AG-induced hair cell damage, it is critical to investigate whether pre-existing hearing loss has been well screened and how many patients received ototoxic agents in spite of pre-existing hearing loss at DR-TB initiation. In addition, there is a need to identify the association between pre-existing hearing loss and other risk factors for developing hair cell damage in DR-TB-infected populations. Thus, the purpose of this study was to determine the prevalence of pre-existing hearing loss and AG ototoxicity risk factors prior to the initiation of treatment for DR-TB in South Africa where high burdens of TB, HIV, and malnutrition coexist.

METHODS

Study Design and Setting

This was a nested cross-sectional study using baseline data collected on treatment initiation as part of an ongoing cluster-randomized trial investigating the effects of nurse case management in improving treatment outcomes in individuals with DR-TB coinfection in the Eastern Cape and KwaZulu-Natal provinces of South Africa. Data were collected at 10 public TB hospitals in the Eastern Cape and KwaZulu-Natal provinces. The trial has been registered at clinical trials.gov where full details regarding the parent study have been reported (NCT02129244).[19,20]

Participants

For this sub-study, we included participants enrolled in the parent study from November 2014 to June 2017. The following participants were included: older than 13

years, with microbiologically confirmed rifampicin-resistant TB, who were tested by GeneXpert and eligible for an AG regimen, and signed informed consent within seven days of treatment initiation. If participants had been confirmed with drug-sensitive or extensively drug-resistant TB from drug sensitivity tests during the 6-month intensive phase, they were excluded from the study. Participants were also excluded if they had started DR-TB treatment at a different facility or were enrolled in a clinical trial changing the standard DR-TB or HIV regimen.

Assessment & Measures

Pre-existing hearing loss was defined as follows: (1) *pre-existing audiometric hearing loss*: a hearing threshold outside of the normal range (above 25dB) in one or both ears at any frequencies in the range from 250 to 8,000 Hz, tested by either a standard audio booth or by a computer-based portable audiometer at baseline audiometry (KUDUwave®); or (2) *pre-existing auditory symptoms:* self-reported symptoms of hearing loss or tinnitus prior to DR-TB treatment initiation. Auditory symptoms were assessed through face-to-face interviews by either nurse case managers (NCMs) or research assistants (RAs) of the parent study with the language the patient preferred. Participants were asked the following questions at the initial screening visit: “In the last month (30 days), before your treatment began, have you had ringing in the ears?” and “In the last month (30 days) before your treatment began have you had hearing loss?” The responses were collected as five-ordinal variables based on severity of symptoms—from no symptom (grade 0) to severely severe (grade 4)—and then it was dichotomized into whether auditory symptoms were present or not.

Potential risk factors of ototoxicity from clinical and laboratory evaluations—nutritional status, HIV status, CD4 count, renal function, and audiometry—were measured before DR-TB treatment initiation. Particularly, clinical parameters of nutritional status included body mass index (BMI) calculated by weight and height, and serologic nutritional status of serum albumin. These baseline data were extracted by medical chart review and the National Health Laboratory System (NHLS) online laboratory portal. Medical history and sociodemographic data, including age, sex, and prior TB history, were collected by baseline interviews on the day of the DR-TB treatment program admission by NCMs or RAs of the parent study.

Statistical Analysis

We initially conducted descriptive statistics to present distributions of data using frequencies and proportions for categorical variables and mean, and standard deviation (SD) or median and interquartile range (IQR) as appropriate for continuous variables. We performed bivariate analyses using Pearson's chi-square test for categorical variables and the Wilcoxon rank-sum tests for continuous variables to compare the presence of potential risk factors by comparing between-study participants, those who had pre-existing hearing loss, and those not at baseline. A Poisson regression model with robust variance was used to adjust for potential confounding variables and to account for clustering of TB hospitals. The model compared the prevalence of risk factors by baseline hearing status and presented the prevalence ratio. All statistical analyses were performed at a significance level of 0.05 using Stata/IC 15 software.[21]

Ethical Approval

The parent study was approved by the Provincial Health Research Committee of the Eastern Cape and KwaZulu-Natal Provincial Departments of Health in South Africa. The parent study and this sub-study were both approved by the Biomedical Research and Ethics Committee of the University of KwaZulu-Natal in South Africa and the Institutional Review Board of the Johns Hopkins Medical Institutions (NA_00078899 / CIR00024657).

RESULTS

Participants' Characteristics

Of the 1,279 participants enrolled in the parent study, 936 were eligible for the present study to be assessed for pre-existing hearing loss (Figure 1). Mean age was 36.15 (SD=11.04) and median was 33.99 (IQR=28.11-41.08); 54% were male; 75% were coinfected with HIV (n=697). While the majority (63.0%) had a normal renal function (eGFR > 90 ml/min/1.73m^2), 7.1% had renal impairment (eGFR < 60 ml/min/1.73m^2); 35.2% (n=329) were underweight (BMI < 18.5 kg/m^2); 58.9% (n=551) had hypoalbuminemia (serum albumin < 35 g/L) and of those, 19.1% had severe hypoalbuminemia (serum albumin < 25 g/L). Half of the sample (n=421) had a prior history of TB infection in the form of either drug-sensitive or resistant (50.0%), and of those, 11.4% had prior DR-TB history treated with second-line anti-TB treatment.

Of the 697 participants with HIV coinfection, 602 had available baseline CD4 count data. Median baseline CD4 count was 188 cells/mm^3 (IQR= 76-340). More than half of HIV-infected patients (53.2%) had a baseline CD4 count below 200 cells/mm^3 and

18.1% were extremely immunocompromised (CD4 count < 50 cells/mm^3). See Table 1 for additional participant characteristics.

Prevalence of pre-existing hearing loss

Of 936 participants, 15.2% reported baseline auditory symptoms (n=142), specifically having hearing loss only (n=48), tinnitus only (n= 48), and both (n=46). Audiometric outcomes were available at baseline for only 51.5% of participants (n=482). More than half (60.2%) of them (n=290) had audiometry-confirmed hearing loss of any level at frequencies from 250 to 8,000Hz. About half (50.6%) were tested by computer-based portable audiometry; the remaining half were tested by standard audio-booth audiometry. There was a strong association between the presence of baseline auditory symptoms and audiometry-confirmed hearing loss in this study population ($\chi^2(1)=12.98$, $p< .001$).

Comparison by pre-existing hearing loss status

In the bivariate analysis, the following variables had significantly higher prevalence among those with pre-existing auditory symptoms than among those without: older than 50 years of age (PrR=3.53, p<.001) and prior TB history with second-line treatment (PrR= 1.95, p<.001). In the multivariable Poisson regression model, the prevalence of pre-existing auditory symptoms among those in their 20s was 2.89 times higher (p=.006); for those in their 30s, the prevalence was 5.10 times higher (p<.001); for those in their 40s, the prevalence was 3.33 times higher (p<.001); and for those in their 50s or older, the prevalence was 5.53 times higher (p<.001) than for teenagers after

adjusting for sex, prior TB history, albumin, BMI, and HIV status with CD4 count. In the same model, the prevalence of pre-existing auditory symptoms was 1.73 times higher among those who had prior TB history with second-line treatment than among those who never had TB (p <.001); overweight or obese patients had 32% lower prevalence of pre-existing auditory symptoms than underweight patients (p <.001) (Table 2).

In the sub-group analysis of those who had baseline audiometric data, the bivariate analysis showed that an age older than 50 was associated with 46% higher prevalence of pre-existing audiometric hearing loss than among teenagers (p=.001); females had 9% lower prevalence than males (0<.001); those with prior DR-TB with second-line treatment history had 33% higher prevalence than new TB patients (p=.031); and those who had HIV coinfection with CD4 count less than 200 had 21% higher prevalence than those without HIV coinfection (p=.005). In the multivariable model, age older than 50 had 1.63 times higher prevalence than teenagers (p<.001), and 1.18 times higher prevalence among those with severe hypoalbuminemia (< 25 g/L) than those with normal albumin level (p <.001), after adjusting for sex, prior TB history, BMI, and HIV status with CD4 count (Table 2).

DISCUSSION

This study found that the prevalence of pre-existing hearing loss—both auditory symptoms and audiometry-confirmed hearing loss—was significantly high among DR-TB patients in Eastern Cape and Kwazulu-Natal provinces in South Africa. Not surprisingly, the higher prevalence of pre-existing hearing loss among patients ≥ 50 years of age is cogent evidence that presbycusis is prevalent in this sample even at age 50 or

older. This should be accounted for in the selection of DR-TB regimens to prevent substantial hearing loss from treatment as both advanced age and pre-existing hearing loss aggravate AG-induced hearing loss. A history of previous exposure to second-line DR-TB treatment was associated with higher prevalence of pre-existing auditory symptoms and with unadjusted prevalence of pre-existing audiometric hearing loss. Such findings may be explained by some level of irreversible hair cell damage that had already occurred due to previous AG exposure. While none of the models found differences in the prevalence of pre-existing hearing loss between BMI categories, the adjusted prevalence of pre-existing hearing loss among those with severe hypoalbuminemia was 18% higher than among those with normal albumin levels. This is an important finding because protein-energy malnutrition—as a clinical manifestation of TB infection when albumin synthesis is compromised—changes colloid oncotic pressure, resulting in abnormal accumulation of fluid in the interstitium of hair cells.[22,23] Since excessive AG molecular accumulation can occur in the interstitium of hair cells because AG is water-soluble,[24] DR-TB providers should be fully aware that the co-existence of pre-existing hearing loss and hypoalbuminemia synergistically enhance the incidence of AG-induced hearing loss after the initiation of a DR-TB regimen. In this study population, while the prevalence of pre-existing hearing loss and HIV coinfection were not associated, the unadjusted prevalence of pre-existing audiometry-confirmed hearing loss among HIV-coinfected patients with immunosuppression (CD4 count < 200) was 21% higher than among HIV-uninfected DR-TB patients. HIV infection not only weakens the host immune system—increasing the chance of opportunistic infections and additional use of ototoxic agents—but also causes excessive oxidative stress in cellular levels that

accelerates oxidative hair cell damage.[25] Thus, further development of AG-induced hearing loss should be closely monitored during AG treatment for those who are HIV coinfected.

We also found that the evaluation of adherence to practical recommendations for baseline hearing screening needs more attention in this study setting. The South African Department of Health MDR-TB guidelines instruct that audiometry should be performed prior to initiation of treatment and repeated at least monthly throughout the injectable phase of treatment.[2] However, we found that baseline audiological evaluation was inconsistently performed, and only 51.5% in this sample were screened for their baseline hearing capacity through audiometry. Moreover, the availability of trained audiologists and well-functioning audiometers at TB hospitals should be audited on a regular basis to make early detection of AG-induced hearing loss possible in practical settings.

This study has several limitations. As this is a nested study using secondary data, the selection of variables was limited based on the parent study collected data. Since the parent study was not designed to primarily inquire about hearing loss from DR-TB treatment, other risks of hearing loss, such as noise exposure, conductive hearing loss, and family history of hearing loss or ototoxicity, were not collected. Since AG is water-soluble and the molecular concentration is influenced by body size,[26] future studies must consider including not only the aforementioned risk factors but also a wider range of measures of nutritional status including: (1) body size measured by anthropometric parameters (e.g., arm/waist/hip/calf circumferences and triceps/subscapular skinfold), and (2) body composition measured by bioelectrical impedance (e.g., fat mass, fat-free mass, muscle mass, fat area, muscle area, total body water, intracellular water, and extracellular

water). In addition, a comprehensive measure of HIV-related variables, such as duration of living with HIV infection, the specific ART combination given and its frequency, needs to be considered in future studies. Since height, weight, and audiometry were measured by TB hospital staff who had not been trained by the parent study, measurement errors might have occurred equally across all sites. These programmatic measurements were used by healthcare providers to make clinical decisions including TB medication dosing, so they are clinically relevant. Finally, as a cross-sectional study, our findings of the associations between pre-existing hearing loss and other ototoxicity risk factors can be suggestive of possible risk factors of AG-induced hearing loss, but they cannot reflect causal relationships. Thus, further longitudinal studies exploring the incidence of AG-induced hearing loss according to the presence of risk factors should be conducted to fill such gaps.

CONCLUSION

This study found a high prevalence of pre-existing hearing loss in DR-TB and HIV-endemic settings in South Africa. Advanced age and prior TB history with use of second-line anti-TB drugs were significantly associated with a higher prevalence of pre-existing hearing loss. DR-TB providers should not only adhere to DR-TB treatment guidelines to screen those at higher risk for developing AG-induced hearing loss but also consider the use of less ototoxic DR-TB regimens for those with advanced age and a prior TB treatment history who display pre-existing hearing loss at baseline.

Table 1. Participants' Baseline Characteristics

	Overall (n=936)	Audiometry available (n= 482)	p-value
Sex: N (%)			.425
Male	505 (53.95)	261 (54.15)	
Female	431 (46.05)	221 (45.85)	
Age*: N (%)			.653
13-19	45 (4.81)	27 (5.60)	
20-29	241 (25.75)	125 (25.93)	
30-39	355 (37.93)	189 (39.21)	
40-49	172 (18.38)	87 (18.05)	
≥ 50	123 (13.14)	54 (11.20)	
Smoking: N (%)			.962
Non-smoker	621 (66.42)	315 (65.49)	
Light smoker (<10 cigarettes/day)	187 (20.00)	98 (20.37)	
Heavy smoker (≥10 cigarettes/day)	83 (8.88)	43 (8.94)	
Alcohol use: N (%)			.588
Non-drinker	552 (59.04)	278 (57.80)	
Less than once per week	290 (31.02)	151 (31.39)	
More than twice per week	83 (8.88)	48 (9.98)	
Poverty: N (%)			.649
Not poor	589 (87.91)	439 (91.27)	
Poor	68 (10.15)	38 (7.90)	
HIV status & CD4 count†: N (%)			.086
HIV negative	239 (25.53)	117 (24.27)	
HIV positive with CD4 ≥ 200	282 (30.13)	152 (31.54)	
HIV positive with CD4 < 200	320 (34.19)	169 (35.06)	
Unknown CD4 count	95 (10.15)	44 (9.13)	
Prior history of TB: N (%)			.422
New TB	478 (51.07)	224 (50.62)	
Prior TB with 1st line treatment	373 (39.85)	192 (39.83)	
Prior TB with 2nd line treatment	48 (5.13)	29 (6.02)	
Unknown	37 (3.95)	17 (3.53)	
BMI‡: N (%)			.444
Underweight (<18.5)	329 (35.15)	173 (35.89)	
Normal (18.5-24.9)	445 (47.54)	240 (49.79)	
Overweight or Obese (≥25)	153 (16.35)	69 (14.32)	
Unknown	9 (0.96)	0 (0.00)	
Serum Albumin§: N (%)			.455
Normal (≥ 35)	193 (20.62)	97 (20.12)	
Mild hypoalbuminemia (25-34.9)	372 (39.74)	190 (39.42)	
Severe hypoalbuminemia (< 25)	179 (19.12)	92 (19.09)	
Unknown	192 (20.51)	103 (21.37)	

eGFR[ǀ]: N (%)			.345
≥ 90	590 (63.03)	313 (64.94)	
60-89	196 (20.94)	104 (21.58)	
< 60	66 (7.05)	32 (6.64)	
Unknown	84 (8.97)	33 (6.85)	

*Age unit=years old; [†]CD4 count unit=cells/mm^3; [‡]BMI unit=kg/m^2; [§]serum albumin unit= g/L; [ǀ]eGFR unit=$mL/min/1.73m^2$

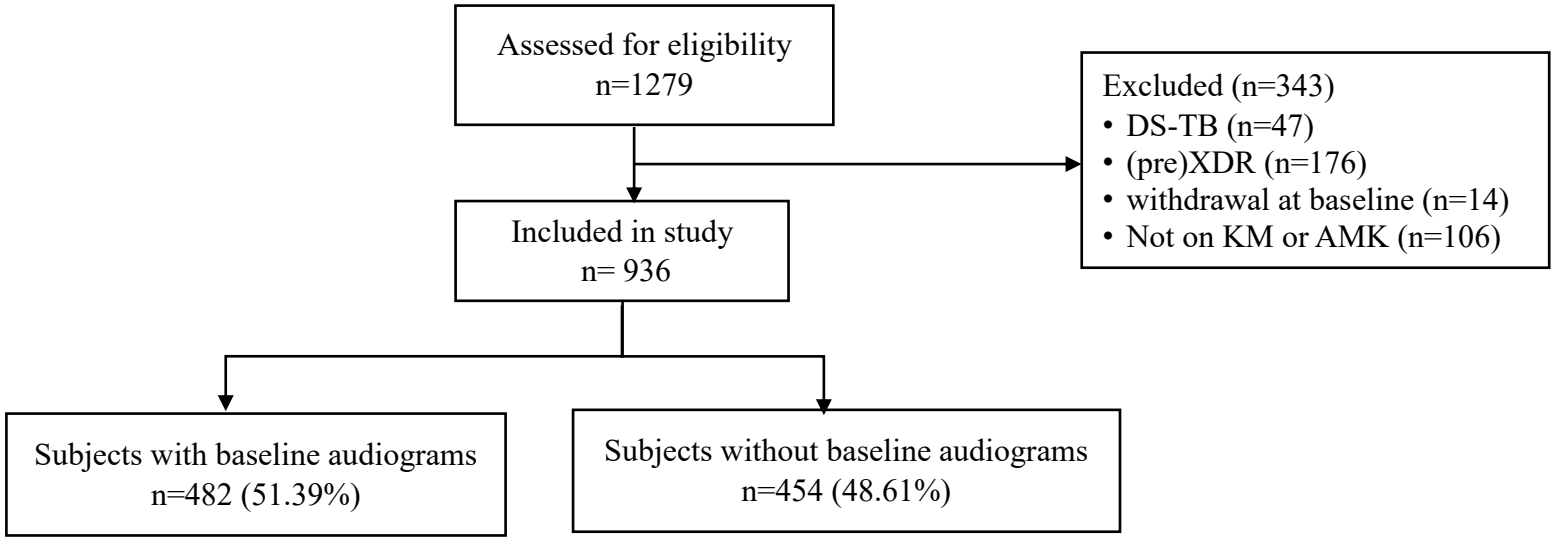
Abbreviations: ART=Anti-retroviral therapy; BMI=body mass index; CD4=cluster of differentiation 4; DR-TB=drug-resistant tuberculosis; eGFR=estimated glomerular filtration rate; HIV=human immunodeficiency virus; TB=tuberculosis.

Table 2. Comparison of the Prevalence Ratios of Ototoxicity Risk Factors by Pre-existing Hearing Loss among Patients with DR-TB

	Pre-existing auditory symptoms (N=936)		Pre-existing audiometric hearing loss (N=482)	
Variable	Unadjusted PrR (95% CI)	Adjusted PrP (95% CI)	Unadjusted PrR (95% CI)	Adjusted PrR (95% CI)
Age (years old)				
13-19	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
20-29	1.56 (0.78-3.11)	2.89 (1.34-6.22)	0.91 (0.72-1.05)	1.01 (0.66-1.56)
30-39	2.81 (1.06-7.44)	5.10 (2.88-9.03)	1.20 (1.10-1.31)	1.30 (1.19-1.42)
40-49	1.66 (0.89-3.09)	3.33 (1.96-5.65)	1.28 (0.93-1.78)	1.49 (0.97-2.29)
≥ 50	3.53 (2.38-5.26)	5.53 (3.363-8.42)	1.46 (1.17-1.83)	1.63 (1.31-2.03)
Sex	0.99 (0.80-1.23)	0.99 (0.73-1.33)	0.91 (0.88-0.93)	1.00 (0.95-1.06)
Prior history of TB				
New TB	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
Prior TB with 1st line treatment	1.27 (0.84-1.94)	0.85 (0.69-1.05)	1.07 (1.02-1.14)	1.00 (0.88-1.15)
Prior TB with 2nd line treatment	1.94 (1.44-2.61)	1.73 (1.66-1.80)	1.33 (1.03-1.73)	1.07 (0.69-1.67)
Serum albumin (g/L)				
Normal albumin	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
Hypoalbuminemia (< 35)	1.06 (0.62-1.82)	1.08 (0.73-1.60)	0.97 (0.87-1.09)	0.95 (0.86-1.03)
Severe hypoalbuminemia (< 25)	1.60 (0.82-3.10)	1.33 (0.69-2.58)	1.21 (0.97-1.50)	1.18 (1.16-1.21)
BMI (kg/m^2)				
Underweight (<18.5)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
Normal (18.5-24.9)	0.86 (0.57-1.32)	0.94 (0.72-1.24)	1.01 (0.99-1.04)	1.10 (1.00-1.22)
Overweight or obesity (≥ 25)	0.56 (0.29-1.09)	0.68 (0.67-0.68)	1.02 (0.95-1.10)	1.01 (0.93-1.09)
HIV status & CD4 count ($cells/mm^3$)				
HIV negative	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
HIV positive with CD4 ≥ 200	1.17 (0.84-1.62)	1.40 (0.81-2.43)	0.92 (0.73-1.15)	0.83 (0.70-0.99)
HIV positive with CD4 < 200	1.18 (0.86-1.64)	1.16 (0.59-2.30)	1.21 (1.06-1.38)	1.09 (0.784-1.43)
Type of audiometer				
Audio booth	-	-	1 [Reference]	1 [Reference]
KUDUwave			1.15 (0.90-1.47)	1.12 (1.01-1.24)

*Abbreviations: BMI=body mass index; CD4=cluster of differentiation 4; HIV=human immunodeficiency virus; PrR=prevalence ratio; TB=tuberculosis

Figure 1. Diagram for Study Flow



Abbreviations: KM= kanamycin; AMK= amikacin; DS-TB= drug-sensitive tuberculosis; XDR= extensively drug-resistant TB

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CHAPTER 4

Increased Risk of Aminoglycoside-Induced Hearing Loss in MDR-TB Patients with HIV Coinfection

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Running head: Impact of HIV on AG-induced hearing loss

Keywords: ototoxicity, sub-Saharan Africa, meta-analysis

ABSTRACT

Setting: A high proportion of individuals with multi-drug-resistant tuberculosis (MDR-TB) develop permanent hearing loss due to ototoxicity caused by injectable aminoglycosides (AGs). The prevalence of AG-induced hearing loss is greatest in tuberculosis (TB) and human immunodeficiency virus (HIV) endemic countries in sub-Saharan Africa. However, whether HIV coinfection is associated with a higher incidence of AG-induced hearing loss during MDR-TB treatment is controversial.

Objective: To evaluate the impact of HIV coinfection on AG-induced hearing loss among individuals with MDR-TB in sub-Saharan Africa.

Design: This was a meta-analysis of articles published in PubMed, Embase, Scopus, Cumulative Index to Nursing and Allied Health Literature, Web of Science, Cochrane Review, and reference lists using search the terms 'hearing loss', 'aminoglycoside', and 'sub-Saharan Africa'.

Results: Eight studies conducted in South Africa, Botswana, and Namibia and published between 2012 and 2016 were included. As the included studies were homogeneous (χ^2=8.84, d.f.=7), a fixed-effects model was used. Individuals with MDR-TB and HIV coinfection had a 22% higher risk of developing AG-induced hearing loss than non-HIV-infected individuals (pooled relative risk=1.22; 95% CI=1.10-1.36) during MDR-TB treatment.

Conclusion: This finding is critical for TB programs with regard to the expansion of injectable-sparing regimens. Our findings lend credibility to using injectable-sparing regimens and more frequent hearing monitoring, particularly in resource-limited settings for HIV-coinfected individuals.

INTRODUCTION

Multidrug-resistant Tuberculosis (MDR-TB), defined as TB resistant to at least isoniazid and rifampicin, is a global health emergency. MDR-TB treatment is prolonged (9–24 months), poorly efficacious (<50% treatment success), poorly tolerated, and quite toxic.[1,2] Despite advances in injectable-sparing regimens, the mainstay of MDR-TB treatment contains one second-line injectable, an aminoglycoside (AG), for at least 4 months in combination with four oral drugs.[2] AGs include amikacin (AMK), kanamycin (KM), and streptomycin (SM), or the mechanistically similar cyclic peptide antibiotic, capreomycin (CPM).[3] One of the main adverse reactions from AGs is sensorineural ototoxicity: SM is mainly vestibulotoxic, causing dizziness, ataxia, or nystagmus; AMK, KM, and CPM are predominantly cochleotoxic, resulting in tinnitus or hearing loss.[4]

AG-induced hearing loss begins at high frequencies, can progress even with AG discontinuation, and is permanent unless quickly identified.[4] Hearing loss leads to social isolation, reduced quality of life, and threatens employment stability and family prosperity.[5,6] The risk of AG-induced hearing loss may be impacted by human immunodeficiency virus (HIV) coinfection. Although the exact mechanism of AG ototoxicity is not known, it has been hypothesized that excessive AG accumulation in the inner ear catalyzes the formation of reactive oxygen species (ROS).[7,8] When ROS formation overwhelms the capacity of the intrinsic protective and repair system, the sensory hair cells undergo apoptotic death, resulting in irreversible hearing loss.[4,9] As chronic immune activation in HIV coinfection triggers massive ROS formation, people living with HIV (PLHIV)—particularly those who are antiretroviral therapy (ART) naïve—may be more vulnerable to AG ototoxicity.[10,11]

Paradoxically, HIV treatment may also be associated with an increased risk of ototoxicity. Nucleoside reverse transcriptase inhibitors (NRTIs), a class of ART drugs, are mitochondrial-toxic and cause mitochondrial damage in outer hair cells.[12,13] Moreover, one NRTI, *tenofovir disoproxil fumarate*, is also nephrotoxic, and can compound AG-induced ototoxicity, as AGs are eliminated through the kidneys.[12,13] Poly-pharmacy is common in MDR-TB and HIV treatment, with additional medications added to manage opportunistic infections or adverse drug reactions.[14] This complexity may result in additional drug-drug interactions, pill fatigue and resultant non-adherence, or drug-induced renal impairment, any of which can affect the risk of ototoxicity.[15]

People in resource-limited settings are more likely to be at high risk for AG ototoxicity. Protein-energy malnutrition caused by insufficient intake of protein and calories is prominent in sub-Saharan Africa due to food insecurity.[16,17] In the case of protein-energy malnutrition, albumin synthesis is impaired and changes in oncotic pressure lead to abnormal accumulation of fluid in the interstitium of hair cells,[18,19] thereby worsening AG ototoxicity because AG is water-soluble.[20] Furthermore, a dietary deficiency of protein and calories reduces the synthesis of antioxidant enzymes and antioxidant concentrations, leading to ROS overproduction.[19,21] Due to the financial costs involved in frequent audiological assessment or therapeutic drug monitoring (i.e., daily blood tests for AG concentration), early detection of hearing loss is impractical in most sub-Saharan African countries, which leads to missed opportunities to prevent hearing loss.[1,22]

Despite these known risks, whether HIV coinfection leads to a higher incidence of AG-induced hearing loss during MDR-TB treatment is controversial. The objective of the

present study was to systematically review the literature and estimate the effect size of the association between HIV coinfection and AG-induced hearing loss among MDR-TB-infected individuals in sub-Saharan Africa.

METHODS

The review process was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards.[23] Institutional review board approval was not required for this meta-analysis.

Inclusion/exclusion Criteria

The inclusion criteria for participants were: (1) known or presumptive TB with isoniazid resistance, rifampicin resistance, or MDR-TB on microbiologic tests (determined either on culture with drug susceptibility testing or using cartridge-based Xpert® MTB/RIF; Cepheid, Sunnyvale, CA, USA), and (2) use of second-line injectable anti-tuberculosis drugs (AMK, KM, SM, or CPM). Hearing loss in study participants should have been observed either prospectively or retrospectively during and/or after treatment with injectables. All ages and both sexes were included in our analyses.

The following diagnoses of AG-induced hearing loss were accepted: (1) audiometric hearing loss, defined as worsening of hearing threshold confirmed using audiometry; (2) self-reported hearing loss, defined as symptomatic hearing loss reported by patients after AG initiation; and (3) clinician-identified hearing loss, diagnosed by clinicians in the absence of audiometry. In our analysis, a broader definition of AG-induced hearing loss was accepted because regular audiological assessments are rarely

conducted in many sub-Saharan African countries due to the shortage of trained audiologists or testing equipment. This definition of hearing loss was supported by a recent study that concluded that patient self-report of hearing loss was highly concordant with clinician-identified hearing loss in the setting of monthly audiological testing.[24] Only studies written in English were included.

Studies were excluded if they did not include participants' HIV status as a study variable. We also excluded studies if full-text versions were not available (e.g., conference abstracts), if the study did not have a quantitative design, or if studies reported the protocol only with no measured outcomes.

Search and Selection Process

PubMed, Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Scopus, Web of Science, and Cochrane Review were searched using the following MeSH terms: 'hearing loss', 'aminoglycosides' and 'Africa South of the Sahara'. Our initial search was not limited by the year of publication. Electronic searches were supplemented by manual searches of references found in identified articles and bibliographies.

Our initial database search, conducted on 19 December 2016, resulted in 367 citations. After removing duplicates, 79 titles with abstracts were reviewed for relevance by HH. Twenty-one articles were passed onto the next full-text review process. Of the 12 full-text articles that were selected by HH and confirmed by CB, six studies reporting the number of participants who developed AG-induced hearing loss and their baseline HIV coinfection status provided useful data for a meta-analysis. We contacted the six

corresponding authors of the eligible studies to request unpublished descriptive statistical data to calculate the cumulative incidence of hearing loss and prevalence of HIV coinfection; of these, two authors provided the requested data, which were finally added to the study data set on 10 July 2017. Eight studies were included in our analysis; four studies were excluded due to lack of useful data required for a meta-analysis (Appendix Figure A.1).

Data Quality Assessment

The Newcastle-Ottawa Quality Assessment Scale (NOS) was used to evaluate the quality of the original studies.[25] Three main themes were evaluated: selection of samples (four items), comparability of cohorts (one item), and ascertainment of outcome (three items).

In this meta-analysis, comparability was assessed as to whether the original studies isolated conductive hearing loss (e.g., cerumen impaction or middle ear infection) using otoscopy or tympanometry, because AG mainly causes cochlear-toxic sensorineural hearing loss. One point was awarded for each quality item; a total of eight points thus indicated the highest quality. In general, as positive findings are more likely to be published, we also tested for publication bias to estimate the possibility of distortion of synthesized meta-analysis results.[26]

Statistical Analysis

Cumulative incidence (absolute risk, i.e., the total number of events divided by the total number of people at risk) of each study was initially calculated because of the

different follow-up durations and formats used to measure events across studies.[27] Heterogeneity was tested using Cochran's Q statistic, along with summary estimates using the *metan* command. Due to non-significance of heterogeneity (χ^2 =8.84, d.f.=7, p=0.26; I^2= 20.9%), which suggested that the differences between the studies were explicable by random variation,[28] we used the Mantel-Haenszel fixed-effects method with the *metan* command in Stata/IC 14 (StataCorp, College Station, TX, USA) to combine the different results and obtain a pooled estimate of the effect size.[28,29] The cumulative incidence ratio (relative risk [RR]) was used as a pooled measure of association to interpret the synthesized impact of the prevalence of HIV coinfection on the risk of AG-induced hearing loss, with variance presented by 95% confidence intervals (CIs). The funnel plot—a graphic plot to diagnose publication bias and other small-study effects (the tendency for smaller studies in a meta-analysis to show larger treatment effects)—was used using the *funnel* command.[28,29]

RESULTS

Overview of studies included in the meta-analysis

This meta-analysis comprised eight studies that met the inclusion and exclusion criteria (Table). All eight studies were published between 2012 and 2016.[13,24,30–35] Most were prospective and retrospective cohort studies; one study retrospectively collected study outcomes from medical records and then compared these to cross-sectional patient interview outcomes.[24] The studies were conducted in specialist TB hospitals (n=7)[13,24,30,32–35] and community-based HIV-TB clinical settings (n=1).[31] Seven studies had a cohort sample of adults aged ≥14 years;[13,24,31–35] only one study had a sample of

children aged <15 years.[30] Sample size was between 50 and 99 individuals in four studies,[30–32,34] between 100 and 299 in two studies,[13,24] and >300 in two studies.[33,35] All studies were conducted in southern Africa: four studies were conducted in South Africa,[13,24,30,31] two in Botswana[33,34] and two in Namibia.[32,35] NOS scores ranged between 5 and 8; the mean was 6.75 out of 8.

The outcomes of hearing loss diagnosis were categorized by audiometric hearing loss (n=3)[13,30,35] and composite hearing loss, including both clinician-identified hearing loss confirmed using audiometry (n=4)[24,31,33,34] and self-reported hearing loss (n=1).[32] Audiometric hearing loss was assessed using either pure tone audiometry in adults and children aged >7 years or distortion product otoacoustic emissions in children aged <6 years.[30] Of the five studies that used audiometry testing of both air and bone conductions to diagnose drug-induced sensorineural hearing loss, only two studies confirmed and differentiated conductive hearing loss by assessing outer and middle ears through tympanometry or otoscopy.[13,30] Finally, the risk of hearing loss during injectable anti-tuberculosis treatment ranged from 23% to 69%. The prevalence of HIV coinfection at TB treatment initiation ranged from 30% to 83%.

Effect of human immunodeficiency virus coinfection on aminoglycoside-induced hearing loss

MDR-TB and HIV-coinfected individuals had a 22% higher risk of developing AG-induced hearing loss than non-HIV-infected individuals (pooled RR=1.22, 95% CI=1.10-1.36, p<.001) during MDR-TB treatment (Figure 1).[13,24,30–35] No significant differences were found in subgroup analysis of studies for which audiometric hearing loss

data were available (n=5). Such analyses demonstrated that the risk of hearing loss was 24% higher among HIV-coinfected individuals than among non-HIV-infected individuals (pooled RR=1.24, 95% CI=1.11-1.38, p<.001) (Figure 2). As shown in Figure 3, three studies compared the effect of participants' ART status on AG-induced hearing loss, although the type of ART was not specified; the risk of developing AG-induced hearing loss did not differ, regardless of ART status in PLHIV (pooled RR=1.01, 95% CI=0.72-1.41, p=0.97).[24,31,33] Baseline CD4 count was available from only one study, and patients whose baseline CD4 count was <200 cells/mm^3 did not have a significantly increased risk of hearing loss compared to those with a baseline CD4 count ≥200 cells/mm^3 (RR=1.16, 95% CI=0.95-1.42, p=0.15).[33]

Publication bias

The asymmetric distribution funnel plots suggested some visual evidence of publication bias (Appendix Figure A.2); however, the effect size of AG-induced hearing loss was considered to be small. The homogeneity from Q statistics and significant P values for effect size supported the characteristics of stability, suggesting reasonably low levels of publication bias.

DISCUSSION

Questions are frequently raised about the risk of treatment-induced hearing loss. However, few studies have focused on the factors that might result in a higher risk of AG ototoxicity during MDR-TB treatment in sub-Saharan Africa. Although mitochondrial mutations in MT-RNR1 may increase genetic susceptibility,[36,37] this is more prevalent in

Europeans and Asians and not in sub-Saharan Black Africans, among whom the prevalence of this mutation is extremely low (0–0.09%).[37–40]

We found that individuals with MDR-TB and HIV coinfection had a higher risk of AG-induced hearing loss than non-HIV-infected MDR-TB patients. It is therefore likely that the high burden of HIV coinfection in sub-Saharan Africa may be the reason for the staggeringly high prevalence of AG-induced hearing loss (23–69%) compared with less burdened countries, such as the United States (13%),[41] the Netherlands (18%),[42] the United Kingdom (28%),[43] and India (10–25%).[44–46]

We also revealed that AMK was the most common choice of AG for MDR-TB treatment across all eight studies. However, one of the included studies found that the risk of ototoxicity with AMK was four times higher than with KM (adjusted odds ratio 4.0, 95%CI 1.5–10.8).[35] These findings will assist healthcare providers develop personalized interventions, for example by choosing less ototoxic drugs, changing to an AG-sparing regimen, or scheduling more frequent hearing monitoring in PLHIV where AG is required for MDR-TB treatment, especially in sub-Saharan Africa.

A new short-course MDR-TB treatment regimen recommended by the World Health Organization (WHO) reduces treatment from 20–24 months to 9–12 months; however, an injectable AG remains part of this recommendation, in part because of the low cost as well as potent antibacterial activities.[2,4] To qualify for substitution of less or non-ototoxic drugs (e.g., bedaquiline) for AGs, many TB programs currently require evidence of treatment-related hearing loss. All patients' hearing should therefore be carefully monitored while using second-line injectable AGs through routine audiological assessments for the early detection of hearing loss. Regular audiological assessments may

prevent severe or complete hearing loss because, by the time a symptom of hearing loss is detected, it is often too late to reverse hair cell damage.[4]

In our meta-analysis, only three studies used an audiometric definition of hearing loss for all study participants,[13,24,35] while others embraced self-reported or clinician-identified hearing loss as a surrogate outcome of hearing loss. Our meta-analysis also found that only two of eight studies conducted tympanometry and otoscopy to confirm drug-induced sensorineural hearing loss by differentiating it from conductive hearing loss.[13,30] These findings suggest that regular and comprehensive audiological assessment may be impractical in many study sites due to insufficient resources.

The present study has several strengths. First, we used PRISMA criteria to increase the transparency of reporting and avoid selection bias during the study selection phase.[23] Second, we conducted a comprehensive search of all potentially relevant studies with the help of an academic librarian to ensure a systematic approach to capture all the evidence that may pertain to the question of interest. Third, the NOS tool was used to assess the quality of all included studies so that results could be interpreted in the context of their quality. Finally, we used a meta-analysis, a rigorous statistical method, to consolidate research findings from studies addressing a similar topic but conducted in diverse settings.[47,48] This approach enabled the analysis to draw more decisive conclusions on effect size for a relationship between AG-induced hearing loss and HIV coinfection because of its greater statistical power and external validity.[47]

While our study findings contributed to the risk analyses of AG-induced hearing loss, there were several limitations. First, despite our expanded search criteria, only a small number of studies met the inclusion criteria due to the lack of published studies. As

very few studies reported the ART status of participants, we were unable to conclude whether concomitant administration of ART affected the risk of AG-induced hearing loss during injectable MDR-TB treatment. Second, samples of included studies were not necessarily representative of the variety of people living in sub-Saharan Africa, as the geographical sites of the included studies were mostly limited to southern Africa, and participants were predominantly adults. Finally, this meta-analysis did not control for potential confounders, such as age or use of ototoxic or nephrotoxic drugs, during injectable treatment.

Future studies aiming to find AG-induced hearing loss risk factors or prevent AG-induced hearing loss must consider including a wide range of HIV-related variables, such as CD4 count, viral load, duration of living with HIV infection, as well as the specific ART combination given and its frequency. Future studies need to consider the influences of time-dependent variables, such as weight, serum creatinine, and AG accumulation on the risk of AG-induced hearing loss. Because conductive hearing loss commonly results from otitis media or cerumen impaction that can threaten construct validity, conductive hearing loss must be ruled out by comprehensive audiological assessment, including audiometry, tympanometry, and otoscopy.[49] Finally, children need to receive more attention in AG-induced hearing loss studies, as children with hearing loss may suffer from delayed communicational development and literacy compared with children with normal hearing.[50,51]

CONCLUSION

The WHO recommends a new short-course MDR-TB treatment regimen, which includes an AG. The present study lends credibility to using injectable-sparing regimens and more frequent hearing monitoring—particularly in resource-limited settings for PLHIV in sub-Saharan Africa. Such strong evidence of AG-induced hearing loss risk may help healthcare providers make clinical decisions when initiating MDR-TB treatment for PLHIV.

Table. Descriptive Analysis of Included Studies

Author, Year (Country)	Design (NOS score), Sample Size, Age	Study Purpose	Diagnostic Methods of HL	Absolute risk of HL	HIV Prev.	ART Status	Type of AGs (%)	Major Findings
Harris et al.[13] 2012 (South Africa)	Prospective cohort (8) N= 153 Adults [range=14-70y]	To document the incidence of ototoxicity in MDR-TB patients with and without HIV, and develop clinical guidelines relating to ototoxicity in such patients	• Audiometric HL by PTA + Tympanometry + otoscopy	87/153 (57%)	86/153 (56%)	86/86 (100%)	AMK(1), KM (94), SM(4), CPM(1)	• 57% developed high-frequency HL within 3m. • Of those who developed HL, 69% were HIV positive and 31% were HIV negative.
Seddon et al.[30] 2012 (South Africa)	Prospective cohort (8) N=93 *(Confirmed MDR-TB n= 50)* Children [IQR=20-110m]	To determine the extent of hearing loss in children treated for MDR-TB	• Audiometric HL by PTA • Audiometric HL by DPOAE + Tympanometry + otoscopy	23/93 (24%)	28/93 (30%)	20/28 (71%)	AMK(88), SM(10), CPM(1)	• 64% had audiometric HL and had progression of HL after finishing the injectable drug.
Brust et al.[31] 2013 (South Africa)	Retrospective cohort (7) N=89 Adults [IQR= 29-41y]	To examine the frequency and severity of AEs in patients with MDR-TB and HIV coinfection treated at an integrated MDR-TB/HIV home-based treatment program	• Composite HL (audiometric + clinician-identified HL)	31/89 (34%)	76/89 (84%)	66/76 (87%)	KM (100)	• 34% developed HL during treatment. • 69% had some degree of HL; 11% had severe HL; and 10% patients required dose reductions of kanamycin for HL.
			• Audiometric HL by PTA	24/35 (69%)				
Sagwa et al.[32] 2013 (Namibia)	Retrospective cohort (6) N=57 No age restriction [range= 11-55y]	To compare the absolute risks and risk factors for commonly observed adverse events (occurring in >20 % of patients) during DR-TB treatment	• Self-reported HL	13/57 (23%)	31/57 (54%)	13/31 (42%)	AMK(36), KM(51), SM(5), CPM(7)	• 23 % developed HL during treatment. • The absolute risk of HL was 8/31 (26 %) in HIV-

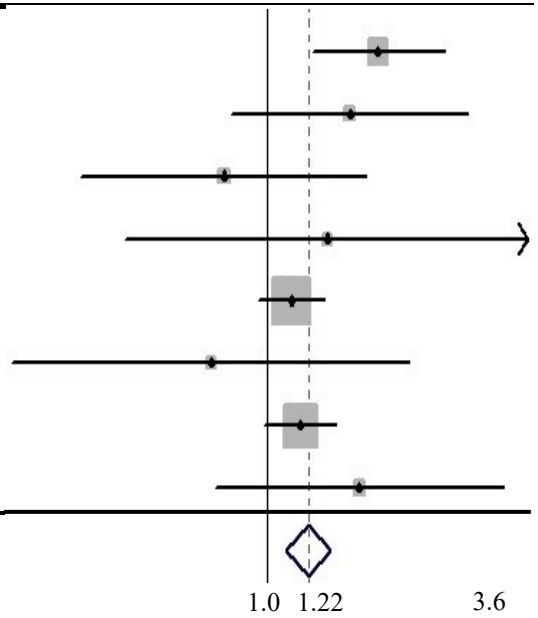
		in HIV-infected and HIV-uninfected patients.						coinfected and 5/26 (19 %) in HIV-uninfected group.
Modongo et al.[33] 2014 (Botswana)	Retrospective cohort (7) N=437 Adults [IQR= 31-49y]	To determine the effect of amikacin on treatment outcomes and development of hearing loss in MDR-TB patients	• Composite HL (audiometric + clinician-identified HL)	270/437 (62%)	288/437 (66%)	267/288 (93%)	AMK(100)	• HIV infection was not associated with increased risk of HL (aOR= 1.32, 95% CI: 0.83-2.12). • The most important HL risk factors were treatment duration in month (aOR 1.98, 95% CI 1.86-2.12) and dosage per mg/kg/month (aOR 1.15, 95% CI 1.04-1.28).
			• Audiometric HL by PTA	147/437 (34%)				
			• Clinician-identified HL	123/437 (28%)				
Modongo et al.[34] 2015 (Botswana)	Retrospective cohort (6) N=28 Adult [mean(SD)= 44y(18)]	To identify clinical factors, including amikacin concentration thresholds that predicted audiometry-confirmed ototoxicity among MDR pulmonary TB patients	• Composite HL (audiometric + clinician-identified HL)	11/28 (39%)	12/28 (43%)	12/12 (100%)	AMK(100)	• A 10% probability of ototoxicity occurred with a threshold cumulative AUC of 87,232 days·mg·h/liter, while that of 20% occurred at 120,000 days·mg·h/liter.
			• Audiometric HL by PTA	7/28 (25%)				
Sagwa et al.[35] 2015 (Namibia)	Retrospective cohort (7) N=353 No age restriction	To compare the cumulative incidence of hearing loss among patients treated for MDR-TB with amikacin or kanamycin-based	• Audiometric HL by PTA	206/353 (58%)	164/353 (46%)	132/164 (80%)	AMK(14), KM(86)	• Patients received Am had a higher risk of developing more severe HL than those used Km

	[mean (SD)= 35.69y (9.56) in Am; 36.47y (11.57) in Km group]	regimens, and to identify the most-at-risk patients, based on the real-life clinical practice experiences						(aOR= 4.0, 95% CI 1.5-10.8). • HIV coinfection (OR= 3.4, 95% CI 1.1-10.6), male sex (OR= 4.5, 95% CI 1.5-13.4) and lower baseline body weight (40-59 kg, OR= 2.8, 95% CI 1.1-6.8) were associated with increased risk of HL.
Kelly et al.[24] 2016 (South Africa)	Retrospective cohort + cross-sectional (5) N=121 Adults [range=17-63y]	To describe concordance between patient report and clinician documentation of ADR from MDR-TB treatment	• Self-reported HL	39/121 (32%)	90/121 (74%)	79/90 (88%)	N/S	• Among ADRs from MDR-TB treatment, the highest degree of concordance was found between patient-reported and audiometric HL (kappa= 0.23).
			• Audiometric HL by PTA	32/121 (26%)				

Abbreviations: NOS=Newcastle-Ottawa Quality Assessment Scale; HIV=human immunodeficiency virus; ART=antiretroviral therapy; AG=aminoglycoside; MDR-TB=multidrug-resistant tuberculosis; PTA=pure tone audiometry; AMK=amikacin; KM=kanamycin; SM=streptomycin; CPM=capreomycin; IQR=interquartile range; DPOAE=distortion product otoacoustic emissions; AE=adverse effect; DR-TB=drug-resistant tuberculosis; aOR=adjusted OR; CI=confidence interval; SD=standard deviation; AUC=area under the curve; OR=odds ratio; ADR=adverse drug reaction.

Figure 1. Effect of HIV Coinfection on Risk of AG-Induced Hearing Loss

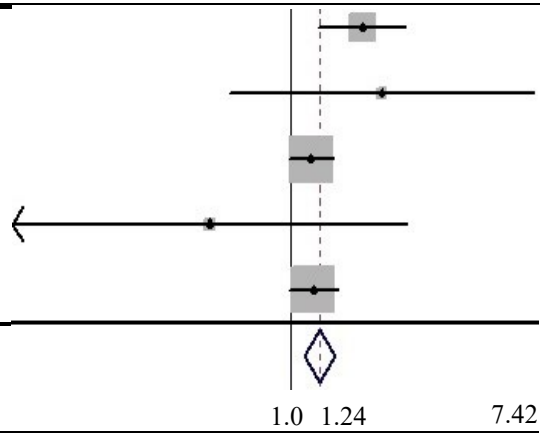
Author	Year	Country	N	AG Type	RR	95% CI		Weight (%)
Harris et al.[13]	2012	South Africa	151	AMK, KM, SM, CPM	1.73	1.25	2.39	11.01
Seddon et al.[30]	2012	South Africa	93	AMK, SM, CPM	1.50	0.84	2.68	3.05
Brust et al.[31]	2013	South Africa	91	KM	0.83	0.42	1.67	3.63
Sagwa et al.[32]	2013	Namibia	57	AMK, KM, SM, CPM	1.34	0.50	3.61	1.97
Modongo et al.[33]	2014	Botswana	437	AMK	1.13	0.96	1.33	40.64
Modongo et al.[34]	2015	Botswana	28	AMK	0.76	0.29	2.02	2.18
Sagwa et al.[35]	2015	Namibia	342	AMK, KM	1.18	0.99	1.40	33.75
Kelly et al.[24]	2016	South Africa	121	N/S	1.58	0.78	3.20	3.78
Mantel-Haenszel pooled RR					1.22	1.10	1.36	100.00



Abbreviations: HIV=human immunodeficiency virus; N=sample size; AG=aminoglycoside; RR=relative risk; CI=confidence interval; AMK=amikacin; KM=kanamycin; SM=streptomycin; CPM=capreomycin; N/S= Not specified

Figure 2. Effect of HIV Coinfection on Risk of AG-Induced Hearing Loss Confirmed by Audiometry

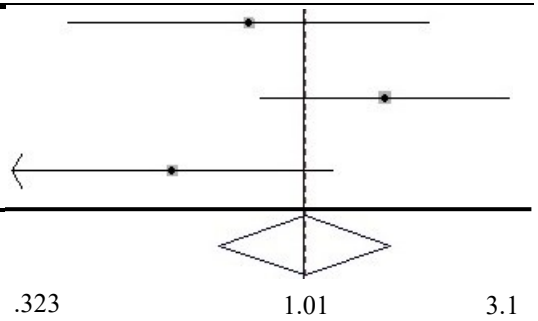
Author	Year	Country	N	AG Type	RR	95% CI		Weight (%)
Harris et al.[13]	2012	South Africa	151	AMK, KM, SM, CPM	1.68	1.22	2.31	13.49
Seddon et al.[30]	2012	South Africa	93	AMK, SM, CPM	1.94	0.64	5.82	1.58
Modongo et al.[33]	2014	Botswana	437	AMK	1.16	0.99	1.36	42.30
Modongo et al.[34]	2015	Botswana	28	AMK	0.56	0.14	2.33	1.83
Sagwa et al.[35]	2015	Namibia	342	AMK, KM	1.18	0.99	1.40	40.80
Mantel-Haenszel pooled RR					1.24	1.11	1.38	100.00



Abbreviations: HIV=human immunodeficiency; virus; N=sample size; AG=aminoglycoside; RR=relative risk; CI=confidence interval; AMK=amikacin; KM=kanamycin; SM=streptomycin; CPM=capreomycin.

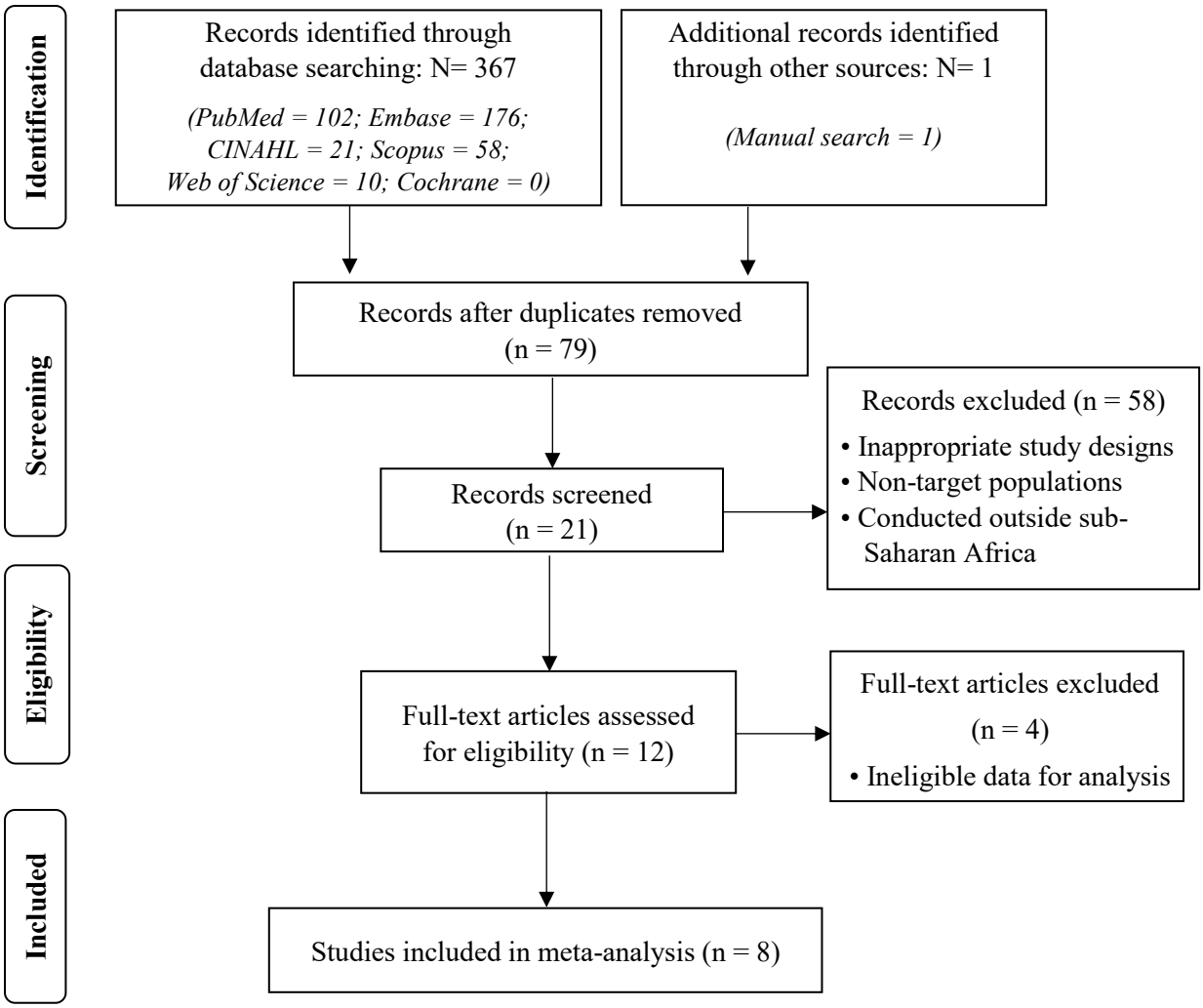
Figure 3. Effect of ART status on Risk of AG-induced Hearing Loss

Author	Year	Country	N	AG type	RR	95% CI		Weight (%)	Forest Plot
Brust et al.[31]	2013	South Africa	91	KM	0.81	0.40	1.64	26.74	
Modongo et al.[33]	2014	Botswana	286	AMK	1.38	0.85	2.23	45.03	
Kelly et al.[24]	2016	South Africa	90	N/S	0.60	0.32	1.13	28.23	
Mantel-Haenszel pooled RR					1.01	0.72	1.41	100.00	

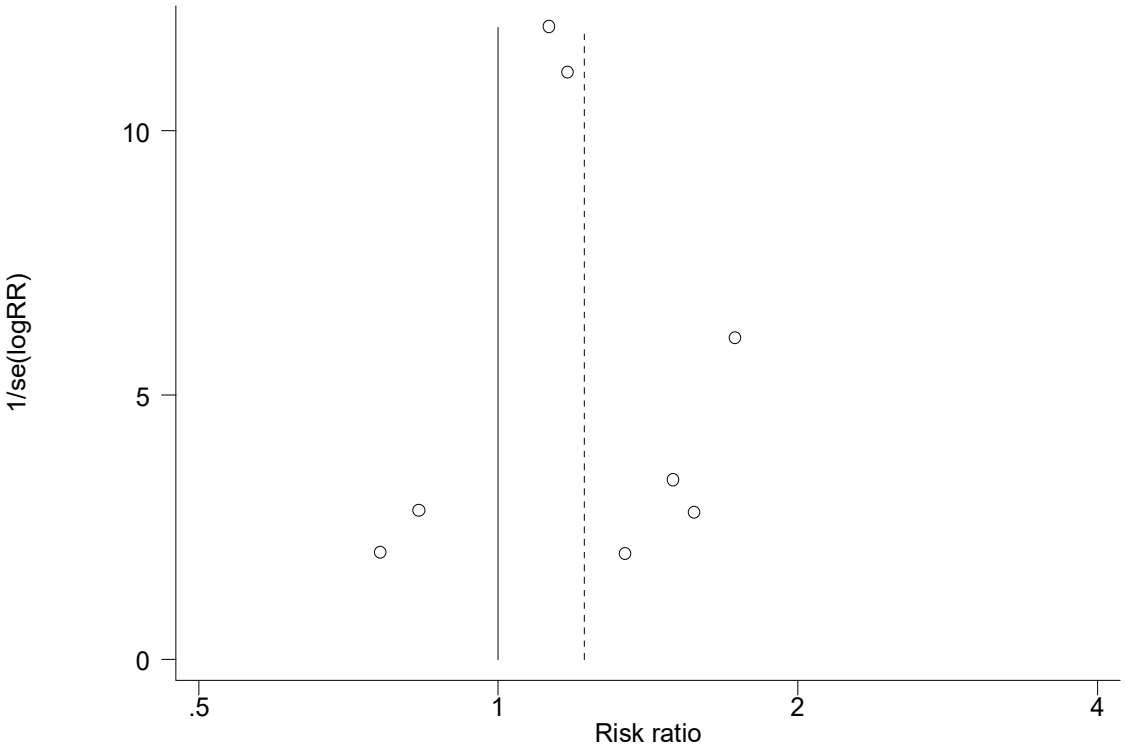


Abbreviations: ART=antiretroviral therapy; N=sample size; AG=aminoglycoside; RR=relative risk; CI=confidence interval; KM=kanamycin; AMK=amikacin; N/S= Not specified.

Appendix Figure A. 1. PRISMA Flow Diagram for Data Selection



Appendix Figure A. 2. Funnel Plot for Publication Bias



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The first author conducted the study and led study design, data collection, data interpretation, article preparation, article review and correspondence as well as contributed to statistical analysis. The second author led statistical analysis and contributed to data collection, statistical analysis, data interpretation, and article review. The last author contributed to article preparation, data interpretation, and review. The authors declared no conflict of interest.

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CHAPTER 5

Hazard of Hearing Loss among Drug-Resistant TB Patients According to Cumulative Aminoglycoside Exposure

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Running head: Survival analysis of AG ototoxicity

Keywords: survival analysis, cumulative hazard, ototoxicity

ABSTRACT

Setting: Aminoglycosides (AGs) are a mainstay of multidrug-resistant tuberculosis (MDR-TB) treatment; however, the ototoxic effects of AGs that lead to permanent hearing loss are a growing concern in MDR-TB treatment. Since AG ototoxicity is dose-dependent, the impact of a surrogate measure of AG concentration on AG-induced hearing loss warrants close attention.

Objective: To explore the prognostic impact of cumulative AG exposure on AG-induced hearing loss in patients following initiation of AG-containing multidrug therapy for MDR-TB.

Design: This prospective cohort study is nested within an ongoing cluster-randomized trial of nurse case management intervention across 10 MDR-TB hospitals in South Africa. The data for this study were collected from November 2014 to June 2017.

Results: The adjusted hazard of AG regimen modification that resulted from ototoxicity among the high-exposure group (≥75mg/kg/week) was 1.33 times higher than the low-exposure group (<75 mg/kg/week; p=.006). The adjusted hazard of developing audiometric hearing loss was 1.34 times higher than the low-exposure group (p=.038). Pre-existing hearing loss (aHR=1.71, p<.001) and age (aHR=1.02, p=.031) were also associated with an increased hazard of hearing loss.

Conclusion: MDR-TB patients with high AG exposure with advanced age and pre-existing hearing loss have a significantly higher hazard of AG-induced hearing loss. Those at high risk need to receive more frequent monitoring of hearing loss or an AG-sparing TB regimen.

INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is mycobacterium TB resistant to both first-line anti-TB drugs (isoniazid and rifampicin) and is treated with at least 4-6 months of injectable aminoglycoside (AG), such as kanamycin or amikacin.[1] During the first 4-6 months of treatment, a large proportion of MDR-TB patients develop permanent hearing loss due to irreversible apoptotic hair cell damage in the cochlea.[2,3] AG-induced hearing loss begins with high-frequency hearing loss with or without tinnitus prior to presentation of hearing loss in audible lower frequencies.[4,5] AG ototoxicity may cause early AG regimen modification (i.e., reduced or discontinued), leading to failed or delayed TB culture conversion due to attenuated bactericidal efficacy of AG, particularly in resource-limited settings without a substitute for AG.

Despite the known risk of AG-induced hearing loss, selections and availability of less ototoxic antibiotics or intensive monitoring of hearing loss are constrained because of limited public health resources in South Africa. The following factors worsen a patient's potential risk for AG-induced hearing loss during MDR-TB treatment: excessive AG concentration, pre-existing hearing loss, renal impairment, coinfection with HIV, severe systemic inflammation, malnutrition, advanced age, and several demographic factors. Among these risk factors, cumulative AG exposure needs to receive more attention because ototoxicity is dose-dependent.[6] Maintaining therapeutic level of AG concentration aids in hearing loss prevention and cure of MDR-TB, but frequent blood testing for drug concentration is not feasible in South Africa. Thus, we hypothesized that patients with high cumulative (or weekly) AG exposure—served as a surrogate measure of longitudinal AG concentrations—would have a shorter time to developing hearing loss

than those with lower cumulative exposure. The purpose of this study was to explore the prognostic impact of cumulative AG exposure on AG-induced hearing loss in patients following initiation of AG-containing multidrug therapy for MDR-TB.

METHODS

Study Design and Setting

This prospective cohort study was nested within an ongoing cluster randomized clinical trial of a nurse case management (NCM) intervention to improve MDR-TB treatment outcomes in Eastern Cape and KwaZulu-Natal provinces of South Africa. The parent study enrolled participants in 10 public TB hospitals chosen because they followed national MDR-TB treatment guidelines and had access to HIV treatment on-site. From a pilot study, these hospitals were randomized by location and size to a nurse case management intervention.[7] Full details regarding the parent study have been reported (NCT02129244). Participants in the parent study included individuals 13 years of age and older receiving care at the study sites and willing to participate by providing informed consent. Participants were excluded from the parent study if they started MDR-TB treatment at a different facility or were enrolled in another clinical trial that impacted their HIV or MDR-TB treatment regimen.

Standard of Care of DR-TB

According to the South African National Department of Health guidelines, the standard MDR-TB regimen consists of at least 4-6 months of intensive phase treatment (aka injectable phase) with one intramuscular injectable AG (e.g., kanamycin or

amikacin) and at least four oral antimycobacterials (e.g., moxifloxacin, ethionamide, terizidone, and pyrazinamide with or without being further strengthened with high-dose isoniazid and/or ethambutol).[1,8] The initial AG dose is based on the patients' baseline weight—15mg x weight (kg)—and on the weight band-dosing table guides selection of dose (mL) in practice.[8] The frequency of AG dosing varied from one to seven times a week and is determined by physicians' clinical judgement, based on patients' pre-existing conditions at the baseline evaluation. The clinical and laboratory evaluations are conducted at baseline and every month during the intensive phase.

Prospective Cohort Sample

For this prospective cohort, we included participants enrolled in the parent study from November 2014 to June 2017 and of all ages (13 years and older) because adolescents are also at risk for hearing loss from AGs.[9-12] We excluded patients with the following conditions: (1) those receiving neither intramuscular kanamycin nor amikacin injection, (2) participants who were finally confirmed to have drug-sensitive or extensively drug-resistant TB from the baseline drug sensitivity tests that resulted during the intensive phase, and (3) those who transferred to another TB facility during the intensive phase.

Study Procedures and Measures

The following clinical parameters were abstracted from the parent study: TB diagnostic results (i.e., smear, cartridge-based Xpert®, line probe assay, sputum culture, and drug sensitivity test), medical history that included previous TB history and HIV

status, TB treatment regimen and ART (if applicable), weight, height, audiological findings, chest X-ray to confirm cavitary disease, serum creatinine, adverse drug reactions, and treatment adherence.[8] Data for the parent study were mainly collected by NCMs at 5 intervention sites or by research assistants (RAs) at 5 control sites. On the day of admission to the MDR-TB treatment program, patients were interviewed for sociodemographic data, medical history, and self-reported symptoms. Then other data were also collected through medical chart review and the National Health Laboratory System (NHLS) online laboratory portal. All sites record weekly data from baseline to the end of the intensive phase of MDR-TB treatment, including DR-TB medication changes and audiological findings based on chart review and patient interviews. RAs at the control sites collected baseline data from the medical records, NHLS online portal, and baseline patient interviews.

Since the parent study was not designed to inquire about hearing loss from DR-TB treatment, several study variables were additionally collected by the first author. The first author collected albumin levels from NHLS because baseline serum albumin results were available as part of the routine laboratory test for all MDR-TB patients. Also, audiograms were captured by medical chart review to achieve specific audiological data at each frequency to define study outcomes of hearing loss. Hearing threshold—the lowest intensity of sound in decibels (dB) that the person can hear—was tested at baseline, monthly, and whenever the patient's hearing condition worsens as well by an audiometer in a standard audio booth or by KUDUwave® (a computer-based portable audiometer)[13] at frequencies ranging from 250 to 8,000 Hz.[14] Then, the hearing threshold

was categorized as degree of hearing loss to define having outcome (See Table 1 for detailed description of study variables and degree of hearing loss categories).

The present study defined *pre-existing composite hearing loss as*: (1) a hearing threshold outside of the normal range between -10 and 25dB in one or both ears at any frequency in the range from 250 to 8,000 Hz, tested by baseline audiometry (i.e., *pre-existing audiometric hearing loss*); or (2) self-reported auditory symptoms including tinnitus or hearing loss. The outcomes of AG-induced hearing loss were further defined as: (1) *clinically-determined hearing loss* resulting in a change in treatment (i.e., reduced or stopped AG) due to ototoxicity confirmed by either audiological evaluation or self-reported symptoms of hearing loss or tinnitus; or (2) *audiometric hearing loss* defined as a deterioration of at least one category of hearing loss compared to baseline hearing in the same range of frequencies in one or both ears. The proxy measure of cumulative AG exposure following treatment initiation was calculated as: (1) *weekly AG exposure* = prescribed daily AG dose (mg) x frequency of dosing over week (times per week), which was categorized into high- (5000+ mg/week), medium- (3000-4999 mg/week) and low-AG exposure groups (< 3000 mg/week); and (2) *standardized weekly AG exposure* =

$$\frac{\text{prescribed daily AG dose (mg) x frequency of dosing over week (times per week)}}{\text{weight (kg)}}$$

which was dichotomized by high- (≥75 mg/kg/week) and low-exposure (<75 mg/kg/week).

Ethical Approval

The parent study was approved by the Provincial Health Research Committee of the Eastern Cape and KwaZulu-Natal Provincial Departments of Health in South Africa.

The parent study and this sub-study were both approved by the Biomedical Research and Ethics Committee of the University of KwaZulu-Natal in South Africa and the Institutional Review Board of the Johns Hopkins Medical Institutions (NA_00078899 / CIR00024657).

Statistical Analysis

Statistical analysis included descriptive and correlational statistics to explore the prevalence of hearing loss and risk factors and their associations. Initially, bivariate analysis was conducted to examine the impact of cumulative AG exposure on time to developing AG-induced hearing loss and estimate effect size. Bivariate analysis was also conducted on potential confounders including age, AG type, pre-existing hearing loss, CD4 count, eGFR, weight, NCM intervention group assignment, and other demographic factors to assess their relationship with AG-induced hearing loss development. Next, statistically and clinically significant variables were entered into a multivariable model to account for the multicollinearity between confounders and to understand the unique contribution of each risk factor to hearing loss. A Cox proportional-hazard model was also used to explore hazard ratios for the time to developing AG-induced hearing loss adjusting for renal function, age, and pre-existing hearing loss as a primary predictor as well as other covariates significant with a p-value <0.05 in bivariate analysis. We separated our sample as two cohorts based on audiogram availability and outcome of interest. In the clinically-determined hearing loss cohort, using all participants' data regardless of the availability of audiogram, we tested whether weekly AG exposure impacted time to AG regimen modification either discontinued or reduced resulted from

ototoxicity based on providers' clinical evaluation. The subset of the audiometric hearing loss cohort was used to evaluate whether standardized weekly AG exposure impacted time to developing audiometry-confirmed hearing loss.

RESULTS

Of the 1,279 participants enrolled in the parent study, 936 were eligible for the present study and assessed for time to developing clinically-determined hearing loss (See Figure 1). Mean age was 36.15 (SD=11.04) years; 54% were male; 75% were coinfected with HIV (n= 697); and 62% (n=432) had known exposure to anti-retroviral therapy (ART) at baseline. Of the 602 patients with a baseline CD4 count available (median=188 cells/mm^3), over 53% of patients had a CD4 count below 200 cells/mm^3 and 18% had a CD4 count of 50 cells/mm^3 or lower.

Most of the sample (63%) had a normal eGFR (>90 ml/min/1.73m^2); 7% had renal impairment (eGFR < 60 ml/min/1.73m^2); 32% (n=297) were underweight (BMI less than 18.5 kg/m^2); 59% (n=551) had baseline hypoalbuminemia (serum albumin < 35 g/L); 15% (n= 142) reported auditory symptoms either tinnitus or hearing loss at baseline. Among those who were tested for audiometric hearing loss (n= 481) by either audio booth (n= 238) or portable KUDUwave (n=243), 60% (n=289) had at least mild hearing loss (≥ 26dB) at any frequencies from 250 to 8,000Hz. We found that those who had auditory symptoms were more likely to have audiometry-confirmed hearing loss at baseline at any frequencies (χ^2 [1]=14.69, p <0.001). About 35% of participants received highly standardized weekly AG exposure (i.e., ≥75 mg/kg/week, n=330). Kanamycin was the most common choice of AG since nine hospital sites offered kanamycin whereas only

one hospital site offered amikacin, and thereby the majority received kanamycin (90%, n=847).

Of 936 participants, 40% (n=379) were tested for baseline and follow-up audiometric hearing loss and were thereby eligible to be assessed for time to developing audiometric hearing loss (See Table 2 for participant characteristics in each model development and validation cohort).

Clinically-Determined Hearing Loss

Initial AG regimens (n=420) was modified at least once in 44% of participants due to clinically-determined hearing loss during the 6 months of the injectable phase. After adjusting for age, eGFR, pre-existing composite hearing loss, AG type, and NCM intervention assignment, the hazard of AG regimen modification due to ototoxicity among the high-exposure group (≥75mg/kg/week) was 1.33 times as high as among the low exposure group (<75mg/kg/week, p=.006) (See Table 3 and Figure 2).

Further, we tested the healthcare providers' tendency of AG regimen modification when they first identified clinical manifestation of AG ototoxicity. The two most common choices of regimen modification were reducing AG frequency (41%) and stopping AG (40%). In the lowest AG exposure group (<3000 mg/week), in particular, providers tended to stop AG when ototoxicity was observed (62%); they tended to reduce the frequency of AG in the intermediate (3000-4999 mg/week) and highest (5000+ mg/week) AG exposure groups (52% and 42%), respectively ($\chi^2[6]=49.48$, $p < .001$).

Audiometric Hearing Loss

Of 379 subjects who were tested with audiometry, 62.8% developed any level of hearing loss during the first 6-months of the intensive phase. In the final model—after adjusting for age, eGFR, pre-existing audiometric hearing loss, type of audiometer, AG type, and NCM intervention assignment—patients with high AG exposure (≥75mg/week) had 1.34 times higher adjusted hazard of hearing loss than those with low exposure (p=.038). Also, this model estimated that patients with baseline audiometric hearing loss had 1.71 times higher adjusted hazard of hearing loss than those with normal hearing (p <.001), and 1 year increased age was associated with 1.02 times increased adjusted hazard of hearing loss (p=.031) after all other factors held constant (see Table 3 and Figure 3). Last, audiometric hearing loss was more common among those who received kanamycin than amikacin, 58% and 5%, respectively ($\chi^2[1]=5.33$, p=.021).

DISCUSSION

This study found that the initial AG dosage is one of the key elements influencing the risk of AG-induced hearing loss and AG regimen modification during the DR-TB treatment intensive phase. According to the South African National Department of Health guidelines, the standard MDR-TB long-course regimen consists of one intramuscular injectable AG with an average dose of 15mg/kg and a maximum frequency of 5 times per week for at least 6 months.[8] That means that, if a patient received more than 75mg/kg/week ($= \frac{15mg\ x\ weight\ (kg) x\ 5\ times\ per\ week}{weight\ (kg)}$) of AG, he/she received AG more than the average dosing suggested by the guidelines. Those who were exposed to AG more than 75mg/kg/week were at higher risk of AG-induced hearing loss, and that

thereby the risk of AG regimen reduction or discontinuation is higher than those exposed to AG less than 75mg/kg/week. The short-course regiment includes one intramuscular injectable AG with an average dose of 15mg/kg and a maximum frequency of 7 times per week for at least 4 months, but because the short-course regimen initiated after July 2017 in South Africa, no patients have received AG frequency more than 5 times per week in this sample.

In addition, when providers identified audiology-confirmed hearing loss or patients' auditory symptoms of AG toxicity, they tended to stop AG regimen if patients were receiving a low dosage; while they tended to reduce AG frequency rather than dose if patients were receiving medium or high dosage. Other risk factors of hearing loss, such as advanced age and pre-existing hearing loss were also significantly associated with the hazard of audiometric hearing loss and the decision of AG regimen modification. These findings highlight the importance of not only baseline screening of hearing as a routine practice, but also more frequent audiometric hearing monitoring or offering a less ototoxic regimen for elderly patients with pre-existing hearing loss to avoid severe hearing deficits.

The standardized weekly AG exposure may be considered a significant predictor of AG-induced hearing loss. Since excessive AG concentration is a known risk factor for AG ototoxicity, standardized weekly AG exposure may also be considered a proxy surrogate measure of AG concentration in resource-limited settings where therapeutic drug monitoring is impractical. Further, we expect that our findings may guide healthcare providers to develop personalized interventions to prevent AG-induced hearing loss in medically underserved settings.

There were several limitations in this study. There were significant missing data of baseline measures, which limited the power of our analysis. Particularly, missing data of audiograms, BMI, creatinine clearance, and CD4 count reflect a lack of adherence to MDR-TB treatment guidelines for ensuring that patients have baseline labs. We hypothesized that renal function at treatment initiation may directly influence the level of AG accumulation in the inner ear as AGs are excreted by glomerular filtration. However, the impact of renal function was underpowered due to low prevalence of renal failure and large missing data in this sample. We also acknowledge that as AG is a nephrotoxic agent, not only baseline but also follow-up measures of eGFR would contribute to the hazard of hearing loss. However, our analysis was not controlled for renal function as a time-varying exposure due to significant missing data of follow-up creatinine clearance. This study only explored the outcome of hearing loss up to 6-months of follow-up. While this is the time period of greatest incidence of AG-induced hearing loss, we assume that even after an intensive phase with AG discontinuation, hearing loss may progress because AG molecules accumulate rapidly in the interstitium but are eliminated slowly.[15] Thus, future studies need to follow patients' hearing beyond the intensive phase of treatment. It is also vital to support audiological rehabilitation to improve communication for those who have moderate to severe hearing loss during or after the continuous phase. There was a possibility of unmeasured confounders. As an observational study using secondary data, randomization is impossible to inquire the time to develop drug toxicity in human study. Also, the selection of study variables was also limited to those collected by the parent study. Future studies should design in larger, well-defined prospective cohorts that include regular audiological evaluation and comprehensive history-taking.

Since hearing loss is one of the most common cochlea toxicities and debilitating adverse outcomes of AGs from MDR-TB treatment, vestibular ototoxicity, can also be permanent, signs and symptoms of dizziness, ataxia, or nystagmus require more attention in future studies. Last, due to minimal use of amikacin in this sample, this study was not powered to explore the impact of type of AG on hearing loss even though AG type was included in the final model to control for potential confounding effect. Thus, special caution is needed in interpretation because it can lead to potential undercoverage bias.

In South Africa, an AG-sparing regimen, including a non-injectable anti-TB drug such as bedaquiline, is available for those at high risk for developing hearing loss. However, there are no clear guidelines for healthcare providers to screen high-risk individuals for practical and cost-effect allocation of an AG-sparing regimen. Therefore, to prevent AG ototoxicity while maximizing treatment effect, we suggest that providers consider calculating the standardized weekly AG exposure as a reasonable proxy measure of AG concentration without invasive testing.

CONCLUSION

Our analysis found significant impact of a standardized weekly AG exposure on AG-induced hearing loss. We additionally found that the presence of pre-existing hearing loss and advanced age increase the hazard of AG-induced hearing loss. Such findings may assist providers' clinical judgement of selection of MDR-TB regimens.

Table 1. Study Variables and Degree of Hearing Loss

Variable	Measurement		Type
Demographics from parent dataset			
Age	Decade intervals		Ordinal
Sex	Male or female		Binary
MDR-TB history	Treated with AG or not		Binary
Smoking	Current, former, or never		Categorical
Alcohol use	None, light, moderate, heavy		Ordinal
Poverty	Both unemployed + social grant recipient		Binary
Social grant	Recipient before MDR-TB diagnosis		Binary
Employment	Unemployed before MDR-TB diagnosis		Binary
Baseline data from parent dataset			
Pre-existing audiometric hearing loss	Audiometric threshold outside of the normal range between -10 and 25dB		Binary
Pre-existing composite hearing loss	Self-reported auditory symptoms or audiometric threshold outside of the normal range between -10 and 25dB		Binary
HIV status	Positive or negative		Binary
NRTIs	Use of any NRTIs or not		Binary
CD4 (cells/mm^3)	<200 or higher		Binary
Lung cavity	Present or not		Binary
eGFR (ml/min/1.73m^2)	<60; 60-89; or 90+		Ordinal
Weight	In kg		Continuous
BMI (kg/m^2)	<18.5; 18.5-24.9; 25+		Ordinal
Baseline data from NHLS online portal			
Serum albumin (g/L)	<35 or higher		Binary
Longitudinal data from parent dataset			
AG daily dose	mg per day		Continuous
AG frequency	Times per week		Continuous
AG adherence	Received days per week		Continuous
Outcome: Hearing loss during injectable phase			
Audiometric hearing loss	Worsened hearing threshold compared to baseline		Binary
Clinically-determined hearing loss	AG regimen change due to ototoxicity confirmed by either audiological or clinical evaluations		Binary
Degree of hearing loss			
Audiometric hearing threshold (dB)	Normal hearing: 0-25	Moderately-severe: 56-70	
	Mild loss: 26-40	Severe loss: 71-90	Ordinal
	Moderate loss: 41-55	Profound loss: 91+	

Abbreviations: AG= aminoglycoside; BMI= body mass index; CD4= cluster of differentiation 4; eGFR= estimated glomerular filtration rate; HIV= human immunodeficiency virus; MDR-TB= multidrug-resistant tuberculosis; NHLS= National Health Laboratory Service; NRTIs= nucleoside reverse transcriptase inhibitors

Table 2. Baseline Characteristics of Participants in the Model

	Clinically determined HL (n=936)	Audiometric HL (n= 379)	p-value
Sex: N (%)			.642
Male	505 (53.95)	201 (53.03)	
Female	431 (46.05)	178 (46.97)	
Age*: N (%)			.111
13-19	45 (4.81)	23 (6.07)	
20-29	241 (25.75)	99 (26.12)	
30-39	355 (37.93)	151 (39.84)	
40-49	172 (18.38)	68 (17.94)	
50+	123 (13.14)	38 (10.03)	
Smoking: N (%)			.548
Non-smoker	621 (66.42)	250 (65.96)	
Light smoker (<10 cigarettes/day)	187 (20.00)	78 (20.58)	
Heavy smoker (≥10 cigarettes/day)	83 (8.88)	32 (8.44)	
Alcohol use: N (%)			.196
Non-drinker	552 (59.04)	217 (57.26)	
Less than once per week	290 (31.02)	124 (32.72)	
More than twice per week	83 (8.88)	36 (9.50)	
Poverty: N (%)			.096
Not poor	589 (87.91)	345 (91.03)	
poor	68 (10.15)	31 (8.18)	
HIV status & CD4 count†: N (%)			.111
HIV negative	239 (25.53)	86 (22.69)	
HIV positive with CD4 200+	282 (30.13)	127 (33.51)	
HIV positive with CD4 <200	320 (34.19)	130 (34.30)	
Unknown CD4 count	95 (10.15)	36 (9.50)	
ART status among HIV-infected: N (%)	(N= 697)	(N=293)	.202
No ART at baseline	265 (38.02)	107 (36.52)	
On ART at baseline	432 (61.98)	186 (63.48)	
Previous history of DR-TB: N (%)			.485
New DR-TB	478 (51.07)	190 (50.13)	
Ever had prior TB	421 (44.98)	177 (46.70)	
Unknown	37 (3.95)	12 (3.17)	
Pre-existing composite HL‡: N (%)			<.001
Normal hearing	568 (60.68)	183 (48.28)	
Baseline hearing loss	366 (39.10)	196 (51.72)	
Unknown	2 (0.21)	0 (0.00)	
BMI§: N (%)			.383
Underweight (<18.5)	329 (35.15)	139 (36.68)	
Normal (18.5-24.9)	445 (47.54)	184 (48.55)	
Overweight or Obese (>25)	153 (16.35)	56 (14.78)	
Unknown	9 (0.96)	0 (0.00)	
Serum Albumin‖: N (%)			.115
Normal (≥35)	193 (20.62)	86 (22.69)	
Hypoalbuminemia (<35)	551 (58.87)	210 (55.41)	
Unknown	192 (20.51)	83 (21.90)	

eGFR[¶]: N (%)			.173
90+	590 (63.03)	249 (65.70)	
60-89	196 (20.94)	82 (21.4)	
<60	66 (7.05)	20 (5.28)	
Unknown	84 (8.97)	28 (7.39)	
NCM Intervention of parent study: N (%)			.988
Intervention site	430 (45.94)	205 (54.09)	
Control Site	506 (54.06)	174 (45.91)	

*Age unit=years old; [†]CD4 count unit=cells/mm^3; [‡]Pre-existing composite hearing loss defined as confirmed by either audiometry or self-reported auditory symptoms; [§]BMI unit=kg/m^2; [‖]serum albumin unit= g/L; [¶]eGFR unit=$mL/min/1.73m^2$

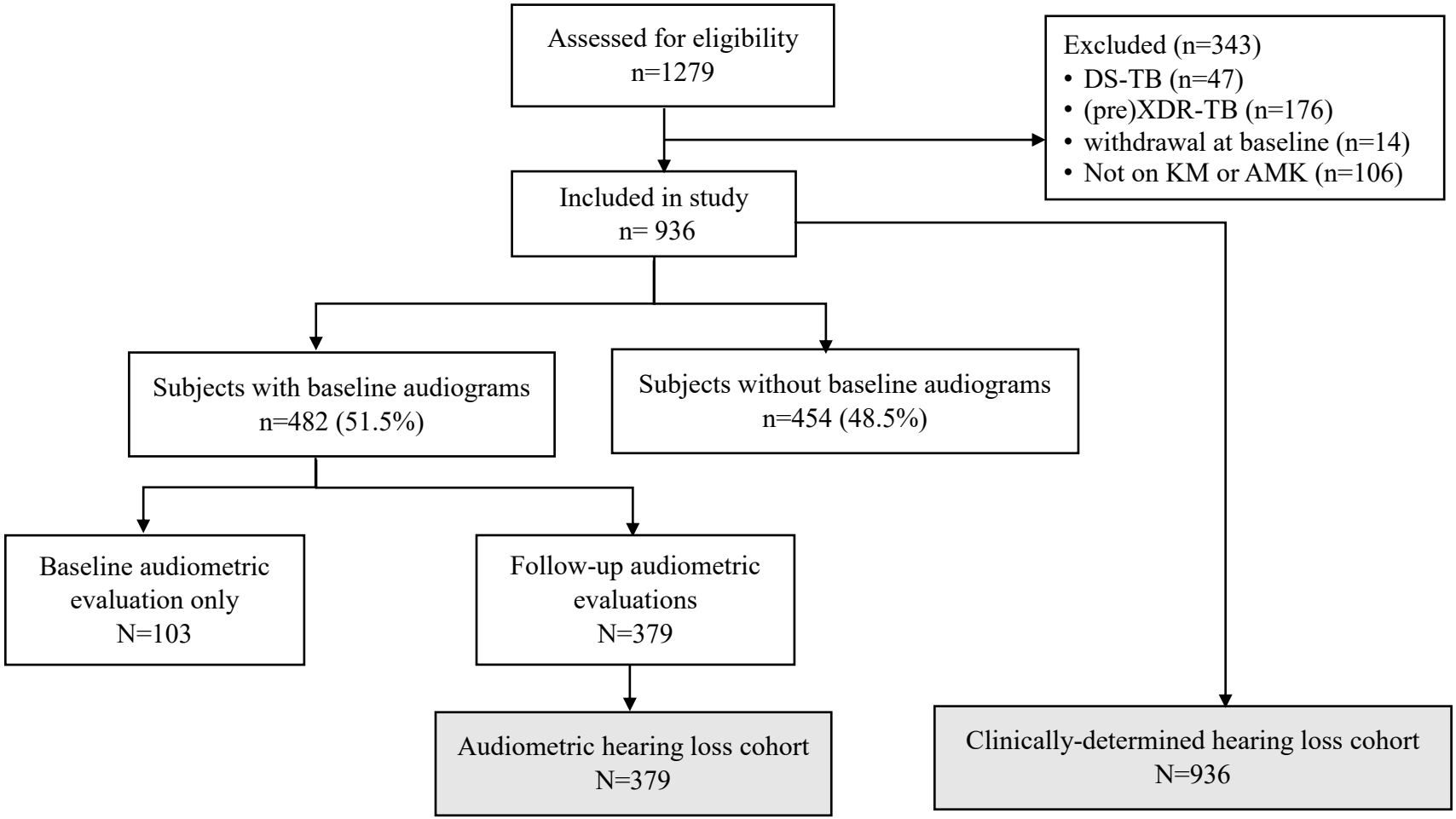
Abbreviations: ART=Anti-retroviral therapy; CD4=cluster of differentiation 4; DR-TB=drug-resistant TB; eGFR=estimated glomerular filtration rate; HIV=human immunodeficiency virus; HL=hearing loss; NCM= nurse case management

Table 3. Cox Proportional Hazard Modeling on AG-induced hearing loss

Variable	Clinically-determined HL (N=936)		Audiometric HL (N=379)	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Standardized weekly AG exposure				
<75 mg/kg/week	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
≥75 mg/kg/week	1.25 (1.03-1.53)	1.33 (1.08-1.64)	1.21 (0.94-1.57)	1.34 (1.02-1.77)
Age (years old)	1.00 (0.99-1.01)	1.00 (1.00-1.01)	1.02 (1.01-1.03)	1.02 (1.00-1.03)
eGFR ($mL/min/1.73m^2$)	1.00 (1.00-1.00)	1.00 (0.99-1.00)	1.00 (0.99-1.00)	0.99 (0.99-1.00)
Pre-existing composite HL*				
Normal hearing	1 [Reference]	1 [Reference]	-	-
Baseline HL	1.67 (1.38-2.02)	1.68 (1.38-2.07)		
Pre-existing audiometric HL				
Normal hearing			1 [Reference]	1 [Reference]
Baseline HL	-	-	1.64 (1.25-2.14)	1.71 (1.29-2.27)
Type of audiometer				
Audio booth			1 [Reference]	1 [Reference]
KUDUwave	-	-	1.21 (0.94-1.56)	1.12 (0.82-1.52)
Type of AG				
Amikacin	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
Kanamycin	2.18 (1.43-3.32)	2.95 (1.68-5.19)	1.64 (1.03-2.62)	1.31 (0.70-2.46)
NCM Intervention				
Intervention site	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
Control site	1.12 (0.92-1.35)	1.01 (0.82-1.24)	1.33 (1.03-1.72)	1.41 (1.03-1.94)

*Pre-existing composite hearing loss defined as confirmed by either audiometry or self-reported auditory symptoms. Abbreviations: AG= aminoglycoside; CI= confidence interval; HR= hazard ratio; TB= tuberculosis; eGFR= estimated glomerular filtration rate; HL= hearing loss; HR= hazard ratio; NCM= nurse case management

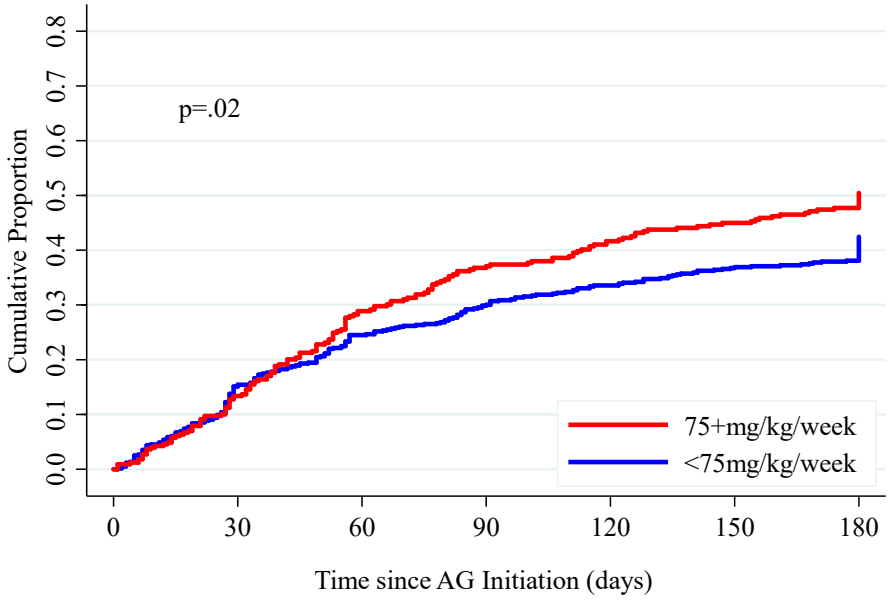
Figure 1. Diagram for Study Flow



Abbreviations: KM= kanamycin; AMK= amikacin; DS-TB= drug-sensitive tuberculosis; XDR-TB= extensively drug-resistant tuberculosis

Figure 2. Kaplan-Meier Cumulative Proportional Hazard of AG-induced Hearing Loss in Clinically-Determined Hearing Loss Cohort

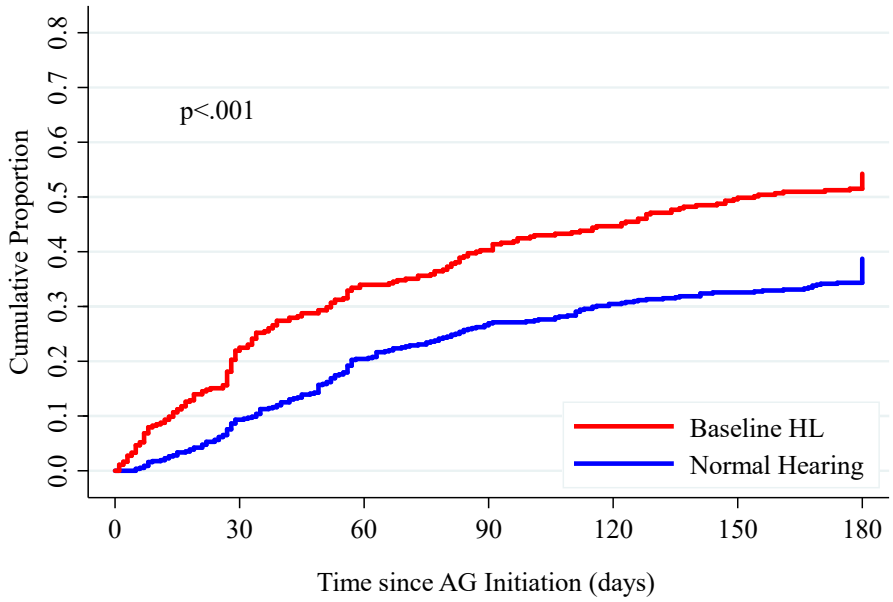
a. By standardized weekly AG exposure



No. at Risk:

<75mg/kg/week	596	(90)	506	(56)	450	(32)	418	(22)	396	(19)	377	(34)	369
75+mg/kg/week	329	(44)	285	(51)	234	(26)	208	(16)	192	(11)	181	(18)	172

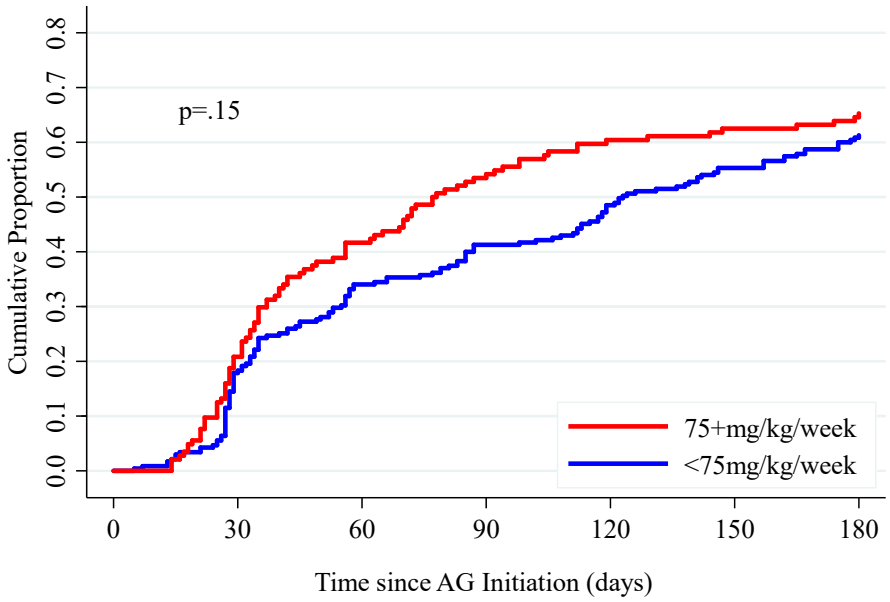
b. By pre-existing composite hearing loss



No. at Risk:													
Normal Hearing	568	(53)	515	(63)	452	(35)	417	(22)	395	(12)	383	(35)	373
Baseline HL	365	(80)	285	(44)	241	(23)	218	(16)	202	(18)	184	(17)	177

Figure 3. Kaplan-Meier Cumulative Proportional Hazard of AG-induced Hearing Loss in Audiometric Hearing Loss Cohort

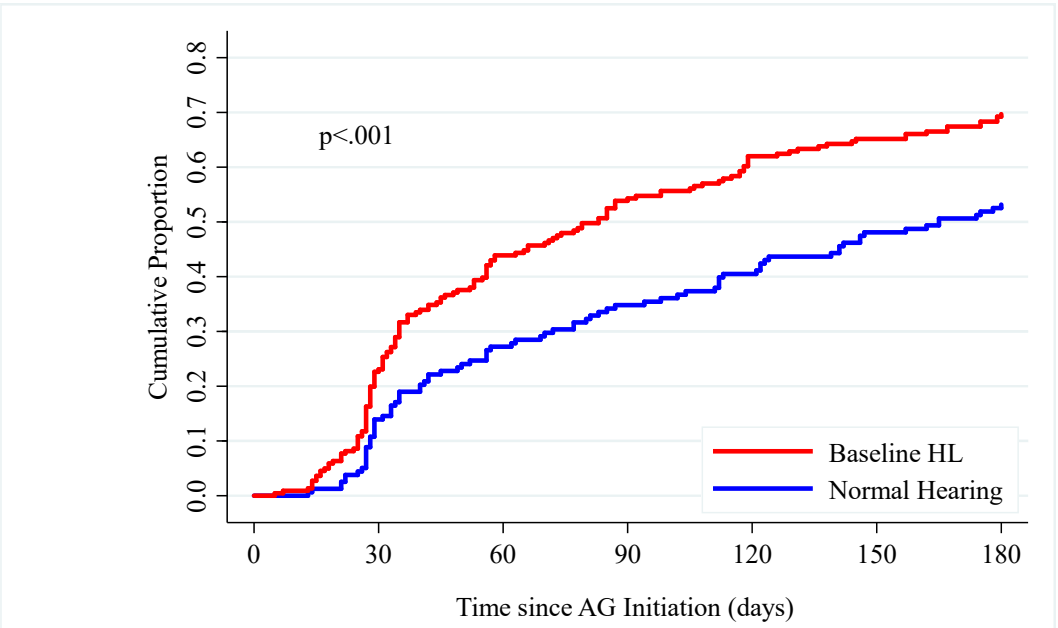
a. By standardized weekly AG exposure



No. at Risk:

<75mg/kg/week	235	(42)	193	(38)	155	(17)	138	(17)	121	(16)	105	(14)	92
75+mg/kg/week	144	(30)	114	(30)	84	(17)	67	(10)	57	(3)	54	(4)	51

b. By pre-existing audiometric hearing loss



No. at Risk:													
Normal Hearing	158	(22)	136	(21)	115	(12)	103	(9)	94	(12)	82	(8)	75
Baseline HL	221	(50)	171	(47)	124	(22)	102	(18)	84	(7)	77	(10)	68

Abbreviations: AG= aminoglycoside; HL= hearing loss

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CHAPTER 6

Predicting Aminoglycoside-Induced Hearing Loss Among Drug-Resistant TB-Infected Individuals in South Africa

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Running head: Predicting ototoxicity in drug-resistant TB

Keywords: Model Development, Model Validation, Ototoxicity

ABSTRACT

Setting: Many individuals being treated for drug-resistant tuberculosis (DR-TB) in resource-limited environments experience permanent hearing loss due to the ototoxic effect of injectable aminoglycosides (AGs). Despite these known risks, there is no practical, cost-effective means to identify those at highest risk for developing hearing loss.

Objective: To develop a prediction model of AG-induced ototoxicity among patients initiating DR-TB treatment in South Africa.

Design: This study used nested prospective cohort data. All participants older than 13 years of age with confirmed DR-TB in South Africa were included. Predictors were collected from clinical, audiological, and laboratory evaluations conducted at the initiation of DR-TB treatment. The outcome of AG-induced hearing loss was identified from audiometric and clinical evaluation by a worsened hearing threshold compared to baseline. Multiple logistic regression was used to develop a prediction model.

Results: The model predicting hearing loss at hearing frequencies from 250 to 8,000Hz included: standardized weekly AG exposure, HIV status with CD4 count, age, serum albumin, BMI, and pre-existing hearing loss. This model demonstrated reasonable discrimination (AUC= 0.715) and calibration (χ^2[8]=6.10, p=.636). The predictive property of ultrahigh-frequency hearing loss—hearing frequency higher than 9,000Hz—(AUC=0.806; χ^2[8]=6.48, p=.593) and clinically determined hearing loss (AUC=0.599; χ^2[8]=4.34, p=.825) were validated. Using a cutoff of 85% predicted the probability of hearing loss; the positive predictive value was 100% and the negative predictive value was 41%.

Conclusion: This model using readily available clinical data may add value in identifying patients with DR-TB who are at high risk of developing ototoxicity during treatment that is utilizable in clinical settings where an AG-sparing regimen is highly limited.

INTRODUCTION

Tuberculosis (TB) is now the leading cause of infectious disease-related deaths worldwide and is particularly common and lethal in HIV/AIDS-endemic areas such as South Africa.[1] A growing concern in South Africa is drug-resistant TB, defined as TB resistant to at least one of the two most powerful first-line anti-TB drugs: rifampicin and isoniazid. Drug-resistant TB (DR-TB) also includes multidrug-resistant TB (MDR-TB), which is TB resistant to both rifampicin and isoniazid, and is an extensively drug-resistant TB (XDR-TB), which is TB resistant to second-line anti-TB drugs: fluoroquinolones and injectable aminoglycosides.[2]

Unfortunately, DR-TB treatment has only a 50% success rate, is costly, and is quite toxic.[3] Hearing loss is the most debilitating adverse drug effect associated with second-line drugs used for DR-TB. Permanent hearing loss is primarily caused by an injectable aminoglycoside (AG) given during the first phase (at least 4-6 months) of DR-TB treatment. It begins with high-frequency hearing loss with or without tinnitus, can progress even with discontinuation of AG treatment, and is often permanent.[4,5] It causes social isolation, threatens quality of life, and puts employment stability and family prosperity at risk.[6-12] Although AG-induced hearing loss is a known adverse reaction that occurs in 23% to 69% of patients, AG is a mainstay of DR-TB treatment recommended by the World Health Organization (WHO).[4,5,13]

There are several risk factors that appear to aggravate AG ototoxicity. High AG plasma concentrations and frequent or prolonged dosing may increase risk; however, monitoring of drug concentrations is impossible in most resource-limited settings.[4,5] The risk of hearing loss is impacted by HIV coinfection as a result of severe immuno-

suppression along with antiretroviral therapy (ART): up to 70% of South African DR-TB patients are living with HIV.[1,13,14] Both ART and anti-TB drugs may also cause renal impairment, which hastens ototoxicity due to decreased renal excretion of AGs.[15-19] Clinical manifestations of TB such as malnutrition and severe, disseminated inflammation may be associated with increased incidence of hearing loss.[20-26] Pre-existing hearing loss, prior use of ototoxic drugs for DR-TB treatment, comorbidities, advanced age, and substance use may increase the risk for subsequent hearing loss.[27,28]

Despite these known risks, there is no cost-effective, practical means in which to translate this knowledge into the risk of hearing loss in DR-TB treatment to identify those at high risk for developing hearing loss. Thus, this study aimed to develop a prediction model of AG-induced hearing loss in DR-TB treatment in South Africa.

METHODS

Study Design and Setting

This prospective cohort study used a secondary analysis nested within an ongoing 5-year cluster-randomized trial in South Africa. The parent study investigated the effects of nurse case management (NCM) in improving treatment outcomes in individuals with DR-TB. Data were collected across 10 public TB hospitals in the Eastern Cape and KwaZulu-Natal provinces. According to Farley et al., these hospitals were randomized by location and size to a nurse case management intervention from a pilot study.[29] Full details regarding the parent study have been reported (NCT02129244).

Participants

The following patients were included: (1) all patients 13 years of age and older; (2) with microbiologically confirmed DR-TB using cartridge-based Xpert®; (3) those enrolled across 10 study sites; (4) those enrolled from November 2014 to June 2017; and (5) those who signed informed consent within seven days of treatment initiation. The following patients were excluded: (1) those receiving neither intramuscular kanamycin nor amikacin injection; and (2) those confirmed for drug-sensitive TB, XDR-TB, and pre-XDR—TB resistant to either fluoroquinolones or aminoglycosides—from baseline drug sensitivity tests that resulted during the first 6-months' injectable phase of treatment.

Predictors and Measures

The following variables were abstracted from the parent study's baseline data: (1) demographics and medical history including previous TB history, comorbidities, prescribed medications, and substance use; (2) presence of lung cavities on chest x-ray at DR-TB diagnosis; (3) serum creatinine levels to calculate estimated glomerular filtration rate (eGFR) for renal function; (4) HIV infection history including use of any ARTs and CD4 count; and (5) nutritional status measured by body mass index (BMI).

Since the parent study did not collect serum albumin levels, baseline albumin results were collected from the South African National Health Laboratory System (NHLS) online portal as a routine laboratory test for DR-TB treatment. Since no instrument exists for measuring poverty in South Africa, the conceptual definition of social deprivation[30] was used to select appropriate study variables to operationalize poverty in this study. South African social grants are given not only to the poor but also

to the elderly, the disabled, or caregivers of a child with a disability.[31] Thus, poverty was measured by a combination of social grant and employment status prior to MDR-TB diagnosis in the context of interdependency between TB and poverty in South Africa.[32,33]

The following variables were abstracted from the parent study's baseline and monthly follow-up data during the injectable phase: (1) DR-TB treatment regimen including type of AG, AG dose, frequency, and adherence; (2) DR-TB confirmation test results including sputum culture and drug sensitivity tests; and (3) auditory symptoms (i.e., hearing loss and tinnitus) and audiometric hearing evaluation results. Weekly measured regimen adherence and dosing information were used to calculate cumulative (or weekly) AG exposure per body weight (this study called *standardized weekly AG exposure*) =

$$\frac{\text{prescribed daily AG dose (mg) x frequency of dosing over week (times per week)}}{\text{weight (kg)}}.$$

Hearing was tested monthly by audiometry to establish the lowest intensity of sound (= *hearing threshold*) in decibels (dB) that the person could hear at frequencies ranging from 250 to 8,000 Hz.[28] Then, the level of hearing threshold for each frequency was transferred to *degree of hearing loss* to define outcome of hearing loss. The degree of hearing loss is categorized in Table 1.

This study defined *pre-existing composite hearing loss as*: (1) a hearing threshold outside of the normal range between -10 and 25dB in one or both ears at any frequencies in the range from 250 to 8,000 Hz, tested by either standard audio booth or computer-based portable audiometer (KUDUwave®) at baseline audiometry (aka *pre-existing audiometric hearing loss*); or (2) self-reported auditory symptoms including tinnitus or hearing loss at baseline. The outcomes of AG-induced hearing loss were further defined

as: *clinically-determined hearing loss* resulting in a change in treatment (i.e., reduced or stopped AG) due to ototoxicity confirmed by either audiological evaluation or self-reported symptoms of hearing loss or tinnitus; or (2) *audiometric hearing loss* defined as a deterioration of at least one category of hearing loss compared to baseline hearing in the same range of frequencies in one or both ears.

Ethical Approval

The parent study was approved by the Provincial Health Research Committee of the Eastern Cape and KwaZulu-Natal Provincial Departments of Health in South Africa. The parent study and this sub-study were both approved by the Biomedical Research and Ethics Committee of the University of KwaZulu-Natal in South Africa and the Institutional Review Board of the Johns Hopkins Medical Institutions (NA_00078899 / CIR00024657).

Statistical Analysis for Model Development and Validation

A multivariable logistic regression model was used to develop prediction models by introducing the following predictors: standardized weekly AG exposure, HIV status, use of ART, CD4 count, presence of lung cavities, renal impairment (eGFR), BMI, serum albumin, pre-existing composite hearing loss, age, sex, comorbidities, previous TB history, poverty, smoking, and alcohol use. Bivariate analyses using all potential variables were conducted initially to guide selection of predictors and the final model. Non-significant variables were excluded to eliminate variables that were not predictive or

highly collinear. All variables in the final model met the assumptions for proportional hazards.

Area under receiver operating characteristics curve (AUC) and Hosmer-Lemeshow χ^2 goodness-of-fit test in a multivariable logistic regression were used as the metrics to assess the model's accuracy including discrimination and calibration, respectively. The AUC and Hosmer-Lemeshow χ^2 goodness-of-fit test were repeated with the remainder sample (i.e., data not used for model development) to test predictive accuracy. All statistical tests were conducted at a significance level of 0.05 using STATA 15.[34]

RESULTS

Overall Descriptive Findings

Of the 1,279 participants enrolled in the parent study, 936 were eligible for the present study (Figure 1). Mean age was 36.19 (SD=11.05) years, 54% were male, 48.5% were unemployed before DR-TB registration, 75% were HIV coinfected, 41% had a prior history of drug-sensitive TB, and only 5% had a prior history of DR-TB infection treated with 2nd line injectable anti-TB drugs. In terms of nutritional status, 32% (n=296) were underweight (i.e., BMI less than 18.5kg/m^2) and 59% (n=551) had hypoalbuminemia (i.e., serum albumin < 35 g/L). Of 697 HIV-coinfected participants, 46% were taking ART, and 34% were severely immunosuppressed (CD4 count < 200 cells/mm^3) at baseline.

Of 936 participants, 51% (n=481) were tested for baseline hearing by either audio booth (n= 238) or portable KUDUwave (n=243); 60% (n=289) had at least mild hearing

loss (≥ 26dB) at any frequencies between 250 and 8,000Hz. Additionally, 157 of 481 participants were tested for ultrahigh-frequency hearing (i.e., hearing threshold from 9,000Hz up to 16,000Hz). Of those, 74% (n=116) had at least mild hearing loss at frequencies from 250 to 16,000Hz and 67% (n=105) had ultrahigh-frequency hearing loss ranging from 9,000 to 16,000Hz. One hundred forty-two of 936 (15%) reported auditory symptoms at baseline, and those who had auditory symptoms were more likely to have audiometry-confirmed hearing loss at baseline at any frequencies from 250 to 8,000Hz (χ^2 [1]= 14.69, p <0.001).

Among those who were tested for baseline hearing threshold (n=481), 379 were tested for follow-up audiometric evaluations during the first 6 months of injectable treatment and of those, 114 were tested for ultrahigh-frequency audiometry (i.e., for frequencies from 250 to 16,000Hz). During this follow-up period, 63% (n=238) developed any level of hearing loss at frequencies from 250 to 8,000Hz; of those, 56% (n=134) who developed audiometric hearing loss experienced AG regimen modification either discontinued or reduced AG treatment due to ototoxicity.

To develop prediction models, we selected data from 265 participants those who were tested for frequencies from 250 to 8,000Hz. The subset of 114 participants, who had been examined for ultrahigh-frequency hearing threshold, were used to validate the model's function to predict hearing loss separately at frequencies from 250 to 8,000Hz and also from 9,000 to 16,000Hz. We also validated the model using 671 participants who were not included in the model development cohort and did not undergo audiometric evaluation to test whether the developed model could predict the risk of modification of

AG regimen that resulted from ototoxicity based on clinical judgement. See Table 2 for participant characteristics in each model development and validation cohort.

Model Development and Validation

In the model development cohort of 265 participants, 62% (n=165) developed audiometric hearing loss. The selection of predictors was initially guided by statistically significant bivariate models, including the following: age (p= .006), age category by decade (p= .022), type of AG (p= .023), eGFR (p= .016), serum albumin (p= .033), previous TB history (p= .016), and pre-existing composite hearing loss (p= .012). We selected clinically significant weekly AG exposure and HIV status with CD4 counts as core predictors (AUC= 0.572; 95% CI= 0.497-0.647). Adding age, serum albumin, BMI, and pre-existing composite hearing loss (full model) led to a better prediction of hearing loss in discrimination (AUC= 0.715, 95% CI= 0.635-0.794) and calibration (χ^2 [8]= 6.10; p= 636). The final model of hearing loss with odds ratios is shown in Table 3. Using a cutoff of 85% predicted the probability of hearing loss; the positive predictive value of this model was 100%; the negative predictive value was 40.91% (See Table 4 and Figure 2-A).

In the audiometric hearing loss validation cohort (n=114), 64% (n=73) developed hearing loss at frequencies from 250 to 8,000Hz and 82% (n=93) developed hearing loss from 9,000 to 16,000Hz. We conducted validation tests at frequencies from 250 to 8,000Hz, and the developed model demonstrated comparable discrimination (AUC= 0.686, 95% CI= 0.564-0.807) and calibration (χ^2 [8]=8.03; p=0.431) (Table 4 and Figure 2-B). Further, in the ultrahigh-frequency audiometric validation cohort, the predictive

accuracy was improved in discrimination (AUC= 0.806, 95% CI= 0.689-0.923) and calibration (χ^2 [8]= 6.48; p= 0.593), signifying the models' good performance in predicting ultrahigh-frequency hearing loss at frequencies from 9,000 to 16,000Hz (Table 4 and Figure 2-B). In the clinically-determined hearing loss validation cohort, we tested predictive property as to whether the model could predict the modification of AG regimen (i.e., reduced or discounted AG regimen) due to ototoxicity as an outcome, representing reasonable discrimination (AUC= 0.599, 95% CI= 0.543-0.655) and calibration (χ^2 [8]= 4.34; p= .825). (Table 4 and Figure 2-D).

DISCUSSION

We developed and validated a simple prediction tool that can be used to estimate DR-TB patients' risk of hearing loss for the first 6 months of AG treatment. Although ototoxicity is dose-dependent, our model suggests that not only initial dosing of AG regimen but also a baseline status of malnutrition (i.e., underweight and hypoalbuminemia), immunosuppression (i.e., HIV coinfection with low CD4 count), advanced age, and pre-existing composite hearing loss were highly associated with the risk of AG-induced hearing loss. We also conducted model validation tests in different clinical settings of utilizability of audiometric evaluation because our model was purposively developed using audiometric data to test clinical standard frequencies from 250 to 8,000Hz.

The validation of our model in ultrahigh-frequency audiometric data, in particular, found that this model may also be useful to predict early manifestations of AG ototoxicity. Such findings are critically important because typical manifestations of

cochleotoxicity begin with ultrahigh-frequency hearing loss with or without tinnitus, which may not be clinically apparent, and are often undetected by standard audiometry testing frequency below 9,000Hz.[4,35] The validation in the clinically-determined hearing loss cohort demonstrated minimal predictive ability in discriminating those at higher risk in incompletion of initial AG regimen due to ototoxicity without audiometric evaluation. Such finding augments generalizability of the model to clinical sites where regular audiometry is impractical. We selected a cutoff of 85% in the development cohort with the goal of screening highest specificity, leading to highest positive predictive value. Thus, healthcare providers can triage patients whose predictive probability is higher than 85% to AG-sparing regimen, which enhances the practicality of the model in clinical sites where an AG-sparing regimen is insufficient.

This study has several limitations. A relatively small sample size was used for model development and validation. We calculated the sample size based on the ratio of the outcome event to the number of predictors, referred to as the events per variable (EPV).[36,37] The rule of thumb is that multivariable logistic models should be used with a minimum of 10-outcome EPV, while an EPV of more than 20 is ideal.[38] The final sample size for model development according to an EPV of 20 was 320 as the number of variables of the prediction model was 16; we used 265 samples to develop models. Since small sample size in developing a prediction model reduces predictive accuracy and increases variance in the validation of model performance, the implication of our models needs special caution in clinical settings. Although we acknowledge that ultrahigh-frequency is more clinically useful for early detection of ototoxicity, data for those who were tested for ultrahigh frequency were not used to develop but to validate the model

due to the small sample size. Since this was a secondary data analysis, the selection of study variables was limited to those collected by the parent study. Since the parent study was not designed to inquire about hearing loss from DR-TB treatment, other risks for hearing loss, such as noise exposure, non-sensorineural hearing loss, and family history of ototoxicity were not collected. However, the impact of these factors on baseline hearing loss was assessed with audiometry in the model development cohort. Additional studies performed in larger, well-defined prospective cohorts and that include regular audiometric evaluation and comprehensive history-taking would be useful to better validate these findings.

This study included samples from both intervention and control sites of the parent study. We acknowledge that there might be potential threats of intervention effects because NCM intervention sites may be more likely to facilitate hearing screening and modification of AG regimen since NCMs are more involved in inpatient care. Although the audiometric validation cohort consisted of more NCM intervention sites, this model was not adjusted for assignment of intervention site to maximize generalizability of the model. Since BMI and audiometry data were collected by hospital staff members who had not been trained by the parent study, measurement errors might occur equally across all sites. These programmatic measurements were used by healthcare providers to make clinical decisions including TB medication dosing, so they are clinically relevant.

Despite these limitations, this study has many strengths. This is the first study to develop a prediction model of AG-induced hearing loss among DR-TB-infected individuals. In 2016, the WHO released new treatment guidelines offering for the first time a shortened MDR-TB treatment of 9-12 months.[83] The regimen includes 7 drugs;

AGs are given at least for the first 4 months.[83] An AG-sparing regimen is reserved for those with substantial risk of hearing loss.[2] Today that risk is based solely on clinical expertise without a tested and validated measure to support those decisions. If the risk of AG-induced hearing loss can be estimated at treatment initiation, healthcare providers can triage high-risk patients to newer, less ototoxic drugs such as bedaquiline, that, while more costly, will eliminate such hearing loss. In July 2017, the South African Department of Health initiated an AG-sparing regimen that includes bedaquiline, and because these study data were collected from November 2014 to June 2017, the clinician' selection of regimen in this study is minimally influenced by bedaquiline availability. In most resource-limited countries, TB programs are suffering from financial constraints to offer an AG-sparing regimen. Thus, our prediction model may be used to avoid this unnecessary adverse event and guide clinical decision-making. Our prediction model was developed by using existing clinical data collected based upon South African national guidelines for DR-TB management. Thus, additional lab tests or clinical evaluations were not required to use the developed model. The study addresses a critical need to predict risk for developing hearing loss in a low-resource setting where therapeutic drug monitoring is not feasible. Although we expect that predicting hearing loss risk will reduce ototoxic drug use for those at highest risk and will thereby reduce hearing loss, other physio-psychological and socioeconomic factors would influence the outcome of hearing loss since each individual is unique. To use this model, providers must consider the impact of other factors unmeasured in this model on AG-induced hearing loss. Finally, cost-effectiveness of prediction model is needed to quantify the gains or setbacks

in DR-TB treatment outcomes by prioritizing the allocation of more expensive AG-sparing regimen based on the model's predictive properties.

CONCLUSION

Our model suggests that patients' initial AG dosing, nutritional status, HIV status, and pre-existing hearing loss at baseline are highly associated with AG-induced hearing loss. The findings have the potential to be very high-impact (informing treatment guidelines) and, moreover, are readily feasible, as the study used existing, quality-assured data. The findings will contribute to improve not only the management of DR-TB and severe clinical complications but also the physical and environmental influences at baseline on impaired hearing and fill key gaps in personalized interventions to prevent drug-induced hearing disability in underserved populations.

Table 1. Degree of Hearing Loss

Degree of hearing loss	ASHA Hearing loss range[39] (dB)	Hearing loss range in this study (dB)
Normal	–10 to 15	-10 to 25
(Slight)	16 to 25	
Mild	26 to 40	26 to 40
Moderate	41 to 55	41 to 55
Moderately severe	56 to 70	56 to 70
Severe	71 to 90	71 to 90
Profound	91+	91+

Abbreviation: ASHA= American Speech-Language-Hearing Association

Table 2. Baseline Characteristics of Participants in the Model

	Audiometric HL 250-8,000Hz (n= 265)	Audiometric HL 250-16,000Hz (n=114)	Clinically Determined HL (n=671)
Sex: N (%)			
Male	145 (54.72)	56 (49.12)	361 (53.80)
Female	120 (45.28)	58(50.88)	310 (46.20)
Age*: N (%)			
Mean (SD)	35.61 (10.56)	33.86 (9.39)	36.43 (11.24)
13-19	14 (5.28)	9 (7.89)	31 (4.62)
20-29	69 (26.04)	30 (26.32)	171 (25.48)
30-39	104 (39.25)	47 (41.23)	251 (37.41)
40-49	44 (16.60)	24 (21.05)	128 (19.08)
50+	34 (12.83)	4 (3.51)	90 (13.41)
Smoking: N (%)			
Non-smoker	168 (63.40)	82 (71.93)	453 (67.61)
Light smoker (<10 cigarettes/day)	58 (21.89)	20 (17.54)	129 (19.25)
Heavy smoker (≥10 cigarettes/day)	22 (8.30)	10 (8.77)	61 (9.10)
Alcohol use: N (%)			
Non-drinker	146 (55.09)	71 (62.28)	406 (60.60)
Less than once per week	92 (34.72)	32 (28.07)	198 (29.55)
More than twice per week	27 (10.19)	9 (7.89)	56 (8.36)
Poverty: N (%)			
Not poor	246 (92.83)	99 (86.84)	589 (87.91)
poor	18 (6.79)	13 (11.40)	68 (10.15)
HIV status & CD4 count†: N (%)			
HIV negative	58 (21.89)	28 (24.56)	181 (26.97)
HIV positive with CD4 ≥200	82 (30.94)	45 (39.47)	201 (29.96)
HIV positive with CD4 <200	100 (37.74)	30 (26.32)	219 (32.64)
Unknown CD4 count	25 (9.43)	11 (9.65)	70 (10.43)
ART status: N (%)	(N=207)	(N=86)	(N=490)
No ART at baseline	75 (36.23)	32 (37.21)	190 (38.78)
On ART at baseline	132 (63.77)	54 (62.79)	300 (61.22)
Previous history of DR-TB: N (%)			
New DR-TB	134 (50.57)	56 (49.12)	345 (51.42)
Ever had prior TB	121 (45.66)	56 (49.12)	299 (44.56)
Unknown	10 (3.77)	2 (1.75)	27 (4.02)
Pre-existing composite HL‡: N (%)			
Normal hearing	117 (44.15)	66 (57.89)	451 (67.21)
Baseline hearing loss	148 (55.85)	48 (42.11)	218 (32.49)
Unknown	0 (0.00)	0 (0.00)	2 (0.30)
BMI§: N (%)			
Underweight (<18.5)	109 (41.13)	27 (23.68)	187 (27.87)
Normal (18.5-24.9)	109 (41.13)	61 (53.51)	277 (41.28)
Overweight or Obese (≥25)	27 (10.19)	24 (21.05)	150 (15.80)
Unknown	20 (7.55)	2 (1.75)	101 (15.05)
Serum Albumin‖: N (%)			
Normal (≥35)	60 (22.64)	26 (22.81)	133 (19.82)
Hypoalbuminemia (<35)	135 (50.94)	75 (65.79)	416 (62.00)

Unknown	70 (26.42)	13 (11.40)	122 (18.18)
eGFR¶: N (%)			
90+	162 (61.13)	87 (76.32)	427 (63.64)
60-89	63 (23.77)	19 (16.67)	133 (19.82)
<60	16 (6.04)	4 (3.51)	51 (7.60)
Unknown	24 (9.06)	4 (3.51)	60 (8.94)
NCM Intervention: N (%)			
Intervention site	101 (38.11)	73 (64.04)	329 (49.03)
Control Site	164 (61.89)	41 (35.56)	342 (50.97)

*Age unit=years old; †CD4 count unit=cells/mm^3; ‡Pre-existing composite hearing loss defined as confirmed by either audiometry or self-reported auditory symptoms; §BMI unit=kg/m^2; lserum albumin unit= g/L; ¶eGFR unit=$mL/min/1.73m^2$

Abbreviations: ART=Anti-retroviral therapy; BMI=body mass index; CD4=cluster of differentiation 4; DR-TB=drug-resistant tuberculosis; eGFR=estimated glomerular filtration rate; HIV=human immuno-deficiency virus; HL=hearing loss; NCM=nurse case management; SD=standard deviation.

Table 3. Multivariable Logistic Regression Model Predicting Hearing Loss

Predictors	Adjusted OR (95% CI)	P Value
Age (years old)	1.04 (1.01-1.08)	.014
BMI (kg/m^2)		
<18.5	1 [Reference]	
18.5-24.9	0.38 (0.18-0.82)	.014
25+	0.28 (0.09-0.87)	.028
Standardized weekly AG exposure (mg/kg/week)		
<60	1 [Reference]	
60-74.9	0.66 (0.26-1.69)	.386
≥75	1.31 (0.52-3.33)	.569
HIV status & CD4 count (cells/mm^3)		
HIV negative	1 [Reference]	
HIV positive with CD4 200+	1.69 (0.68-4.22)	.261
HIV positive with CD4 <200	2.02 (0.82-5.01)	.127
Serum Albumin (g/L)	1.02 (0.97-1.08)	.486
Pre-existing composite hearing loss*	1.17 (0.55-2.46)	.685

Full model

log odds of hearing loss = 0.045 (age) – 0.96 (BMI: 18.5-24.9) – 1.27 (BMI: ≥25) – 0.41 (weekly AG exposure: 60-74.9) + 0.27 (weekly AG exposure: ≥75) + 0.53 (HIV+ with CD4 ≥ 200) + 0.71 (HIV+ with CD4 < 200) + 0.02 (serum albumin) + 0.15 (pre-existing composite hearing loss) – 1.61

*Pre-existing composite hearing loss defined as confirmed by either audiometry or self-reported auditory symptoms

Abbreviations: AG= aminoglycoside; BMI= body mass index; CD4= cluster of differentiation 4; CI= confidence interval; HIV= human immunodeficiency virus; OR= odds ratio

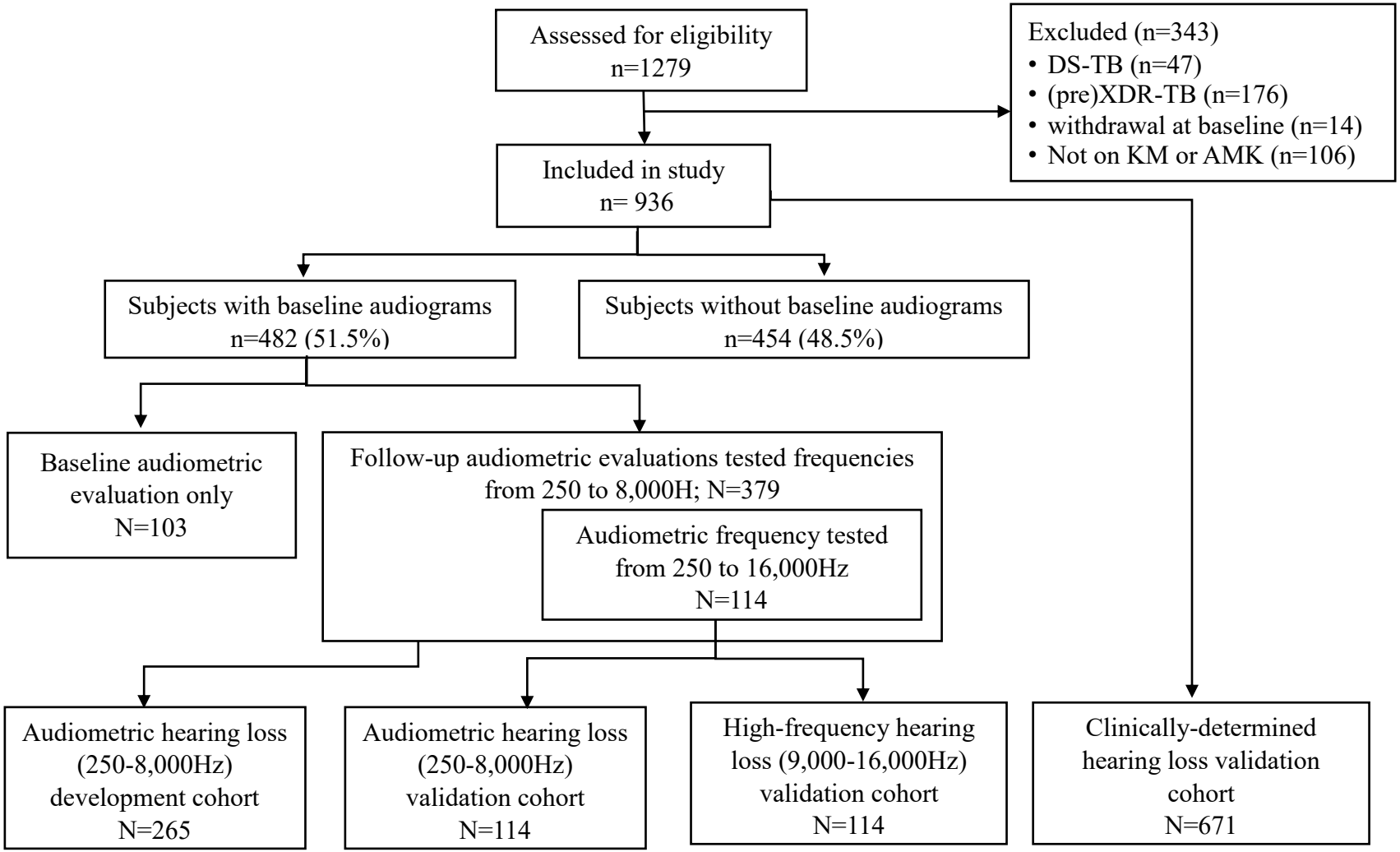
Table 4. Model Performance in Predicting AG-induced Hearing Loss

	Audiometric hearing loss in development cohort (250-8,000Hz)	Audiometric hearing loss in validation cohort (250-8,000Hz)	High-frequency hearing loss in validation cohort (9,000Hz-16,000Hz)	Clinically-determined hearing loss validation cohort
AUC (95% CI)	0.715 (0.635-0.794)	0.686 (0.564-0.807)	0.806 (0.689-0.923)	0.599 (0.543-655)
Hosmer-Lemeshow	χ^2 [8] = 6.10 (p= .636)	χ^2 [8] = 8.03 (p= .431)	χ^2 [8] = 6.48 (p= .593)	χ^2 [8] = 4.34 (p=.825)
Sensitivity*(%)	9.90	14.75	70.13	0.00
Specificity* (%)	100.00	90.63	87.50	100.00
PPV* (%)	100.00	75.00	96.43	.
NPV* (%)	40.91	35.80	37.84	59.95

*Using cutoff of 85% predictive probability.

Abbreviations: AUC=area under receiver operating characteristic curve; PPV=positive predictive value; NPV=negative predictive value.

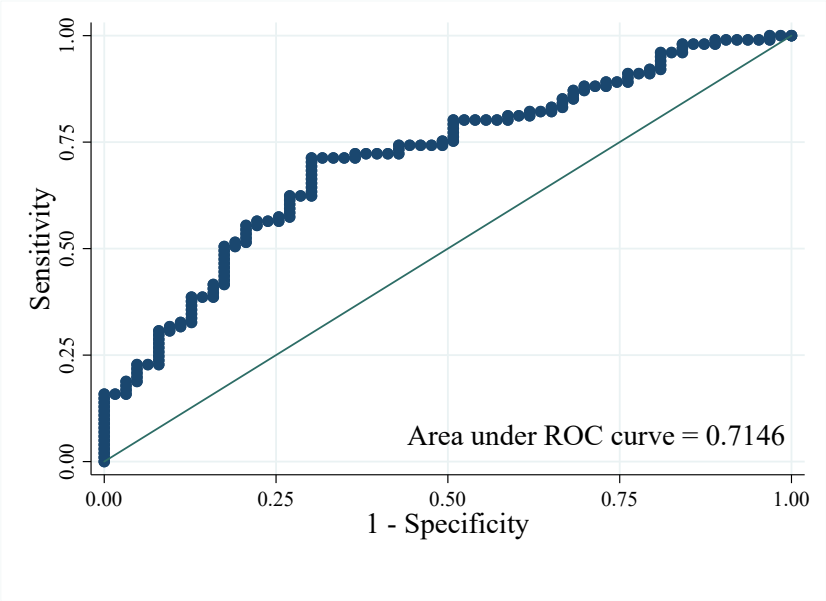
Figure 1. Diagram for Study Flow



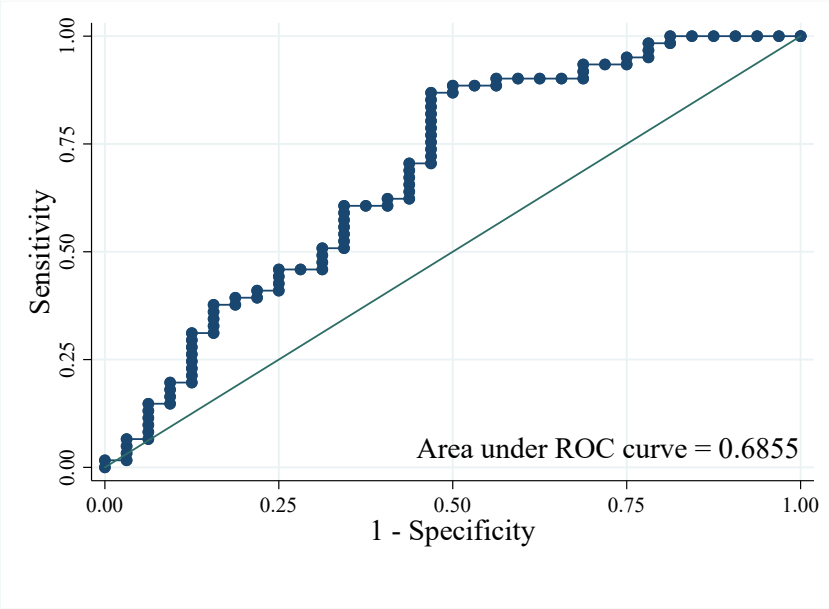
Abbreviations: KM= kanamycin; AMK= amikacin; DS-TB= drug-sensitive tuberculosis; XDR-TB= extensively drug-resistant tuberculosis

Figure 2. Receiver-Operating-Characteristic (ROC) Curves for AG-induced Hearing Loss

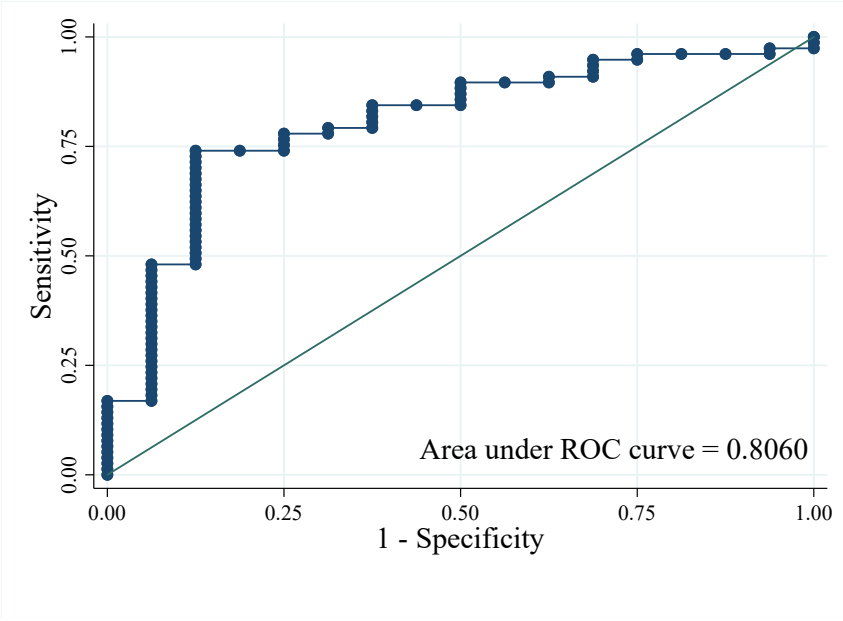
A. Audiometric hearing loss development cohort: AUC= 0.715



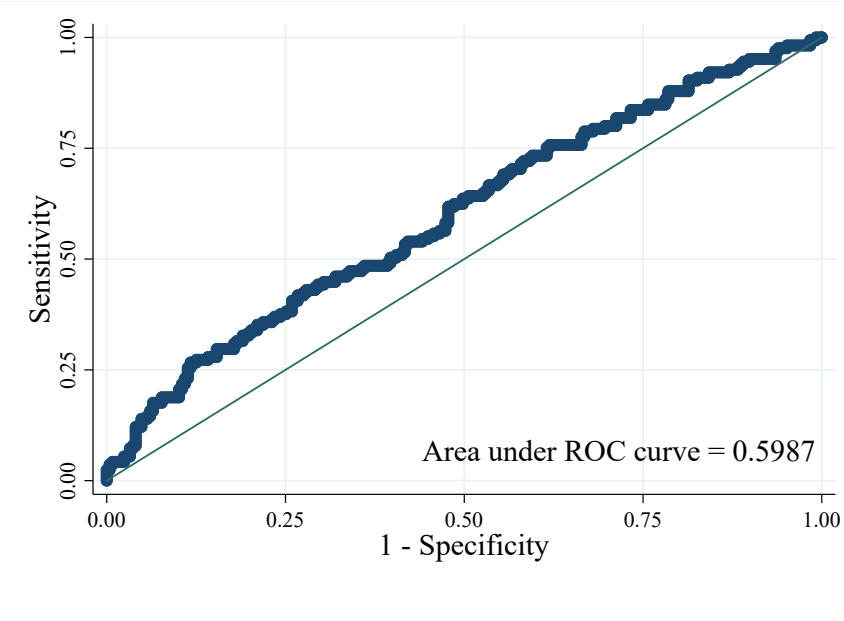
B. Audiometric hearing loss validation cohort (250-8,000Hz): AUC= 0.686



C. High-frequency hearing loss validation cohort (9,000Hz-16,000Hz): AUC= 0.806



D. Clinically-determined hearing loss validation cohort: AUC= 0.599



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CHAPTER 7: DISCUSSION

INTRODUCTION

The goal of this dissertation was to estimate the risk of AG-induced hearing loss for MDR-TB-infected individuals in the Eastern Cape and KwaZulu-Natal provinces of South Africa. To our knowledge, this study is the first to explore the impact of cumulative AG exposure as a surrogate measure of AG concentrations on the risk of AG-induced hearing loss and to develop and validate a prediction model to calculate the risk of AG-induced hearing loss at the initiation of MDR-TB treatment.

This chapter presents a summary of the results of this study presented within the framework of the two study aims. Findings are discussed for each aim of the study separately followed by the strengths and limitations, and then the implications of the study's findings are described. Finally, suggestions for future research are discussed.

The specific aims of this study were:

Aim 1: To explore the prognostic impact of cumulative AG exposure on AG-induced hearing loss in MDR-TB patients following initiation of injectable-containing multidrug therapy for MDR-TB.

Aim 2: To develop a prediction model of AG-induced hearing loss in MDR-TB treatment.

SUMMARY OF FINDINGS

Aim 1

This study found that the initial AG dosage is one of the key elements influencing the risk of AG-induced hearing loss and AG regimen modification during the DR-TB treatment intensive phase. We found that those who were exposed to AG more than

75mg/kg/week—the average dosage that the MDR-TB treatment guidelines suggested—were at higher risk of AG-induced audiometry-confirmed hearing loss, and that thereby the risk of AG regimen reduction or discontinuation was higher than those exposed to AG less than 75mg/kg/week (aHR=1.34, p=0.38). Since excessive AG concentration is a known risk factor for AG ototoxicity, standardized weekly AG exposure may also be considered a proxy surrogate measure of AG concentration in resource-limited settings where therapeutic drug monitoring is impractical. We expect that our findings may guide DR-TB providers to develop personalized interventions to prevent AG-induced hearing loss in medically underserved settings. Further, initial AG dosage was a matter of clinical judgement of regimen adjustment. In cases where AG ototoxicity was detected either by audiological evaluation or by the presence of auditory symptoms of AG toxicity, DR-TB providers tended to stop the AG regimen if patients were receiving low dosage (< 3000 mg/week), while they tended to reduce AG frequency rather than the daily dose if patients were receiving medium or higher dosage (≥ 3000mg/week). Further studies are warranted to evaluate AG concentration between reducing frequency versus a daily dose in this population to maximize therapeutic efficacy and minimize adverse effects.

Other risk factors of hearing loss, such as advanced age and pre-existing hearing loss, were also significantly associated with the hazard of audiometry-confirmed hearing loss and the decision of AG regimen modification. This finding weighted not only practical but also policy-level concerns because, as described in Chapter 3, a high prevalence of pre-existing hearing loss and potential presbycusis was found in this study population. These findings highlight the importance of not only baseline screening of hearing as a routine practice, but also more frequently repeated audiometric hearing

monitoring. Also, offering a less ototoxic regimen for elderly patients with pre-existing hearing loss should be considered to avoid severe hearing loss.

Aim 2

In this aim, we developed and validated a predictive model that can be used to calculate the probability of risk of hearing loss at clinical standard frequencies from 250 to 8,000Hz for the first 6 months of AG treatment among DR-TB patients. Although ototoxicity is dose-dependent, our model suggests that not only the initial dosage of AG regimen but also the baseline status of the following factors were highly impactful in predicting the incidence of hearing loss: malnutrition (i.e., underweight and hypoalbuminemia), immunosuppression (i.e., HIV coinfection with low CD4 count), advanced age, and pre-existing hearing loss. This model demonstrated reasonable discrimination (AUC=0.715) and calibration (χ^2[8]=6.10, p=.636). We also validated that it becomes more practical and generalizable to expand this model based on the availability of audiometric evaluation in different clinical situations. The validation with the audiometric data in ultrahigh frequencies (i.e., ≥ 9,000Hz), in particular, found clinical potentials that this model may also be useful to predict early manifestations of AG ototoxicity (AUC=0.806; χ^2[8]=6.48, p=.593). Such findings are practically meaningful because typical manifestations of cochleotoxicity begin with ultrahigh-frequency hearing loss, which may not be clinically apparent and are often undetected by standard audiometry testing frequencies below 9,000Hz.[2,3] In the clinical settings where audiometric evaluation is impossible, this model may be utilizable to discriminate those at higher risk in incompletion of the initial AG regimen due to ototoxicity without

audiometry, which was validated in the clinically-determined hearing loss cohort (AUC=0.599; χ^2 [8]=4.34, p=.825). This model represented a perfect positive predictive value (100%) at a cutoff of 85%. Thus, healthcare providers can triage patients whose predictive probability is higher than 85% to an AG-sparing regimen, enhancing the practicality of the model in clinical sites where an AG-sparing regimen is insufficient.

STRENGTHS AND LIMITATIONS

A major strength of this dissertation is that it is the first study to estimate the risk of AG-induced hearing loss for MDR-TB-infected individuals, leading to development of a clinically utilizable AG-induced hearing loss prediction model. The WHO recommends that an AG-sparing regimen is reserved for those with substantial risk of hearing loss.[4] However, there is no practical tools to screen those at highest risk for developing AG-induced hearing loss, and thereby that risk is determined by the presence of pre-existing hearing loss or providers' clinical expertise, even without audiological evaluations. If the risk of AG-induced hearing loss can be estimated at treatment initiation by using this predictive model, healthcare providers can triage high-risk patients to AG-sparing regimens. In addition, our prediction model was developed by using existing clinical data collected based upon South African national guidelines for DR-TB management. As a result, additional lab tests or clinical evaluations were not required to use this model. This prediction model would be more useful in a low-resource setting in terms of filling the gaps in personalized interventions to prevent hearing disability where AG-sparing regimens are insufficient. Further, this study suggested that standardized weekly AG exposure may be a reasonable surrogate measure of AG concentration, especially where

therapeutic drug monitoring is not feasible. Our findings suggest that DR-TB providers should closely monitor AG ototoxicity, especially for those who are receiving AG more than 75mg/kg/week because they are exposed to more than the average dosage that the MDR-TB treatment guidelines suggest.

We acknowledge that this study has several limitations. First, selection of study variables was limited to those collected by the parent study. Since the parent study was not designed to inquire about hearing loss from DR-TB treatment, other risks for hearing loss, such as noise exposure, non-sensorineural hearing loss, and a family history of ototoxicity, were not collected for this sub-study. However, the impact of these factors on pre-existing hearing loss was assessed by both audiological and clinical evaluations.

A relatively small sample size was used for prediction model development and validation. We evaluated the sample size based on the ratio of the outcome event to the number of predictors, referred to as the events per variable (EPV).[5,6] The rule of thumb is that a minimum of 10-outcome EPV should be used in multivariable logistic models, while an EPV of more than 20 is ideal.[7] The final sample size for model development according to an EPV of 15 was 240 and an EPV of 20 was 320 as the number of variables of the prediction model was 16; we used 265 samples to develop models. Since a small sample size in developing a prediction model reduces predictive accuracy and increases variance in the validation of model performance, the implication of our models needs special caution in clinical settings. Although we acknowledge that ultrahigh-frequency is more clinically useful for early detection of ototoxicity, the model was not developed with ultrahigh-frequency audiometric data due to the small sample size. Therefore,

further validation and refinement of the prediction model to estimate ultrahigh-frequency hearing loss must be considered.

This study included samples from both the NCM intervention and control sites of the parent study. We acknowledge that there might be potential threats of intervention effects because the NCM intervention sites may be more likely to facilitate hearing screening and modification of AG regimen since NCMs are more involved in inpatient care. An NCM interaction effect was found in the survival analysis because the adjusted hazard of audiometric hearing loss in NCM intervention sites was 1.41 times higher than in the control sites (p=.032). Although the audiometric validation cohort consisted of more NCM intervention sites, the prediction model was not adjusted for assignment of the intervention site to maximize generalizability of the model. Since several study variables, such as BMI and audiometry data, were collected by hospital staff members who had not been trained by the parent study, measurement errors might occur equally across all sites. These programmatic measurements were used for clinical decisions including TB medication dosage, so they are clinically relevant.

Due to significant missing data of baseline measures, the power of our analysis was limited. Particularly, missing data of audiogram, creatinine clearance (or eGFR), and CD4 count reflects a lack of adherence to MDR-TB treatment guidelines for ensuring that patients have baseline labs. We hypothesized that baseline renal function may be a significant predictor in the prediction model, but the impact of renal function was underpowered due to the low prevalence of renal failure and large missing data in this sample. For this study, missing height was imputed using the mean height for those with a height by sex and age categories. We also acknowledge that since AGs are nephrotoxic

agents, not only baseline but also follow-up measures of creatinine clearance would contribute to the risk of AG-induced hearing loss. However, our analysis was not considered renal function as a time-varying exposure due to significant missing data of follow-up creatinine clearance. Furthermore, as an observational study using secondary data, there was also the possibility of unmeasured and uncontrolled confounders because randomization is impossible to inquire the time to developing drug toxicity in human study.

Finally, this study only explored the outcome of hearing loss up to 6 months of follow-up. While this is the time period of greatest incidence of AG-induced hearing loss, hearing loss may progress even after AG discontinuation because AG molecules accumulate rapidly in the interstitium but are eliminated slowly.[8] Thus, future studies need to follow patients' hearing beyond the intensive phase of treatment.

IMPLICATIONS

There are far-reaching implications from this study for healthcare providers, policy makers, and researchers. South Africa has made tremendous strides in improving DR-TB treatment including introduction of new regimens such as 9-month short-course regimens or injection-sparing regimens. The developed AG-induced hearing loss prediction model (discussed in Chapter 6) may be useful to allocate AG-sparing regimens cost-effectively in clinical sites where an AG-sparing regimen is insufficient. Predictors in the model were selected from existing clinical data collected based upon South African national guidelines, so there is no need to conduct additional lab tests or clinical evaluations to use the developed model. Since this model represented 100% positive

predictive value at a cutoff of 85%, healthcare providers can prioritize patients whose predictive probability is higher than 85% to an AG-sparing regimen. Although we expect that predicting hearing loss risk will reduce ototoxic drug use for those at highest risk and will thereby reduce hearing loss, other physio-psychological and socioeconomic factors would influence the outcome of hearing loss since each individual is unique.

This study warrants an urgent awareness of the fact that the incidence of AG-induced hearing loss in South Africa (62.8%) is much higher than other countries, such as the United States (13%),[9] the Netherlands (18%),[10] the United Kingdom (28%),[11] and India (10–25%).[12-14] There are several possible explanations for this finding. Financial considerations may, in part, explain the higher incidence of AG-induced hearing loss in resource-limited countries than in high-resource countries. As discussed in Chapter 4, the high burden of HIV coinfection in South Africa may be one of the reasons for the significantly higher incidence of AG-induced hearing loss.[15] HIV coinfection leads to higher risk of otologic opportunistic infections, such as seborrheic dermatitis of the external ear, otitis externa with otomycosis, serous otitis media, causing acute or chronic conductive hearing loss before or during AG treatment media.[16-18] Also, because HIV-infected individuals with severe immunosuppression have increased levels of oxidative DNA damage[19] that accelerate hair cell damage, frequent systematic hearing monitoring and repeated measure of CD4 counts during the intensive phase of treatment are strongly suggested. Chapter 3 found that not only the incidence of hearing loss but also the prevalence of pre-existing hearing loss was substantially higher in this study setting as the prevalence of pre-existing audiometric hearing loss was 60%. Pre-existing hearing loss was more prevalent among those who were 50 years of age and older and had

previous AG exposure. Since conductive hearing loss was inconsistently evaluated only at several sites, pre-existing hearing loss must be screened and treated by comprehensive audiological assessment, including audiometry, tympanometry, and otoscopy.

This study suggests the need to evaluate hospital and provider adherence to national guidelines of audiometric evaluation for those are on injectable-containing regimens for DR-TB treatment. The South African Department of Health MDR-TB guidelines instruct that audiometry should be performed prior to initiation of treatment and repeated at least monthly throughout the intensive phase of treatment. However, this study (in Chapter 3) found that only 51% of study participants' baseline hearing was tested as a routine practice. What is worse is that, of those tested for baseline audiometric hearing, only 78.8% were tested for follow-up hearing at least once during the intensive phase. The availability of on-site audiologists and well-functioning audiometers at TB hospitals must be audited on a regular basis to make early detection of AG-induced hearing loss possible. Further, additional financial support is required to maintain regular audiometric evaluation a continuous phase of treatment because ototoxicity may worsen even after AG discontinuation. Also, audiological rehabilitation is needed to improve communication skills and quality of life for those who have moderate to severe hearing loss during or after the continuous phase of treatment.

It is also critical to understand that poverty and other social determinants of health play a significant role in hearing loss from DR-TB treatment. Since no instrument exists for measuring poverty in South Africa, the conceptual definition of social deprivation[20] was used to select appropriate study variables to operationalize poverty in this study. South African social grants are given not only to the poor but also to the elderly, the

disabled, or to caregivers of a child with a disability.[21] Thus, poverty was measured by a combination of social grant and employment status prior to MDR-TB diagnosis in the context of interdependency between TB and poverty in South Africa.[22,23] However, since measurement of poverty was not validated, the combination of social grant and unemployment status may not precisely calibrate patients' poverty levels in this study. Most patients with MDR-TB experience some level of poverty, so patients who already have grant access may already have social networks that connect them to this resource. Therefore, it is critical for policy makers to design government support systems that are accessible for DR-TB patients to maintain their treatment and to optimize health outcomes.

RECOMMENDATIONS FOR FUTURE RESEARCH

There is a need for external validation and refinement of the prediction model to achieve better generalizability by using a larger sample size and well-defined prospective cohorts, which include regular audiometric evaluation and comprehensive history-taking. It also is necessary to follow patients' hearing beyond the intensive phase of treatment, because hearing loss may progress with AG discontinuation even after the intensive phase. In addition, cost-effectiveness of the prediction model is needed to quantify the gains or setbacks in DR-TB treatment outcomes by prioritizing the allocation of more expensive AG-sparing regimen based on the model's predictive properties. Further, additional observational research or clinical trials are warranted to evaluate the importance of audiological rehabilitation on improving communication skills and quality

of life for those who have moderate to severe hearing loss during or after the continuous phase of TB treatment.

A comprehensive measure of nutritional status must be considered for future studies because it is a significant predictor of AG-induced hearing loss. In both the bivariate Cox regression model and the logistic model, the impact of baseline underweight (i.e., BMI < 18.5 g/m^2) was significantly associated with the risk of AG-induced hearing loss, and adding BMI and albumin level significantly improved the predictive property of the model. However, this study was limited to inquiry regarding the impact of nutritional status on AG-induced hearing loss because only BMI and serum albumin were introduced in the model. Since AG molecular concentration is influenced by body size,[24] future studies may need to include the following physiological parameters: (1) body size measured by anthropometric parameters such as body weight, BMI, arm/waist/hip/calf circumferences, and triceps/subscapular skinfold; or (2) body composition measured by bioelectrical impedance such as fat mass, fat-free mass, muscle mass, fat area, muscle area, total body water, intracellular water, and extracellular water, etc. In addition, future observational studies or clinical trials should be considered to offer nutritional supplements for malnourished patients to boost their antioxidant concentrations and immune responses.

CONCLUSION

This study found that the majority of DR-TB patients receiving AG experienced some level of hearing loss during the first 6 months of the intensive phase of treatment. Comprehensive risk analyses and systematic literature review led to the development of a

prediction model of AG-induced hearing loss, and the selection of predictors was guided by the conceptual framework of the adverse outcome pathway on AG ototoxicity in MDR-TB treatment. An AG-sparing regimen is available for those at higher risk for developing hearing loss in South Africa, but many TB hospitals in South Africa are suffering from a shortage of a substitute for AG. Since there are no clear guidelines for healthcare providers to screen high-risk individuals for practical and cost-effective allocation of AG-sparing regimens, we suggest that providers consider using our prediction model, especially in resource-limited settings. It would help to develop personalized interventions by simply calculating the probability of hearing loss of each individual and triaging high-risk patients to AG-sparing regimens at treatment initiation to avoid AG ototoxicity and maximize treatment outcomes.

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15. Hong H, Budhathoki C, Farley JE. Increased risk of aminoglycoside-induced hearing loss in MDR-TB patients with HIV coinfection. *The International Journal of Tuberculosis and Lung Disease.* 2018;22(6):667-674.

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21. Ferreira L. FACTSHEET: Social grants in South Africa – separating myth from reality. In: Check A, ed2015.
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Date Revised: 11/27/2018

CURRICULUM VITAE

Hyejeong Hong, PhD, MSN, FNP-BC

Part I

PERSONAL DATA

Johns Hopkins University School of Nursing
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Baltimore, MD 21205, USA
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EDUCATION

2018	Doctor of Philosophy, Johns Hopkins University, Baltimore, MD, USA (Expected)
2013	Master of Science in Nursing, University of Illinois at Chicago, Chicago, IL, USA
2006	Bachelor of Science in Nursing, Pusan National University, Busan, Republic of Korea

CURRENT LICENSE AND CERTIFICATION

2014	Certified Registered Family Nurse Practitioner, Maryland, USA; No. R213320
2014	Registered Professional Nurse, Maryland, USA; No. R213320
2013	Advanced Practice Registered Nurse, Illinois, USA; No. 209010839
2013	Family Nurse Practitioner, Board-Certified, American Nurses Credentialing Center, USA; No. 2013015194
2011	Registered Professional Nurse, Illinois, USA; No. 041395424
2008	Registered Professional Nurse, New York, USA; No. 596371
2006	Registered Professional Nurse, Republic of Korea; No. 220526

PROFESSIONAL EXPERIENCE

2015–present	Research Resident, Johns Hopkins University School of Nursing, Baltimore, MD

2016–2017	Research Assistant, Johns Hopkins University School of Nursing, Baltimore, MD
2015–2016	Research Assistant, Towson University, Towson, MD
2015	Teaching Assistant, Principles of Pharmacology, Johns Hopkins University School of Nursing, Baltimore, MD
2015	Research Assistant, Johns Hopkins Hospital Moore Clinic, Baltimore, MD
2014–present	Family Nurse Practitioner, MinuteClinic, LLC., Rockville, MD
2013–2014	Family Nurse Practitioner, Korean American Community Services, Chicago, IL
2011–2013	Research Assistant, University of Illinois at Chicago, Chicago, IL
2011	Teaching Assistant, Biostatistics, University of Illinois at Chicago, Chicago, IL
2010	Clinical Research Nurse, Busan Paik Hospital, Busan, Republic of Korea
2006–2009	Registered Nurse, Kangbuk Samsung Hospital, Seoul, Republic of Korea

HONORS AND AWARDS

2018	Dr. Scholl Foundation Dissertation Scholarship
2017–2018	F31 Ruth L. Kirschstein National Research Service Award Predoctoral Fellowship, NIH
2017	Sigma Theta Tau International Global Nursing Research Grant
2017	Sigma Theta Tau International Association of Nurses in AIDS Care Grant
2017	Global Korean Nursing Foundation Scholarship
2017	Dean's Travel Award, Center for Global Initiatives, Johns Hopkins School of Nursing
2017	International 3MT® Exhibition at the USETDA/NDLTD Symposium, Third Place
2017	Maryland State Thesis Showcase, Second Place
2017	Johns Hopkins University Three-Minute Thesis (3MT®) Competition Finalist
2016–2017	Johns Hopkins School of Nursing Graduate Research Fellowship
2015–2016	TL1 Predoctoral Clinical Research Training Program Trainee, NIH

2015	Johns Hopkins University Global Health Established Field Placement Award
2015	Johns Hopkins School of Nursing Professional Development Award
2013	Virginia M. Ohlson Scholarship
2013	Midwest Nursing Research Society Student Poster Award, First Place
2012	Academy of International Leadership Development Award
2012	Induced, Sigma Theta Tau International Honor Society, Alpha Lambda Chapter
2012	Korean American Scholarship Foundation Award
2011	Academy of International Leadership Development Award
2011–2013	Nursing Scholarship, University of Illinois at Chicago College of Nursing
2004–2006	Scholarships for Academic Achievement, Pusan National University

RESEARCH

Dissertation Research

2016−2018 Predicting Aminoglycoside-Induced Hearing Loss Among Multidrug-Resistant Tuberculosis (MDR-TB)-Infected Individuals in South Africa

The goal of this study is to develop a prediction model of aminoglycoside-induced hearing loss for MDR-TB patients in South Africa. This secondary data analysis uses a prospective cohort study design nested within an ongoing 5-year cluster-randomized trial in South Africa (R01 AI104488, PI: J. Farley).

Agency: NIH/NINR: F31 NR016910-01A1

Role: PI

Sponsored Projects

2016−2017 Implementing HIV Evidence-Based Interventions for African American Women in Church Settings

The purpose of this church-based intervention study was to test the effect of the addition of a peer education network intervention—Self-Help in Eliminating Life-Threatening Disease (SHIELD)—as an implementation strategy to promote acceptability and maintenance of Sister to Sister, an

evidence-based HIV risk-reduction intervention for African American women in church settings, and to examine the role of organizational variables on implementation.

PI: Jennifer M. Stewart, PhD, Johns Hopkins University School of Nursing

Agency: NIH/NIMH: K23 MH106378

Role: Research Assistant–Recruitment, enrollment, retention and follow-up with church readers and educators, and leading church leader training as an interventionist, as well as in manuscript preparation.

2015−2018 A Nurse Case Management Intervention to Improve MDR-TB/HIV Coinfection Outcomes

The purpose of this cluster-randomized trial is to evaluate the outcome of nurse case management on treatment outcomes for MDR-TB/HIV coinfected patients in South Africa.

PI: Jason E. Farley, PhD, Johns Hopkins University School of Nursing

Agency: NIH/NIAID: R01AI104488
Role: Research Resident- Conducting preliminary data QA/QC, data entry with cleaning/management, data analysis and manuscript preparation.

2015 Stop Community MRSA Colonization Among Patients (SUSTAIN)

This randomized controlled trial tested a household-level intervention for MRSA decolonization in people living with HIV

PI: Jason E. Farley, PhD, Johns Hopkins University School of Nursing

Agency: Robert Wood Johnson Foundation

Role: Research Assistant- Recruitment, enrollment, retention/follow-up, biospecimen collection, and preliminary data entry

2011−2013 Dispelling Cultural Myths to Promote Early Detection of Breast Cancer in African American Women

This cross-sectional study collected large-scale survey data related to cultural beliefs contributing to disparities in breast cancer among African American women to understand the beliefs and perceptions about breast cancer screening and treatment, and the barriers to initiating treatment.

PI: Carol E. Ferrans, PhD, University of Illinois at Chicago College of Nursing

Agency: The Institute for Health Research & Policy, University of Illinois at Chicago
Role: Research Assistant- Conducting preliminary data entry, data cleaning and management, and data analysis

2009−2010 Establishment of Nationwide Prospective Registration System for Amyotrophic Lateral Sclerosis (ALS)

This multicenter cross-sectional study collected large-scale survey data to identify the current status of ALS diagnosis and management in Korea and to compare the results with those from other countries.

PI: Jong Seok Bae, MD, Department of Neurology, Busan Paik Hospital, Busan, Republic of Korea

Agency: Korea Healthcare Technology Research and Development Project, Ministry for Health, Welfare, and Family Affairs, Republic of Korea [A091049].

Role: Clinical Research Nurse- Study protocol development with IRB approval, recruitment, enrollment, retention/follow-up, and primary data entry.

Unsponsored Research

2015−2016 Parenting Experience Among Mothers Defecting from North Korea

The purpose of this mixed-methods study was to explore North Korean defectors' characteristics on the range of mother role, parenting style, as well as parenting and health-related knowledge levels.

PI: Hyunjeong Park, PhD, Towson University Department of Nursing

Role: Research Assistant- Managing/analyzing quantitative data, merging both quantitative data and qualitative themes to create new or consolidated variables, and writing manuscripts.

SCHOLARSHIP

Publications *(*data-based)*

Hong, H., Budhathoki, C., and Farley, J.E. (2018). Increased risk of aminoglycoside-induced hearing loss in MDR-TB patients with HIV coinfection. *The International Journal of Tuberculosis and Lung Disease*. 22(6), 667-674. doi: 10.5588/ijtld.17.0830.

Hong, H., Budhathoki, C., and Farley, J. E. (2018). Effectiveness of macronutrient supplementation on nutritional status and HIV/AIDS progression: A systematic review and meta-analysis. *Clinical Nutrition ESPEN,* 27, 66-74. doi:https://doi.org/10.1016/j.clnesp.2018.06.007.

*Stewart, J. M., **Hong, H.**, and Powell, T. W. (2018). Strategies to Promote African-American Church Leadership Engagement in HIV Testing and Linkage to Care. J Racial Ethn Health Disparities. doi:10.1007/s40615-018-0527-5.

Han, H.-R., **Hong, H.**, Starbird, L. E., Ge, S., Ford, A. D., Renda, S., . . . Stewart, J. (2018). eHealth Literacy in People Living with HIV: Systematic Review. JMIR Public Health Surveill, 4(3), e64. doi:10.2196/publichealth.9687.

*Stewart, J.M., **Hong, H.**, and Melton, M. (2017). HIV Testing, Stigma, and Risk: A Comparison of Church Leaders and Their Congregants. *AIDS education and prevention.* 29(6), 503-515. doi: 10.1521/aeap.2017.29.6.503.

*Stewart, J.M., **Hong, H.**, and Powell, T. W. (2017). African American Church Engagement in the HIV Care Continuum. *The Journal of the Association of Nurses in AIDS Care.* 29(3), 406-416. doi: 10.1016/j.jana.2017.11.004.

Publications under review *(*data-based)*

*Farley, J.E. McKenzie-White, J., Bollinger, B., **Hong, H.,** Lowensen, K., Chang, L.W., Stamper, P., Berry, L., Olsen, F., Isherwood, L., Ndjeka, N., Stevens, W. Evaluation of the miLINC solution to shorten time from clinical presentation to treatment initiation for drug-resistant tuberculosis. Submitted to *The International Journal of Tuberculosis and Lung Disease*.

Starbird, L.E., **Hong, H.**, Farley, J.E., Sulkowski, M. Management of the patient with HIV/hepatitis C drug-drug interactions: A guide for nurses. Submitted to *The Journal for Nurse Practitioners.*

Publications in progress *(*data-based)*

Hong, H., Dooley, K.E., Starbird, L.E., Francis, H.W., Farley J.E. Adverse Outcome Pathway on Aminoglycoside Ototoxicity in Drug-Resistant Tuberculosis Treatment.

***Hong, H.**, Dowdy, D.W., Dooley, K.E., Francis, H.W., Budhathoki, C., Han, H., Farley J.E. Prevalence of pre-existing hearing loss among drug-resistant tuberculosis patients in South Africa. A cross-sectional study.

*__Hong, H.__, Dowdy, D.W., Dooley, K.E., Francis, H.W., Budhathoki, C., Han, H., Farley J.E. Hazard of hearing loss among drug-resistant TB patients according to cumulative aminoglycoside exposure. A prospective cohort study.

*__Hong, H.__, Dowdy, D.W., Dooley, K.E., Francis, H.W., Budhathoki, C., Han, H., Farley J.E. Predicting aminoglycoside-induced hearing loss among drug-resistant TB-infected individuals in South Africa. A prospective cohort study.

Presentations *(*data-based)*

International

Hong, H., Dowdy, D.W., Dooley, K.E., Budhathoki, C., Han, H., Farley J.E. Development and Validation of a Predictive Model for Aminoglycoside Ototoxicity. *Conference on Retroviruses and Opportunistic Infections.* March 6, 2019. Seattle, WA. USA. Accepted for poster presentation.

Hong, H., Budhathoki, C., Mlandu, N., Lowensen, K., Stamper, P.D., Farley, J.E. Symptom Presentation of Drug Resistant Tuberculosis: Implications of Nurse-Led Active Screening. *Association of Nurses in AIDS Care 2018 Annual Conference.* November 9, 2018. Denver, CO. USA. Podium Presentation.

Hong, H., Dowdy, D.W., Whitehouse, E., Lowensen, K., Stamper, P.D., Farley. J.E. Predicting Aminoglycoside-Induced Ototoxicity among DR-TB-Infected Individuals in South Africa. *The 49th Union World Conference on Lung Health.* October 27, 2018. The Hague, Netherlands. Poster Presentation.

Hong, H., Starbird, L.E., Dooley, K.E., Francis, H.W., Farley J.E. Development of an Adverse Outcome Pathway for Aminoglycoside-Induced Hearing Loss from Drug-Resistant Tuberculosis Treatment. *The 3rd Global Korean Nursing Foundation International Nursing Conference.* June 28, 2018. New York, NY, USA. Poster Presentation.

Hong, H., Budhathoki, C., Farley J.E. Substantial increase in risk for HIV patients with MDR-TB for aminoglycoside-induced hearing loss. *The 48th Union World Conference on Lung Health.* October 12, 2017. Guadalajara, Mexico. Podium Presentation.

Hong, H., Budhathoki, C., Farley J.E. Risk of baseline hearing loss among DR-TB patients in South Africa. *The 48th Union World Conference on Lung Health.* October 13, 2017. Guadalajara, Mexico. Poster Presentation.

Hong, H., Dowdy, D.W., Dooley, K.E., Francis, H.W., Budhathoki, C., Han, H., Farley J.E. Predicting Aminoglycoside-Induced Hearing Loss among Multidrug-

Resistant Tuberculosis Infected Individuals in South Africa: Dissertation Proposal. *The 2nd Global Korean Nursing Foundation International Nursing Conference.* July 13, 2017. Chicago, IL, USA. Poster Presentation.

National

Hong, H. Effectiveness of Nutritional Supplements on Reducing Adverse Drug Reactions from MDR-TB Treatment. *Association for Clinical and Translational Science 2016 Annual Conference.* April 14, 2016. Washington, DC, USA. Poster Presentation.

Hong, H., Budhathoki, C., Farley, J.E. The Effectiveness of Macronutrients on Nutritional Status and HIV/AIDS Progress in Sub-Saharan Africa: a Meta-Analysis. *Association of Nurses in AIDS Care 2015 Annual Conference.* October 30, 2015. Chicago, IL, USA. Podium Presentation.

Regional

Hong, H. Are All Eyes on the Tuberculosis Treatment Effect? *Johns Hopkins University Center for Global Health.* April 7, 2016. Baltimore, MD, USA. Poster Presentation.

Hong, H., McCreary, L. Association of Iron Supplementation with or without Folic Acid with Anemia and Malaria Incidence in Sub-Saharan Africa: a Meta-Analysis. *Midwest Nursing Research Society 2013 Annual Conference*. March 8, 2013. Chicago, IL, USA. Poster Presentation.

EDITORIAL ACTIVITIES

2015	Ad-hoc reviewer, *Journal of the Association of Nurses in AIDS Care*
2018	Ad-hoc reviewer, *PLoS ONE*
2018	Abstract reviewer, *The 49th Union World Conference on Lung Health*
2018	Peer reviewer, *International Journal of Tuberculosis and Lung Disease*

PROFESSIONAL ACTIVITIES

2017	Member, Southern Nursing Research Society
2017−	Member, Sigma Theta Tau International, Nu Beta Chapter
2016−	Member, Global Korean Nursing Foundation, USA
2015−	Member, Association of Nurses in AIDS Care, Chesapeake chapter
2013–2014	Scholarship Committee, Korean Nurses Association of Chicago
2012–2014	Member, Midwest Nursing Research Society
2012–2016	Member, Sigma Theta Tau, Alpha Lambda Chapter
2006–	Member, Korean Nurse Association

Curriculum Vitae

Hyejeong Hong

Part II

EDUCATIONAL ACTIVITIES

Classroom Instruction

Fall 2015 — Johns Hopkins University School of Nursing
NR 110.314 Principles of Pharmacology (3 credits)
Undergraduate, 120 students
Provided 4 in-class lectures, facilitated in-class and on-line discussions, and graded assignments and examinations

Fall 2011 — University of Illinois at Chicago College of Nursing
NURS 525, Intermediate Statistics for Master's Program (3 credits)
Masters, 100 students
Facilitated on-line discussions, provided exam reviews, and graded assignments and examinations

ACADEMIC SERVICE

2015−2016 — Representative of School of Nursing, Korean Graduate Student Association, Johns Hopkins University

2012−2013 — International Student Representative, Graduate Student Nurse Organization, University of Illinois at Chicago

2004−2005 — Vice-President, College of Nursing Student Association, Pusan National University

2002−2003 — Student Representative of the Nursing Student Council, Pusan National University