1 **TITLE PAGE**

2 **The adverse effects and choice of injectable agents in MDR-TB: amikacin or capreomycin**

3 **Running title: Adverse events of injectable agents in MDR-TB**

4

5 **Amber Arnold,** [#a] Graham S. Cooke,[b] Onn Min Kon,[c] Martin Dedicoat,[d] Marc Lipman,[e] Angela
6 Loyse, [a] Irina Chis Ster, [a] Thomas S. Harrison, [a]

7 [a]Institute for Infection and Immunity, St. George's University of London, London, United
8 Kingdom; [b]Division of Medicine, Imperial College London, United Kingdom; [c]Tuberculosis
9 Service, St Mary's Hospital, Imperial College Healthcare NHS Trust, United Kingdom;
10 [d]Department of Infectious diseases, Heart of England Foundation Trust, Birmingham, United
11 Kingdom; [e]Royal Free London NHS Foundation Trust and UCL Respiratory, Division of
12 Medicine, University College London, United Kingdom

13 #a Address correspondence to Amber Arnold: Institute for Infection and Immunity, St.
14 George's University of London, London, United Kingdom, SW17 0RE,
15 amber.arnold@doctors.org.uk,

16

17

18

The adverse effects and choice of injectable agents in MDR-TB: amikacin or capreomycin

Abstract

Background: The prolonged use of injectable agents in an MDR-TB regimen is recommended by the WHO despite association with ototoxicity and nephrotoxicity.

Objective: We undertook this study to look at the relative adverse effects of capreomycin and amikacin.

Methods: We reviewed the case notes of 100 consecutive patients treated at 4 MDR-TB treatment centres in the UK.

Results: The median total duration of treatment with an injectable agent was 178 (IQR 109-192, n=73) days for those with MDR-TB, 179 (104-192, n=12) days for those with MDR-TB plus fluoroquinolone resistance and 558 (324-735, n=8) days for those with XDR-TB. Injectable use was longer for those started with capreomycin at 183 (IQR 123-197) days compared to 119 (IQR 83-177) days with amikacin (p=0.002). Excluding XDR-TB, 51 (51/85, 60%) patients were treated with an injectable for over 6 months and 12 (12/85, 14%) for over 8 months. 40 % of all patients discontinued the injectable due to hearing loss. 55% of patients experienced ototoxicity: 5 times (hazard ratio (HR) 5.2, CI 1.2-22.6, p=0.03) more likely in those started on amikacin compared to treatment with capreomycin only. Amikacin was associated with less hypokalemia than capreomycin (Odds ratios: 0.28 (0.11-0.72)), with 5 (5/37, 14%) patients stopping capreomycin due to recurrent electrolyte loss. There was no difference in the number experiencing a creatinine rise of > 1.5 times baseline.

Conclusion: Hearing loss is frequent in this cohort, though significantly lower in those starting capreomycin which should be given greater consideration as a first line agent.

Main Text

Introduction

Treatment of multidrug resistant tuberculosis (MDR-TB) is challenging requiring extensive multidrug combinations for up to two years associated with significant adverse effects(1) Current treatment for MDR-TB is largely dependent on the World Health Organisation (WHO) guidelines(2-4) which are based on cohort, meta-analysis data and expert opinion. These recommend that all patients should be initially (intensive phase) treated with an injectable agent in the form of an aminoglycoside (kanamycin/amikacin) or polypeptide (capreomycin). The duration of the intensive phase recommended by the WHO rose from a minimum of 6 months to 8 months in 2011(3, 4) with even longer durations recommended for cases with more extensive resistance. The recommendation was based on a large meta-analysis of patient outcomes and did not take into account the side effects or other costs of these drugs.(5)

The injectable agents have significant side effects in the form of permanent and potentially progressive post cessation ototoxicity and usually reversible nephrotoxicity. (6-9) The frequency of ototoxicity and nephrotoxicity experienced by patients varies between studies, and most focus on the side effects of the aminoglycosides rather than the polypeptide,

capreomycin. Limited evidence suggests that capreomycin may be less ototoxic than amikacin.(10)

No randomised controlled trial of different injectable agents has been performed but better data is needed to inform policy. We performed a detailed service evaluation cohort study within four specialist UK MDR-TB treatment centres to compare the outcomes with different injectable agents in a real world setting.

Methods

Setting

Retrospective data were collected through clinical records and hospital database review at 4 tuberculosis (TB) treatment centres; St Mary's Hospital, Imperial College NHS Trust, London (centre 1), Heartlands Hospital, Birmingham (centre 2), the Royal Free Hospital, London (centre 3), St George's Hospital, London (centre 4). These centres act as regional referral hubs for MDR-TB treatment . Data were also collected at referring hospitals if patients were treated under a shared care model. Standard definitions were used for MDR-TB and extensively drug resistant tuberculosis (XDR-TB), pulmonary (PTB), extra pulmonary tuberculosis (EPTB)(11) and treatment was based on the WHO guidelines.(2, 3) At sites 1-2 amikacin is the preferred injectable agent, site 3 uses a mix and site 4 predominantly uses capreomycin (all intravenous). All sites switched injectable at the physician's discretion. All injectable agents are dosed initially at 15mg/kg once a day with trough drugs levels for amikacin at least weekly. Reduced frequency of dosing is used if side effects occur. Duration

of 6 months or more was defined as over 160 days and duration of 8 months was defined as over 220 days.

Study population and eligibility criteria

The first 100 consecutive patients, over 14 years of age, with a diagnosis of MDR-TB made in the UK, initiating MDR-TB treatment at the four sites between 2008 and 2014, were reviewed. Seven patients were excluded due to: lack of injectable agent use (2), streptomycin use at start (2), and over three initiations on MDR-TB medications (n=3). The cohort was split into two according to date of treatment start (the 51st patient started treatment in spring 2011) which corresponded to the change in WHO advice regarding injectable duration.

Renal function monitoring

To be included in analysis of renal function patients required at least weekly blood results available for review. Renal impairment was defined as mild at 1.5 times baseline creatinine and severe at over 3 times baseline(12). Hypokalaemia was defined as any drop below 3.5mmol/L.(13) Hypomagnesaemia was defined as any measurement below 0.7mmol/L.(12)

Audiological monitoring

All patients underwent pure tone audiometry (PTA) performed to the standards of the British Society of audiology (14) at the start of the injectable therapy. All sites performed PTA if hearing loss/change symptoms/any concern about hearing arose on treatment and sites 1, 2 and 3 had a policy of monthly PTA in addition (limited by patient adherence to protocol). Centre 3 performed audiograms at frequencies above 9- 20khz for a proportion of

the study period. Significant deterioration between audiograms was determined by the American speech and hearing association (ASHA) criteria which were as follows for frequencies tested between 250-8khz: (i) 20dB decrease ay any one test frequency, (ii) 10 dB decrease at any two adjacent frequencies, (iii). Loss of response at any three adjacent frequencies where responses were previously obtained .(15) Two end points relating to hearing were chosen: an audiogram definition (ototoxicity) and a composite definition encompassing audiogram results and clinically reported hearing loss (hearing loss (composite)) (**Table 1)**. Patient reported 'hearing impairment' was defined as any report by the patient of a negative change in hearing while on injectable agents or after stopping the injectable as documented by a nurse or doctor. 'Tinnitus' was defined as any symptoms reported by the patient that were interpreted as tinnitus by a doctor or nurse and documented in the records. Reasons for stopping injectable agents were collated from the medical notes according to what was written by the consultant in charge of treatment.

Statistics

Patients were grouped according to the injectable agent they were exposed to: 1. capreomycin only, 2. amikacin start (includes those only treated with amikacin and those treated with amikacin and switched to capreomycin or streptomycin because hearing loss was the main driver of this switch), 3. capreomycin then switch to amikacin (none switched due to hearing loss). Hearing loss was analysed within survival settings using Cox proportional hazard models, modelling the time since treatment start to point of hearing loss. Raised creatinine and hypokalaemia were investigated using logistic regression.

Univariate analyses were initially undertaken which included all variables collected *(age, gender, baseline creatinine, baseline creatinine clearance (Cockroft Gault equation), dose of drug, MDR-TB type, number of amikacin troughs, centre, and amikacin and capreomycin group)*. Associations with resulting p-values less than 0.1 were further considered to form a multivariable/adjusted models based on similar numbers of complete observations. Model selection was undertaken by choosing the most parsimonious model using Akaike information criteria (AIC) and Bayesian information criteria (BIC). The final models were further refined using multiple imputation methodologies assuming missing at random model to account for approximately 15% of the original data that was missing (16). Further details on statistical methodologies are given in appendix 1. STATA software was employed for data analyses (StataCorp.2015 S*tata Statistical Software: Release 14. College Station,* TX:StataCorp LP).

Ethics

The study was deemed to be a service evaluation at the NHS ethics board (NRES committee London- City and East). Consent was given by the Confidentiality Advisory Group (GAG) for access to clinical records review. The data were anonymised onsite for off sites analyses.

Results

Fifty-four patients were started on amikacin and 39 were started on capreomycin (total, n=93). Nineteen patients switched injectable agent for the reasons stated in **Figure 1.** Background demographics and tuberculosis characteristics can be seen in **Table 2.**

Total duration of treatment with an injectable agent

The median total duration of treatment with an injectable agent was 178 (IQR 109-192, n=73) days for those with MDR-TB, 179 (104-192, n=12) days for those with MDR-TB plus fluroquinolone resistance (MDR-TB +FLQ) and 558 (324-735, n=8) for those with XDR-TB.

Excluding those with XDR-TB, 51 (51/60, 60%) patients were treated for 6 months or more and 12 (12/85, 14%) for 8 months or more. In the early cohort the median duration of treatment was 165 (107-187, n=42) days, of which 23 (23/42, 55%) achieved the target of 6 months and 3 (3/42, 7%) were treated for 8 months plus. In the latter cohort the median duration of treatment was 183 (109-210, n=43) days, of which 28 (28/43, 65%) were treated for 6 months or more and 9 (9/43, 21%) achieved the target of 8 months or more. There was no statistical difference in duration between the early and late cohort (p=0.19).

Seven (7/8, 87%) patients with XDR-TB were treated for 6 months or more and 6 (6/8, 75%) for 8 months or more.

The reasons for not achieving 6 months of treatment or more for all groups of patients were hearing loss (composite) 14 (14/35, 40%), physician choice 8 (8/35, 23%), resistance 4 (4/35, 11%), compliance concerns 3 (3/35, 9%) other 6 (6/35, 17%).

The median duration of the first line injectable agent was 160 (IQR 91-186) days for all patients. The median total duration was 183 (IQR 123-197) days for those started on capreomycin and 119 (IQR 83-177) days for those started on amikacin (p=0.002).

Ototoxicity

The proportion of cases that met the criteria for ototoxicity assessment was 55 (55/93, 59%) (Table 1) of whom 39 were started on amikacin and 16 started on capreomycin. Clinical notes were available for all 55 patients. Ototoxicity occurred in 30 patients (30/55, 55%), at a median duration of 112.5 days (IQR 91-177) and 18 (18/55, 60%) had bilateral changes. Deterioration was seen at the frequencies 6 -8 kHz only in 19 (19/55, 63%) cases, in the frequencies 4-8kHz only in 3 (3/55, 10%) cases, in frequencies 2-8kHz only in 6 (6/55, 20%) cases and across all frequencies tested (250Hz-8kHz) in 2 (2/55, 7%) cases. The median maximum change from baseline hearing at the worst effected frequency was 40 dB (IQR 25-55). At the time that ototoxicity was detected 8 (8/55, 27%) patients reported new onset hearing disturbance and tinnitus, 8 (8/55, 27%) reported tinnitus only, 3 (3/55, 10%) reported hearing disturbance only and 11 (11/55, 37%) did not report any symptoms.

Ototoxicity occurring on Amikacin

Twenty-eight cases of ototoxicity occurred while on treatment with amikacin (n=23) or after stopping treatment with amikacin (n=5). The median total number of amikacin trough levels did not differ between those with ototoxicity (1.03 IQR 0.77-1.28) and those without (1.21 IQR 1-1.43) (p=0.10). The proportion of one or more amikacin trough levels above 2.5 was 12/28 (40%) for those with ototoxicity and 5/14 (36%) for those treated with amikacin and no ototoxicity (P=0.66).

Antimicrobial Agents and Chemotherapy

188 3 cases experienced ototoxicity on amikacin and had initially been treated with
189 capreomycin. They had been switched to amikacin due to electrolyte disturbance (n=2) or
190 resistance (n=1). Two of the patients had normal audiograms (and same as their baseline) at
191 the time of switch (174 and 164 days) and the third had a normal audiogram at the start of
192 capreomycin followed by an abnormal audiogram after 282 days of amikacin treatment
193 when newly reported tinnitus lead to testing. Fifteen (15/28, 54%) patients had sufficient
194 audiograms to assess deterioration after stopping amikacin; 10 (10/15, 67%) progressed, 1
195 (1/28, 7%) improved and 4 (4/15, 27%) did not change.

196

197 ***Ototoxicity occurring on Capreomycin***

198 Two cases of ototoxicity occurred on capreomycin. Both were in patients with XDR-TB in
199 whom stopping the regimen would have reduced the number of active drugs below 4 and so
200 despite early detection, treatment was continued with monitoring. Neither case
201 experienced any permanent symptoms. Both cases had normal audiograms on first
202 assessment and sensorineural hearing loss was identified on the second audiogram to be
203 performed after the baseline which was at day 33 (performed due to vague symptoms of
204 muffled hearing which went away) and day 112 (performed for screening no symptoms) of
205 treatment respectively. Changes were seen bilaterally in both cases at the 6KHz and 8KHz
206 frequency. There was a drop of 10-20db in case 1 and a drop of 30-55db in case 2. A further
207 3 and 4 audiograms were performed until days 434 (case 1) and 447 (case 2) of treatment
208 and no further deterioration was seen. Both patients continued treatment after this period
209 of monitoring with no change in symptoms but no further audiograms were performed.

210

Multivariable analysis using only the patients who fitted the ototoxicity criteria showed that ototoxicity was five times more likely for patients started on amikacin than for those treated with only capreomycin (HR 5.2, CI 1.2-22.6, p=0.03).

Hearing loss (composite)

Three patients (3/93) did not have sufficient medical notes (n=1) or could not express loss of hearing (psychosis n=1, intubated n=1) to be included in this analysis. Thirty-four (34/90, 38%) of those meeting criteria for inclusion experienced hearing loss (composite). The multivariable analysis showed that the likelihood of hearing loss (composite) was 14 times greater for patients started on amikacin compared to those treated with capreomycin only (Hazard ratio 13.9 CI 3.25-59, P<0.001) **(Table 3) .** Predicted survival analysis also showed that the probability of not developing hearing loss beyond 90 days was 0.99 (0.95- 1.00) in those on capreomycin only compared to 0.85 (0.73-0.92) for those starting amikacin. Furthermore the probability of surviving without hearing loss beyond 180 days was 0.97 (0.86-0.99) for those on capreomycin only compared to 0.58 (0.41, 0.72) for those started on amikacin **(Figure 2).**

Nephrotoxicity

Over the first 3 months renal function monitoring was performed a median of 19 times (IQR: 14-25) and over months 4-6, 9 times (IQR: 4-15).

Raised creatinine

233 Eighty-five cases had complete set of creatinine blood results. 25% (21/85) had a rise of 1.5
234 times or more from baseline of which 3 (3.5% =3/85) had a rise of 3 times baseline. The
235 creatinine returned to baseline (under 1.5 times normal) in 19 (19/21) cases, 16 before the
236 end of the injectable and 3 before the end of MDR-TB treatment. In patients where the
237 creatinine did not return to baseline; one required haemodialysis after the amikacin was
238 stopped (he already had chronic kidney disease at the start of therapy for MDR-TB and a
239 baseline creatinine of 313 µmol/L which peaked at 846µmol/L) and the other due to death
240 from advanced HIV (CD4=5). A multivariable model including baseline creatinine, duration
241 on injectable agent and choice of injectable agent at start showed that there was no
242 significant difference in the odds of raised creatinine between the two injectable agents
243 chosen at the start (p=0.178) when adjusted for the total duration of the treatment.
244 However, some evidence suggests that increasing duration may increase the odds of raised
245 creatinine, i.e. 30 days increase is associated with 15% (95%CI(25, 32%)) raise in the odds of
246 raised creatinine (p=0.04) (Table 4).
247
248 ***Electrolyte disturbance***
249
250 Eighty-six patients had a complete set of potassium results, 37 started on capreomycin and
251 49 amikacin. Hypokalaemia was found in 38 (38/86, 44%) patients while on an injectable
252 agent: 23 (23/38, 61%) were on capreomycin and 15 (15/38, 39%) amikacin.Eighteen cases
253 (18/38) resolved alone without potassium replacement. Seventeen required replacement
254 with oral potassium (13/17 on capreomycin), 7 required replacement with intravenous

potassium (all capreomycin), 4 had their dose reduced to 3 times per week (all capreomycin) and 3 required a switch in injectable agent (all capreomycin to amikacin). A multivariable model including duration of injectable agent and initial injectable agent indicated that the odds of hypokalaemia were approximately 4 times lower in those starting amikacin than for those starting capreomycin (Odds ratios: 0.28 (0.11-0.72). **(Table 4)**

Regular magnesium testing was performed for 15 of the capreomycin and none of the amikacin patients. Thirteen (13/15) were hypomagnesemic (11/13 with a reading below 0.5 mmol/L) of which 10 were treated with oral replacement, 9 with intravenous replacement and 4 required a switch to amikacin (3 of these also had reduced potassium and are inclusive of the 3 above). One stopped injectable earlier than planned due to hypomagnesaemia.

Switching from capreomycin to amikacin or stopping capreomycin early for electrolyte disturbance occurred in 5 patients (5/37) at a median of 132 (range 53-207, n=5) days. Of the four cases switched from capreomycin to amikacin one subsequently suffered ototoxicity on amikacin.

Discussion

We present data showing that ototoxicity is very frequent and that in England a third of patients do not reach the original 2008 WHO treatment guideline advising at least 6 months of an injectable agent. Even fewer reach the newer target of 8 months for the intensive

277 phase. In a sub-cohort analysis capreomycin is associated with less ototoxicity and/or
278 hearing loss than amikacin though its use is sometimes limited by electrolyte disturbance.
279 Those starting capreomycin were also able to tolerate injectable treatment for much longer.

280 Hearing loss during MDR-TB treatment is reported to be anywhere between 4.4% (1, 17)and
281 62% (18) (19) dependent on duration, drug choice, dose(6) and type of monitoring. Studies
282 with a clinical definition (patient reporting symptoms) show lower levels than those with an
283 audiogram based definition (20) and the majority of studies have been performed in the
284 presence of the aminoglycosides, amikacin or the more commonly used worldwide and
285 closely related kanamycin (15mg/kg/day). Our level of 55% ototoxicity is similar to the
286 findings of others using intense monitoring and aminoglycosides at 15mg/kg, (15) (7, 18, 19,
287 21) Retrospective cohort analysis suggests that Kanamycin use is associated with less
288 ototoxicity than amikacin. (21)

289 There are few recent MDR-TB studies investigating hearing loss associated with
290 capreomycin possibly as its cost and need for electrolyte monitoring put it out of reach for
291 many low income countries. However, although clearly defined methods for monitoring are
292 not always described, there is a suggestion that levels of hearing loss are lower for
293 capreomycin with proportions affected ranging from 0.7%-25%.(6, 22-25) Studies comparing
294 amikacin to capreomycin are limited to a small retrospective study by this group which
295 showed in univariate analysis that hearing loss was associated with amikacin use over
296 capreomycin(10) and a pharmacovigilance reporting study showing spontaneous reports of
297 deafness were disproportionately associated with amikacin followed by kanamycin

compared to capromycin. (26) Our study has larger numbers than our earlier study and is not limited by reporting bias and other issues inherent in pharmacovigilance reporting. The main limitation of our study arises from the differing audiogram policies at the sites. In the hearing loss (composite) analysis there is the possibility of underestimating hearing loss caused by capreomycin due to asymptomatic cases with ototoxicity being less likely to be identified (ascertainment bias) than those in the amikacin group who had more routine audiograms. However, to counter this possible bias we performed the ototoxicity analysis including in the denominators only those who had had an audiogram within a month of ending the injectable agent. Although the numbers of patients is smaller, in this analysis, the possible bias works in the opposite direction because patients at capreomycin sites who had audiograms were more likely to be those with a perceived risk of ototoxicity. These issues probably account for the difference between the hazard ratio for the ototoxicity outcome (5 times more likely with amikacin) compared to 15 times more likely for the composite hearing loss outcome with amikacin, and the real value may lie between the two numbers. We also consider that the character as well as the likelihood of occurrence of hearing loss can differ with capreomycin. The evidence for this suggestion is that the audiograms of the two patients who experienced ototoxicity on capreomycin did not display progressive hearing loss despite on-going exposure (lack of alternative drugs) which would be extremely unlikely for amikacin. (8) However, further investigations on the type of and degree of hearing loss caused by capreomycin in a randomised controlled trial is required. Reducing the proportions of patients experiencing hearing loss treated with amikacin may be possible with lower doses (7.5mg/kg) and AUC monitoring. (27) However the efficacy of this dose is

320 unclear and it is not currently recommended. Other possibilities include the co-
321 administration of N-acetyl cysteine or other antioxidants,(28) and genetic testing for
322 mutations in the mitochondrial gene encoding 12S rRNA (*MT-RNR1*) and avoiding
323 aminoglycosides in these cases, (29-31) though the prevalence of these mutations is low.

324 However, our findings support the initial use of capreomycin over amikacin as a means of
325 reducing hearing loss. Capreomycin use first line has also been advocated for, when
326 onwards resistance patterns are considered; amikacin activity is often spared after the
327 evolution of capreomycin resistance but not the other way round.(32, 33) The disadvantage
328 of capreomycin is the associated electrolyte disturbance which led to
329 discontinuation/switch in 14% of patients treated with it in our study. Of note, however,
330 electrolyte abnormalities were managed effectively in all patients with no long term
331 consequences. The association of capreomycin with electrolyte disturbance and renal
332 impairment during treatment for TB is well reported. (13, 34, 35) In settings where regular
333 rapid and reliable blood monitoring is not feasible, the nephrotoxicity of capreomycin may
334 lead to deaths due to hypokalaemia and renal failure.(13, 23) Our data demonstrate that
335 this is not the case in a well-resourced setting.

336 In summary we provide retrospective cohort evidence of high levels of ototoxicity and
337 hearing loss in a UK MDR-TB cohort. Hearing loss was 14 times more likely with amikacin
338 than capreomycin, while capreomycin was associated with electrolyte disturbance leading
339 to cessation of the drug in 14% of those treated with it. Given the significance and
340 irreversibility of hearing loss, in settings where blood monitoring is possible, we would

favour starting with capreomycin rather than amikacin, until such time as short course and injectable drug -free regimens incorporating the newer drugs have been shown to be effective .(17)

Acknowledgements

Maria Mercer and Vera Pavlova (Respiratory medicine, St George's Hospital, London), Marie O Donoghue (Division of Medicine, Imperial College Hospital NHS Trust, London), Angelita Solamalai (Respiratory Medicine, Royal Free Hospital, London), Veronica White (Respiratory Medicine, Royal London NHS Trust), Lucy Baker (Respiratory Medicine, University Hospital Lewisham).

Funding

This work was supported by St George's Healthcare NHS Trust and The Jefferiss Charitable Trust (no grant number). GC is supported by BRC of Imperial College NHS Trust.

Transparency and Declaration of competing interests statement

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: AA has received a research studentship funding from The Jefferiss Charitable Trust (no grant number), PB is funded by INNOVATE UK (UK Government Agency) in collaboration with QuantuMDx Ltd. No further information is declared.

Ethics

The study was deemed to be a service evaluation at the NHS ethics board (NRES committee London- City and East). Consent was given by the Confidentiality Advisory Group (GAG) for access to clinical records review. The data were anonymised onsite for off sites analyses. Data sharing with public health was according to Caldicott principles.

Contributorship statement

AA, TSH, MD, GSC, OMK, AL, PDB contributed to the design of the work. The acquisition of data was undertaken by AA, MD, OMK, ML, AL and acknowledged persons MM, VP, MOD and AS. ICS undertook statistical analysis with AA and TSH. All authors contributed to data interpretation, drafting and are responsible for content.

Contributors were as follows:

The conception and design of the work	Amber Arnold, Tom Harrison, Martin Dedicoat, Graham Cooke, Onn Min Kon, Angela Loyse,
The acquisition of data	Amber Arnold, Martin Dedicoat, Onn Min Kon, Marc Lipman, Angela Loyse Acknowledged persons: Maria Mercer, Vera Pavlova, Marie O Donoghue, Angelita Solamalai
Analysis and interpretation of data.	All Authors
Drafting the work or revising it critically for	All Authors

important intellectual content	
Final approval of the version published	All Authors
Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.	All Authors

370

371 **References**

372 1. **Wu S, Zhang Y, Sun F, Chen M, Zhou L, Wang N, Zhan S.** 2013. Adverse Events
373 Associated With the Treatment of Multidrug-Resistant Tuberculosis: A Systematic
374 Review and Meta-analysis. Am J Ther 23(2):e521-30 https://doi.org/10.1097/01
375 mjt.0000433951.09030.5a.
376 2. **World Health Organization**. 2008 Guidelines for the programmatic management of
377 drug-resistant tuberculosis. Geneva, Switzerland.
378 3. **World Health Organisation. 2011.** Guidelines for the programmatic management of
379 drug-resistant tuberculosis-2011 update. Geneva, Switzerland.
380 4. **World Health Organisation.** 2016. The WHO treatment guidelines for drug-resistant
381 tuberculosis (2016 update). Geneva, Switzerland.
382 5. **Ahuja SD, Ashkin D, Avendano M, Banerjee R, Bauer M, Bayona JN, Becerra MC,**
383 **Benedetti A, Burgos M, Centis R.** 2012. Multidrug resistant pulmonary tuberculosis

384 treatment regimens and patient outcomes: an individual patient data meta-analysis
385 of 9,153 patients. PLoS Med **9:**1212.
386 6. **Duggal P, Sarkar M.** 2007. Audiologic monitoring of multi-drug resistant tuberculosis
387 patients on aminoglycoside treatment with long term follow-up. BMC Ear, Nose
388 Throat Disord **7:**5. **DOI:** 10.1186/1472-6815-7-5
389 7. **Melchionda V, Wyatt H, Capocci S, Garcia Medina R, Solamalai A, Katiri S, Hopkins**
390 **S, Cropley I, Lipman M.** 2013. Amikacin treatment for multidrug resistant
391 tuberculosis: how much monitoring is required? Eur Respir J **42:**1148-1150.
392 8. **Seddon JA, Godfrey-Faussett P, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS.** 2012.
393 Hearing loss in patients on treatment for drug-resistant tuberculosis. Eur Respir J
394 **40:**1277-1286.
395 9. **Seddon JA, Godfrey-Faussett P, Hesseling AC, Gie RP, Beyers N, Schaaf HS.** 2012.
396 Management of children exposed to multidrug-resistant Mycobacterium
397 tuberculosis. Lancet Infect Dis **12:**469-479.
398 10. **Sturdy A, Goodman A, Jose RJ, Loyse A, O'Donoghue M, Kon OM, Dedicoat MJ,**
399 **Harrison TS, John L, Lipman M, Cooke GS.** 2011. Multidrug-resistant tuberculosis
400 (MDR-TB) treatment in the UK: a study of injectable use and toxicity in practice. J
401 Antimicrob Chemother **66:**1815-1820.
402 11. **World Heath Organisation. 2014.** Definitions and reporting framework for
403 tuberculosis – 2013 revision (updated December 2014). Geneva, Switzerland.
404 12. U.S.Department of Health and Human Services. Common terminology criteria for
405 adverse events version 4.03. 2010

https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf accessed 28th Novemeber 2016.

13. **Shin S, Furin J, Alcántara F, Hyson A, Joseph K, Sánchez E, Rich M.** 2004. Hypokalemia among patients receiving treatment for multidrug-resistant tuberculosis. Chest **125:**974-980.

14. **British Society of Audiology.** Pure tone air and bone conduction threshold audiometry with and without masking and determination of uncomfortable loudness levels. 2011. Reading, UK.

15. **American Speech Language hearing Association**. Audiologic Management of Inidividuals Receiving Cochleotoxic Drug Therapy (Guideline). 1994. Rockville, USA

16. **Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR.** 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ **338:**b2393.

17. **Nunn AJ, Rusen I, Van Deun A, Torrea G, Phillips PP, Chiang C-Y, Squire SB, Madan J, Meredith SK.** 2014. Evaluation of a standardized treatment regimen of anti-tuberculosis drugs for patients with multi-drug-resistant tuberculosis (STREAM): study protocol for a randomized controlled trial. Trials **15:**353. doi: 10.1186/1745-6215-15-353

18. **Modongo C, Sobota RS, Kesenogile B, Ncube R, Sirugo G, Williams SM, Zetola NM.** 2014. Successful MDR-TB treatment regimens including amikacin are associated with high rates of hearing loss. BMC Infect Dis **14:**542.

19. **Harris T, Bardien S, Schaaf HS, Petersen L, De Jong G, Fagan JJ.** 2012. Aminoglycoside-induced hearing loss in HIV-positive and HIV-negative multidrug-resistant tuberculosis patients. S Afr Med J **102:**363-366.

20. **Ramma LD, Ibekwe TS.** 2012. Efficacy of utilising patient self-report of auditory complaints to monitor aminoglycoside ototoxicity. Int J Tuberc Lung Dis **16:**283.

21. **Sagwa EL, Ruswa N, Mavhunga F, Rennie T, Leufkens HGM, Mantel-Teeuwisse AK.** 2015. Comparing amikacin and kanamycin-induced hearing loss in multidrug-resistant tuberculosis treatment under programmatic conditions in a Namibian retrospective cohort. BMC Pharmacol and Toxicol **16:**36

22. **Donomae I.** 1966. Capreomycin in the treatment of pulmonary tuberculosis. Ann of N Y Acad of Sci **135:**1011-1038.

23. **Shean K, Streicher E, Pieterson E, Symons G, van Zyl Smit R, Theron G, Lehloenya R, Padanilam X, Wilcox P, Victor TC.** 2013. Drug-associated adverse events and their relationship with outcomes in patients receiving treatment for extensively drug-resistant tuberculosis in South Africa. PloS One 7;8(5):e63057. doi: 10.1371/journal.pone.0063057

24. **Miller JD, Popplewell AG, Landwehr A, Greene ME.** 1966. Toxicology studies in patients on prolonged therapy with capreomycin. Ann of N Y Acad of Sci. **135:**1047-1056.

25. **Kass I.** 1965. Chemotherapy regimens used in retreatment of pulmonary tuberculosis: Part II. Observations on the efficacy of combinations of ethambutol, capreomycin and companion drugs, including 4–4 diisoamyloxythiosemicarbanilide. Tubercle **46:**166-177.

26. **Sagwa EL, Souverein PC, Ribeiro I, Leufkens HG, Mantel-Teeuwisse AK.** 2016. Differences in VigiBase(R) reporting of aminoglycoside and capreomycin-suspected ototoxicity during tuberculosis treatment. Pharmacoepidemiol Drug Saf doi:10.1002/pds.4125.

27. **van Altena R, de Vries G, Haar C, de Lange W, Magis-Escurra C, van den Hof S, van Soolingen D, Boeree M, van der Werf T.** 2015. Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000–2009. Int J Tuberc Lung Dis **19:**406-412.

28. **Kranzer K, Elamin WF, Cox H, Seddon JA, Ford N, Drobniewski F.** 2015. A systematic review and meta-analysis of the efficacy and safety of N-acetylcysteine in preventing aminoglycoside-induced ototoxicity: implications for the treatment of multidrug-resistant TB. Thorax **70:**1070-1077.

29. **Hutchin T, Haworth I, Higashi K, Fischel-Ghodsian N, Stoneking M, Saha N, Arnos C, Cortopassi G.** 1993. A molecular basis for human hypersensitivity to aminoglycoside antibiotics. Nucleic Acids Res **21:**4174-4179.

30. **Bindu LH, Reddy PP.** 2008. Genetics of aminoglycoside-induced and prelingual non-syndromic mitochondrial hearing impairment: a review. Int J Audiol **47:**702-707.

31. **Bardien S, Human H, Harris T, Hefke G, Veikondis R, Schaaf HS, van der Merwe L, Greinwald JH, Fagan J, de Jong G.** 2009. A rapid method for detection of five known mutations associated with aminoglycoside-induced deafness. BMC Med Genet **10:**1-9.

471 32. **Tsukamura M.** 1969. Cross-resistance relationships between capreomycin,
472 kanamycin, and viomycin resistances in tubercle bacilli from patients. Am Rev Respir
473 Dis **99:**780-782.
474 33. **Caminero JA, Sotgiu G, Zumla A, Migliori GB.** 2010. Best drug treatment for
475 multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis
476 **10:**621-629.
477 34. **Holmes AM, Hesling CM, Wilson TM.** 1970. Capreomycin-induced serum electrolyte
478 abnormalities. Thorax **25:**608-611.
479 35. **Aquinas M, Citron KM.** 1972. Rifampicin, ethambutol and capreomycin in pulmonary
480 tuberculosis, previously treated with both first and second line drugs: the results of 2
481 years chemotherapy. Tubercle **53:**153-165.

482

483

484 **Table 1: Ototoxicity and Hearing loss (composite) definitions**
485

	Hearing loss	No hearing loss	Unable to classify
Ototoxicity	A significant deterioration (as determined by ASHA criteria) between an audiogram performed before or during therapy and one performed later during therapy or after completing therapy in the presence of normal tympanograms.*	A normal audiogram in the last month or after completing injectable therapy.*	An abnormal audiogram without an earlier audiogram for comparison*
		No significant deterioration (ASHA criteria) between an audiogram performed in the last month or after injectable therapy stopped and one performed within the first month of therapy.*	A normal final audiogram before the last month of therapy (unless performed after 365 days on therapy).
		No significant deterioration (ASHA criteria) between an audiogram performed after 365 days of injectable therapy and one performed within the first month of therapy.	

Hearing loss (composite)	As for ototoxicity	No report of 'hearing impairment' or 'tinnitus' and does not fit the criteria for ototoxicity.	Unable to report symptoms (intubated, extreme psychosis) or full set of medical or nursing notes missing.
	A clinical report of new 'hearing impairment' or 'tinnitus' during or after therapy with an injectable agent in association with an abnormal audiogram. No prior audiogram required.	A clinical report of new 'hearing impairment' or 'tinnitus' during or after therapy with an injectable agent in association with a normal audiogram or no deterioration in audiograms performed within a month of starting and at the time or after the onset of symptoms.	
	A clinical report of new 'hearing impairment' or 'tinnitus' during or after therapy with an injectable agent in association with a significant deterioration (ASHA criteria) between an audiogram performed before or during therapy and one performed later during therapy or after completing therapy above 8khz range.		
Worsening ototoxicity after stopping injectable agent	A significant deterioration (as determined by ASHA criteria) between an audiogram performed 30 days or more after the end of injectable therapy to one performed in the month before the end of therapy or on the stop date.	No significant deterioration (as determined by ASHA criteria) between an audiogram performed 30 days or more after the end of injectable therapy to one performed in the month before the end of therapy or on the stop date.	Any case not fitting either of the definitions.

PTA= pure tone audiometry, Normal audiogram=all frequencies better than 25 dB, abnormal audiogram = ASHA criteria, ASHA=American speech and Hearing Association. *based on definitions of hearing loss proposed by Seddon et al 2012[9]

Table 2: Background characteristics and demographics of patients (n=93)

Characteristic		Number (% unless otherwise indicated)
Median age in months (IQR*) (n=93)		28 (24-38)
Male gender (n=93)		64 (68)
HIV infected (n=93)		5 (5)
Country of birth (n=93)	UK	9 (10)
	Western and Northern Europe other	1 (1)
	Chinese subcontinent	10 (10)
	Indian subcontinent	36 (38)
	Africa	15 (16)
	Eastern Europe + Russia	22 (24)
Type of TB (n=93)	MDR-TB	73 (78)
	MDR-TB +FLQr**	12 (13)
	XDR-TB	8 (9)
Location of TB (n=93)	Pulmonary	41 (44)
	Extra-pulmonary only	31 (33)
	Both pulmonary and extra-pulmonary	21 (23)
Injectable agent (n=93)	Capreomycin	31 (33)
	Amikacin	43 (46)
	Amikacin and capreomycin (sequentially, either order)	18 (19)
	Amikacin followed by streptomycin	1 (1)
Baseline creatinine µmol/L (n=87)(IQR)		66 (58-75)
Creatinine clearance (n=81, median/IQR)		116.2 (75.7, 179.1)
Median initial dose of injectable agent (mg/kg) (n=82) (IQR)		14.81 (14.06-16.13)
Median number of Amikacin troughs/week (those on amikacin) (n=58) (IQR)		1.01 (0.76-1.29)

*IQR-interquartile range, FLQr=fluroquinolone resistance.

505 **Table 3: Multivariable (adjusted) analysis investigating the predictors of hearing loss (composite)**

VARIABLES		Hearing loss (%) n=34 (38%)	No hearing loss (%) n=56 (62%)	Univariate analysis		Multivariable analysis	
				Hazard ratio	p	Hazard ratio	p
Choice at start:	Amikacin (n=53) versus Capreomycin (n=37)	29 (55) / 5* (14)	24 (45) / 32 (86)	5.80(2.23-15.04)	<0.001		
Grouping	Starting amikacin (n=53) versus Capreomycin only (n=30)	29 (55) / 2 (7)	24 (45) / 28 (93)	11.70 (2.78-49.20)	0.001	13.85 (3.25-58.99)	<0.001
	Capreomycin followed by amikacin (n=7) versus Capreomycin only (n=30)	3 (43) / 2 (7)	4 (57) / 28 (93)	6.29 (1.05-37.65)	0.044	4.03 (0.66-24.63)	0.13
	Starting amikacin (n=53) versus Capreomycin followed by amikacin (n=7)	29 (55) / 3 (43)	24 (45) / 4 (57)	1.86 (0.56-6.13)	0.307	3.44 (0.97-12.18)	0.06
MDR-TB Type	MDR+ FLQ-TB (n=12) versus MDR-TB (n=70)	8 (67) / 22 (31)	4 (33) / 48 (69)	3.26(1.44-7.36)	0.005		
	XDR-TB (n=8) versus MDR TB (n=70)	4 (50) / 22 (31)	4 (50) / 48 (69)	1.62 (0.55-4.73)	0.378		
	XDR-TB (n=8) versus MDR+FLQ-TB (n=12)	4 (50) / 8 (67)	4 (50) / 4 (33)	0.55 (0.17-1.83)	0.331		
	FLQ resistance (n=20) versus MDR TB (n=70)	12 (60) / 22 (31)	8 (40) / 48 (69)	2.43 (1.20-4.93)	0.013	3.15(1.45-6.88)	0.004
Median dose of injectable at start (mg/kg) (IQR)		14.58 (13.82-15.51)	14.94 (14.07-16.63)	0.84 (0.71-1.00)	0.047		
Median creatinine baseline µmol/L (log scale)		4.25(4.13, 4.30)	4.17 (4.04, 4.32)	4.37 (1.12-17.11)	0.034		
Median creatinine clearance		114.1 (99.5, 122.9)	119.3 (105.8-134.5)	0.98 (0.97, 1.00)	0.055	0.99 (0.97-1.00)	0.11
Median Age (1 year effect) (IQR)		28.5 (25-39)	27.5 (22.5-33.5)	1.03 (0.99-1.07)	0.127		

506 *Only two of these cases occurred on capreomycin. The other three occurred on amikacin after they

507 had been switched off capreomycin for other reasons. 2 had normal pure tone audiograms (PTA) at

508 the start of amikacin.

509

510

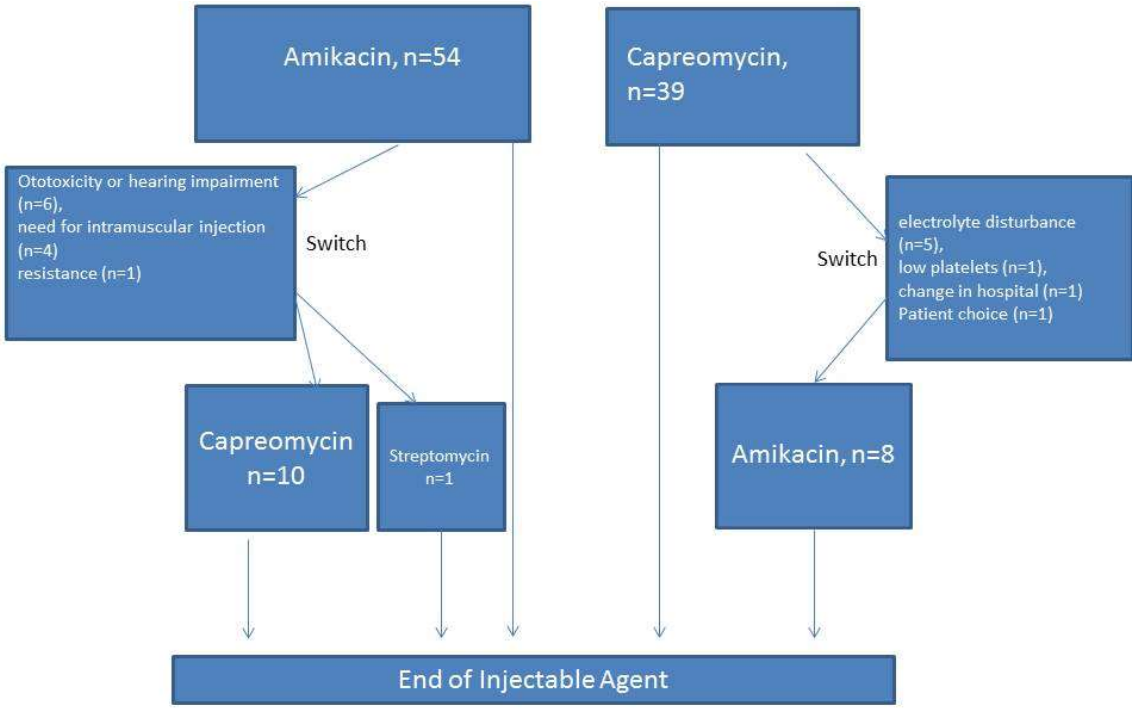
511

512 **Table 4: Multivariable model for creatinine rise to over 1.5 times baseline and**
513 **hypokalaemia**

	MV model for cratinine rise > 1.5x baseline		MV model for hypokalaemia	
variable	Odds Ratios	P value	Odds ratios	P value
Creatinine baseline	1.02 (0.99-1.05)	0.145		
Amikacin verses capreomycin at start	0.44 (0.14-1.45)	0.178	0.28 (0.11-0.72)	0.008
Total duration (30 days effect)	1.15 (1.02-1.32)	0.040	1.00 (0.91-1.08)	0.869

514

515



516

517

518 **Figure 1: Flow diagram showing injectable agent use in cohort**

519

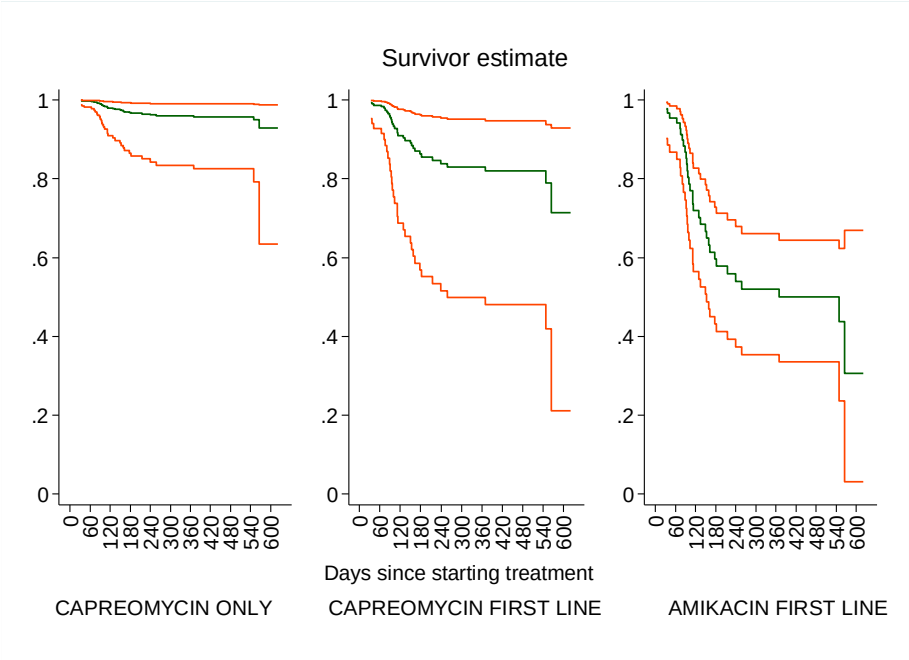


Figure 2: Predicted proportion surviving without hearing loss by initial choice of injectable agent. Middle line (black) represents the predicted proportion and outer lines represent 95% confidence intervals (red).