Primary Capreomycin Resistance Is Common and Associated With Early Mortality in Patients With Extensively Drug-Resistant Tuberculosis in KwaZulu-Natal, South Africa

Max R. O'Donnell, MD, MPH,†‡ Melendhran Pillay, BMedSc (Hons),§ Manormoney Pillay, PhD,‖ Lise Werner, MSc,‡ Iqbal Master, MBChB,¶ Allison Wolf, MPH,* Barun Mathema, PhD,† Yacoob M. Coovadia, MBcHB, FF Path,§‖ Koleka Mlisana, MBChB,§‖ Charles Robert Horsburgh, MD, MUS,# and Nesri Padayatchi, MD, MSc†*

Background: Capreomycin is a key antimycobacterial drug in treatment of extensively drug-resistant tuberculosis (XDR-TB). Drug-susceptibility testing (DST) for capreomycin is not routinely performed in newly diagnosed XDR-TB in South Africa. We performed this study to assess the prevalence, clinical significance, and molecular epidemiology of capreomycin resistance in newly diagnosed patients with XDR-TB in KwaZulu-Natal, South Africa.

Methods: Retrospective cohort study of consecutive patients with XDR-TB admitted to a TB referral hospital without previous XDR-TB treatment. A subset of isolates had extended DST (including capreomycin), mutational analysis, and IS6110 restriction fragment length polymorphism assays.

Results: A total of 216 eligible patients with XDR-TB were identified. The majority were treated with capreomycin (72%), were young (median age: 35.5 years), and were female (56%). One hundred five (76%) were HIV+, and 109 (66%) were on antiretroviral therapy. A subset of 52 patients had full DST. A total of 47/52 (90.4%) patients with XDR-TB were capreomycin resistant. Capreomycin-resistant patients experienced worse mortality and culture conversion than capreomycin susceptible, although this difference was not statistically significant. The A1401G mutation in the *rrs* gene was associated with capreomycin resistance. The majority of capreomycin-resistant strains were F15/LAM4/KZN lineage (80%), and clustering was common in these isolates (92.5%).

Conclusions: Capreomycin resistance is common in patients with XDR-TB in KwaZulu-Natal, is predominantly because of ongoing province-wide transmission of a highly resistant strain, and is associated with high mortality. Capreomycin should be included in routine DST in all patients with XDR-TB. New drug regimens that do not include injectable agents should be operationally tested as empiric treatment in XDR-TB.

Key Words: extensively drug-resistant tuberculosis, capreomycin resistance, South Africa, drug-susceptibility testing

(*J Acquir Immune Defic Syndr* 2015;69:536–543)

INTRODUCTION

Drug-resistant tuberculosis exacerbated by endemic HIV has been well described in KwaZulu-Natal, South Africa.[1–5] Extensively drug-resistant tuberculosis (XDR-TB) is the most drug-resistant form of TB and is defined as *Mycobacterium tuberculosis* (MTB) resistant to isoniazid, rifampicin, any fluoroquinolone drug, and at least one of the 3 second-line injectable agents (kanamycin, amikacin, and capreomycin).[6] A hospital-based outbreak of XDR-TB and HIV in Tugela Ferry, KwaZulu-Natal, in 2005 attracted significant global attention to this syndemic.[7]

A key component of the current XDR-TB treatment regimen is capreomycin, an injectable antimycobacterial agent in the cyclic peptide class. Globally, capreomycin is included in XDR-TB treatment regimens for its excellent bactericidal activity and lack of availability of alternative bactericidal agents for densely drug-resistant MTB. Since 2006, in KwaZulu-Natal, South Africa, capreomycin has been available and is restricted for treatment of XDR-TB. Cross-resistance between aminoglycosides and capreomycin associated with polymorphisms within *rrs* gene has been

Received for publication October 23, 2014; accepted February 27, 2015.

From the *Department of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine, Columbia University College of Physicians and Surgeons, New York, NY; †Department of Epidemiology, Columbia Mailman School of Public Health, New York, NY; ‡Centre for AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa; §Department of Microbiology, National Health Laboratory Service (NHLS), Durban, South Africa; ‖Department of Medical Microbiology, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa; ¶DR-TB Department, King Dinuzulu Hospital, Department of Health, Sydenham, South Africa; and #Department of Epidemiology, Boston University School of Public Health, Boston, MA.

Supported by a career development award from the Albert Einstein College of Medicine/Montefiore Medical Center Institute for Translational Research; by the Stony-Wold Herbert Foundation; and the Albert Einstein Center for Global Health to M.R.O. This work was also supported by the Centre for AIDS Programme of Research (CAPRISA), which was established by the National Institutes of Health and US Department of Health and Human Services (Grant A1069469) to M.R.O. and N.P.

The authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

Correspondence to: Max R. O'Donnell, MD, MPH, Columbia University College of Physicians and Surgeons, PH-8 East, Room 101, 622 West 168th Street, New York, NY 10032 (e-mail: mo2130@columbia.edu).

TABLE 1. Demographic Characteristics of Eligible Patients With XDR-TB Admitted to King DinuZulu Hospital During the Study Period (N = 216) and the Subset With Extended DST and Genotyping (N = 52)

	All Patients With XDR-TB, N = 216 (%)	Patients With XDR-TB With Isolates for DST and Genotyping, N = 52 (%)	Patients With XDR-TB Without Isolates for DST and Genotyping, N = 164 (%)	*P* Patients With DST vs. No DST
Sex				
Male	95 (44)	26 (50)	69 (42)	0.32
Female	121 (56)	26 (50)	95 (58)	
Age, yrs				
≤35	108 (50)	24 (46)	84 (51)	0.52
>35	108 (50)	28 (54)	80 (49)	
Median age	35.5	36.5		
Body mass index				
Obtained	99 (46)	25 (48)	74 (45)	0.71
Not obtained	117 (54)	27 (52)	90 (55)	
Median (IQR)	19 (16–23)	18 (16–23)		
HIV status				
Infected	165 (77)	44 (84)	121 (74)	0.19
Uninfected	32 (14)	5 (10)	27 (16)	
Unknown	19 (8)	3 (6)	16 (10)	
CD4 T-cell* count, cells/mm^3				
Known	93 (56)	25 (57)	68 (56)	0.40
Not determined	72 (44)	19 (43)	53 (44)	
Median (IQR)	195 (105–302)	192 (112–234)		
ARV*				
Yes	114 (69)	31 (70)	83 (69)	0.82
No	51 (31)	13 (30)	38 (31)	
Previous TB treatment				
Yes	207 (96)	51 (98)	156 (95)	0.37
No	9 (4)	1 (2)	8 (5)	
Initial AFB smear				
Positive	108 (50)	30 (58)	78 (47)	0.38
Negative	98 (45)	22 (42)	76 (46)	
Unknown	10 (5)	0	10 (6)	
Health care worker				
Yes	16 (7)	3 (6)	13 (8)	0.61
No	200 (93)	49 (94)	151 (92)	

*Among HIV infected.

described, but the population-level prevalence of capreomycin resistance is unknown.[8,9]

Drug-susceptibility testing (DST) is an essential aspect of the management of drug-resistant TB. However, full DST to second-line antimycobacterial agents is not routinely performed globally even in patients with known multidrug-resistant tuberculosis (MDR-TB).[10] Until recently, capreomycin DST was not routinely performed in KwaZulu-Natal outside the research setting because of resource constraints. Current World Health Organization guidelines on treatment of XDR-TB recommend that in the setting of resistance to aminoglycosides to use an injectable agent, which the patient has not used before since clinical data on the efficacy of DST is limited.

We performed this study to determine the prevalence of capreomycin resistance in patients with XDR-TB before treatment with capreomycin-containing TB regimens in KwaZulu-Natal, South Africa, to elucidate the mechanism of capreomycin drug resistance and understand the clinical implications of capreomycin resistance for patients with XDR-TB.

METHODS

Clinical

We performed a retrospective cohort study of all newly diagnosed, microbiologically confirmed, adult patients with XDR-TB admitted from January 2008 to September 2010 in a public TB referral hospital in KZN. Eligible patients were adults with microbiologically confirmed XDR-TB by DST without previous therapy for XDR-TB. Demographics and clinical data were collected by retrospective chart review. TB culture results and routine first- and second-line

drug-susceptibility results were collected retrospectively through the clinical laboratory system. Treatment regimen was determined by the attending physician. The standard of treatment for XDR-TB was individualized therapy according to DST and patient tolerance. TB sputum culture conversion was defined as having >2 negative consecutive sputum cultures 30 days apart after initiation of treatment. Mortality was defined as all-cause mortality.

All eligible patients in the study were included in an analysis of risk factors for survival and culture conversion. Proportional hazards regression analysis was performed to assess factors associated with death and culture conversion. Cox proportional hazards were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) at 6 months after initiation of XDR-TB treatment. Significant variables or variables that caused >10% change in the univariate HR were included in the multivariate model. We calculated 95% CIs using a normal approximation of the binomial distribution. The Fisher exact test or χ^2 test was used to compare categorical variables. Kaplan–Meier survival curves for death and for time to culture conversion were calculated using standard techniques from time of XDR-TB treatment initiation with appropriate anti-TB drugs. Time to culture conversion and mortality were censored at 6 months because long-term follow-up data are not available. Statistical analysis was performed using SAS version 9.2 software (SAS Institute, Cary, NC).

Microbiology

MTB isolates were sought for all patients from a time before initiation of XDR-TB treatment. If isolates before treatment were not available, we included isolates up to 3 months after initiation of treatment. These isolates were subcultured from stocks from the regional TB laboratory. Full DST including capreomycin testing was performed using the 1% proportional method on Middlebrook 7H11 agar using standard antibiotic concentrations (10 μg/mL cut point). Extended DST to determine moxifloxacin minimum inhibitory concentration (MIC) using clinically relevant cut points (0.125, 0.25, 0.5, 1, 2, 4, and 8 μg/mL) was also performed.[11,12] A commercial kit (Geno Type MTBDRsl; Hain Lifescience, GmbH, Neheren, Germany) was used to probe for known resistance conferring mutations in the *rrs* gene.

Genotyping

Single MTB isolates of patients with capreomycin-resistant XDR-TB were genotyped by performing IS6110 restriction fragment length polymorphism (RFLP).[13,14] Briefly, cetyltrimethylammonium bromide (CTAB)-extracted genomic DNA was restricted with the restriction endonuclease Pvu*II*, separated in a 1% agarose gel and immobilized onto a Hybond-N+ nylon membrane (Amersham). IS6110-fragments were hybridized and detected using enhanced chemiluminescence (Amersham). Banding patterns were analyzed with BioNumerics version 6.6 (Applied Maths). Isolates were considered clustered if they had greater than 3 bands identical without discordant bands.

Ethics approval was obtained through the Boston University Medical Center Institutional Review Board and the Biomedical Research Ethics Committee of the University of KwaZulu-Natal.

RESULTS

We identified 216 adult patients during the study period with bacteriologically confirmed TB, DST consistent with XDR-TB, who had not undergone previous treatment for XDR-TB (Table 1). Patients were predominantly female (56%), with a median age of 35.5 years, and TB treatment experienced (96% previously treated for TB). Eighty-four percent (165/197) of patients with a known HIV status were HIV coinfected. Forty-five percent of all patients (98/216) were acid-fast bacilli (AFB) smear negative at treatment start. The majority of HIV-coinfected patients with XDR-TB (65%) were on antiretroviral therapy, and median CD4 T-cell count was 195 [interquartile range (IQR), 105–302] at commencement of XDR-TB treatment. Patients were tested for resistance to 6 drugs (kanamycin, ofloxacin, isoniazid, rifampicin, streptomycin, and ethambutol) and on average were resistant to 5.7 drugs. Capreomycin-susceptibility testing was not

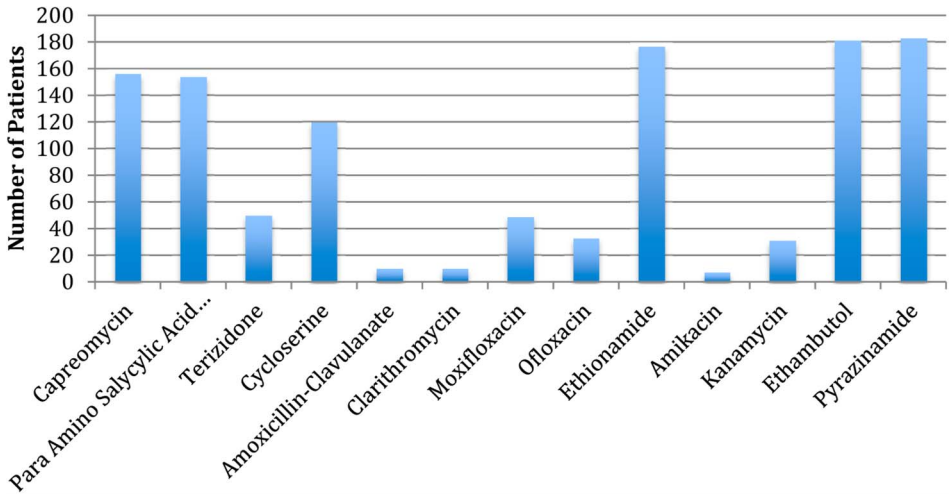


FIGURE 1. Initial XDR-TB treatment regimen for patients with XDR-TB on treatment (N = 216).

performed on these isolates, as it was not clinically available during the study period. Patients were started on XDR-TB treatment regimens that included a median of 5 drugs (Fig. 1). Capreomycin (72%) and para-aminosalicylic acid (73%) were components of the XDR-TB treatment regimen for most of the patients. Treatment strategy was determined by the resident physician, and 60/216 (28%) did not receive capreomycin. Among those who did not receive capreomycin as part of their regimen, 25/60 received an aminoglycoside and 25/60 received an alternative fluoroquinolone (moxifloxacin or ofloxacin).

During the first 6 months of treatment, 49/216 (22.7%) patients died and 21.6% converted their sputum TB culture to negative. On multivariate analysis, the presence of moxifloxacin in the initial treatment regimen was associated with improved survival [HR, 0.13 (95% CI: 0.031 to 0.54)] in the first 6 months of treatment (Table 2). Older age was significantly associated with increased mortality [HR, 1.18 (95% CI: 1.02 to 1.37) per every 5 years of increased age]. There were no factors independently associated with 6-month culture conversion (data not shown).

Fifty-two XDR-TB isolates (52/216, 24%) were identified and found to be viable for further testing. MIC testing for capreomycin was performed using agar dilution method on 52 clinical isolates. Forty-seven (90%) patients with XDR-TB were capreomycin resistant. A total of 37/52 specimens were collected before initiation of XDR-TB treatment. Of the 37 samples collected before XDR treatment, 94% were capreomycin resistant. Of the 15 samples collected after initiation of XDR-TB treatment, 80% were capreomycin resistant. This difference was not statistically significant (P = 0.13). Samples collected after XDR treatment were collected mean 72 days after initiation (SD 45.5 days). Of note, 4/52 patients had been treated for MDR-TB with aminoglycosides previously. The Geno Type MTBDRsl assay codons 1401, 1402, and 1484 of the *rrs* gene were analyzed. Mutations tested for included A1401G, C1402T, and G1484T. Forty-seven (100%) of the capreomycin-resistant isolates harbored the *rrs* A1401G mutation. None of the 5 capreomycin-susceptible isolates had this mutation. Neither C1402T nor G1484T mutations were detected in any isolate. Despite the fact that all XDR-TB isolates were resistant to ofloxacin, 47% had MICs of less than 2 μg/mL for moxifloxacin (the proposed "high" critical concentration for moxifoxacin drug susceptibility) and 27% had MICs of 0.50 μg/mL or less (the proposed "low" critical value) (see Figure S1, Supplemental Digital Content, http://links.lww.com/QAI/A672).

Patients with capreomycin-resistant isolates (N = 47) had lower rates of 6-month TB culture conversion (25% vs. 60%) and higher mortality (20% vs. 0%) compared with patients with capreomycin-susceptible isolates, but this difference was not statistically significant (P = 0.35 and P = 0.0573, respectively) (Figs. 2A, B).

Of the 47 capreomycin-resistant isolates, 44 (83%) were successfully genotyped using RFLP; no RFLP fingerprint patterns were obtained for 4 isolates despite DNA being present. Five different strain types were identified among the remaining 40 isolates (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A672). The F15/LAM4/KZN genotype, which was identified in 35/40 (87.5%) patients, could be divided into 4 subclusters separated by copy number of a few IS6110 elements. None of the patients was infected with a Beijing strain.

TABLE 2. Risk Factors for Mortality Among Patients With XDR-TB on Treatment 6 Months After Initiation of Treatment

		Univariate Analysis		Multivariate Analysis	
Variable	**Mortality, % (n/N)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Capreomycin					
No	18.3 (11/60)	1.00 (ref)	—	1.00 (ref)	—
Yes	24.4 (38/156)	1.39 (0.71 to 2.72)	0.3387	1.68 (0.83 to 3.41)	0.1486
Moxifloxacin					
Yes	4.1 (2/49)	0.127 (0.032 to 0.54)	0.0049	0.13 (0.031 to 0.54)	0.0051
No	28.1 (47/167)	1.00 (ref)	—	1.00 (ref)	—
Gender					
Male	16.8 (16/95)	1.00 (ref)	—	1.00 (ref)	—
Female	27.3 (33/121)	1.70 (0.94 to 3.09)	0.0806	1.83 (0.96 to 3.49)	0.0675
Age (by 5-yr increase)		1.12 (0.97 to 1.29)	0.1153	1.18 (1.02 to 1.37)	0.0310
Previously treated for TB					
Yes	22.2 (46/207)	1.00 (ref)	—	1.00 (ref)	—
No	28.6 (2/7)	1.25 (0.30 to 5.14)	0.7589	0.97 (0.23 to 4.03)	0.9629
HIV status					
Negative	15.6 (5/32)	1.00 (ref)	—	1.00 (ref)	—
Positive	23.6 (39/165)	1.55 (0.61 to 3.94)	0.3535	1.85 (0.65 to 5.26)	0.2471
Unknown	26.3 (5/19)	1.73 (0.50 to 5.97)	0.3877	3.63 (0.94 to 14.09)	0.0623
ARV (HIV+ only)					
Yes	22.0 (24/109)	1.00 (ref)	—		
No	26.8 (15/56)	1.23 (0.65 to 2.35)	0.5264		

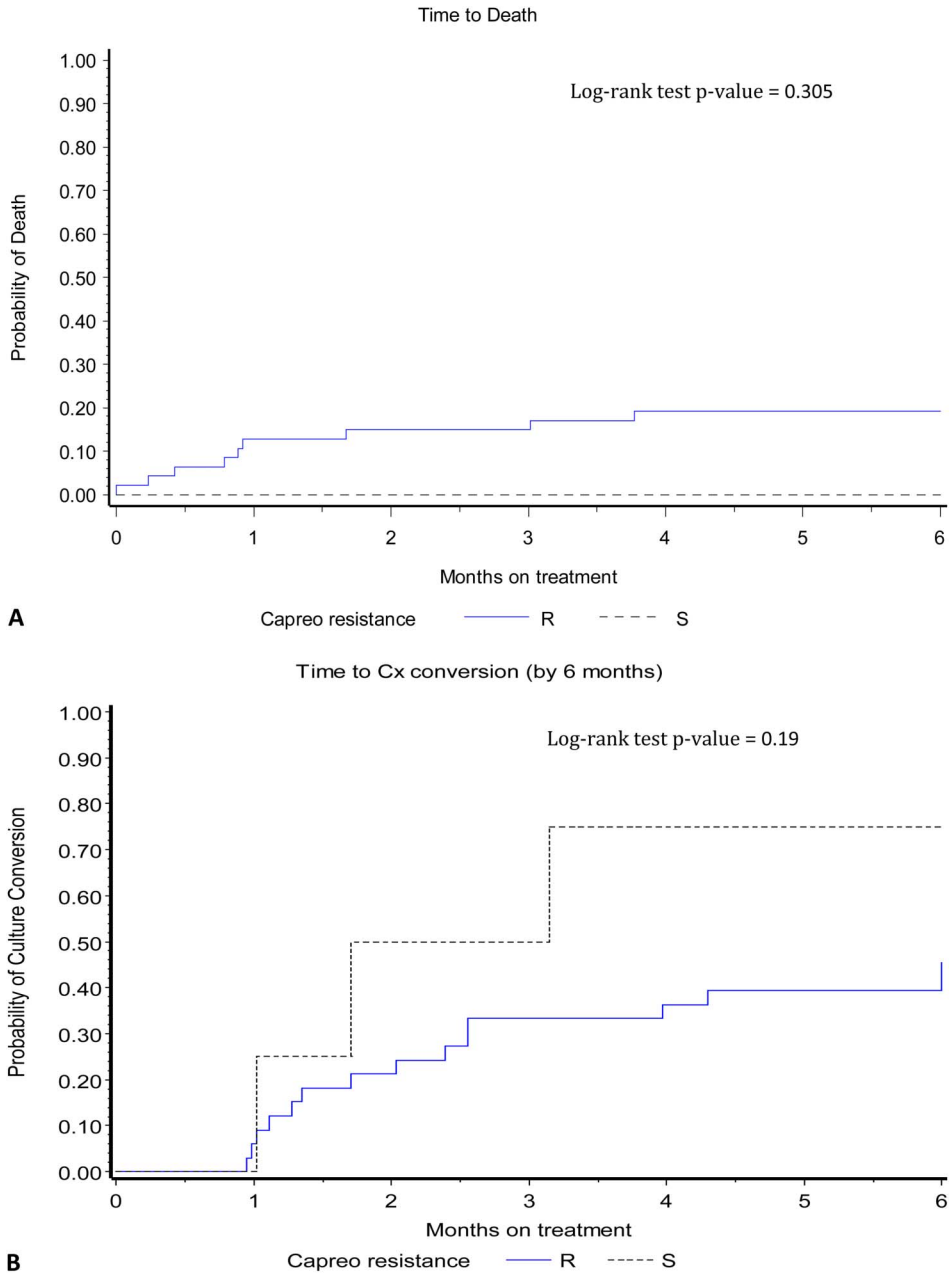
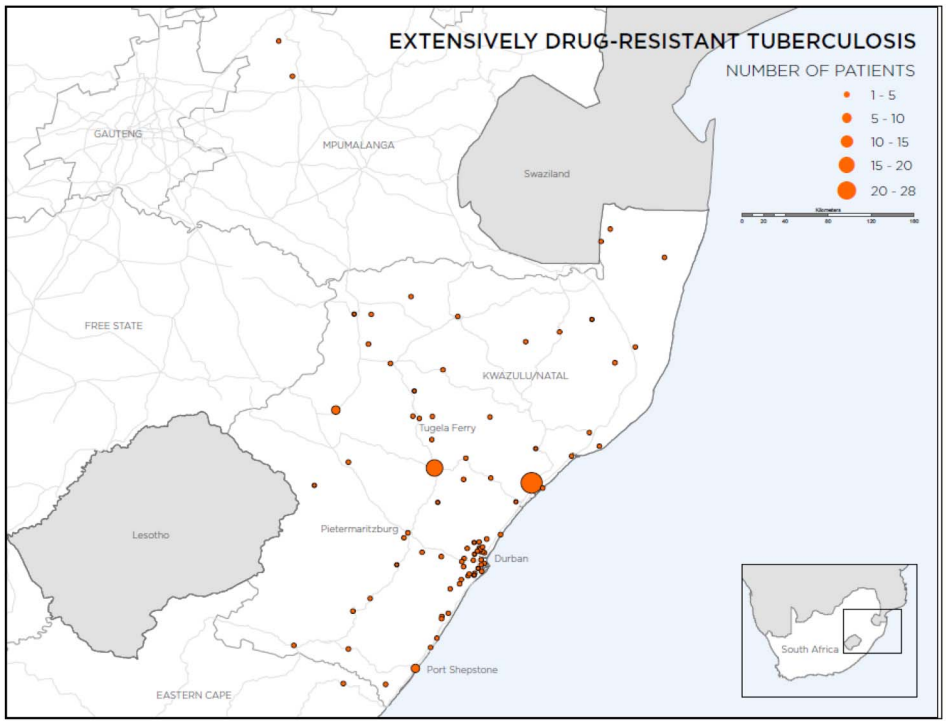


FIGURE 2. A, Kaplan–Meier mortality among patients with XDR-TB with full DST (N = 52) stratified by capreomycin resistance. B, Kaplan–Meier time to culture conversion among patients with XDR-TB with full DST (N = 52) stratified.

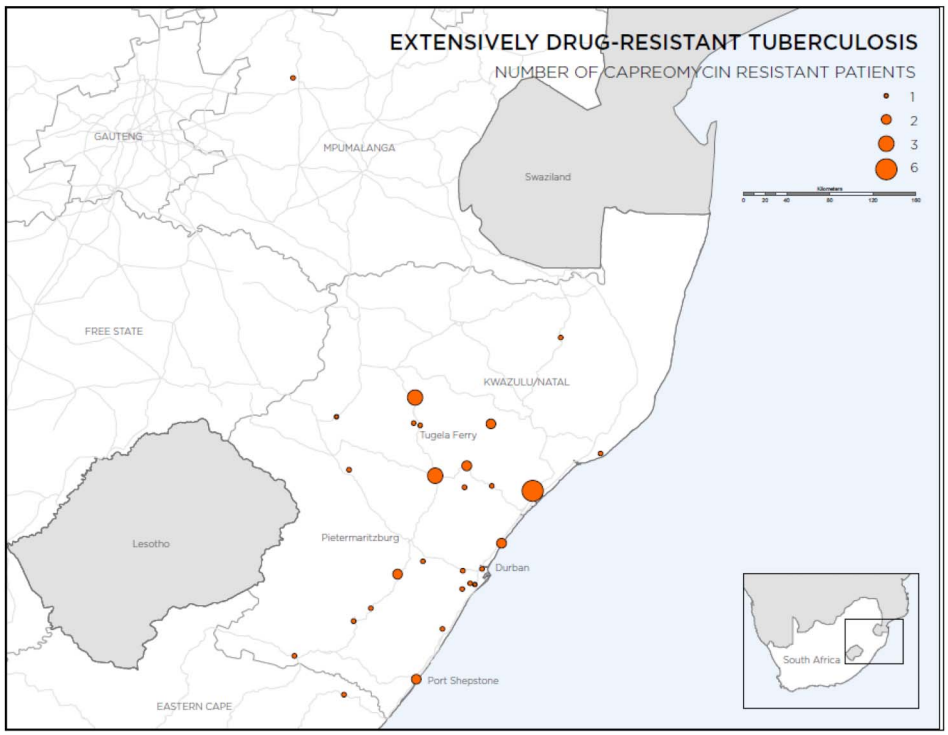
XDR-TB cases were mapped according to the home address of each patient. The initial reported XDR-TB outbreak was considered to be a point source outbreak in the Msinga subhealth district (Tugela Ferry). However, in our cohort, patients reported home address in all 11 provincial health districts. In terms of RFLP genotyping, the F15/LAM4/KZN genotype was predominant and widespread (Figs. 3A, B).

CONCLUSIONS

Our study highlights several important findings. A high percentage (90%) of newly diagnosed patients with XDR-TB in KwaZulu-Natal were found to have primary capreomycin resistance (ie, MTB isolates resistant to capreomycin, either before, or soon after first exposure to a capreomycin-containing regimen). Capreomycin resistance was widespread with cases in all 11 provincial health districts. All capreomycin-resistant isolates characterized in this cohort had the same A1401G mutation in *rrs* gene, a mutation known to confer capreomycin resistance (and cross-resistance to aminoglycosides), whereas no susceptible MTB isolates had this mutation. Capreomycin-resistant XDR-TB isolates were predominantly (80%) of the same MTB lineage (F15/LAM4/KZN genotype).[15] Although most of the samples were in clusters (92.5%) by RFLP, there did not seem to be clustering at the patient's home locations, unlike the original Tugela Ferry outbreak.[7] Although the



A



B

FIGURE 3. Geographic distribution of the origin of XDR-TB cases in KwaZulu-Natal, South Africa. A, Distribution of all XDR-TB cases by home address (N = 210). B, Distribution of capreomycin-resistant XDR-TB cases by home address (N = 47).

majority were previously treated for TB (92%), few (8%) had documented previous treatment with aminoglycosides, making acquired capreomycin resistance in the setting of cross-resistance with aminoglycosides unlikely. Taken together, these data suggest that capreomycin resistance is predominantly because of primary transmission of a highly drug-resistant strain of MTB in KwaZulu-Natal.[15–18] This hypothesis needs further confirmation in a population-based study of drug-resistant TB using whole-genome sequencing.

Predictably, patients with capreomycin-resistant XDR-TB experienced poor treatment outcomes with lower rates of TB culture conversion and higher mortality than patients with capreomycin-susceptible strains through 6 months. On multivariate analysis, moxifloxacin was associated with improved early mortality. When we tested a subset of the MTB isolates for susceptibility to moxifloxacin, 27% were moxifloxacin susceptible (based on World Health Organization recommended critical concentration) despite being resistant to ofloxacin in standard testing.[12] This suggests that the mortality benefit seen with moxifloxacin treatment in this cohort may be due to partial or complete susceptibility to moxifloxacin in a subset of patients with XDR-TB in the context of near-universal capreomycin resistance.

A recent study of patients with XDR-TB in the Tugela Ferry area identified a high proportion of capreomycin resistance (89.5%), but only 19 isolates were tested and treatment outcomes were not reported.[18] All patients were capreomycin resistant before treatment with either an amikacin or capreomycin suggesting primary transmission of a capreomycin-resistant strain. Our study demonstrates that this phenomenon is considerably more widespread than previously believed. A European study identified capreomycin resistance as an independent risk factor for poor outcome in MDR-TB patients, but it was not clear how patients were treated, whether resistance was primary or secondary, or the molecular mechanism of resistance.[19]

One study of XDR-TB treatment in South Africa identified moxifloxacin as significantly associated with survival in the first 12 months of treatment[20]; however, this survival benefit was not seen with prolonged follow-up.[6] Moxifloxacin has been recommended by experts for treatment of XDR-TB despite flouroquinolone resistance,[21] and a meta-analysis of XDR-TB treatment supported the inclusion of later generation flouroquinolones in XDR-TB treatment.[22] Laboratory studies have been inconclusive regarding the degree of cross-resistance between early and later generation fluoroquinolones, although mouse studies support the use of moxifloxacin in the treatment of XDR-TB. The clinical benefit of later generation fluoroquinolones in isolates, which are resistant to earlier generation quinolones, is poorly characterized.[23]

Our study has several limitations to generalizability including having only a subset of isolates for extended DST and molecular testing. We attempted to retrieve all available MTB isolates from an operational provincial TB laboratory, but only 24% were located and culturable. Another limitation is that the small number of patients with capreomycin-susceptible XDR-TB (N = 5) means that we have insufficient power to show a difference between the groups. Since complete genomic sequencing was not performed, it is possible that the *rrs* mutation reported was not the only contributor to capreomycin resistance. It is possible that with whole-genome sequencing, we could further discriminate the genotypic clustering seen on RFLP, or that with a larger sample other patterns of RFLP clustering would emerge. Finally, because we did not have access to long-term follow-up data, the 6-month outcome results obtained may not reflect treatment outcomes at 24 months, or end of treatment relapse free survival.

In conclusion, primary capreomycin-resistant XDR-TB in KwaZulu-Natal, South Africa, is common, geographically widespread, associated with RFLP clustering, is predominantly of a single MTB lineage, and seems to be associated with poor 6-month culture conversion and survival. Full first- and second-line DST, including capreomycin susceptibility, should be included in routine DST of all patients with XDR-TB. New drug regimens, which do not depend on capreomycin, should be operationally tested urgently as empiric treatment in XDR-TB.

REFERENCES

1. Padayatchi N, Abdool Karim SS, Naidoo K, et al. Improved survival in multidrug-resistant tuberculosis patients receiving integrated tuberculosis and antiretroviral treatment in the SAPiT trial. *Int J Tuberc Lung Dis.* 2014;18:147–154.
2. Loveday M, Padayatchi N, Voce A, et al. The treatment journey of a patient with multidrug-resistant tuberculosis in South Africa: is it patient-centred? *Int J Tuberc Lung Dis.* 2013;17:56–59.
3. O'Donnell MR, Padayatchi N, Kvasnovsky C, et al. Treatment outcomes for extensively drug-resistant tuberculosis and HIV co-infection. *Emerg Infect Dis.* 2013;19:416–424.
4. Brust JC, Shah NS, Scott M, et al. Integrated, home-based treatment for MDR-TB and HIV in rural South Africa: an alternate model of care. *Int J Tuberc Lung Dis.* 2012;16:998–1004.
5. Cooke GS, Beaton RK, Lessells RJ, et al. International spread of MDR TB from tugela ferry, South Africa. *Emerg Infect Dis.* 2011;17:2035–2037.
6. Pietersen E, Ignatius E, Streicher EM, et al. Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study. *Lancet*. 2014;383:1230–1239.
7. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet*. 2006;368:1575–1580.
8. Rodwell TC, Valafar F, Douglas J, et al. Predicting extensively drug-resistant Mycobacterium tuberculosis phenotypes with genetic mutations. *J Clin Microbiol.* 2014;52:781–789.
9. Sirgel FA, Tait M, Warren RM, et al. Mutations in the rrs A1401G gene and phenotypic resistance to amikacin and capreomycin in Mycobacterium tuberculosis. *Microb Drug Resist.* 2012;18:193–197.
10. Dowdy DW, O'Brien MA, Bishai D. Cost-effectiveness of novel diagnostic tools for the diagnosis of tuberculosis. *Int J Tuberc Lung Dis.* 2008;12:1021–1029.
11. Sirgel FA, Warren RM, Streicher EM, et al. gyrA mutations and phenotypic susceptibility levels to ofloxacin and moxifloxacin in clinical isolates of Mycobacterium tuberculosis. *J Antimicrobial Chemother.* 2012;67:1088–1093.
12. van Ingen J, Simons S, de Zwaan R, et al. Comparative study on genotypic and phenotypic second-line drug resistance testing of Mycobacterium tuberculosis complex isolates. *J Clin Microbiol.* 2010;48:2749–2753.
13. van Embden JD, Cave MD, Crawford JT, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol.* 1993;31:406–409.
14. van Soolingen D, Borgdorff MW, de Haas PE, et al. Molecular epidemiology of tuberculosis in the Netherlands: a nationwide study from 1993 through 1997. *J Infect Dis.* 1999;180:726–736.
15. Gandhi NR, Brust JC, Moodley P, et al. Minimal diversity of drug-resistant Mycobacterium tuberculosis strains, South Africa. *Emerg Infect Dis.* 2014;20:426–433.
16. Moodley P, Shah NS, Tayob N, et al. Spread of extensively drug-resistant tuberculosis in KwaZulu-Natal province, South Africa. *PLoS One*. 2011;6:e17513.
17. Pillay M, Sturm AW. Evolution of the extensively drug-resistant F15/LAM4/KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa. *Clin Infect Dis.* 2007;45:1409–1414.
18. Shah NS, Richardson J, Moodley P, et al. Increasing drug resistance in extensively drug-resistant tuberculosis, South Africa. *Emerg Infect Dis.* 2011;17:510–513.

19. Migliori GB, Lange C, Centis R, et al. Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drug-resistant tuberculosis cases. *Eur Respir J.* 2008;31:1155–1159.
20. Dheda K, Shean K, Zumla A, et al. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. *Lancet*. 2010;375: 1798–1807.
21. Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis. World health Organization, 2014. Available at: http://www.who.int/tb/publications/pmdt_companionhandbook/en/http://www.who.int/tb/publications/pmdt_companionhandbook/en/. Accessed February 9, 2015.
22. Jacobson KR, Tierney DB, Jeon CY, et al. Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. *Clin Infect Dis.* 2010;51:6–14.
23. Dookie N, Sturm AW, Moodley P. Moxifloxacin resistance in the F15/LAM4/KZN extensively drug-resistant strain of Mycobacterium tuberculosis. *Infect Drug Resist.* 2014;7:223–228.