Assessment of epidemiological and genetic characteristics and clinical outcomes of resistance to bedaquiline in patients treated for rifampicin-resistant tuberculosis: a cross-sectional and longitudinal study

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Summary

Background: Bedaquiline improves outcomes of patients with rifampicin-resistant and multidrug-resistant (MDR) tuberculosis; however, emerging resistance threatens this success. We did a cross-sectional and longitudinal analysis evaluating the epidemiology, genetic basis, and treatment outcomes associated with bedaquiline resistance, using data from South Africa (2015–19).

Methods: Patients with drug-resistant tuberculosis starting bedaquiline-based treatment had surveillance samples submitted at baseline, month 2, and month 6, along with demographic information. Culture-positive baseline and post-baseline isolates had phenotypic resistance determined. Eligible patients were aged 12 years or older with a positive culture sample at baseline or, if the sample was invalid or negative, a sample within 30 days of the baseline sample submitted for bedaquiline drug susceptibility testing. For the longitudinal study, the first surveillance sample had to be phenotypically susceptible to bedaquiline for inclusion. Whole-genome sequencing was done on bedaquiline-resistant isolates and a subset of bedaquiline-susceptible isolates. The National Institute for Communicable Diseases tuberculosis reference laboratory, and national tuberculosis surveillance databases were matched to the Electronic Drug-Resistant Tuberculosis Register. We assessed baseline resistance prevalence, mutations, transmission, cumulative resistance incidence, and odds ratios (ORs) associating risk factors for resistance with patient outcomes.

Findings: Between Jan 1, 2015, and July 31, 2019, 8041 patients had surveillance samples submitted, of whom 2023 were included in the cross-sectional analysis and 695 in the longitudinal analysis. Baseline bedaquiline resistance prevalence was 3·8% (76 of 2023 patients; 95% CI 2·9–4·6), and it was associated with previous exposure to bedaquiline or

clofazimine (OR 7·1, 95% CI 2·3–21·9) and with rifampicin-resistant or MDR tuberculosis with additional resistance to either fluoroquinolones or injectable drugs (pre-extensively-drug resistant [XDR] tuberculosis: 4·2, 1·7–10·5) or to both (XDR tuberculosis: 4·8, 2·0–11·7). *Rv0678* mutations were the sole genetic basis of phenotypic resistance. Baseline resistance could be attributed to previous bedaquiline or clofazimine exposure in four (5·3%) of 76 patients and to primary transmission in six (7·9%). Odds of successful treatment outcomes were lower in patients with baseline bedaquiline resistance (0·5, 0·3–1). Resistance during treatment developed in 16 (2·3%) of 695 patients, at a median of 90 days (IQR 62–195), with 12 of these 16 having pre-XDR or XDR.

Interpretation: Bedaquiline resistance was associated with poorer treatment outcomes. Rapid assessment of bedaquiline resistance, especially when patients were previously exposed to bedaquiline or clofazimine, should be prioritised at baseline or if patients remain culture-positive after 2 months of treatment. Preventing resistance by use of novel combination therapies, current treatment optimisation, and patient support is essential.

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Introduction

Bedaquiline, a novel diarylquinoline, has positively affected the outcomes of patients with drug-resistant tuberculosis, achieving a median time to culture conversion of 2 months, success in more than 70% of cases, and a three-times reduction in the adjusted hazard ratio for mortality.[1, 2, 3] A study substituting the second-line injectable antituberculosis drugs with

bedaquiline showed improved patient outcomes at 12 months with sustained culture conversion.[4] WHO classifies bedaquiline as a group A agent for multidrug-resistant (MDR) tuberculosis, and it is recommended for use in both the shorter all-oral treatment regimen and the long rifampicin-resistant tuberculosis treatment regimen.

South Africa has been an early implementer of bedaquiline and, as of March, 2020, accounted for just over half (50·7%) of the global usage. Bedaquiline was available in South Africa through a clinical access programme as of December, 2012,[5] and in 2015, it was introduced for use in routine care at specialised drug-resistant tuberculosis initiation sites following qualifying indications. A policy that guided the use of the new drugs and regimens was introduced, which required the establishment of a national surveillance programme to monitor drug resistance emergence to bedaquiline. The data generated through this programme forms the basis of this Article. Bedaquiline-based treatment was subsequently decentralised from the specialised drug-resistant tuberculosis initiation sites and, in 2018, South Africa took the bold step of introducing a patient-friendly, injection-free, all-oral, short regimen, which included bedaquiline as a core drug for most patients with drug-resistant tuberculosis. The other drugs in the regimen were linezolid (for the first 2 months of treatment), high-dose isoniazid, levofloxacin, clofazimine, pyrazinamide, and ethambutol. The detail of the changes has been previously described.[6] Exposure to bedaquiline treatment before 2015 was possible through research studies dating back to 2007 or programmatically through the access programme in 2012. Clofazimine was used for the treatment of extensively-drug resistant (XDR) tuberculosis since 2010 in South Africa.

Research in context

Evidence before this study

We searched PubMed for original research that presented results on bedaquiline and drug resistant tuberculosis published in English between Jan 1, 2012, and March 15, 2021. We searched using the terms "bedaquiline" and "resistance" and looked for studies indicating bedaquiline resistance, minimum inhibitory concentration, mutations, risk factors, and outcomes associated with bedaquiline resistance. We identified 158 articles, 53 of which were basic science, including microbiology and test methods; 45 were clinical studies on the efficacy, safety, and pharmacological aspects, including use in special populations; 23 were opinion pieces or commentaries; and 15 were reviews, resulting in 22 relevant articles. Bedaquiline resistance emergence was first described in 2015, followed by multiple case reports and series, raising concerns for the future survival of the drug. Mutations in *atpE* (target-based) and *Rv0678* (non-target efflux-based) are now well established mechanisms of resistance. Mutations in *Rv0678* are the dominant mechanism and are associated with clofazimine cross-resistance. Mutations in this gene predate the discovery of bedaquiline and are ubiquitous. They have also been identified in clones in both Africa and Asia. Baseline *Rv0678* mutations often do not lead to minimum inhibitory concentration increases above the resistant range in individuals with no bedaquiline exposure, whereas hetero-resistance at low frequency is often undetected. Patient outcomes and risk factors associated with bedaquiline resistance emergence are poorly described in the literature. A recent cohort study from China showed that patients with acquired bedaquiline resistance on treatment had worse outcomes than those with bedaquiline resistance at baseline; however, the number of patients in the study was small.

Added value of this study

Our study provides insights on bedaquiline resistance and its emergence under programmatic conditions over a 5-year period. We showed the prevalence of bedaquiline resistance to be 3·8% (76 of 2023 patients; 95% CI 2·9–4·6) and similar to that in other studies despite a rapid expansion of use during the study period. Previous exposure to bedaquiline and clofazimine, as well as baseline resistance to fluoroquinolones, were significant risk factors for bedaquiline resistance but not age, sex, HIV status, or rifampicin-resistant or multidrug resistant (MDR) tuberculosis, providing a means for risk stratification. Baseline bedaquiline phenotypic resistance was associated with poorer patient outcomes, and it was not limited to treatment-emergent resistance alone. Mutations in *Rv0678* were the sole basis of phenotypic resistance; however, isolates with *Rv0678* mutations at baseline but phenotypically susceptible were equally common and not sustained while on combination therapy when hetero-resistant variants are present at low frequency (<20%). Using a sequencing-only based approach at baseline might lead to overestimating resistance. Treatment-emergent bedaquiline resistance developed at a median of 3 months post-initiation of bedaquiline treatment, primarily in patients with additional resistance to core second-line drugs.

Implications of all the available evidence

Bedaquiline resistance, even in patients naive to rifampicin-resistant or MDR tuberculosis treatment, should be expected. Upfront use of effective novel combination therapies is essential to curtail resistance emergence. Fluoroquinolone resistance is an important risk factor and should be tested for universally. Patients re-entering care after bedaquiline exposure and those who do not have culture conversion after 2 months of treatment require comprehensive drug susceptibility testing for individualised treatment.

As the uptake of bedaquiline expanded, individual patient reports of emerging resistance were recorded globally and in South Africa. In-vitro studies showed that the *atpE* gene variants were the primary target of drug resistance emergence to bedaquiline,[7] but this has been uncommon in clinical isolates.[8]

An unexpected non-target-based mechanism of resistance emerged and has dominated low-level and high-level resistance to bedaquiline in clinical isolates. Mutations in the *mmpR* (*Rv0678)* gene encoding the MmpL5-MmpS5 efflux pump repressor have been shown to confer bedaquiline resistance and clofazimine cross-resistance.9, 10 The presence of baseline

Rv0678 mutations observed in patients not exposed to bedaquiline is concerning, and its relationship to resistance is uncertain.[11] Criteria to define phenotypic resistance were formally established by WHO in 2018 for liquid culture and agar-based methods,[12] whereas for the broth microdilution (BMD) method, criteria were proposed[13] and later confirmed in a multicountry study.[14]

In this observational study, we aimed to analyse results from the bedaquiline resistance surveillance programme in South Africa between 2015 and 2019 to determine the baseline prevalence, risk factors, and molecular characteristics of bedaquiline resistance and its effect on patient outcomes. We also aimed to evaluate the proportion of patients that develop treatment-emergent bedaquiline resistance.

Methods:

Study design and participants

All patients starting bedaquiline treatment as part of a drug-resistant tuberculosis regimen in South Africa were required to have sputum samples sent to the WHO supranational tuberculosis reference laboratory at the National Institute for Communicable Diseases (NICD) in Johannesburg, an ISO15189-accredited laboratory. Three samples were requested: a baseline sample (at bedaquiline treatment initiation), one at month 2 of treatment, and one at month 6, although only culture-positive samples could be tested phenotypically for bedaquiline resistance. Samples were sent with the following basic information included: age, sex, location, and previous bedaquiline or clofazimine exposure. Patients eligible for a bedaquiline-based treatment regimen varied over time on the basis of evolving evidence and policy changes.[11] By June, 2018, the policy extended to most patients with rifampicin-resistant

tuberculosis based on specific criteria, leading to a substantial scale-up of bedaquiline use, and the surveillance programme was reduced to limited sites for operational reasons. The evolution of bedaquiline use within rifampicin-resistant tuberculosis regimens and the number of surveillance samples received over the different periods are shown in figure 1.

Our analysis was separated into baseline cross-sectional and longitudinal studies. For the cross-sectional study, the inclusion criterion was having a positive culture sample at baseline or, if the sample was invalid or negative, a sample within 30 days of the baseline sample submitted for bedaquiline drug susceptibility testing (DST). For the longitudinal study, the inclusion criterion was similar, but the first surveillance sample had to be phenotypically susceptible to bedaquiline; additionally, patients had to have at least one post-baseline surveillance sample (irrespective of timepoint) with a culture-positive isolate for bedaquiline DST. For patients with more than one sample submitted that met the inclusion criteria, the first sample was included in the analysis. All samples from patients younger than 12 years were excluded, as these patients were not eligible for bedaquiline treatment at the time.

The project was part of the national essential surveillance programme in South Africa, and ethics approval was obtained from the University of Witwatersrand Human Research Ethic Committee (M160667).

Procedures

We cultured surveillance samples using Mycobacterium Growth Indicator Tube (MGIT) 960 system (Becton Dickinson, Franklin Lakes, NJ, USA). Culture-positive isolates confirmed to be *Mycobacterium tuberculosis* complex had bedaquiline DST done, with interpretation based on WHO criteria for MGIT and agar,[12] whereas multicountry study criteria were used for interpretation of broth microdilution assays.[14] We did additional Sanger sequencing of *atpE*

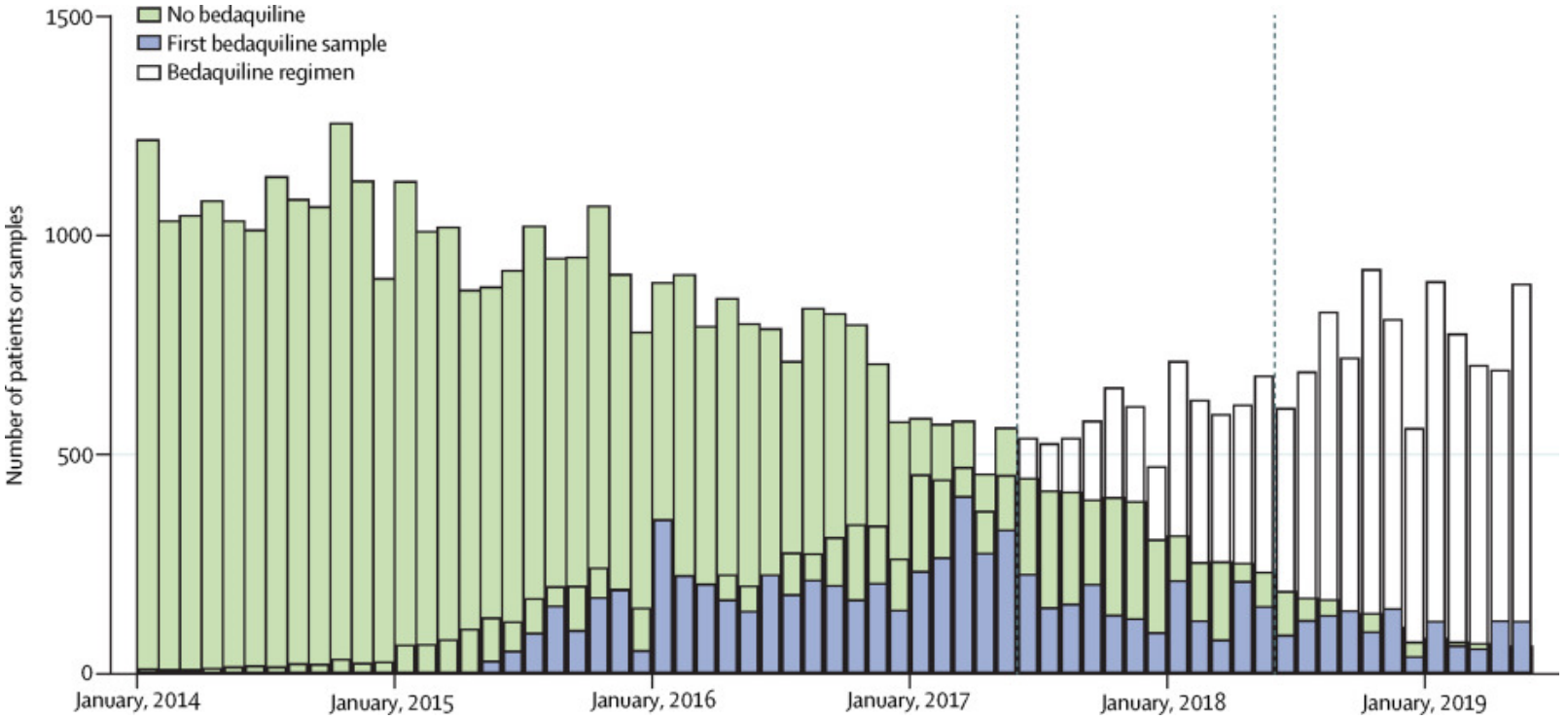

Figure 1. Histogram over time of patients on a bedaquiline-based or non-bedaquiline-based regimen compared with the first bedaquiline surveillance sample

Green shaded bars and unshaded bars are from the Electronic Drug-Resistant Tuberculosis Register and represent counts of patients not on a bedaquiline-based regimen compared with those on a bedaquiline-based regimen. Blue shaded bars are counts of first sample for bedaquiline surveillance received. Counts are monthly totals. The dotted lines separate the graph by three periods that were the following, by chronological order: the first period was centralised treatment of patients with pre-XDR, XDR, or MDR tuberculosis with either dual isoniazid mutations or adverse events associated with the injectable treatment, who received a bedaquiline-based regimen; the second period was the same group, but they received treatment at centralised and decentralised sites; the third period was treatment of most patients with rifampicin-resistant tuberculosis with bedaquiline, but with specific exceptions. MDR=multidrug-resistant. XDR=extensively-drug resistant.

and *Rv0678* for isolates with minimum inhibitory concentrations in the intermediate range or in cases of discordance between methods.

To evaluate genomic relatedness, determine a complete genetic resistance profile, and assess resistance emergence, we did whole-genome sequencing (WGS) as previously described.[15] The threshold for variant calling was lowered to a minimum frequency of 10%, and coverage was aimed for at least 100x (100 reads, half in one sense and half in the opposite sense, for each fragment). We chose 10% frequency as a conservative threshold because frequencies higher than 30% have been reported to be clinically significant whereas thresholds lower than 5% are reported to be clinically irrelevant.[16] We did WGS on a subset of baseline isolates in three groups: all bedaquiline-resistant, all bedaquiline-susceptible with previous or unknown bedaquiline or clofazimine exposure history, and all isolates irrespective of previous exposure history with at least a baseline and one paired post-baseline culture-positive isolate. This last group was included to evaluate the molecular basis of treatment-emergent resistance. Details on WGS methods, resistance determination, and phylogenetic analysis are provided in the appendix (p 3). We used a threshold of fewer than six single-nucleotide polymorphisms to infer primary transmission.[17] The full list of mutations identified are shown in the Three databases were linked and used in the analysis: the NICD tuberculosis laboratory database, which included the bedaquiline phenotypic DST and the laboratory request form data; the NICD national tuberculosis surveillance database, with the routine culture monitoring data; and the Electronic Drug-Resistant Tuberculosis Register database, to obtain information on HIV status and antiretroviral treatment, previous tuberculosis treatment, tuberculosis drug resistance classification, additional laboratory results, and treatment outcomes.

Outcomes and statistical analysis

We determined baseline bedaquiline resistance prevalence estimates among patients in the cross-sectional study stratified by previous bedaquiline or clofazimine exposure and calculated 95% CIs. We analysed risk factors for bedaquiline resistance using χ^2 test and determined odds ratios (OR) using logistic regression. Statistical analysis was done on STATA, version 15. The variables available and included in the risk factor analysis were the following: age, sex, HIV and antiretroviral treatment status, geographical area, type of treatment facility, year, guideline period, previous drug exposure to bedaquiline or clofazimine, drug-resistant profile, and resistance to other core second-line agents. As this was a surveillance study and not designed to explore causality, we did only a univariate analysis.[18] For completeness, two multivariable models were done. Lastly, we assessed outcomes including culture conversion and treatment success among patients with and without bedaquiline resistance. We determined time to culture conversion and used a Wilcoxon rank-sum test to compare the equivalence of medians. The definition for these outcomes followed South African tuberculosis guidelines used for routine reporting and were based on WHO definitions applicable during the study period.[19] We determined the cumulative incidence of resistance and time to resistance development after initiation of treatment with bedaquiline. We also reviewed outcomes for patients who developed resistance on treatment. The STROBE checklist for this study is included in the appendix (p 23).

Role of the funding source

The funder of the study and the sponsor of the bedaquiline test consumables had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Jan 1, 2015, and July 31, 2019, 8041 patients had surveillance samples submitted, of which 6018 were excluded: 2282 were rejected due to poor quality, 1720 were not baseline samples, 1408 were culture-negative baseline samples, 527 were samples taken more than 30 days after the baseline sample, 49 had non-tuberculosis mycobacteria cultured, and 32 were from children younger than 12 years. Therefore, the final cross-sectional analysis set comprised 2023 patients with bedaquiline phenotypic DST results from baseline sputum samples. The flow diagrams for both analyses are shown in the appendix (pp 6–7). Many patients were already on treatment and switched to bedaquiline due to renal impairment, ototoxicity, or both,[20] resulting in baseline-negative cultures; or patients were unable to produce quality sputum for analyses. Sputum was not induced for those not able to provide it. Furthermore, the 8041 patients represent a subset of all patients enrolled on a bedaquiline-based regimen (figure 1). During the first guideline period, 4803 (65·7%) of 7305 patients on a bedaquiline-based regimen were represented, decreasing to 1882 (26·3%) of 7162 and, finally, to 1356 (14·1%) of 9630 in the subsequent two periods, as the rollout expanded. We did not have the precise start date of bedaquiline treatment in the regimen, only the initial treatment start date, and the percentages should be interpreted in this context.

The baseline bedaquiline resistance prevalence was 3·8% (76 of 2023 patients; 95% CI 2·9–4·6). Among those with no previous exposure (1987 patients) or unknown previous exposure (17 patients) to bedaquiline or clofazimine, the prevalence of bedaquiline resistance was 3·6% (72 of 2023; 2·8–4·6), whereas among those with previous exposure, prevalence was 21·1% (four of 19; 6·1–45·6).

Age, sex, or HIV status were not associated with bedaquiline resistance (table 1). We found no association between bedaquiline resistance and province, although patients treated at specialised centres had significantly higher odds of having bedaquiline resistance. Specialised centres received referrals of patients who were less stable or had a more complex disease state (eg, extrapulmonary tuberculosis). Additionally, no association occurred between bedaquiline resistance and previous drug-resistant tuberculosis treatment; however, if patients had previous bedaquiline or clofazimine exposure, they had significantly higher odds of having bedaquiline resistance at baseline (table 1). There were higher odds of bedaquiline resistance observed for exposure to either drug, but there was nearly complete overlap of previous exposure to both drugs, with eight of nine patients having had bedaquiline and clofazimine exposure.

We observed no association by guideline period, which included periods of centralised and decentralised care and the use of bedaquiline for most patients with rifampicin-resistant or MDR tuberculosis. The odds of bedaquiline resistance were significantly higher among patients with pre-XDR and XDR tuberculosis compared with those with rifampicin-resistant tuberculosis (table 1). Two multivariable models were developed and did not change our conclusions (appendix pp 4, 8–14). The proportion of resistance to other drugs among patients with bedaquiline resistance was 75% (43 of 57 patients) for fluoroquinolones, 44% (25 of 57) for second-line injectable agents, 7% (three of 44) for linezolid, 70% (31 of 44) for clofazimine, and 81% (13 of 16) for rifabutin.

Table 1: Odds ratios of risk factors associated with bedaquiline resistance

Factor		Bedaquiline-susceptible (n=1947)	Bedaquiline-resistant (n=76)	Total (N=2023)	OR (95%CI)
Sex (n=2022)					
	Female	794 (96%)	33 (4%)	827	1
	Male	1152 (96%)	43 (4%)	1195	0.9 (0.6 - 1.4)
Age (IQR)		37 (30-37)	34 (28-45)	37(30-47)	
HIV status (n=1917)					
	Negative	632 (95%)	30 (5%)	662	1
	Positive	1211 (96%)	44 (4%)	1255	0.8 (0.5 - 1.2)
Started on ART (n=1221)					
	No	14 (100%)	0 (0%)	14	1
	Yes	1164 (96%)	43 (4%)	1207	0.8 (0.5 - 1.4)
Province (n=2009)					
	Eastern Cape	458 (95%)	23 (5%)	481	1
	Free State	0 (0%)	0 (0%)	0	NA
	Gauteng	579 (96%)	24 (4%)	603	0.8 (0.4 - 1.4)
	KwaZulu-Natal	232 (95%)	13 (5%)	245	1.3 (0.6 - 2.7)
	Limpopo	11 (100%)	0 (0%)	11	NA
	Mpumalanga	66 (99%)	1 (1%)	67	0.6 (0.1 - 2.6)
	North West	65 (98%)	1 (2%)	66	0.3 (0.1 - 2.4)
	Northern Cape	144 (97%)	5 (3%)	149	0.6 (0.2 - 1.7)
	Western Cape	378 (98%)	9 (2%)	387	0.5 (0.2 - 1.1)
Specialised treatment centre (n=2023)					
	No	379 (99%)	4 (1%)	383	1
	Yes	1568 (96%)	72 (4%)	1640	4.35 (**1.6 - 11.9**)
Previous multidrug resistant TB (n=2006)					
	No	1681 (96%)	65 (4%)	1746	1
	Yes	249 (96%)	11 (4%)	260	1.1 (0.6 - 2.2)
Previous bedaquiline exposure (n=2006)					
	No	1922 (96%)	75 (4%)	1997	1
	Yes	8 (89%)	1 (11%)	9	3.2 (0.4 - 25.9)
Previous clofazimine exposure (n=2006)					
	No	1916 (96%)	72 (4%)	1988	1
	Yes	14 (78%)	4 (22%)	18	7.6 (**2.4 - 23.7**)
Previous bedaquiline/clofazimine exposure (n=2006)					
	No	1915 (96%)	72 (4%)	1987	1
	Yes	15 (79%)	4 (21%)	19	7.1 (**2.3 - 21.9**)
Guideline period[#]					
	XDR/pre-XDR-TB	1074 (96%)	36 (4%)	1110	1
	Decentralisation	523 (95%)	25 (5%)	548	1.4 (0.8 - 2.4)
	All RR-TB patients	350 (96%)	15 (4%)	365	1.3 (0.7 - 2.4)
EDR DR-TB Classification* (n=1924)					
	Rif-R	433 (98%)	7 (2%)	440	1

	MDR	608 (97%)	18 (3%)	626	2.0 (0.8 - 5.1)
	Pre-XDR	406 (95%)	23 (5%)	429	4.2 (**1.7 - 10.5**)
	XDR	403 (94%)	26 (6%)	429	4.8 (**2.0 - 11.7**)
Individual Drug resistance profiles					
Fluoroquinolone Resistance (n=1515)					
	Susceptible	887 (98%)	14 (2%)	901	1
	Resistant	571 (93%)	43 (7%)	614	4.8 (**2.6 - 8.8**)
Second line injectables (n=1513)					
	Susceptible	1016 (97%)	32 (3%)	1048	1
	Resistant	440 (95%)	25 (5%)	465	1.8 (**1.1 - 3.1**)
Linezolid (n=1093)					
	Susceptible	1038 (96%)	41 (4%)	1079	1
	Resistant	11 (79%)	3 (21%)	14	6.9 (**1.9 - 25.7**)
Clofazimine (n=1092)					
	Susceptible	942 (99%)	13 (1%)	955	1
	Resistant	106 (77%)	31 (23%)	137	21.2 (**7.3 - 41.7**)
Rifabutin (n=438)					
	Susceptible	209 (99%)	3 (1%)	212	1
	Resistant	213 (94%)	13 (4%)	226	4.3 (**1.2 - 15.1**)

Row percentages instead of column percentages are presented allowing the reader to observe the percentage bedaquiline-R by risk factor. S= Susceptible, R=Resistant, OR = odds ratio, 95%CI = 95% confidence interval. Bold font indicates 95% confidence interval that does not cross 1.*DR-TB classification is based on the EDR-TB classification: Rif-R (rifampicin resistant), MDR (resistance to rifampicin and isoniazid), Pre-XDR (MDR with resistance to fluoroquinolones or second line injectables), XDR (MDR with resistance to both fluoroquinolones and second line injectables). [#] Guideline periods: Extensively drug-resistant TB (XDR TB), pre-XDR TB, adverse events (AE) was the period before 01 June 2017; Decentralisation was the period 01 June 2017 to 30 May 2018; and all RR-TB patients was from 01 June 2018 onwards.

WGS was completed for 270 baseline isolates on the basis of the inclusion criteria: 76 (100%) of 76 phenotypically bedaquiline-resistant isolates and 194 (10·0%) of 1947 phenotypically susceptible isolates. Of the 76 bedaquiline-resistant isolates, four (5·3%) were from patients with previous bedaquiline or clofazimine exposure and all four had insertion events in *Rv0678* between codon positions 139–144 (table 2, figure 2). Genetic analysis did not infer primary transmission events of bedaquiline-resistant mutations, although three belonged to a single large cluster and harboured the 144insC insertion (figure 2). Of the 72 bedaquiline-resistant isolates from patients without previous bedaquiline or clofazimine-exposure history, none

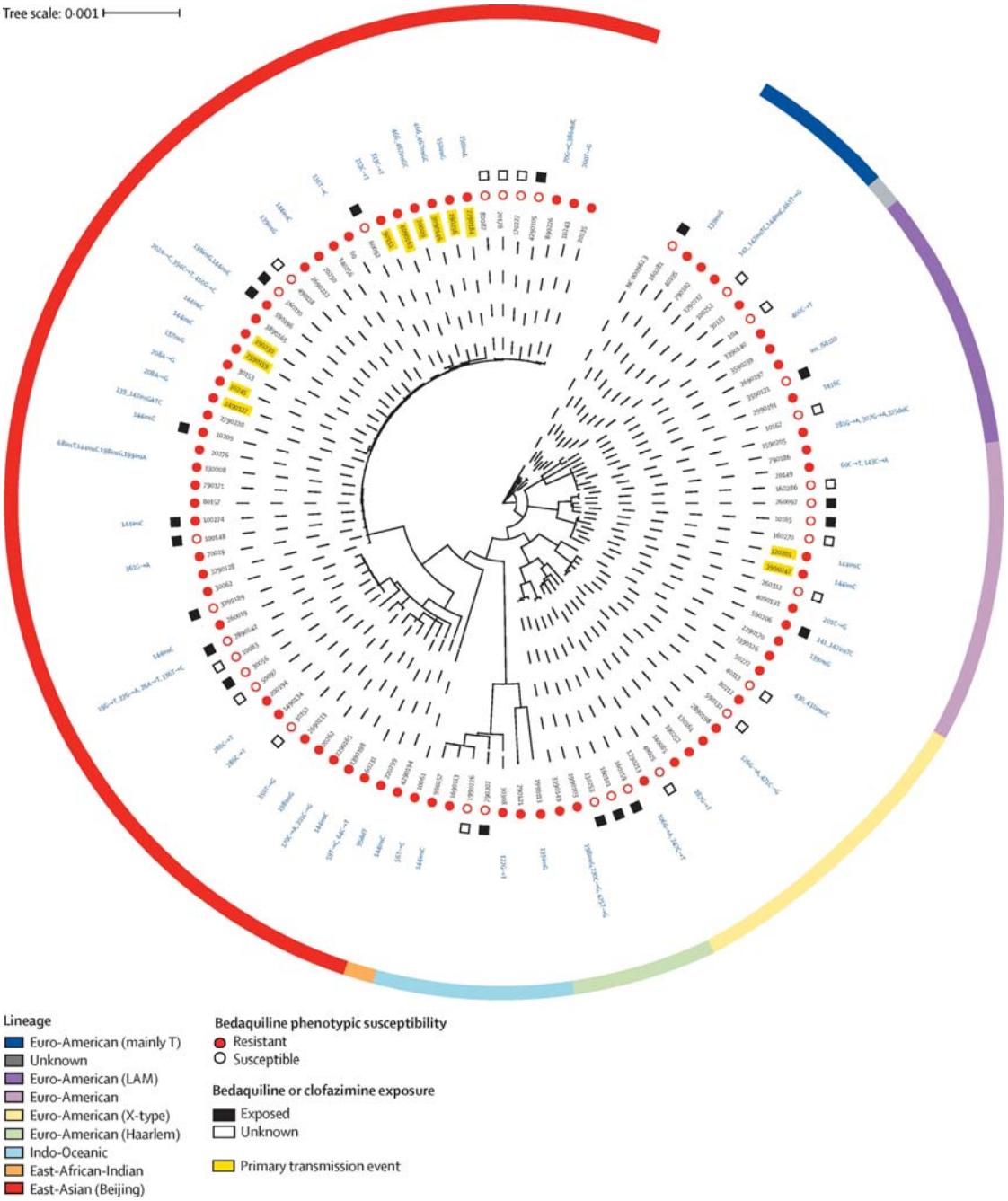

Figure 2. Maximum likelihood phylogenetic tree of 104 baseline *Mycobacterium tuberculosis* strains

104 strains are shown: 76 baseline bedaquiline-resistant strains and 34 strains with either a documented or unknown exposure to bedaquiline or clofazimine before bedaquiline treatment initiation. From inner ring to the outer, the figure shows the study identifier for each strain, bedaquiline susceptibility, bedaquiline or clofazimine exposure, *Rv0678* mutations detected, and lineage. Primary transmission events are highlighted in yellow.

had a mutation in the *atpE* or *pepQ* genes, whereas 55 (76·4%) had a mutation in the *Rv0678* gene. Primary transmission events were attributable in six (7·9%) of 76 isolates, due to being one of a pair genetically linked with identical *Rv0678* mutations (figure 2). Five of the six pairs occurred in the same province and were sampled within a year. The remaining bedaquiline-resistant isolates that were genetically clustered all had unmatched *Rv0678* mutations relative to the cluster, indicating independent acquisition of bedaquiline resistance, at least on the basis of the cohort in this study. Overall, 59 (2·9%) of 2023 patient isolates (95% CI 2·2–3·7) were phenotypically resistant and had a *Rv0678* mutation at baseline.

Table 2: Association between baseline bedaquiline phenotypic drug susceptibility testing, mutations and prior exposure (N=270)

	Phenotypic bedaquiline drug susceptibility testing					
	Resistant (N=76)			Susceptible (N=194)		
	Prior BDQ/CFZ exposure (%)	No Prior BDQ/CFZ exposure	Unknown	Prior BDQ/CFZ exposure	No Prior BDQ/CFZ exposure	Unknown
Mutation						
Rv0678	4 (100%)	55 (76.4%)		1 (5.9%)	5 (3.1%)	
atpE					1 (0.6%)	
WT: *Rv0678* or *atpE*		17 (23.6%)		16 (94.1%)	156 (96.3%)	15 (100%)
Total	4 (100%)	72 (100%)	0	17 (100%)	162 (100%)	15 (100%)

WT: wild-type

Among the 194 susceptible isolates, 17 were from patients with previous bedaquiline or clofazimine exposure, 15 from those with unknown exposure, and 162 from those with no previous exposure history. Overall, mutations were observed in seven bedaquiline-susceptible isolates, six with *Rv0678* mutations and one with an *atpE* mutation (table 2). Of the 17 isolates from patients with previous bedaquiline or clofazimine exposure, only one harboured *Rv0678* mutations, with four variants having frequencies ranging between 13%

and 54% in addition to the upstream −11 mutation (98·7% frequency) associated with hyper susceptibility. Overall, six (3·1%) of 194 patient isolates (95% CI 1·1–6·6) had a *Rv0678* mutation at baseline and were phenotypically susceptible.

Table 3: Culture conversion and treatment outcomes among patients with and without bedaquiline resistance

Outcome	Bedaquiline-S	Bedaquiline-R	OR (95%CI)
Culture conversion after bedaquiline baseline sample (n=1962)			
	1889	73	
No	238 (13%)	15 (21%)	1
Yes	1651 (87%)	58 (79%)	0.6 (0.3 - 1)
Days to negative culture (median, IQR; n=1709)*			
	32 (21 - 62)	47 (26 - 66)	
Successful patient outcome (n=1140)			
	1103	37	
No	309 (28%)	16 (43%)	1
Yes	794 (72%)	21 (56%)	0.5 (0.3 - 1)

S= Susceptible, R=Resistant, OR= odds ratio, 95%CI = 95% confidence interval. *p=0.02

Evaluating the effect of bedaquiline resistance on patient outcomes, 238 (12·6%) of 1889 patients with bedaquiline susceptibility did not have sputum culture conversion compared with 15 (20·5%) of 73 patients with bedaquiline resistance, and the odds of culture conversion was lower in those with bedaquiline resistance (OR 0·6, 95% CI 0·3–1·0; table 3). Time to culture conversion, although delayed, was not significantly different (p=0·20; table 3). We observed lower odds of successful patient outcomes (OR 0·5, 95% CI 0·3–1·0) among those with bedaquiline resistance. In absolute terms, the proportion of patients with a successful outcome was 72% (794 of 1103) among those with bedaquiline susceptibility compared with

57% (21 of 37) among those with bedaquiline resistance. Regarding outcomes by phenotype and mutation, our findings suggest that the combination of phenotypic resistance with the presence of a mutation was most important (appendix pp 5, 20). However, this was a subset analysis and not powered to show a difference.

We evaluated treatment-emergent resistance in the longitudinal analysis, with a total of 695 patients meeting the criteria for inclusion, 16 (2·3%) of whom developed phenotypic resistance; 12 (75%) of these 16 were patients with pre-XDR or XDR tuberculosis. The median time to develop bedaquiline resistance from baseline was 90 days (IQR 62–195), and the range was 21–654 days post baseline. The outlier was a patient who returned to care after being lost to follow-up and resistance was detected on re-entry. The sampling was sparse, and thus we acknowledge that time to resistance could not be precisely determined.

WGS data for one of the 16 patients with treatment-emergent resistance were excluded from further analysis because the isolate was mixed with a background non-tuberculosis mycobacterium. Phylogenetic analysis of the 15 patients showed that 13 acquired resistance during treatment, with the emergence of an *Rv0678* mutation not present at baseline as strains were confirmed to be the same at the different sampling time (figure 3), except for one (40325), which was a new strain, suggesting primary transmission (one [6·7%] of 15) in the longitudinal analysis (figure 2). Two patients had pre-existing *Rv0678* mutations at the onset of treatment that were not identified phenotypically. Of the four patients without pre-XDR or XDR tuberculosis who developed bedaquiline resistance, one was lost to follow-up (resistance detected on re-entry), one had a new resistant strain that was acquired post-baseline but achieved cure, one had acquired two low-frequency mutations (15% and 25%)

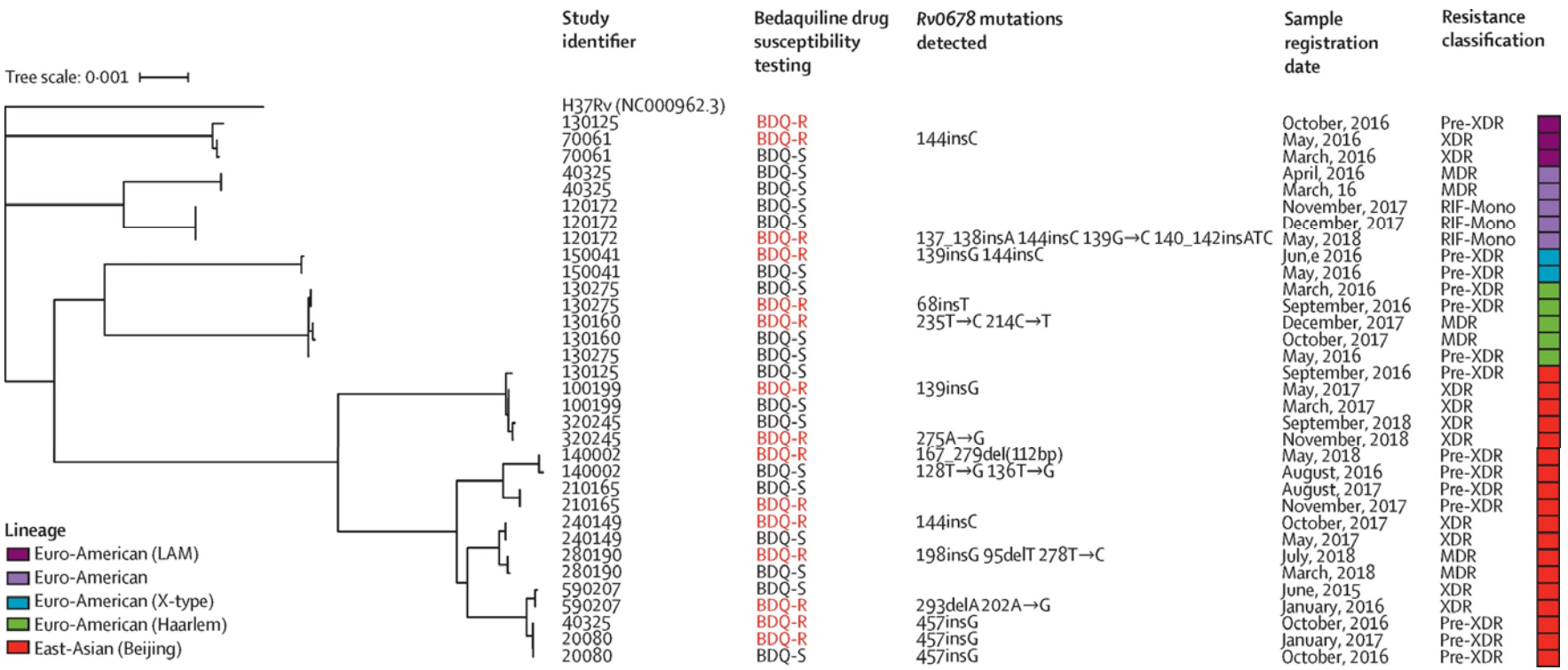

Figure 3. Maximum likelihood phylogenetic tree of 34-paired baseline and post-baseline patient isolates

BDQ-R=bedaquiline-resistant. BDQ-S=bedaquiline-susceptible. MDR=multidrug-resistant. RIF-Mono=rifampicin resistant and isoniazid susceptible. XDR=extensively-drug resistant.

and was cured, and the last patient developed multiple low-frequency mutations and had a poor treatment outcome.

Among the 162 patients with paired phenotypically susceptible isolates, mutations were found in seven isolates, six in *Rv0678* (three baseline and three post-baseline) with low frequencies (10–19%) and one in *atpE* at baseline, with a frequency higher than 99%. Of the five baseline genotypic mutant isolates (3·1%), four had a subsequent wild-type genetic profile post-baseline.

Discussion

Over the surveillance period (2015–19) and under programmatic conditions, 3·8% of patients had baseline isolates resistant to bedaquiline, correlating with data from clinical trials done in South Africa (16 [4%] of 391).[11] A multicountry surveillance study using isolates from patients without exposure to bedaquiline across 28 countries showed a prevalence of 2·2% (95% CI 1·1–3·9),[21] and an identical point prevalence was observed in a multicentre study from China.[22] Bedaquiline resistance was associated with *Rv0678* mutations across all studies, including our study.

The South African data, with a slightly higher prevalence of bedaquiline resistance, are expected because patients with pre-XDR or XDR, an identified risk factor, were the primary group to receive bedaquiline-based regimens at the onset. In 2019, 13 005 laboratory-confirmed cases of rifampicin-resistant or MDR tuberculosis were diagnosed in South Africa and, on the basis of 95% CI estimates (2·9–4·6) in the surveillance, an imputed burden between 380 and 600 patients with bedaquiline resistance can be expected per annum. The

lower estimate is likely to be more appropriate, reflecting the overall population with rifampicin-resistant or MDR tuberculosis (2–3%; table 1).

Genetic analysis did not show primary transmission to be the dominant route, nor was previous exposure to bedaquiline or clofazimine a major contributor, although previous exposure to bedaquiline or clofazimine was a significant risk factor. It is possible that the period for this study was not long enough to fully identify transmission events, and not all patients were enrolled in the surveillance. Acquired resistance was evident by the phylogenetic distribution of mutations in the cross-sectional analysis (figure 2) and was confirmed by the longitudinal analysis, with the identical strains acquiring new mutations (figure 3). Previous bedaquiline or clofazimine exposure was an important risk factor and is of concern when patients are lost to follow-up; such patients need to be assessed for bedaquiline resistance on re-entry. Notably, even in this higher risk group, 15 of 19 patients who were tested were susceptible, and thus empirical treatment while awaiting results is appropriate. Minimising treatment interruption and loss to follow-up, which can be relatively high (up to 33%),[23] is important when bedaquiline and clofazimine are used, as they have long terminal elimination half-lives (6 months for bedaquiline and 1 month for clofazimine)24, 25 that could lead to unintentional monotherapy. Other relevant factors leading to poor therapeutic drug exposure are not taking bedaquiline with food and low albumin levels, collectively highlighting the need for holistic patient support.[26]

Phenotypic bedaquiline resistance was associated with poorer outcomes, and most patient isolates with bedaquiline resistance had an *Rv0678* mutation. However, *Rv0678* mutations were also found in phenotypically susceptible isolates and, for some, these baseline mutations were not sustained at a later time while on combination therapy. We should note

that these mutations were hetero-resistant, with low frequencies (10–19%) at baseline. The proportion with an *Rv0678* mutation was similar among phenotypically susceptible (3·1%) and resistant (2·9%) strains.

Using a sequencing approach upfront could detect all mutants; however, almost half of these might benefit from continued bedaquiline use as they were phenotypically susceptible. Upfront sequencing would nonetheless provide a very good screen to exclude resistance. A study from China showed that poor response to treatment was observed in patients who developed resistance on treatment correlating with phenotypic resistance (however, these were mainly patients with pre-XDR or XDR tuberculosis),[22] whereas baseline resistance or the presence of an *Rv0678* mutation did not consistently lead to poor outcomes.

The odds of bedaquiline resistance in our study were higher among patients who had highly resistant forms of drug-resistant tuberculosis (eg, pre-XDR or XDR tuberculosis), had a complex disease history as inferred by management at a specialised treatment site, and were resistant to core second-line agents. This highlights the need for early and rapid detection of resistance to bedaquiline and core companion drugs such as fluoroquinolones. Such interventions are crucially important because resistance was associated with lower odds of successful outcomes. However, these interventions would mitigate the effect of, rather than prevent, bedaquiline resistance.

Strategies to prevent resistance are, however, not straight forward as directly attributable factors (previous exposure and primary transmission) contributed very little in absolute terms to bedaquiline resistance at baseline. The surveillance might have missed index cases in the community, and primary transmission might be underrepresented in our study. However, in the longitudinal cohort, primary transmission was 6·7%, which is similar to the 7·9% observed

in the cross-sectional analysis. The occurrence of bedaquiline resistance at baseline at a prevalence of 2–4% in multiple regions among patients without previous exposure,[21] which is not sufficiently explained directly by the factors we identified, suggests a generalised occurrence.

Sporadic development of bedaquiline resistance is plausible, as *Rv0678* mutations have been shown to have emerged before the introduction of bedaquiline or clofazimine for treatment of tuberculosis.[11] Although the use of azoles has been postulated to lead to *Rv0678* mutations,[27] these drugs are commonly used during the management of patients with HIV and no relationship was observed between HIV status and bedaquiline resistance. Reduced concentrations of bedaquiline associated with efavirenz, a strong inducer of CYP3A4, is a potential risk;[26] however, efavirenz is contra-indicated for concomitant use in South Africa, and no association was observed between resistance and antiretroviral treatment. The *Rv0678* gene encodes for an efflux pump that is not specific to bedaquiline, further strengthening the argument for alternative factors that contribute to resistance among patients with no previous exposure to bedaquiline, which require additional research. Expansion of the drug pipeline and regimens with novel drug combinations, rather than single new drugs added to existing regimens, should be a key focus. Furthermore, therapeutic drug monitoring for fluoroquinolones and linezolid should be considered.[26]

Treatment-emergent resistance to bedaquiline occurred in 2·3% of patients at a median of 90 days. Expectedly, most of these cases occurred among patients with pre-XDR or XDR tuberculosis and occurred early in the treatment. However, the samples from the last patients to be enrolled might not have had a long enough period for resistance to develop, and the median time could have been longer than 90 days. Patients on bedaquiline who remained

culture-positive at month 2 or had a reversal of culture status should be retested for bedaquiline resistance. The finding of primary transmission is another crucial concern, and serious attention to infection prevention and control is required to prevent onward transmission of tuberculosis that would be very difficult and costly to treat. An important finding of our study was that treatment-emergent resistance to bedaquiline in South Africa was similar to sporadic frequencies and might be explained by the empirical combination treatment of linezolid and clofazimine to prevent resistance emergence when bedaquiline is used.

Our findings provide important new insights but need to be seen considering certain limitations. The surveillance could not capture all patients who started bedaquiline-based treatment, particularly in the later years of the study, and primary transmission is probably under-estimated, requiring further research. Previous bedaquiline or clofazimine exposure might have been underreported, but the findings are consistent with other data. Only a subset of bedaquiline-susceptible isolates underwent WGS, and this might have been biased towards isolates from patients with poor response to treatment, such as those with at least a single post-baseline positive culture. Therefore, we might have overrepresented resistance emergence frequency, and the findings might represent a worst-case scenario. Additionally, outcomes were not always interpretable, for example, for patients who were transferred out of a treatment centre. We were only able to analyse those with clearly classifiable outcomes, which might have led to further bias; however, this study represents real-world and national data, which provides important insights not otherwise observable.

In conclusion, bedaquiline-resistance prevalence has remained relatively low during the surveillance period in SA; however, this is likely to increase over time, as more TB strains are

exposed to bedaquiline. Efforts supporting patients through treatment early when the bacterial load is highest are paramount. The need for early detection of bedaquiline and fluoroquinolone resistance is essential and ensuring that robust upfront regimens include new or re-purposed drugs with minimal background drug resistance. The poorer outcomes observed in patients with bedaquiline resistance is an important warning sign that this issue needs to be addressed if we are to sustain the current improved outcomes in the management of drug-resistant tuberculosis.[28]

Contributors

NAI, SVO, HM, FI and NN were involved in the conception and design,

SVO, FI, ZB, HF, YK, VL, DN, NO, XP, AR, RR, HSS, JTR, EV and MvM were involved in the implementation

SVO, LJ, HM did the data curation and formal analysis

SVO and HM accessed and verified all data

NAI, SVO, FI, HM and NN wrote the first draft

NAI, SVO, HM, FI, NN, HSS, GRM, GM, JH, AR, RR, EV, XP interpreted the data, provided intellectual input including editing and reviewing the final draft

Conflicts of interest

NAI: Partial support was received from Janssen Pharmaceuticals to institution for the consumables used to perform bedaquiline drug susceptibility testing. The rest of the authors declare that they have no conflicts of interest.

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Data sharing

Raw sequencing reads are uploaded onto National Center for Biotechnology Information (NCBI) under Sequence Read Archive (SRA) submission: SUB9806910, Bedaquiline Surveillance, Jun 06 '21

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