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Early View

Research letter

Bedaquiline resistance in drug-resistant tuberculosis HIV co-infected patients

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Title page

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Bedaquiline resistance in drug-resistant tuberculosis HIV co-infected patients

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Take home message

Genetic mutations linked to bedaquiline resistance were found before starting treatment and acquired during treatment in patients with drug-resistant TB and HIV in KwaZulu-Natal, South Africa. Routine bedaquiline resistance testing needs to be accelerated.

Plain language summary

Bedaquiline is a highly effective drug for tuberculosis (TB) and is an important part of current regimens for drug-resistant TB. Bedaquiline resistance is rarely tested in

operational settings. In a prospective cohort of 292 patients with drug-resistant TB and HIV starting bedaquiline in KwaZulu-Natal, South Africa, we whole genome sequenced isolates from 92 baseline culture positive patients. Five patients were infected with strains harbouring mutations associated with bedaquiline resistance, and an additional five acquired resistance mutations during treatment. Among the baseline culture positive patients, those with baseline or acquired resistance mutations had substantially worse clinical outcomes compared to patients without. Given the importance of bedaquiline in current and future treatment, routine bedaquiline resistance monitoring is urgently needed and programmes require strengthening to ensure full adherence and prevent widespread transmission of bedaquiline-resistant tuberculosis.

Main text

Global tuberculosis (TB) control is threatened by drug resistance, with over 500,000 cases resistant to first-line drugs in 2018[1]. Bedaquiline is a highly effective TB drug and has improved drug-resistant TB (DR-TB) outcomes in trial and programmatic settings[2,3]. The World Health Organization (WHO) recommends its inclusion in most DR-TB regimens[4] and it is under further evaluation in clinical trials. There have been several reports of clinical bedaquiline resistance[5–8]. Resistance-associated variants (RAVs) in clinical isolates identified to date are almost exclusively caused by *Rv0678* mutations which can raise *Mycobacterium tuberculosis* (*Mtb*) minimum inhibitory concentrations (MICs) for bedaquiline and clofazimine[9].

The South African province of KwaZulu-Natal was the site of an extensively drug-resistant TB (XDR-TB) outbreak among HIV co-infected patients[10,11]. Despite extensive bedaquiline use in KwaZulu-Natal, routine phenotypic or genotypic drug susceptibility testing (DST) is not performed, leaving the potential for unidentified bedaquiline resistance to spread.

The clinical significance of baseline *Rv0678* variants is unclear[12]. Emergence of *Rv0678* mutations during treatment has been documented but their incidence is unknown. We report the frequency of baseline and emergent bedaquiline RAVs and associated clinical outcomes in a prospective DR-TB cohort treated with bedaquiline and clofazimine.

Adult patients with DR-TB and HIV presenting at a public TB referral hospital in KwaZulu-Natal, South Africa were enrolled within two weeks of starting bedaquiline in the PRAXIS study (NCT03162107) between November 2016 and January 2019. Treatment with antiretroviral therapy was a mandatory inclusion criterion. Clinical data, questionnaires, and sputum were collected at monthly visits over the first six months of treatment, with end of treatment follow-up. The study was approved by the University of KwaZulu-Natal Biomedical and the Columbia University ethics review boards. All sputa were inoculated into mycobacterial growth indicator tubes and cultured in a BACTEC 960 (BD, MD, USA). Positive cultures underwent whole genome sequencing (WGS) and bedaquiline MIC testing was performed for isolates with *Rv0678* variants using the proportion method on 7H11 agar. Culture conversion at six months was defined as ≥2 consecutive negative monthly cultures. Outcomes at the end of treatment were assigned according to standard definitions[13].

Of 965 adult TB patients who presented during the study period, 297 were eligible to participate and consented to enrolment. The most common reasons for ineligibility were not being HIV co-infected (n=160) and starting bedaquiline >2 weeks previously (n=126). Positive baseline cultures and WGS results were available for 92 patients who are the subjects of this report. The remaining 205 patients were culture negative at baseline (n=198) or had isolates that failed WGS (n=7). The sequenced cohort was 51% female and median age was 36 years (IQR 30-43); 66% had a previous history of any TB and 23% of DR-TB. The median CD4 count was 276 (IQR 134-452). Patients with sequenced isolates were more likely to have second-line drug resistance (53/92 [57.6%] vs 82/205 [40.0%], p=0.006) but otherwise had similar baseline characteristics.

Baseline *Rv0678* variants were identified in 5.4% (5/92) patients with a sequenced positive baseline culture prior to initiating bedaquiline treatment (Figure). Although none of the MICs for samples with baseline *Rv0678* variants exceeded the bedaquiline critical concentration, 3/5 had MICs at the top of the wild-type range (0.25μg/mL). The bedaquiline MIC of patient B, who had a baseline *Rv0678* variant, increased from 0.03μg/mL at baseline to 0.25μg/mL at month 2 with an increase in *Rv0678* variant allele frequency from 72% to 96%. Additional emergent *Rv0678* variants occurred in 5.7% (5/87) patients during treatment (Figure). Emergent *Rv0678* variants were associated with a >8-fold increase in bedaquiline MIC.

All patients with baseline or emergent *Rv0678* variants had resistance to fluoroquinolones or second-line injectables (pre-XDR-TB/XDR-TB) and 60% had previously been treated for DR-TB. Patients with *Rv0678* mutations were more likely to have XDR-TB (60%) than those without (29%) (p=0.07). None were previously treated with bedaquiline or clofazimine. No *pepQ* or *atpE* mutations were identified. No further phenotypic or genotypic resistance to drugs other than bedaquiline/clofazimine emerged during treatment. Baseline drug resistance profiles and regimens for patients with *Rv0678* variants are shown (Figure).

In 4/5 cases, emergent bedaquiline resistance occurred due to within-patient evolution of the infecting strain, while in one case (patient G) resistance was suggestive of superinfection with a bedaquiline-resistant strain from patient F. Both patients were hospitalised during the same period and this appears to represent nosocomial transmission as there was no evidence of cross-contamination.

Median follow-up duration for the entire cohort was 12.8 months (IQR 6.0-18.9) and mortality was 20.7% (19/92). Overall, 73/92 (79.3%) patients culture converted by six months. Among patients without *Rv0678* mutations, 70/82 (85%) culture converted; while in patients with baseline *Rv0678* mutations 2/5 (40%) culture converted. Among five patients with baseline *Rv0678* variants, 3/5 (60%) had an unsuccessful outcome (two deaths and one loss to follow-up) compared to 16/82 (18.4%) in patients without *Rv0678* variants (p=0.058). Patients with emergent *Rv0678* variants were more likely to be culture-positive at six months than those without (4/5, 80.0% v 12/82, 14.6%, p=0.004) and 4/5 (80.0%) died or were lost to follow-up compared to 15/82 (18.3%) without (p=0.007). Among patients with a baseline or emergent *Rv0678* variant, 70% (7/10) had an unsuccessful outcome (death, treatment failure, or loss to follow-up) compared to 18% (15/82) without (p=0.001).

The distribution of MICs in patients with *Rv0678* variants in this study are consistent with other reports, finding that many variants are associated with raised MICs below or at the critical concentration[14]. All isolates with MICs >0.25μg/mL in this study had C46 or D47 frameshift mutations and were only seen in acquired drug resistance. Strains with higher (but technically susceptible) MICs for other TB drugs have also been linked to a higher risk of relapse[15]. The key question is whether bedaquiline MICs at or just below the critical concentration of 0.25μg/mL (on 7H11 agar) have clinical consequences and undermine guidelines on bedaquiline phenotypic DST for clinical decision making and monitoring resistance transmission.

It is concerning that all five patients with emergent resistance had ≥4 active drugs in their regimen. Interestingly, no other emergent resistance was found during follow-up. Bedaquiline may represent such a key drug within treatment regimens that resistance develops either to bedaquiline or to none at all, and due to its long half-life, resistance may occur as a result of prolonged exposure to subtherapeutic levels when adherence is suboptimal. The combination of bedaquiline and clofazimine may also have contributed to selection of resistance as *Rv0678* mutations confer cross-resistance and all patients received a combination of both drugs[9].

The percentage of baseline *Rv0678* variants identified in our study was similar to the 6.6% identified in the C208 and C209 bedaquiline clinical trials[8]. C209 reported >4-fold MIC increases associated with *Rv0678* variants in 12/205 (4.4%) patients, similar to the 4/92 (4.3%) in our study, but no association with outcome[12]. Presence of baseline *Rv0678* mutations may indicate current transmission of bedaquiline and clofazimine resistant strains in the community.

Limitations of this study include the relatively small number of patients evaluable. The number of patients with *Rv0678* variants was also small, limiting causal interpretation of clinical outcome differences. While South Africa is an important early adopter of bedaquiline, the high HIV prevalence and unique TB epidemic may limit generalisation of our findings. Factors related to poor outcome tend to cluster (e.g. low medication adherence, suboptimal HIV control and substance abuse), making it difficult to disentangle to what extent bedaquiline resistance is causative of poor outcome or merely a co-variate. Strengths of this study include its prospective design, longitudinal follow-up, and carefully collected clinical outcomes.

This study identifies an important subpopulation of DR-TB HIV patients with baseline and emergent BDQ RAVs associated with poor clinical outcomes. We highlight a role for active genotypic monitoring to identify bedaquiline resistance as well as re-evaluation of phenotypic DST critical concentrations. This report also raises concerns surrounding the overall strategy of empiric treatment regimens for DR-TB, even when constructed with novel agents, and suggests individualised treatment regimens, guided by sequencing may be required to achieve optimal treatment outcomes in all patients and prevent the emergence of bedaquiline resistant DR-TB strains.

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Figure. (A) Patients with *Rv0678* mutations in positive TB sputum cultures. Resistance profiles are for the current and most recent previous TB episodes. Drugs used in treatment regimen are indicated, with those ineffective due to resistance coloured red. H=isoniazid, Z=pyrazinamide, E=ethambutol, FQ=fluoroquinolones, B=bedaquiline, C=clofazimine, Et=ethionamide, L=linezolid, T=terizidone, PAS=p-aminosalicylic acid, D=delamanid, Im=imipenem. Patient A was phenotypically ethionamide resistant in the absence of ethionamide resistance-associated variants. *Rv0678* variants are categorised as baseline (orange background) or emergent (white background). Amino acid changes at variant sites are specified (fs = frameshift mutation). Bars indicate culture-positive samples without variants (grey), heterozygous variants (light blue) and fixed variants (dark blue). Minimum inhibitory concentrations (MICs) are shown at baseline and at subsequent timepoint if performed (red lines). *In patient J six further low-frequency *Rv0678* variants appeared at month six (A57E, R72T, D88fs, D88A, G121R, L122P). (B) Kaplan-Meier curve for survival probability following initiation of bedaquiline therapy with censoring for loss to follow-up.

A

Patient ID	Resistance profile		Treatment regimen (number of effective drugs)	6 month outcome	Subsequent outcome	Bedaquiline RAVs	Culture positivity and RAV presence by treatment month	Baseline MIC	Subsequent MIC
	Current TB	Previous TB History							
A	Pre-XDR	RR	Z FQ B C Et L Tz PAS (3)	Failure	Lost to follow-up	R109L R159fs		0.25	0.25
B	Pre-XDR	RR	Z FQ B C L Tz PAS Im (4)	Failure	Death	E49fs		0.03	0.25
C	Pre-XDR	None	H Z FQ B C (2)	Culture converted	Successful	V20G		0.25	N/A
D	XDR	None	H Z FQ B C Tz PAS (3)	Failure	Death	F93S		0.25	0.25
E	XDR	RR	Z FQ B C L Tz (3)	Culture converted	Successful	F93L		0.12	N/A
F	XDR	None	Z FQ B C L Tz PAS (6)	Failure	Death	C46fs D47fs		0.06	1.0
G	XDR	RR	Z E FQ B C Et L Tz PAS (6)	Culture converted	Successful	C46fs		0.03	1.0
H	XDR	RR	Z E FQ B C Et L Tz PAS (4)	Failure	Failure	D47fs		0.06	0.5
I	XDR	RR	B C L Tz PAS D (6)	Failure	Death	E147fs		0.03	0.25
J	XDR	None	Z FQ B C Et L Tz PAS (5)	Failure	Death	Q22P D47fs*		0.03	1.0
			Drugs with resistance in red				Baseline, Month 1, Month 2, Month 3, Month 4, Month 5, Month 6		

Baseline bedaquiline RAV

Emergent bedaquiline RAV

Culture positive, *Rv0678* variant present 5-95%

Culture positive, *Rv0678* variant present >95%

Culture positive, *Rv0678* variant absent

MIC measured

B