Vuka! Let's unite towards a TB-free world!

9th SA TB Conference
8 – 11 June 2026

Phenotypic and Genotypic Characterisation of Bedaquiline and Clofazimine Resistance in Drug-Resistant *Mycobacterium tuberculosis* Isolates at a Tertiary laboratory in Pretoria, South Africa

Gogela Ndiitwani Noel[1,2], Mensah Eric[1], Shikwambane-Ntlemo Grace[1,2], Nchabeleng Maphoshane[1,2], Steyn Adrie[3,4], and Makhado Ndivhuho Agnes[1,4].

Department of Microbiological Pathology, Tuberculosis Research Unit, Sefako Makgatho Health Sciences University, Pretoria, South Africa[1]. Department of Medical Microbiology, National Health Laboratory Service Dr George Mukhari Tertiary Laboratory, Pretoria, South Africa[2]. The Department of Medical Microbiology, University of Alabama at Birmingham, USA[3]. Department of Microbiology, Africa Health Research Institute, Basic and Translational Science, Durban, South Africa[4].

SEFAKO MAKGATHO HEALTH SCIENCES UNIVERSITY

NATIONAL HEALTH LABORATORY SERVICE

AHRI AFRICA HEALTH RESEARCH INSTITUTE

NIH National Institutes of Health

Introduction

- Tuberculosis (TB) is an infectious disease that threatens the health of humans worldwide[1].

- Multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB have made treatment more complex, prompting the use of newer and repurposed drugs, such as bedaquiline (BDQ) and clofazimine (CFZ) [2&3].

- Emerging mutations in genes associated with BDQ and CFZ (including *Rv0678*, *pepQ, 1979c,* and *atpE*), may lead to reduced susceptibility, and this may further impact treatment outcomes negatively[4].

- This study investigated phenotypic and genotypic resistance patterns to BDQ and CFZ in *M. tuberculosis* isolates collected at a tertiary laboratory in Pretoria, South Africa.

METHODOLOGY

01

Drug resistant TB isolates

n = 169

02

Sub-cultured

(Middlebrook 7H10 agar)

03

Phenotypic characterization

(Microplate Alamar Blue Assay)

04

Genotypic characterization

(Whole Genome Sequencing)

05

Data analysis

(Statistical and Bioinformatics)

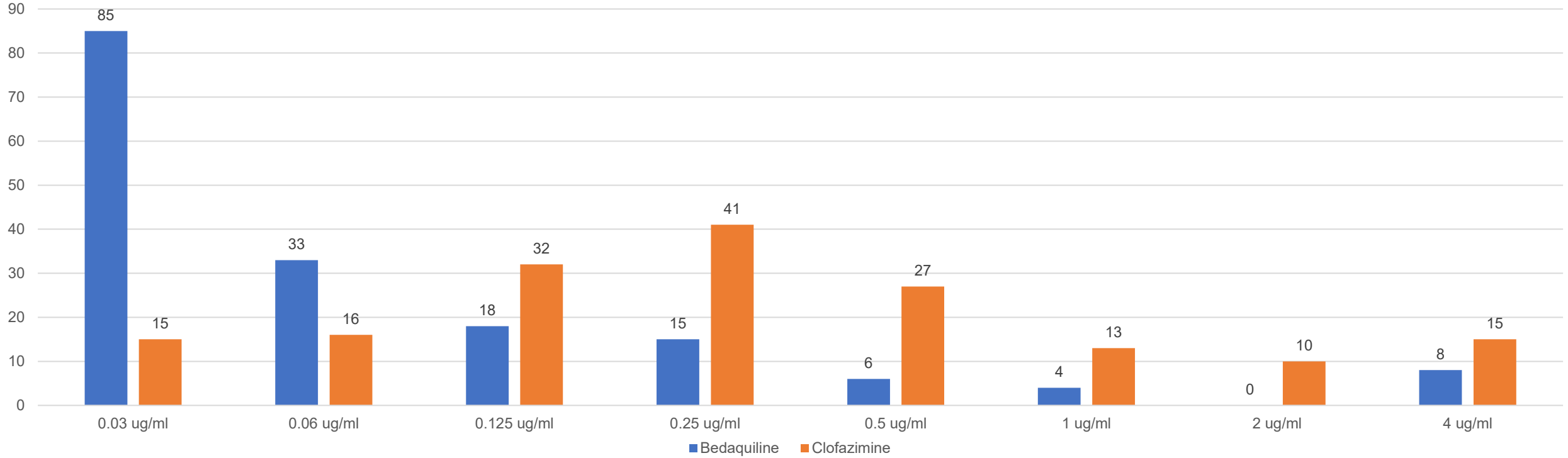
9th SA TB Conference

8 – 11 June 2026

Table 1: Demographic and clinical characteristics of study subjects

Variable		Frequency (169)	Percentage (%)
Age	0 -14	2	1.2
	15-24	10	5.9
	25-44	97	57.4
	45-64	48	28.4
	≥65	4	2.4
	Unknown	8	4.7
Gender	Male	92	54.4
	Female	68	40.2
	Unknown	9	5.3
Province	Gauteng	116	68.6
	Mpumalanga	11	6.5
	Northwest	38	22.5
	Unknown	4	2.3
HIV status	Positive	44	26.0
	Negative	5	3.0
	Unknown	120	71.0
Sample type	Pulmonary	158	93.5
	Extra pulmonary	3	1.8
	Unknown	8	4.7

Overall Minimum Inhibitory Concentrations (N=169)



- **Figure 1**: Bedaquiline and clofazimine minimum inhibitory concentrations

- BDQ showed high potency at very low MICs, in agreement with international studies demonstrating its superior efficacy, whereas CFZ had higher and more variable MICs [3 & 7].

- CFZ MICs were higher in MDR-TB isolates, Pre-XDR-TB, and RIF-R isolates, indicating reduced susceptibility, while XDR-TB isolates have moderate MICs.

Whole Genome Sequence (N=82)

9th SA TB Conference 8 - 11 June 2026

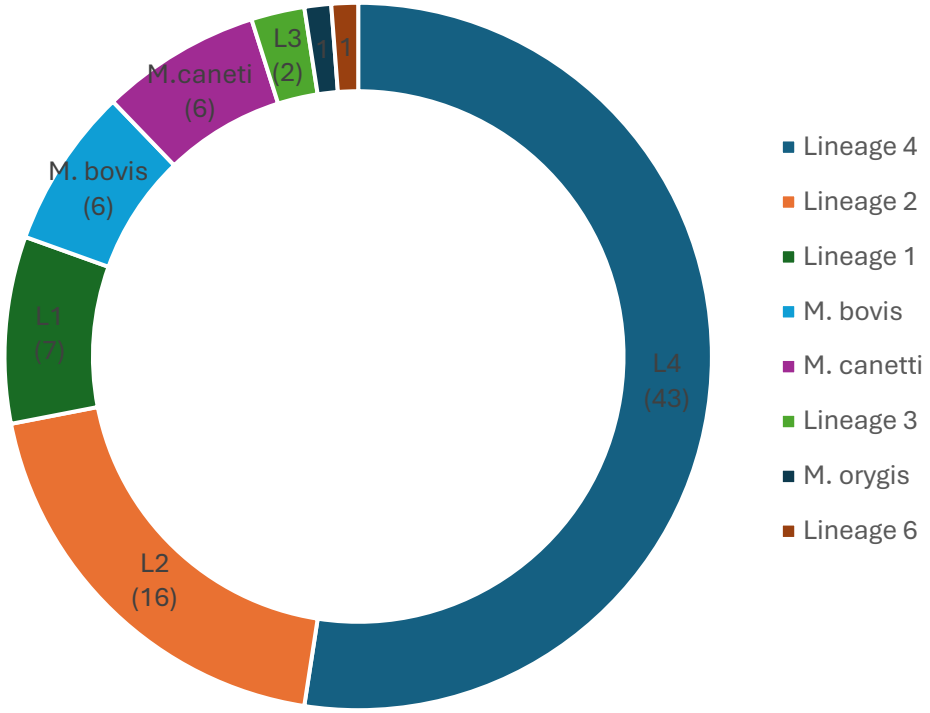


Figure 2: Lineage distribution (N=82)

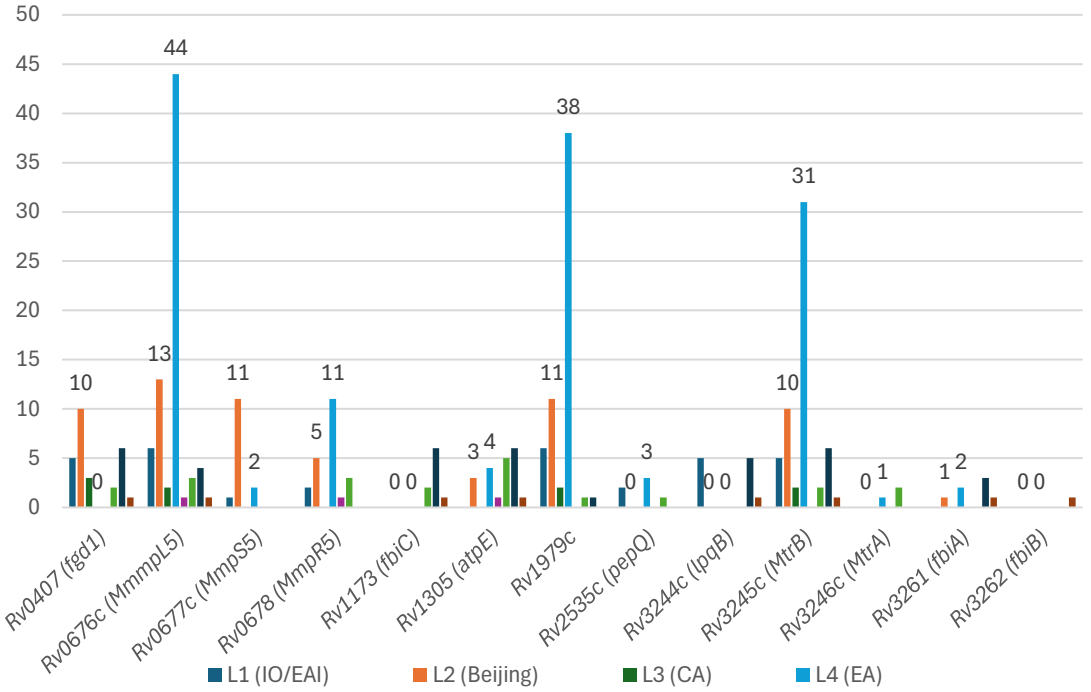


Figure 3: Total isolates mutated in 13 genes (N=82)

- WGS analysis revealed 8 lineages, with L4 [43/82 (52.4%)] and L2 [16/82 (19.5%)] being the most predominant lineages, respectively.
- A total of 319 resistance associated variants were identified in 13 genes that are associated with the BDQ and CFZ resistance, with *Rv0676c*, *Rv1979c* and *Rv3245c* being the highly mutated genes

Mutations Observed

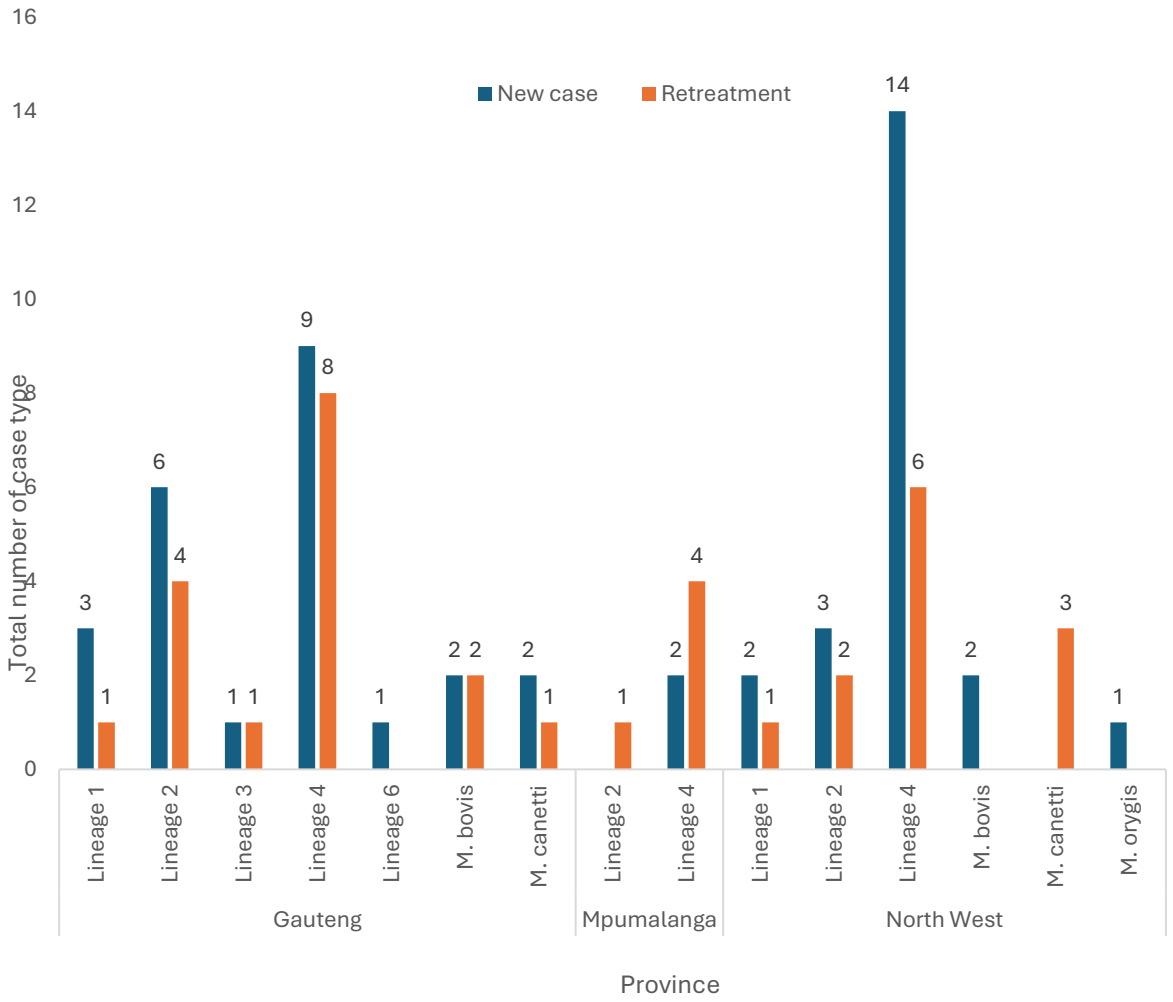


Figure 4: Distribution of lineages per case types in different provinces (N=82).

Table 2: Some of 319 mutations observed in 13 genes (N=82)

Rv0407 (fgd1)	*Rv0676c (MmmpL5)*	*Rv0677c (MmpS5)*	*Rv0678 (MmpR5)*	*Rv1173 (fbiC)*	*Rv1305 (atpE)*	*Rv1979c*	*Rv2535c (pepQ)*	*Rv3244c (lpqB)*	*Rv3245c (MtrB)*	*Rv3246c (MtrA)*	*Rv3261 (fbiA)*	*Rv3262 (fbiB)*
p.Arg212Leu	p.Ile948Val	c.-71C>T	c.141_142dupTC	Glu801Gly	p.Ala18Gly	c.-129A>G	Val214Ser	Asp142Gly	Met517Leu	c.-20delT	Ile208Val	c.1095C>G
c.18A>C	p.Thr794Ile	c.-710C>G	c.424dupC; c.390dupG		p.Val30Ile	c.-389C>A	Gln213Ala	c.657T>C	Val273Met	c.111C>T	Thr302Met	
c.291C>T	p.Asp767Asn	c.-720G>A	c.238delA; c.38dupA		p.Ile36Val		Ala196Thr	c.879T>C	Pro18Ser	c.324G>A	Gly264Arg	
c.960T>C	p.Trp598Arg		c.198dupG		p.Val39Ile		Asp8Gly		c.958T>C		c.-7C>G	
	c.2652C>G		p.Ser68Asn; p.Ser53Ala		p.Ser37Ala		c.621G>C		c.147G>A			
	p.Ala883Ser		p.Gln159Lys; p.Ser68Gly									
	p.Ser583Asn		p.Arg94Gln; p.Arg134*									
			p.Glu138Gly									

MIC distribution of WGS isolates (N=42)

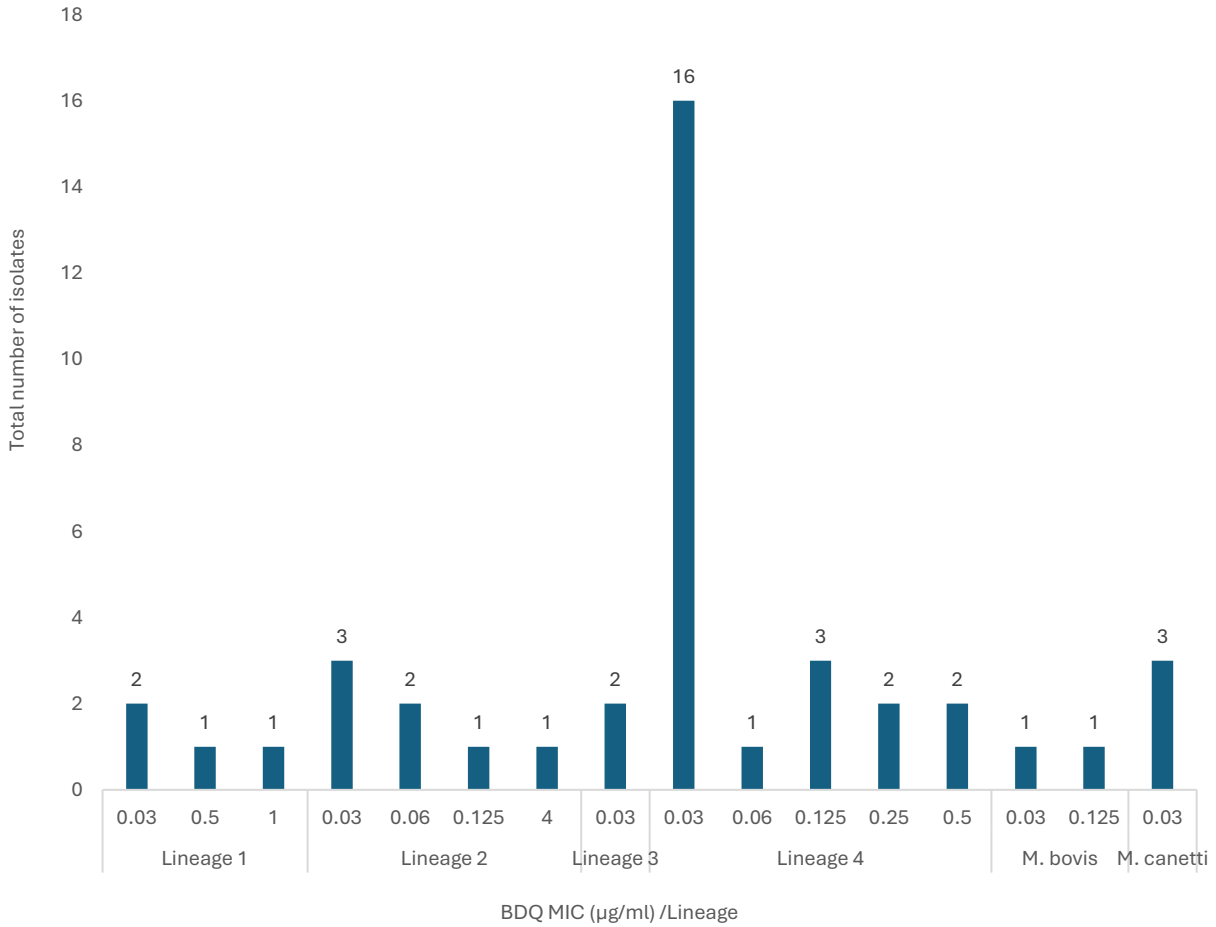


Figure 5: Distribution of Bedaquiline MICs within lineages

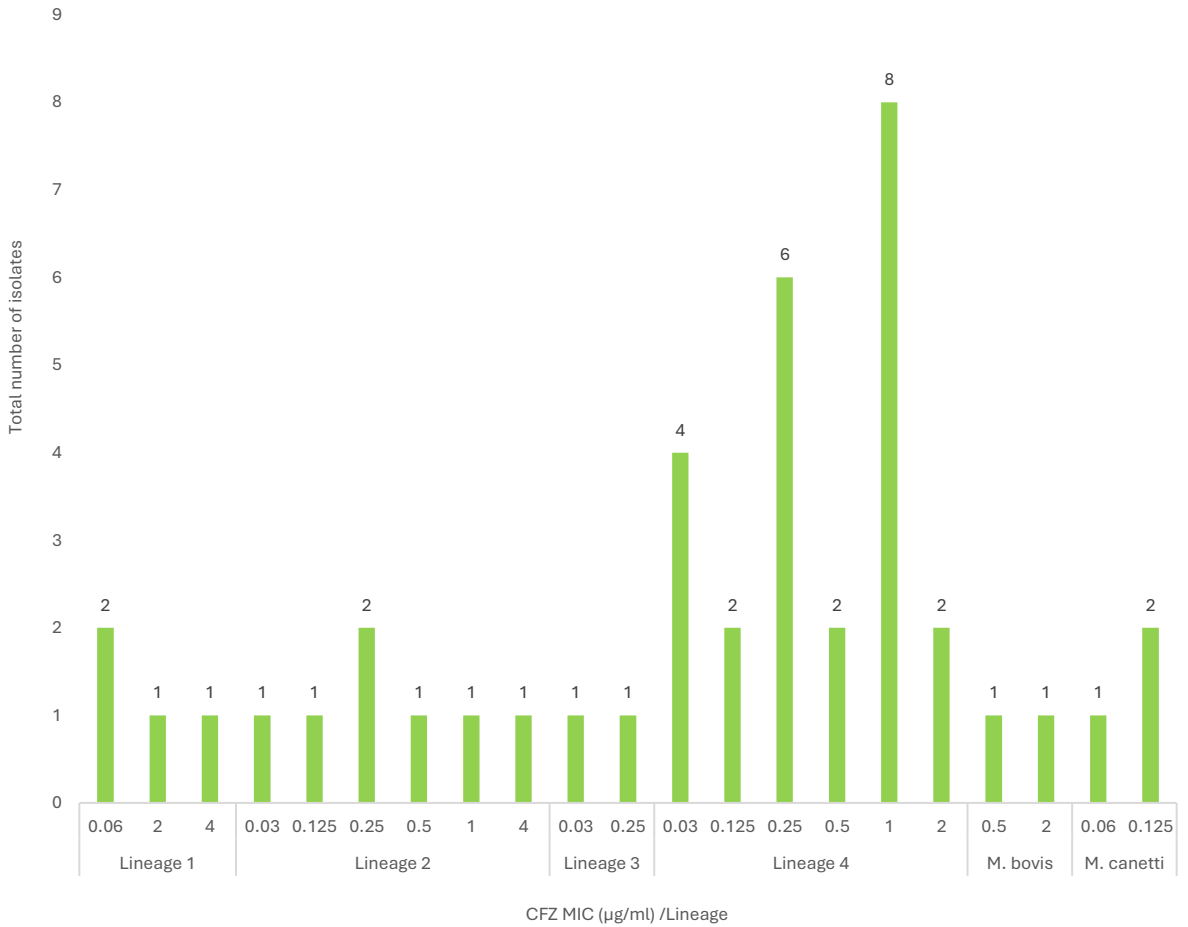


Figure 6: Distribution of clofazimine MICs within lineages

Most isolates from lineages had the MICs of 0.03ug/ml,
For CFZ, Lineage 4 isolates 1µg/ml.

9th SA TB Conference
8 - 11 June 2026

Discussion

- Most TB cases occurred among males and adults aged 25–44 years, consistent with prior studies in South Africa and other high-burden countries showing higher TB incidence in working-age men [5 & 6].

- Lineage 4 predominates across isolates, mutations, and provincial distributions, highlighting its major role in transmission and drug resistance.

- Variants in *Rv3245c* and *Rv0407* were also identified, similar to a few recent reports of emerging RAVs, but these specific variants are not yet in the WHO catalogue.

- BDQ maintained strong activity across resistance profiles, reinforcing global findings, while the emergence of variants in *Rv3245c* and *Rv0407* underscores the need for ongoing surveillance.

Conclusion

9th SA TB Conference 8 - 11 June 2026

- Lineage 4 and lineage 2 were the most predominant lineages in this study.
- BDQ remained highly potent across resistance profiles, while CFZ showed variable activity, especially in MDR and Pre-XDR isolates.
- The emergence of novel RAVs and detection of animal lineages, highlight an ongoing drug-resistance evolution and potential zoonotic transmission, underscoring the need for continued surveillance.

References

1. Faye LM, Hosu MC, Oostvogels S, Dippenaar A, Warren RM, Sineke N, Vasaikar S, Apalata T. The Detection of Mutations and Genotyping of Drug-Resistant *Mycobacterium tuberculosis* Strains Isolated from Patients in the Rural Eastern Cape Province. Infect Dis Rep. 2023 Jul 10;15(4):403-416. doi: 10.3390/idr15040041. PMID: 37489395; PMCID: PMC10366782.
2. Shafipour M, Mohammadzadeh A, Mahmoodi P, Dehghanpour M, Ghaemi EA. Distribution of lineages and type II toxin-antitoxin systems among rifampin-resistant Mycobacterium Tuberculosis Isolates. PLoS One. 2024 Oct 24;19(10):e0309292. doi: 10.1371/journal.pone.0309292. PMID: 39446830; PMCID: PMC11500941.
3. Nimmo, C., Millard, J., van Dorp, L., Brien, K., Moodley, S., Wolf, A., Grant, A.D., Padayatchi, N., Pym, A.S., Balloux, F. and O'Donnell, M., 2020. Population-level emergence of bedaquiline and clofazimine resistance-associated variants among patients with drug-resistant tuberculosis in southern Africa: a phenotypic and phylogenetic analysis. *The Lancet Microbe*, *1*(4), pp.e165-e174.
4. Hu, X., Wu, Z., Lei, J. *et al*. Prevalence of bedaquiline resistance in patients with drug-resistant tuberculosis: a systematic review and meta-analysis. *BMC Infect Dis* **25**, 689 (2025). https://doi.org/10.1186/s12879-025-11067-2.
5. Saeed, D.K., Shakoor, S., Razzak, S.A., Hasan, Z., Sabzwari, S.F., Azizullah, Z., Kanji, A., Nasir, A., Shafiq, S., Ghanchi, N.K. and Hasan, R., 2022. Variants associated with Bedaquiline (BDQ) resistance identified in Rv0678 and efflux pump genes in Mycobacterium tuberculosis isolates from BDQ naïve TB patients in Pakistan. *BMC microbiology*, *22*(1), p.62.
6. Yang, J., Pang, Y., Zhang, T., Xian, X., Li, Y., Wang, R., Wang, P., Zhang, M. and Wang, J., 2020. Molecular characteristics and in vitro susceptibility to bedaquiline of Mycobacterium tuberculosis isolates circulating in Shaanxi, China. *International Journal of Infectious Diseases*, *99*, pp.163-170.
7. Seung KJ, Keshavjee S, Rich ML. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harb Perspect Med. 2015 Apr 27;5(9):a017863. doi: 10.1101/cshperspect.a017863. PMID: 25918181; PMCID: PMC4561400.
8. World Health Organization, 2020. *WHO consolidated guidelines on tuberculosis: tuberculosis preventive treatment*. World Health Organization.

Thank You