

Engineered Phages to illuminate Mycobacteria like Miniature Flashlights

Usefulness in BPaL drug susceptibility testing

Saranathan Rajagopalan Ph.D.

Research Assistant Professor

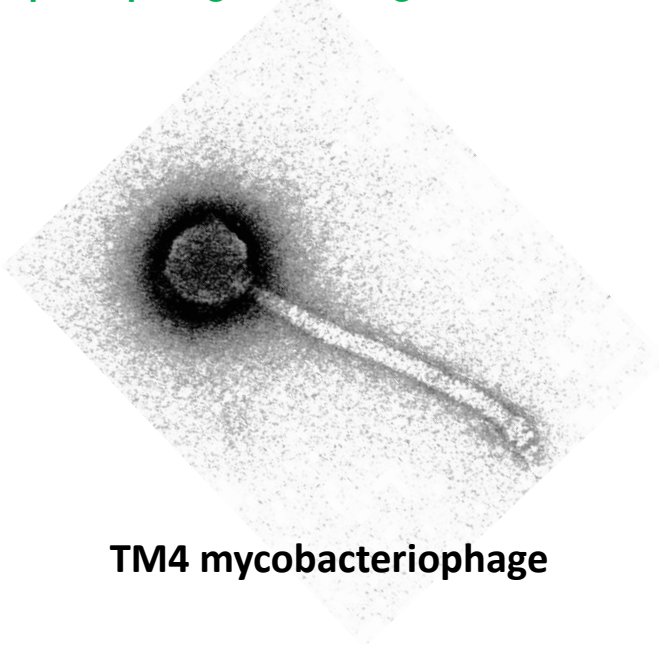
Laboratory of **William R Jacobs Jr.**

Albert Einstein College of Medicine, Bronx, New York

Mycobacteriophages

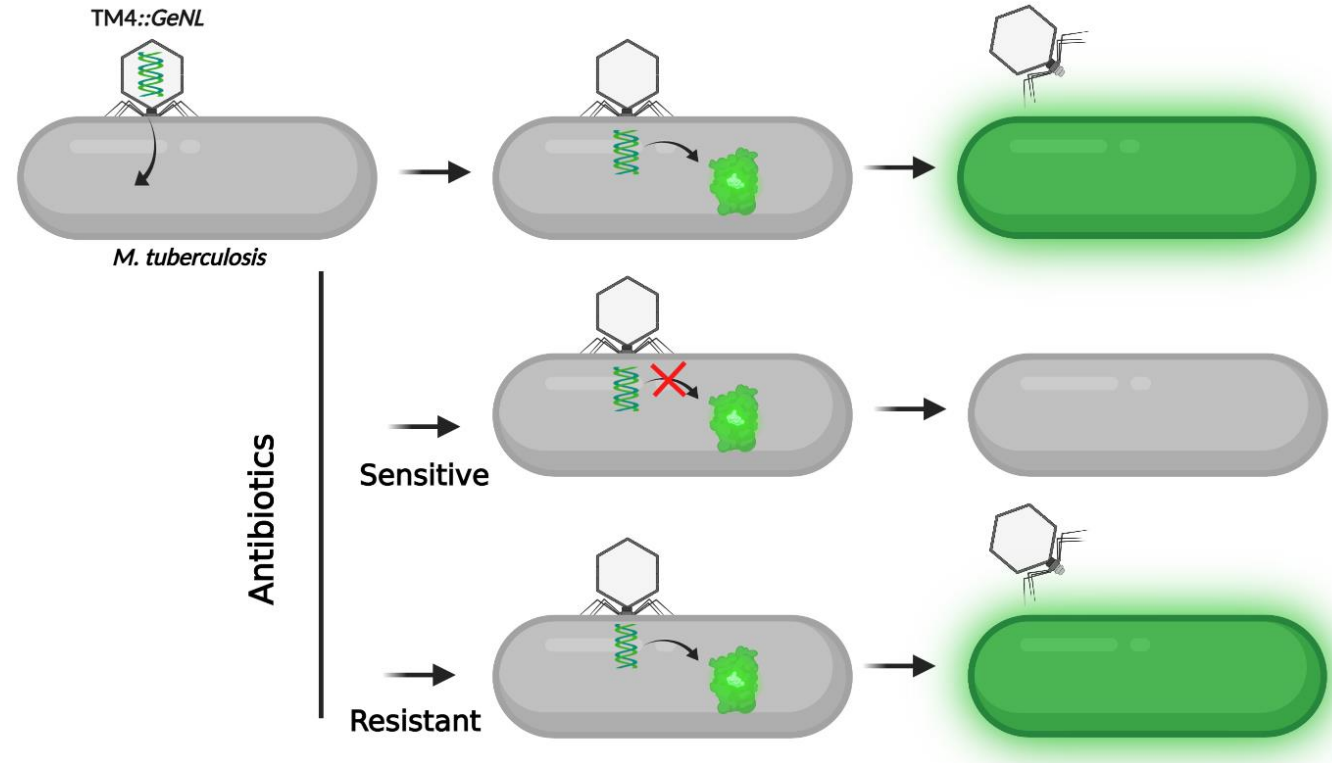
- Bacteriophages (viruses) that infect mycobacteria
- Replication is host dependent
- Highly diverse, 21000+ isolated, 3900+ sequenced

[\(https://phagesdb.org/\)](https://phagesdb.org/)



TM4 mycobacteriophage

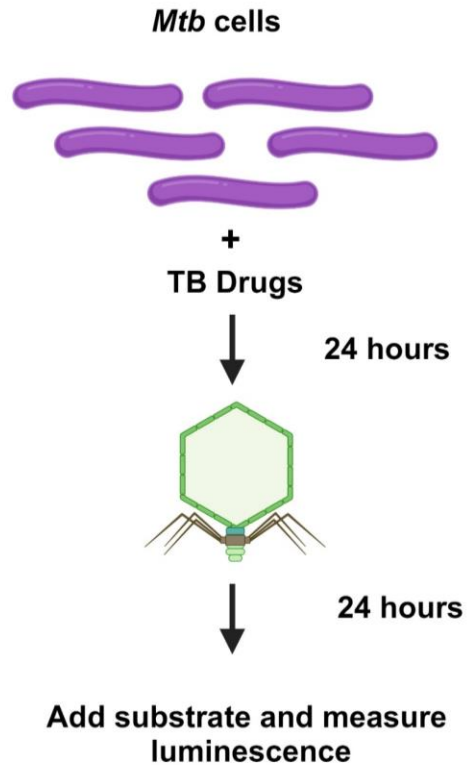
Drug sensitivity testing



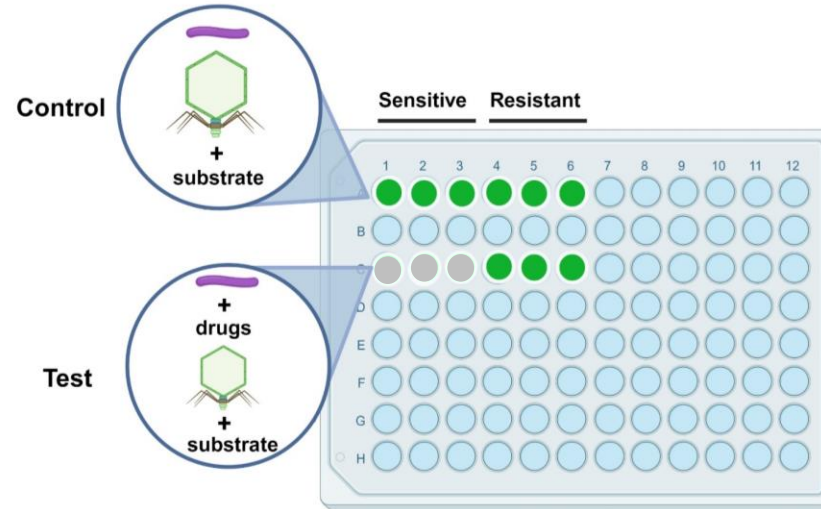
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Typical layout of testing

a)



b)

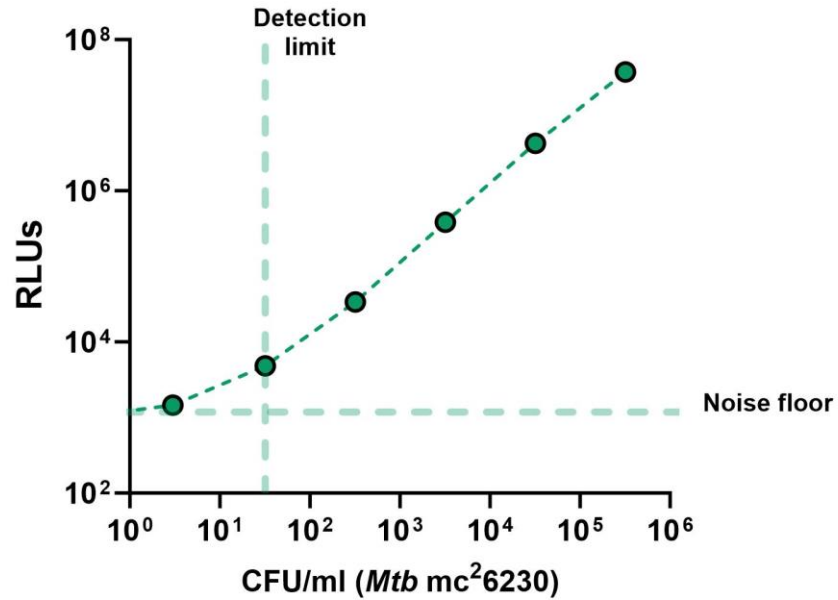


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Consumable and reagents: Culture/clinical specimen, 96 well plate, phage, substrate, media and other consumables

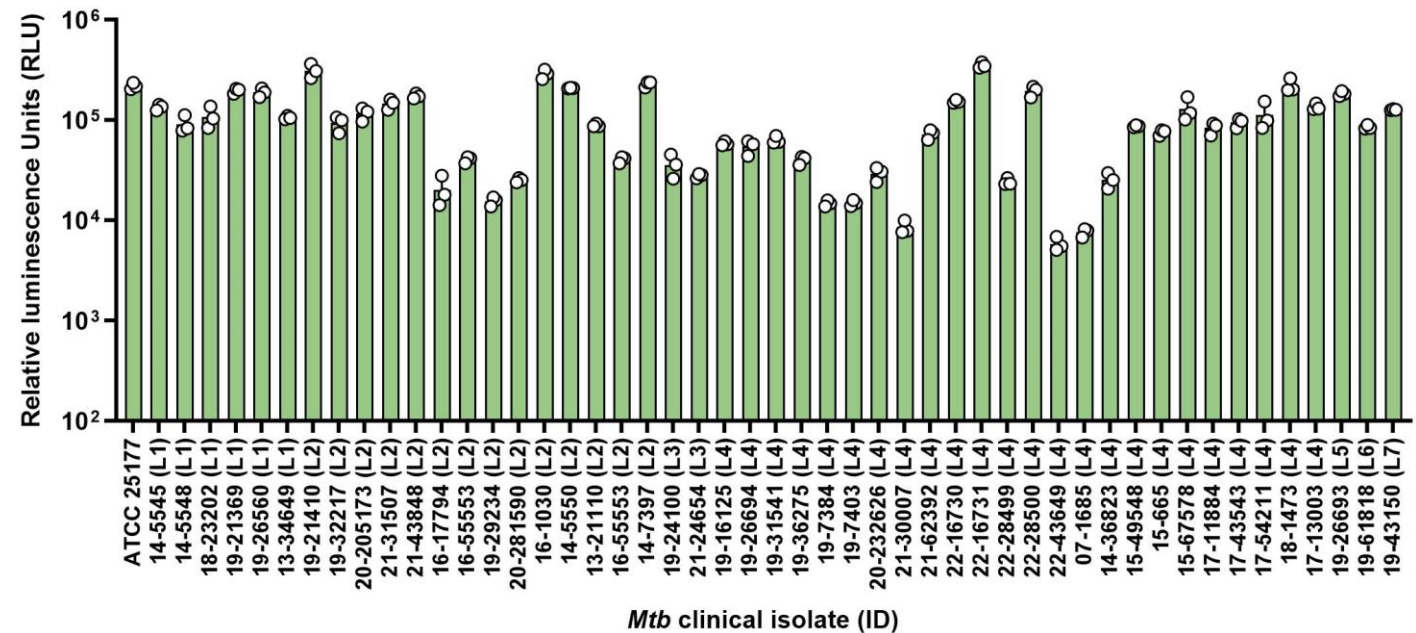
Equipment: Incubator, Plate reader

M. tuberculosis Limit of detection



This assay can detect as little as 30 tubercle bacilli

Reporter phage infects clinical isolates of *M. tuberculosis*



Our phage could infect and light up all the clinical isolates tested so far - All lineages

i. Bedaquiline, pretomanid, linezolid and clofazimine drug susceptibility testing

| Drug | Tier 1 | Tier 2 | Number of resistant mutations |
|-------------|--|---------|-------------------------------|
| Bedaquiline | <i>pepQ, Rv0678, mmpL5, mmpS5, atpE</i> | Rv1979c | 80+ |
| Pretomanid | <i>fgd1, ddn, fbiA, fbiB, fbiC, Rv2983</i> | None | 100+ |
| Linezolid | <i>rplC, rrl</i> | None | 20+ |
| Clofazimine | <i>pepQ, Rv0678, mmpL5, mmpS5</i> | Rv1979c | 80+ |

ii. Occult/dispute/low-level/borderline Rifampicin resistance

Leu430Pro, Asp435Tyr, His445Asn, His445Leu, Leu452Pro, Ile491Phe

iii. Heterogenous variants of alleles (Mixed infections)

Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, WHO 2022

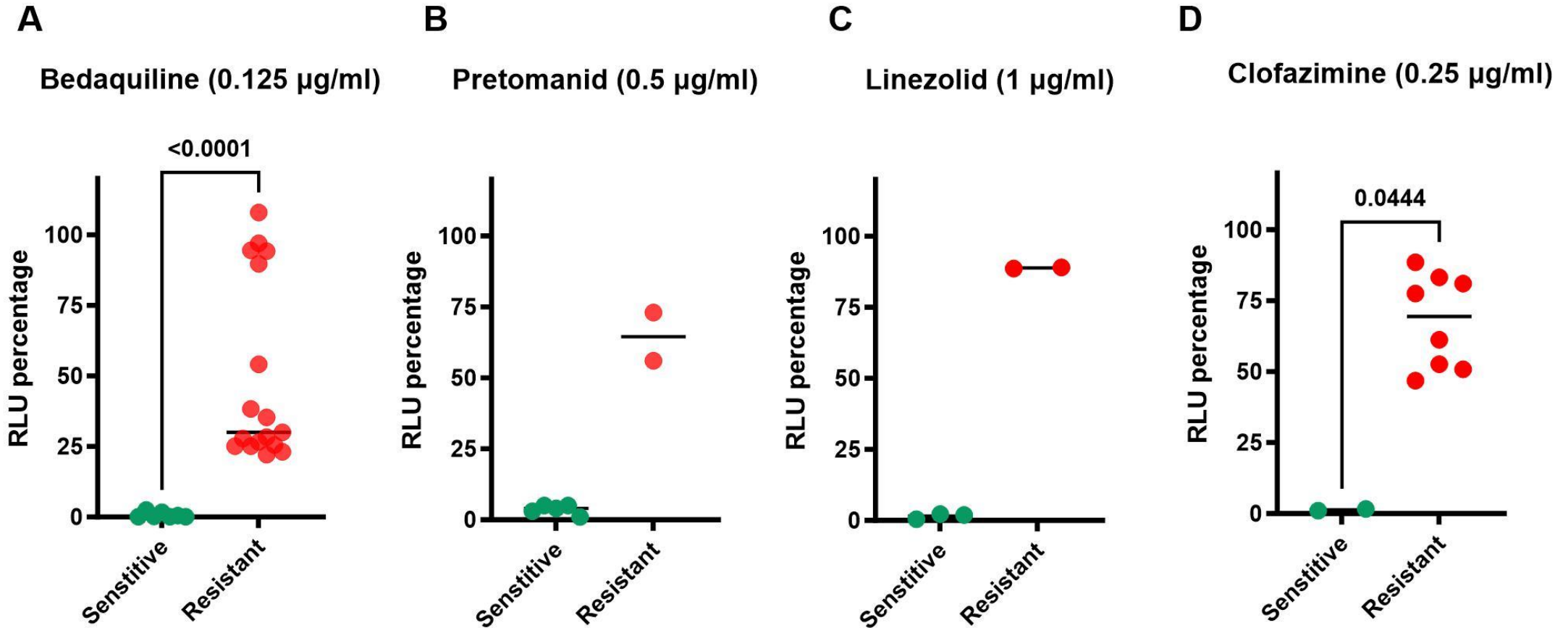
Makhado et al., Lancet Infect Dis. 2022

Barilar et al., Lancet Infect Dis. 2024

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Drug susceptibility testing of *Mtb* clinical isolates: BPaL-C



Our results demonstrate that the phage assay can perform drug susceptibility testing (DST) for all drugs used in the BPaL regimen, effectively differentiating between sensitive and resistant isolates

Occult/dispute/low-level/borderline Rifampicin resistance

Table 1. Sensitivity of clinical *Mtb* isolates to Rifampicin

| Sample ID (lineage) | WGS | | Culture based DST | | TM4::GeNL DST (0.125 µg/ml) | |
|---------------------|-------------|----------------------|-----------------------|--------------|-----------------------------|-------------|
| | Gene | Mutation | MGIT DST (1 µg/mL) | MIC µg/ml | % RLU retention (Mean) | Phenotype |
| ATCC 25177 (L4) | None | | Sus | <0.12 | 0.1 | Susceptible |
| 21-24654 (L3) | None | | Sus | <0.12 | 0.9 | Susceptible |
| 19-21369 (L1) | None | | Sus | < 0.12 | 1.1 | Susceptible |
| 19-21410 (L2) | None | | Sus | < 0.12 | 0.5 | Susceptible |
| 19-24100 (L3) | None | | Sus | 0.25 | 1.7 | Susceptible |
| 19-26694 (L4) | None | | Sus | <0.12 | 0.5 | Susceptible |
| 20-205173 (L2) | None | | Sus | <0.12 | 0.5 | Susceptible |
| 18-1473 (L4) | None | | Sus | <0.12 | 0.3 | Susceptible |
| 07-1685 (L4) | <i>rpoB</i> | Ser450Leu | Res | >16 | 96.8 | Resistant |
| 16-17794 (L2) | <i>rpoB</i> | Ser450Leu | Res | >16 | 94.2 | Resistant |
| 16-55553 (L2) | <i>rpoB</i> | Ser450Leu | Res | >16 | 79 | Resistant |
| 20-281590 (L2) | <i>rpoB</i> | His445Tyr | Res | >16 | 90.5 | Resistant |
| 19-26693 (L5) | None | | Sus | < 0.12 | 27.3 | Resistant |
| 19-61818 (L6) | None | | Sus | < 0.12 | 36.6 | Resistant |
| 19-43150 (L7) | None | | Sus | < 0.12 | 16.6 | Resistant |
| 13-34649 (L1) | <i>rpoB</i> | Asp435Tyr | Sus | 8 | 80.4 | Resistant |
| 14-5550 (L2) | <i>rpoB</i> | Ile491Phe, Ala584Asp | Sus | 1 | 74 | Resistant |
| 13-21110 (L2) | <i>rpoB</i> | Leu452Pro, Asp265Gly | Sus | 2 | 49.7 | Resistant |
| 14-5545 (L2) | <i>rpoB</i> | Asp435Tyr | Sus | 8 | 91.6 | Resistant |
| 14-7397 (L2) | <i>rpoB</i> | Leu430Pro | Sus | 4 | 86.5 | Resistant |
| 17-13003 (L4) | <i>rpoB</i> | Ile491Phe | Sus | 1 | 86.8 | Resistant |
| 14-5548 (L1) | <i>rpoB</i> | Asp516Tyr | Sus | >16 | 102.3 | Resistant |
| 19-29234 (L2) | <i>rpoB</i> | Leu430Pro | Sus | 0.5 | 12.5 | Resistant |
| 15-49548 (L4) | <i>rpoB</i> | Asp516Tyr | Res | 2 | 26.1 | Resistant |
| 19-31541 (L4) | <i>rpoB</i> | Leu511Pro | Res | 1 | 19.7 | Resistant |

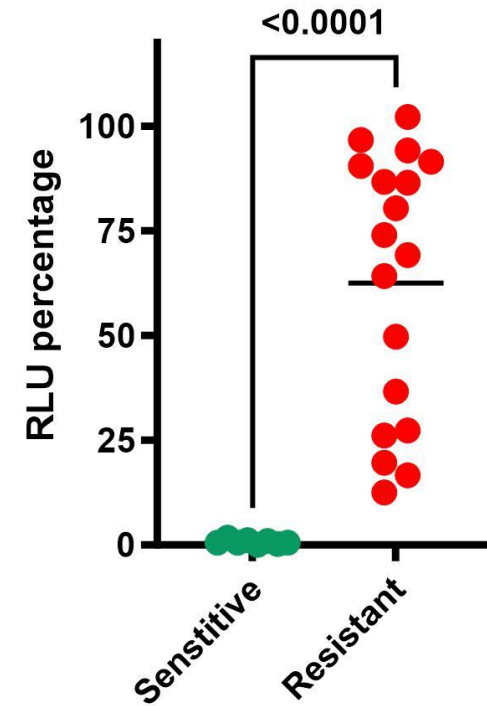
Pan-susceptible

Resistant - classic high level resistant

Lineage specific low-level resistant like phenotype

Occult/dispute/borderline/low-level resistant mutations

Rifampicin (0.125 µg/ml)



Heterogenous variants of alleles (Mixed infections)

| 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 | | | | | | | | |
| P0027 | 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 | G49fs | | 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 |
| P0121 | E | XDR | RR | Z FQ B C L Tz (3) | Culture converted | Successful | F93L | | 0.12 | N/A |

Baseline bedaquiline RAV

Emergent bedaquiline RAV

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

Culture positive, *Rv0678* variant present >95%

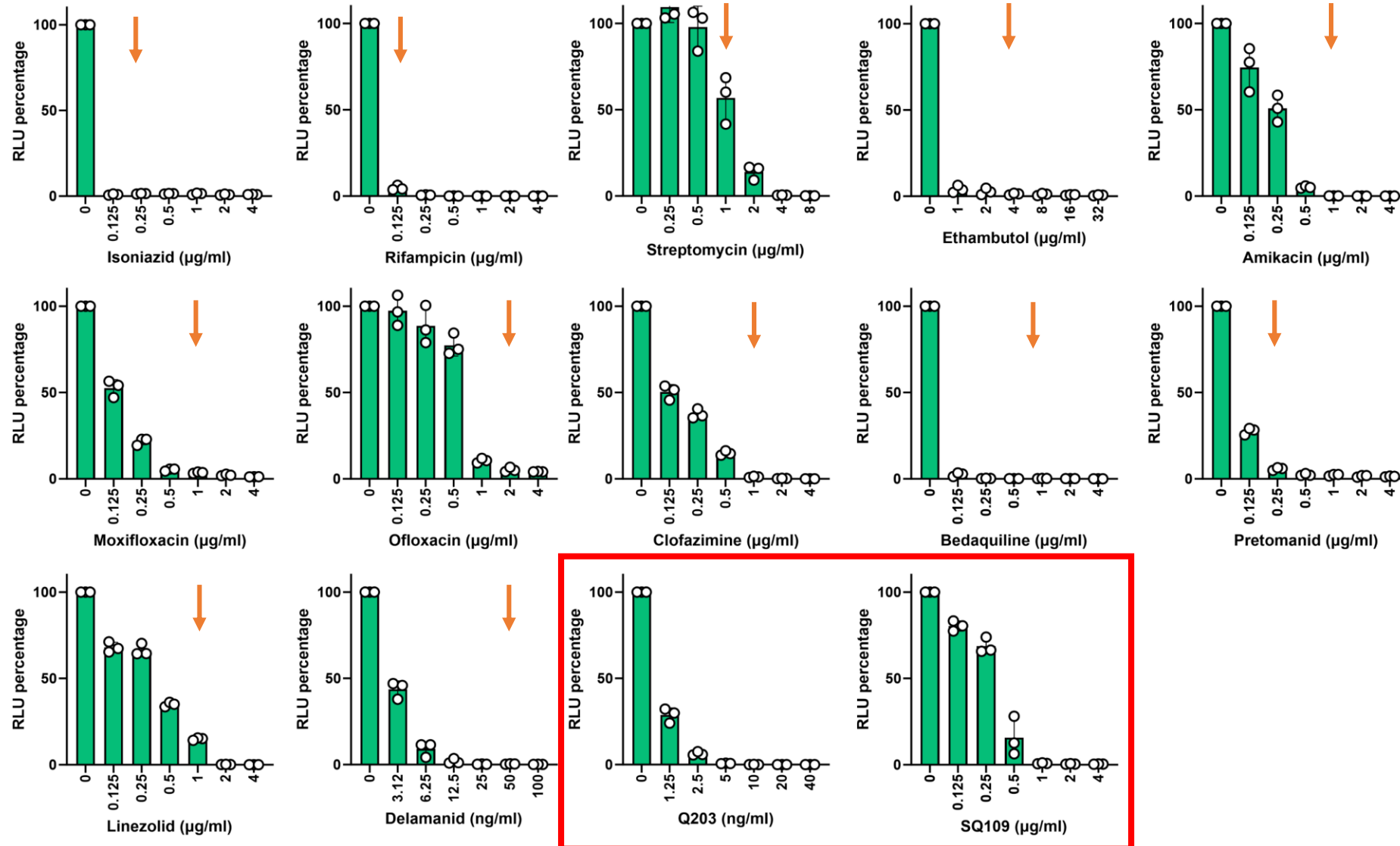
Culture positive, *Rv0678* variant absent

MIC measured

Phage DST data from Senamile Ngema, CAPRISA

| Strains | BDQ (0.125) | PMD (0.5) | LZD (1) | RIF (0.125) | MXF (0.25) | CFZ (0.25) |
|---------|-------------|-----------|---------|-------------|------------|------------|
| H37Rv | S (0.4%) | S | S | S | S | S |
| P 0027 | R (7.2%) | S | S | R | R | R |
| P 0121 | R (32.3%) | S | S | R | R | R |

Drug susceptibility testing of *Mtb mc²6230* with different TB drugs



Summary

- TM4::*GeNL* reporter phage can differentiate the *M. tuberculosis* strains with drug resistant mutations
- Identifies baseline and emerging drug resistance against drugs in BPaL regimen, clofazimine
- Works well in diagnosing low-level drug resistance towards rifampicin and heterozygous resistance
- Holds the potential to facilitate the customization of treatment regimens for drug-resistant TB more effectively
- This assay can work with all the future drugs which are in pipeline and could be implemented in LMIC set up



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