# Clinical application of quantitative susceptibility testing

## (if and when to use MICs)

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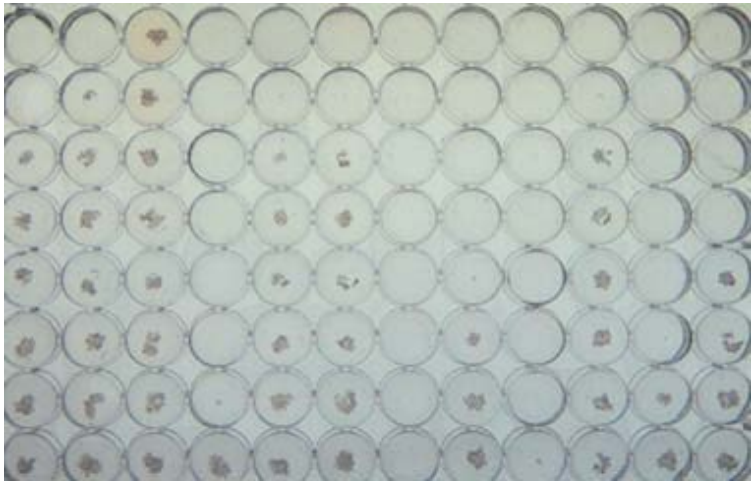
**No disclosures**

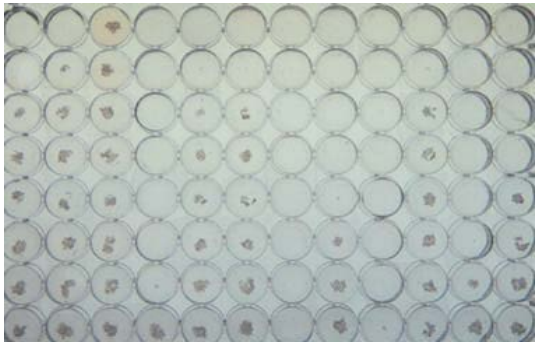
**MIC plate**



**Patient with MDR-TB, Dhaka Bangladesh**

With permission

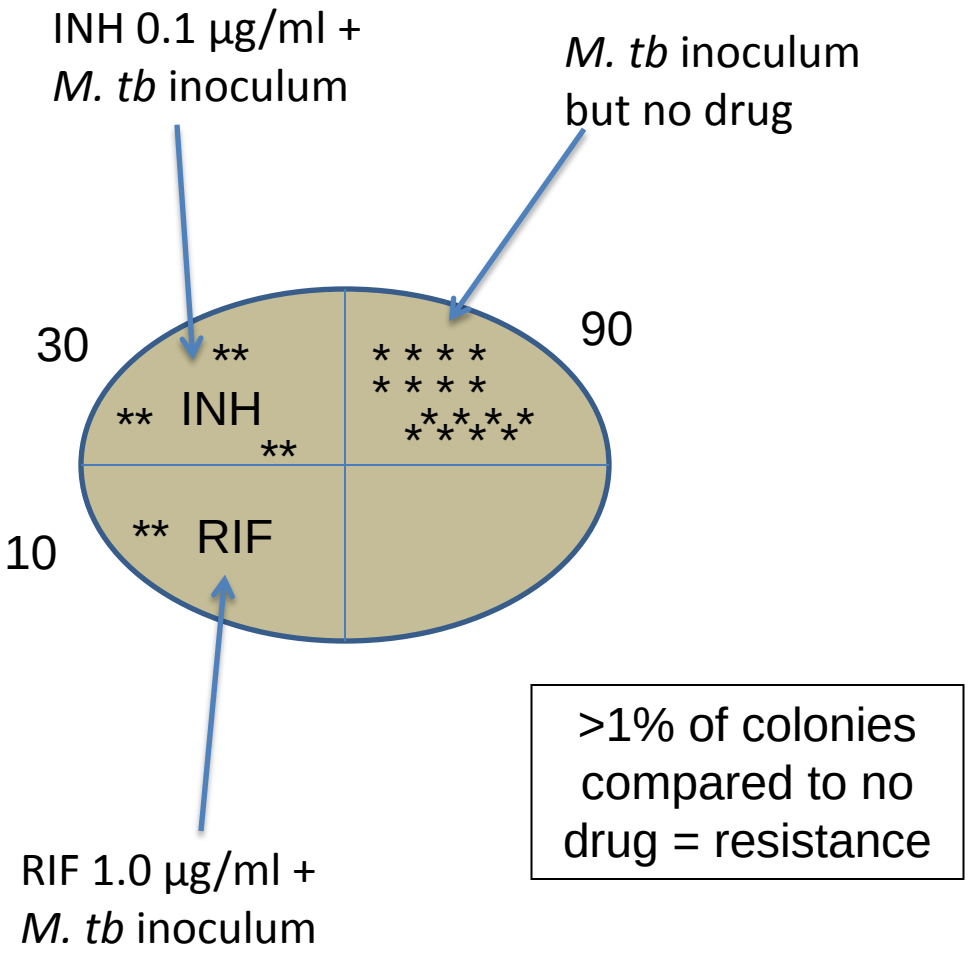
**How can quantitative susceptibility impact care at the bedside?**



# Outline

- Introduction to quantitative susceptibility testing
- The limited role of minimum inhibitory concentration (MIC) testing for fully drug susceptible TB
- The importance of MIC for drug-resistant TB or patients slow-to-respond given new data on individual pharmacokinetic variability
- Advantages/ disadvantages for TB/MDR-TB endemic areas
- Moving from resistance breakpoints: do we need an “intermediate” range?

# Principles of the 1% proportion method




>1% of colonies compared to no drug = resistance

**Single critical concentration with qualitative yes/no resistance (*different than most other infectious diseases*)**

**But *M. tb* is different→ susceptibility testing on only subpopulation of organism in rapid growth phase, regimens used are 4+ different drugs**

**Crit concn can vary by media**

**But some isolates may teeter on Sus/Res, even using same media, same day of prep**

**Media prep at multiple concn for different drugs necessary for true MIC may be tedious, lack reproducibility**

# Minimum inhibitory concentrations- historically used in specialized settings on solid agar

We'll return to this concept

*

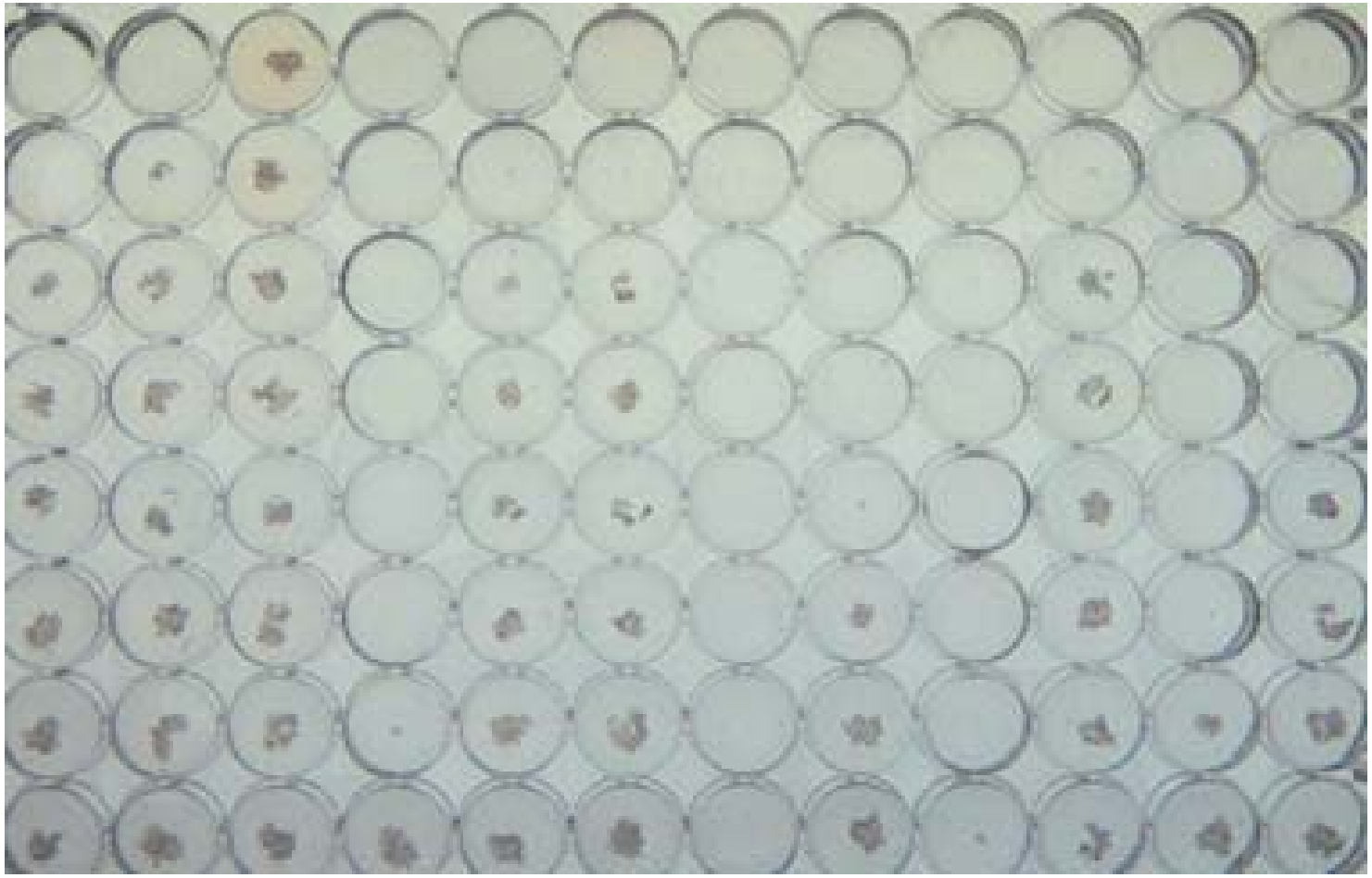
| Drug | Susceptible | Moderately susceptible | Moderately resistant | Resistant |
|---|---|---|---|---|
| **Isoniazid** MIC µg/ml | ≤0.1 | 0.2-1.0 | 2.0 | ≥4.0 |
| **Rifampin** MIC µg/ml | ≤0.5 | 1.0-4.0 | 8.0 | ≥16.0 |

In practice this is uncommon

Majority with *rpoB* mutation

*Adapted from Iseman (LWW 2000) and Heifets, *Am Rev Respir Dis* 1988.

# Now commercial microplate platform available



OFL MXF RIF AMI STR RFB PAS ETH CYC INH KAN EMB

**Lyophilized drug in prefilled wells, shelf-life 2 years at room temperature**

Sensititre MYCOTB, TREK

- 122 *M. tb* isolates
- APM on 7H10
- **94%-100%** categorical agreement using Plate concn nearest to APM crit concn
- ***Very few*** resistant isolates by APM: Eg. Moxi 2 (1.6%), Amik 8 (6.5%)

**TABLE 1** Comparison of the APM critical concentrations and MycoTB plate ranges

| Agent | APM critical concn(s) tested (μg/ml) | MycoTB plate range (μg/ml) | MycoTB plate concn(s) nearest to the APM critical concn(s)[a] (μg/ml) |
|---|---|---|---|
| First-line agents | | | |
| Ethambutol | 5.0, 10.0 | 0.5–32 | 4.0, 8.0 |
| Isoniazid | 0.2, 1.0 | 0.03–4 | 0.25, 1.0 |
| Rifampin | 1.0 | 0.12–16 | 1.0 |
| Second-line agents | | | |
| Amikacin | 5.0 | 0.12–16 | 4.0 |
| Cycloserine | 25.0 | 2.0–256 | 32.0 |
| Ethionamide | 5.0 | 0.3–40 | 5.0 |
| Kanamycin | 5.0 | 0.6–40 | 5.0 |
| Moxifloxacin | 2.0 | 0.06–8.0 | 2.0 |
| Ofloxacin | 2.0 | 0.25–32 | 2.0 |
| *p*-Aminosalicylic acid | 2.0 | 0.5–64 | 2.0 |
| Rifabutin | 0.5 | 0.12–16 | 0.5 |
| Streptomycin | 2.0, 10.0 | 0.25–32 | 2.0, 8.0 |

Hall et al, *J Clin Micro* 2012

**But the real advantage is *not* in another yes/no qualitative resistance test…**

I want to know if an isolate is:

1. borderline susceptible

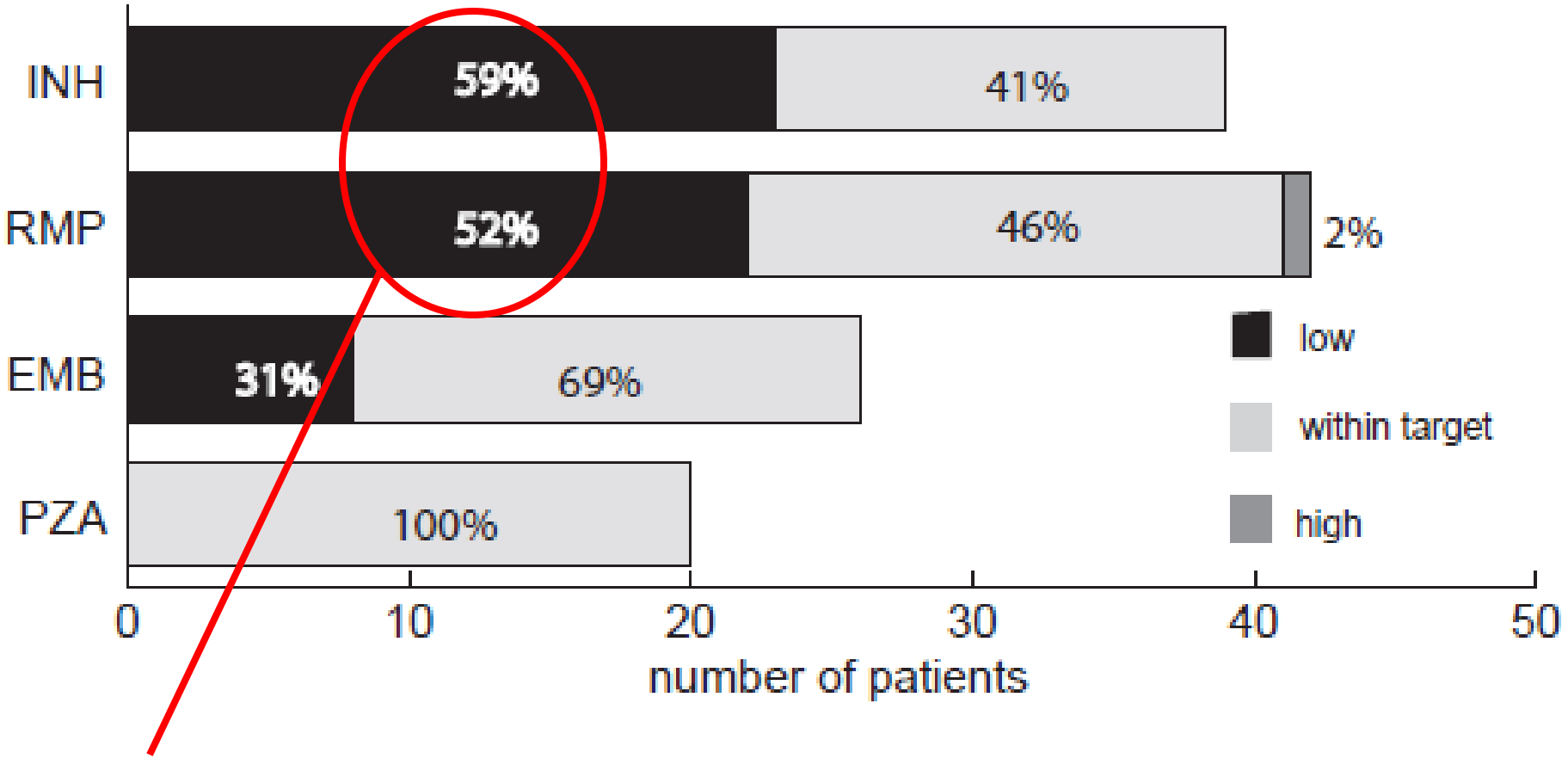
and I can maximize pharmacokinetics, particularly in a slow responder

or…

2. borderline resistant

if the drug options are limited
(complex MDR/XDR-TB)

# Majority of slow responders in Virginia had low $C_{2hr}$ levels of isoniazid (INH) and rifampin (RMP)

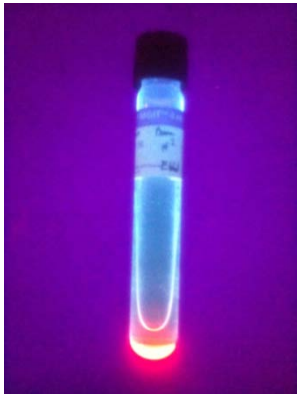



**These are the patients where a borderline susceptible MIC would matter most**

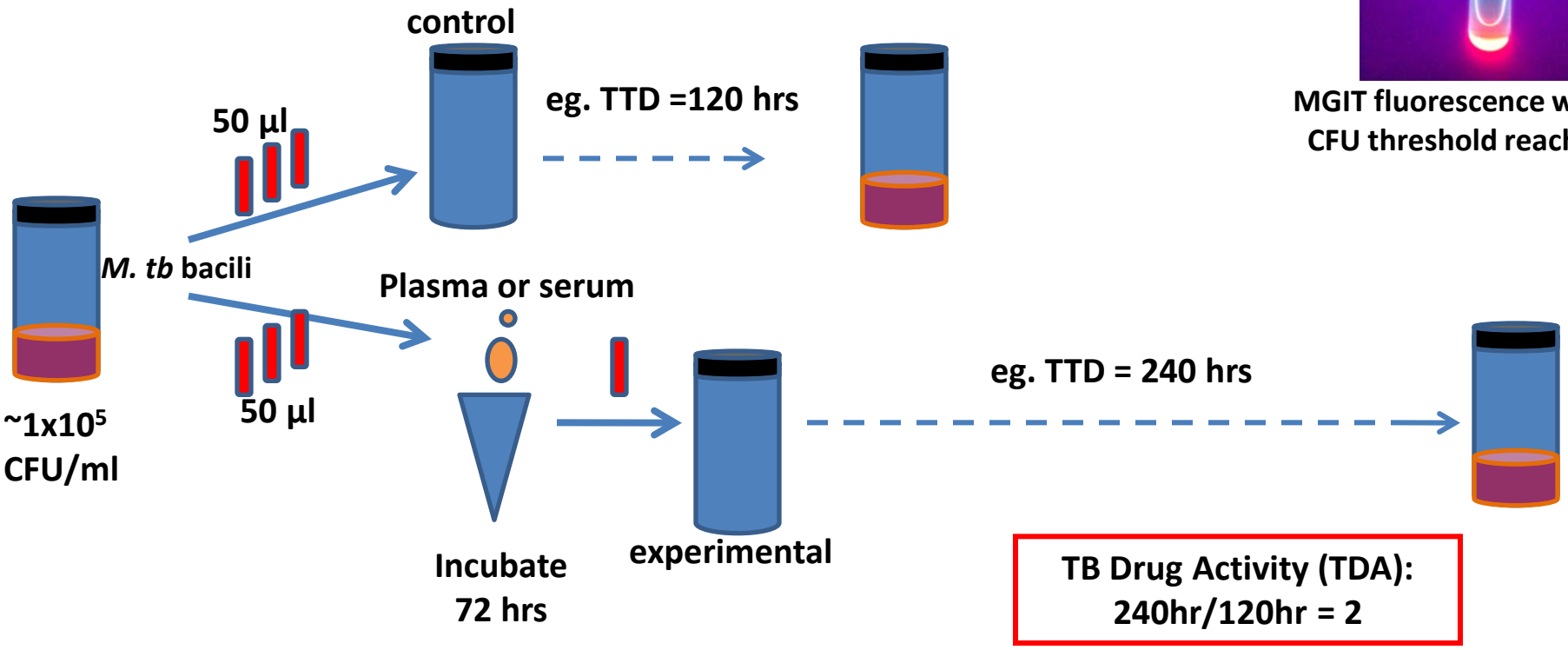
Heysell et al, *Emerg Infect Dis* 2010

## Plasma TB Drug Activity (TDA) assay:

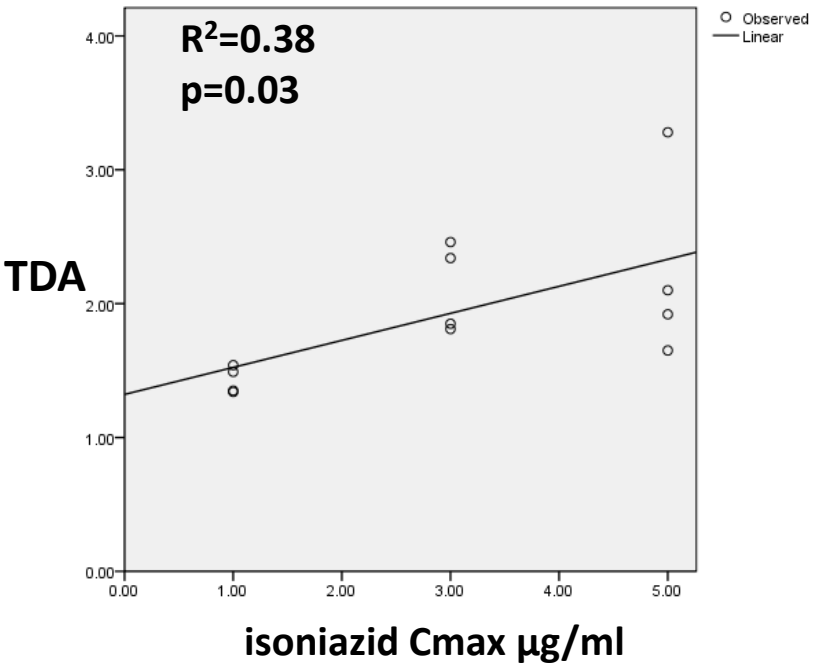
**In BACTEC MGIT tubes quantifiable killing measured as time-to-detection (TTD), accurate and reproducible as colony counting**

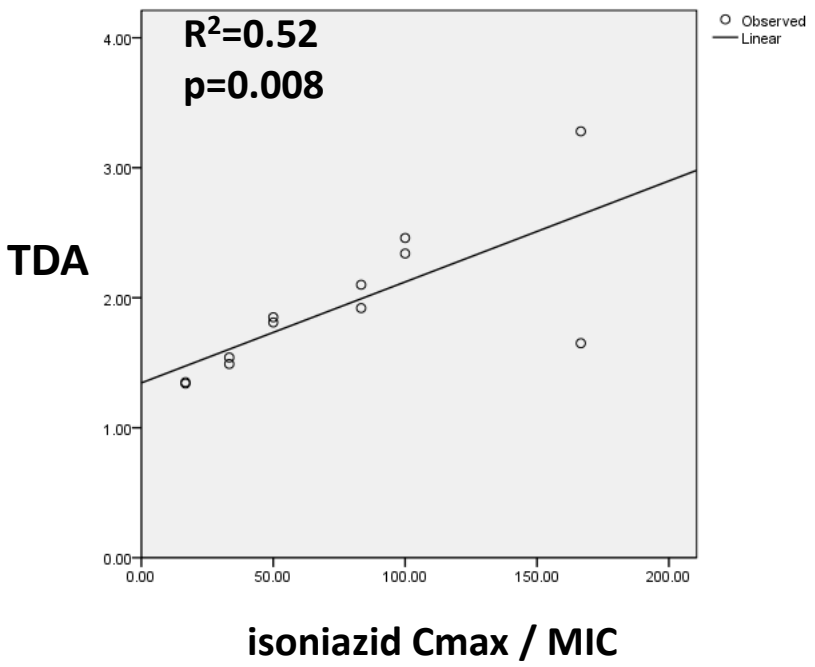


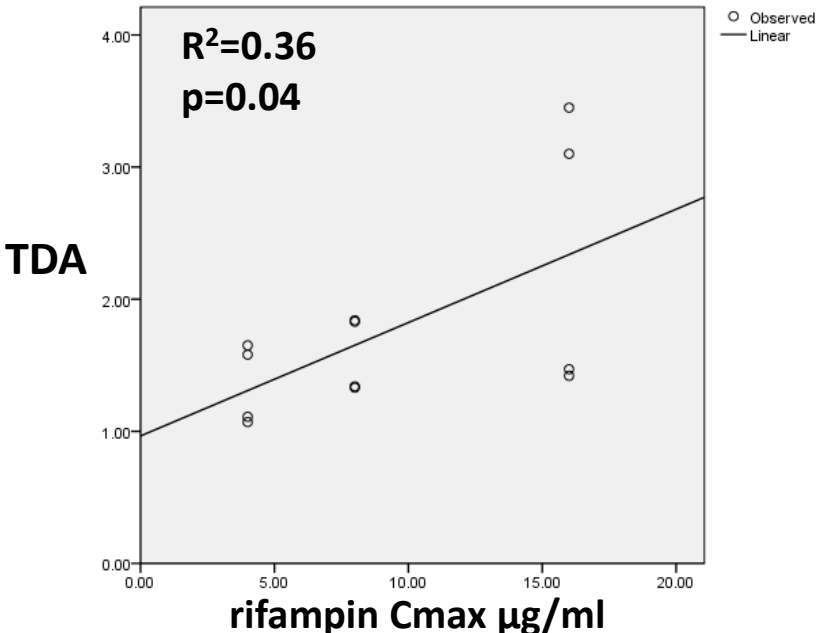
MGIT fluorescence when CFU threshold reached

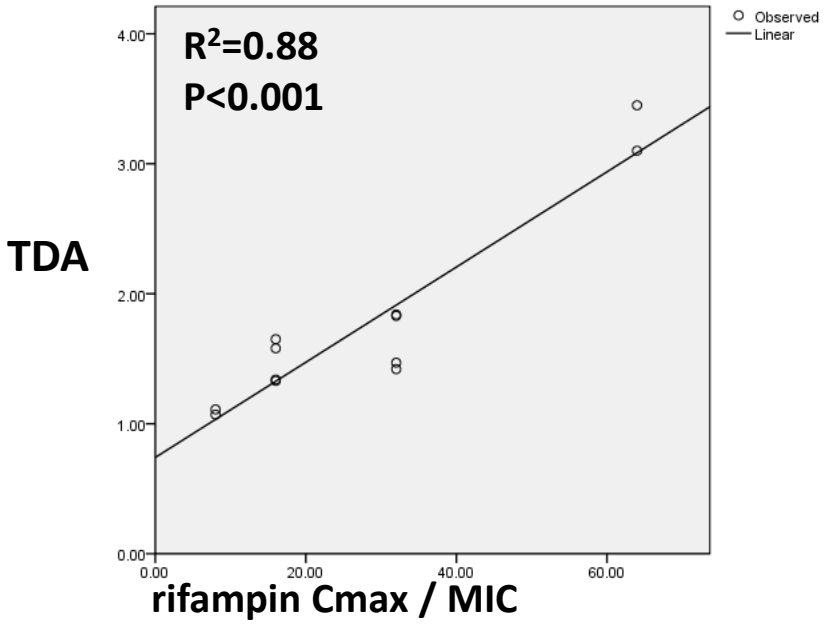


# The TB drug activity assay is a metric for Cmax/ MIC



# Poor plasma TB drug activity (Cmax/MIC) led to worse outcomes in Tanzania

TABLE 2. TB drug activity (TDA) values and $C_{2\ h}$ drug levels at 14 days of TB treatment for Tanzanian patients[a]

| Drug | Mean drug $C_{2\ h}$ ± SD (μg/ml) | | *P* value |
|---|---|---|---|
| | TDA ≤ 2.0 ($n$ = 9) | TDA > 2.0 ($n$ = 7) | |
| Isoniazid | 1.31 ± 1.2 | 2.56 ± 1.2 | 0.05 |
| Rifampin | 0.77 ± 1.3 | 4.65 ± 3.2 | 0.005 |
| Ethambutol | 0.83 ± 0.37 | 1.68 ± 0.93 | 0.03 |
| Pyrazinamide | 20.3 ± 7.3 | 28.0 ± 10.7 | 0.11 |

[a] The plasma samples used for $C_{2\ h}$ drug level and TDA measurements were from same blood draw. Comparisons of $C_{2\ h}$ levels for isoniazid and rifampin were performed by $t$ test.

**Among subjects with the lowest TDA (≤1.5), only 2 (40%) were cured at 6 months compared to 10 (91%) with the higher TDA values (*p=0.06)***

Heysell et al. *Antimicrob Agents Chemother* 2011

# Isolate Worksheet

**Breakpoint:** ◇

**Isolate MIC:** ⬭

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OFL 32 | MXF 8 | RIF 16 | AMI 16 | STR 32 | RFB 16 | PAS 64 | ETH 40 | CYC 256 | INH 4 | KAN 40 | EMB 32 |
| OFL 16 | MXF 4 | RIF 8 | AMI 8 | STR 16 | RFB 8 | PAS 32 | ETH 20 | CYC 128 | INH 2 | KAN 20 | EMB 16 |
| OFL 8 | MXF 2 | RIF 4 | AMI 4 | STR 8 | RFB 4 | PAS 16 | ETH 10 | CYC 64 | INH 1 | KAN 10 | EMB 8 |
| OFL 4 | MXF 1 | RIF 2 | AMI 2 | STR 4 | RFB 2 | PAS 8 | ETH 5 | CYC 32 | INH 0.5 | KAN 5 | EMB 4 |
| OFL 2 | MXF 0.5 | RIF 1 | AMI 1 | STR 2 | RFB 1 | PAS 4 | ETH 2.5 | CYC 16 | INH 0.25 | KAN 2.5 | EMB 2 |
| OFL 1 | MXF 0.25 | RIF 0.5 | AMI 0.5 | STR 1 | RFB 0.5 | PAS 2 | ETH 1.2 | CYC 8 | INH 0.12 | KAN 1.2 | EMB 1 |
| OFL 0.5 | MXF 0.12 | RIF 0.25 | AMI 0.25 | STR 0.5 | RFB 0.25 | PAS 1 | ETH 0.6 | CYC 4 | INH 0.06 | KAN 0.6 | EMB 0.5 |
| OFL 0.25 | MXF 0.06 | RIF 0.12 | AMI 0.12 | STR 0.25 | RFB 0.12 | PAS 0.5 | ETH 0.3 | CYC 2 | INH 0.03 | POS ✓ | POS ✓ |

**Borderline Resistant?**

**Borderline Susceptible?**

**Patient on moxifloxacin… is this even the correct breakpoint?**

# Significant regional variation of MIC, and target concentration/MIC

target

Typical dose

TABLE 4 PTA expectation values, ofloxacin pharmacokinetic study in patients with MDR-TB, Cape Town and Durban, South Africa

| Ofloxacin daily dose (mg) | Overall PTA expectation | Cape Town PTA expectation | Durban PTA expectation |
|---|---|---|---|
| *f*AUC/MIC ≥ 100 | | | |
| 800 | 0.45 | 0.33 | 0.65 |
| 1,000 | 0.57 | 0.46 | 0.76 |
| 1,200 | 0.66 | 0.57 | 0.83 |
| 1,400 | 0.73 | 0.64 | 0.89 |
| 1,600 | 0.77 | 0.70 | 0.91 |
| *f*AUC/MIC ≥ 40 | | | |
| 800 | 0.83 | 0.77 | 0.94 |
| 1,000 | 0.87 | 0.83 | 0.95 |
| 1,200 | 0.90 | 0.87 | 0.96 |
| 1,400 | 0.92 | 0.89 | 0.97 |
| 1,600 | 0.93 | 0.91 | 0.97 |

***With MIC of 2.0 µg/ml (WHO critical concentration), no patient achieved target ≥100**

**PTA**: probability of target attainment

Chigutsa et al, *Antimicrob Agents Chemother* 2012

# In a TB endemic setting, Tanzania, MDR-TB patients (N=25) had a wide range of drug concentration/ MIC

| Drug (expected $C_{2hr}$ range) | $C_{2hr}$ µg/ml Mean ±SD | N below expected $C_{2hr}$ range (% total N) | MIC µg/ml Median (IQR) | $C_{2hr}$ /MIC Mean ±SD |
|---|---|---|---|---|
| Levofloxacin (8-12 µg/ml) | 8.0 ±2.8 | 13 (52) | 0.75 (0.25-1.0) | 15.8 ±14.1 |
| Kanamycin (25-35 µg/ml) | 26.0 ±10.2 | 10 (40) | 1.2 (0.6-2.5) | 22.9 ±18.7 |
| Ethionamide (1-5 µg/ml) | 3.6 ±1.8 | 1 (4) | 2.5 (1.2-5.0) | 1.8 ±1.5 |
| Cycloserine (20-35 µg/ml) | 33.9 ±12.2 | 3 (13)[a] | 8.0 (8.0-16.0) | 4.3 ±3.0 |
| Pyrazinamide (20-60 µg/ml) | 43.1 ±9.7 | 0 | N/A | N/A |

Drugs concentration dependent in activity (like rifampin and isoniazid)

# But drug concentrations (by HPLC) not available in most MDR-TB endemic settings, so…

**Distribution of probable changes based on MIC, for MDR-TB patients (N= 13)**

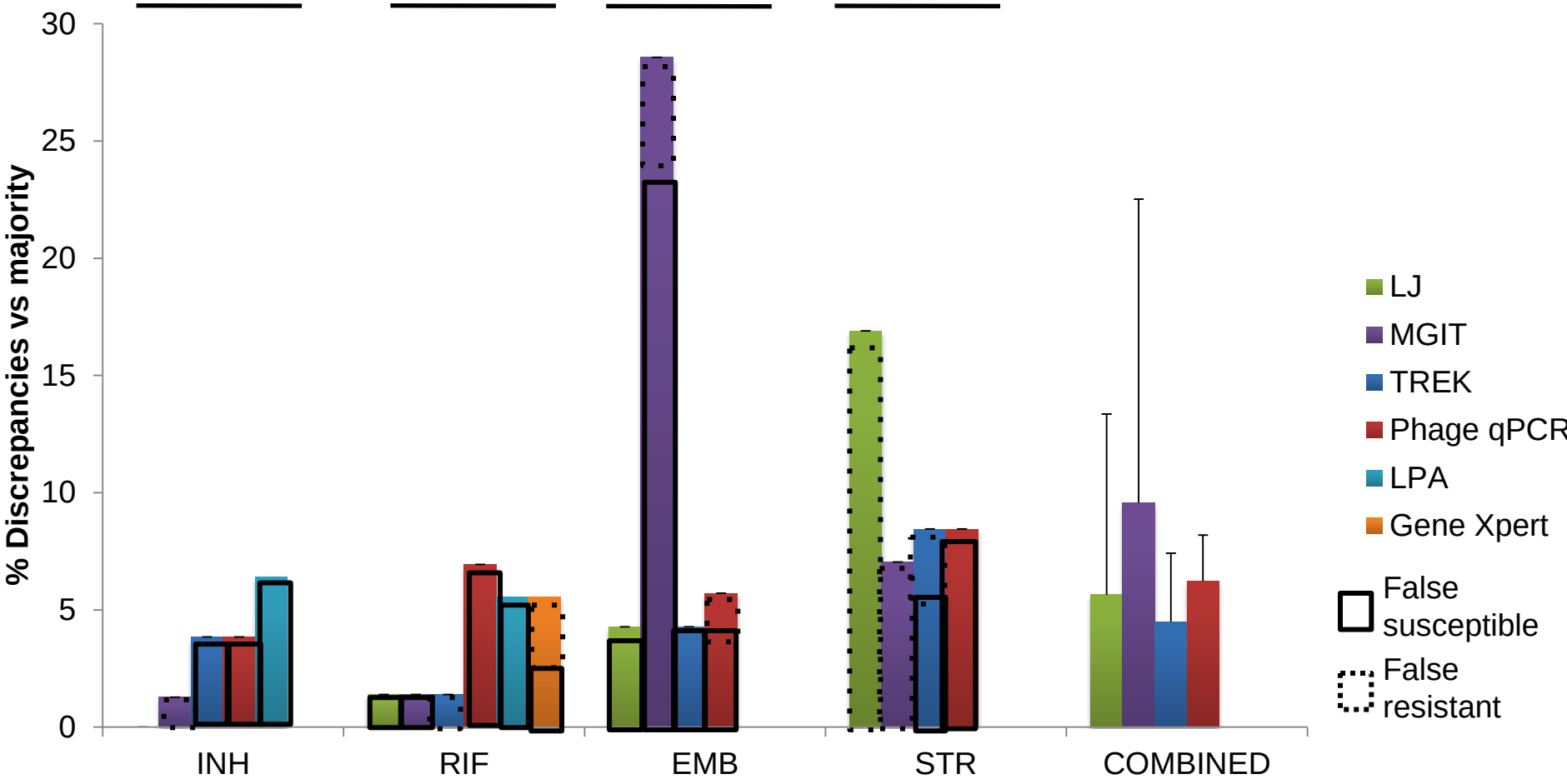
| Modification | Frequency (%N) |
|---|---|
| Ethionamide *change* to para-aminosalicylic acid | 7 (54) |
| Ofloxacin or levofloxacin *change* to high-dose levofloxacin | 6 (46) |
| Kanamycin *change* to amikacin | 3 (23) |
| Amikacin or kanamycin *empiric change* to capreomycin | 3 (23) |
| Amikacin *change* to kanamycin | 1 (8) |

***gyrA* wildtype**

**MIC can inform/alter the standardized MDR-TB regimen in Tanzania, even within a limited formulary**

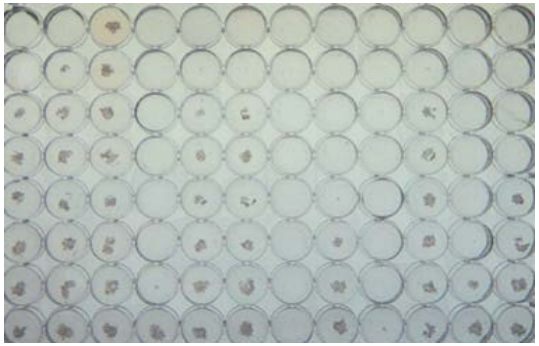
Mpagama et al, submitted

# Even the best qualitative methods will be discrepant: 86 *M tb* isolates (80% MDR) from Bangladesh




**The MIC plate (TREK ), using breakpoints, was the least discrepant when compared to other genotypic and phenotypic methods**

Banu et al, in prep



# Conclusions

- **Commercial microplate MIC is available (and advantageous for many settings inexperienced in second-line DST) *but use is limited* for fully drug-susceptible TB– unless patient is slow-to-respond and drug concentrations can be measured and/or dose increased**

- **Given *significant individual pharmacokinetic variability*, including for MDR-TB drugs (fluroquinolones, aminoglycosides, ethionamide), MIC best applied with drug concentration measurement**

- **In the absence of drug concentration measurement (HPLC), MIC may still inform and alter MDR-TB management within a WHO formulary**

- **Quantitative susceptibility invites "borderline" or "intermediate" ranges but must be studied prospectively on a consistent platform (and informed by drug concentration/MIC targets)**

# Thank You

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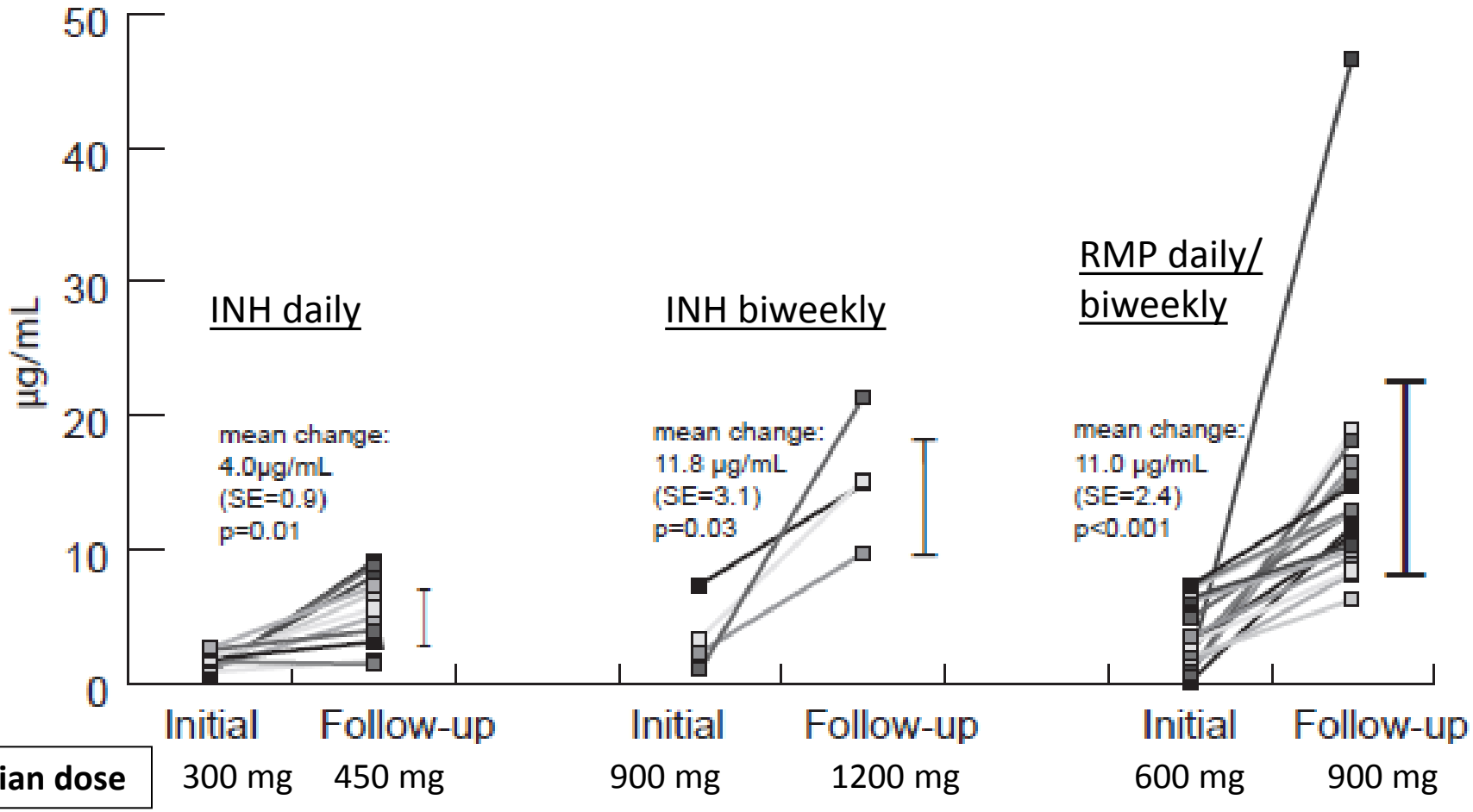
**skh8r@virginia.edu**

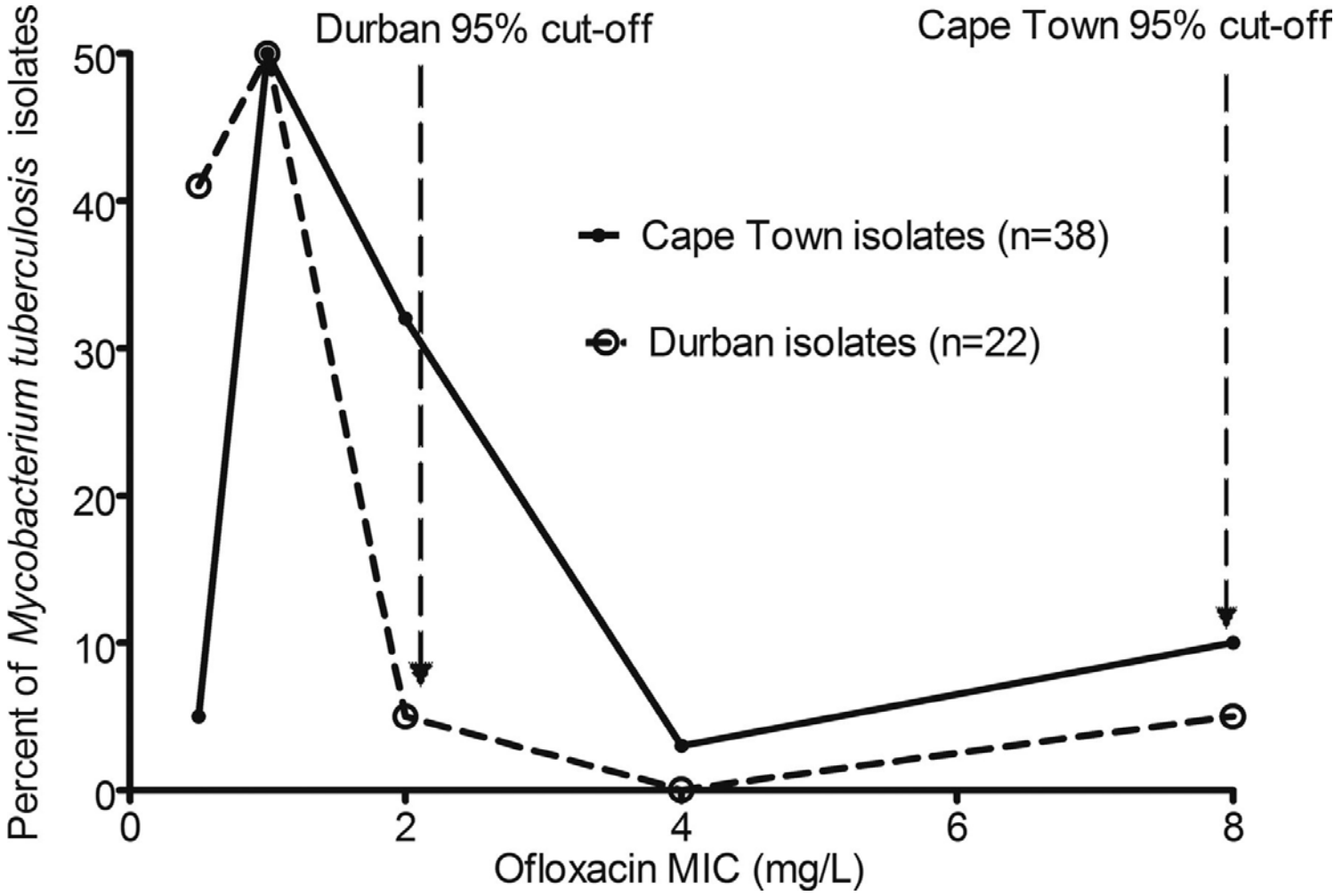
# Drug levels correct easily after first dose adjustment




I spans $C_{2hr}$ expected range

Heysell et al, *Emerg Infect Dis* 2010

# The epidemiologic cut-offs (95%) could be wildly different and miss the subtlety of drug concentration/ MIC




Pasipanodya J et al. *Antimicrob Agents Chemother*, 2012