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Paper Poster Session
Mycobacterium tuberculosis - susceptibility testing methods

Pyrazinamide is active against Mycobacterium tuberculosis at neutral pH at the clinical breakpoint concentration under specific conditions

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Background: Despite being a highly effective component of multi drug anti-tuberculosis therapy, pyrazinamide (PZA) is not active against *Mycobacterium tuberculosis* (MTB) at standard culture conditions and thus performed at low pH complicating drug susceptibility testing (DST). As both the growth of MTB and the effect of PZA are highly dependent on the acidity of the medium, PZA testing is technically challenging reducing test accuracy and reproducibility as compared to rifampicin and isoniazid DST. These technical difficulties make that the susceptibility of patients' isolates to PZA is seldom routinely tested, even though most TB patients receive PZA, and the prevalence of PZA resistance in MDR patients is high. As a low pH has been demonstrated to induce metabolic changes to MTB (Abramovitch et al. 2011), we tested whether other such triggers could induce PZA susceptibility at neutral pH.

Material/methods: MTB H37Ra microcolony culturing was performed at neutral pH on MB7H11 agar with some adaptations in the presence or absence of 100mg/L PZA. Growth of microcolonies was monitored in a system essentially as described earlier (den Hertog et al. 2010). In addition, liquid culture DST was performed concurrently at KIT and Becton Dickinson on 6 MTB complex strains of various PZA susceptibilities with reagents from and inoculum preparation per the BD BACTEC™ MGIT™ 960 PZA Drug Kit. Culture was performed in BD BACTEC MGIT 960 PZA Medium (pH 5.9) and BD BACTEC MGIT Barcoded 7mL Tubes (pH 6.6) with MGIT PZA Growth Supplement with specific adaptations. Inoculated MGIT tubes were read three times per week over 26 days with Micro MGIT fluorescent readers. Full details of the new procedure can only be disclosed after April 10th, because of a patent application.

Results: Using our adapted conditions, 100 mg/L PZA strongly inhibited the growth of MTB H37Ra colonies at neutral pH. Furthermore, using the adapted MGIT assay, all strains could be classified as resistant or susceptible identically to the routine DST assay at low pH. Median time to detection of the liquid cultures was 10 (range 7-17) days in the adapted assay compared to 7 (range 5-10) days at low pH.

Conclusions: Our study together with other recent (Peterson et al 2015) and historical data (McDermott and Tompsett, 1954) demonstrates that PZA activity is independent of acidic pH per se. These data challenge the generally accepted mechanism that a low pH is required for activation of PZA. In addition, we here demonstrated that DST can be performed under neutral pH test conditions, thereby possibly circumventing the issues associated with testing at low pH. Further research and validation are required to fully define the optimal conditions for PZA DST, and explore the implications of this finding for treatment and future drug development.