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O0932 Towards a reference method for susceptibility testing of *Mycobacterium tuberculosis*

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Background: There are many antimicrobial susceptibility testing (AST) methods used for *Mycobacterium tuberculosis* (Mtb) both in solid (Middlebrook 7H10/7H11 and LJ) and liquid media (Middlebrook 7H9 broth microdilution and BACTEC 960 MGIT). The lack of a reference method give rise to confusion about cross resistance, genotype-phenotype correlations and is an obstacle for defining clinical breakpoints and evaluating clinical trials. The EUCAST subcommittee for anti-mycobacterial drug susceptibility testing (AMST) was launched in 2016 with a primary goal to define a reference AST method and subsequently clinical breakpoints for Mtb by using epidemiologic cut-off values (ECOFFs), PK/PD and clinical outcome data according to EUCAST principles.

Materials/methods: A detailed protocol for Middlebrook 7H9 (7H9) and Middlebrook 7H10 (7H10) was developed and evaluated by laboratories (n=4) represented within the AMST. MIC determination was performed for amikacin (AMI), levofloxacin (LEV), isoniazid (INH) using Mtb H37Rv ATCC 27294. Reading of results was done from 7 to 21 days for 7H9 and at 21 days for 7H10 both comparing to a 1:100 diluted control and the visual minimum inhibitory concentration (MIC).

Results: In general, reproducibility was well within ±one MIC-dilutions for all drugs in both methods. For INH, the distribution was 0.016-0.06 mg/L (7H9) and 0.06-0.25 mg/L for 7H10 with >90% of values at 0.03 and 0.12 mg/L, respectively. LEV showed a similar pattern with a peak (range) at 0.25 mg/L (0.25-0.5) and 0.5 mg/L (0.12-0.5) for 7H9 and 7H10 respectively. For AMI, there was a low variation in 7H9 with most observations at 0.5 mg/L whereas the peak was 1 mg/L for 7H10. There was no detectable difference in MICs evaluated visually compared to the indirect proportion method. MICs were systematically higher with increasing inocula.

Conclusions: A detailed protocol was developed for liquid and solid media alternatives as candidate reference AST methods for Mtb. Both methods have so far proven to be reproducible. Further evaluation is ongoing including testing of bedaquiline and clinical strains. A suggestion for a reference method is expected during 2019 which should then be used to define ECOFFs and clinical breakpoints to which the other AST methods in use should be calibrated.