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O0745 Rapid simultaneous testing of multiple antibiotics by the MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOT-MGA)

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Background: Direct-on-target microdroplet growth assay (DOT-MGA) based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been suggested as a universal phenotypic antimicrobial susceptibility testing (AST) method. In this study, we developed and evaluated antibiotic panels for simultaneous testing of multiple antibiotics.

Materials/methods: Eleven *Enterobacteriaceae* comprising *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter aerogenes, Citrobacter koseri* (each n=2) and *Citrobacter freundii* (n=1) as well as twelve *Pseudomonas aeruginosa* consecutive clinical isolates were included. Panels for MALDI-TOF MS AST were created, including 19 relevant antibiotics for *Enterobacteriaceae* and 16 for *P. aeruginosa.* Bacterial suspensions with antibiotics in cation-adjusted Mueller-Hinton broth were placed onto the spots of disposable MALDI-TOF MS targets (MBT Biotarget96, Bruker Daltonics, Germany) as 6-µl microdroplets. The inoculum size was $5x10^5$ cfu/ml and the antibiotics were tested at EUCAST breakpoint concentrations to allow categorization as susceptible, intermediate or resistant isolate. For any single isolate, a separate target was used to test the antibiotic panel. Three spotting replicates of each antibiotic as well as the growth control were prepared. The inoculated targets were incubated in a humidity chamber at 36°C for 8 hours. Subsequently, medium was removed by absorption with a tissue wipe. MALDI-TOF MS spectra were acquired and quantitatively analysed by a dedicated prototype software comparing samples with antibiotics to a correspondent growth control. Medians of triplicates were calculated. Broth microdilution was used as reference method.

Results: For *Enterobacteriaceae*, 100% categorical agreement (CA) with the reference method was achieved with piperacillin/tazobactam, cefotaxime, ceftazidime/avibactam, ceftolozane/tazobactam, ertapenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin, tigecycline, trimethoprim/sulfamethoxazole, fosfomycin, and colistin. CA was 90.9% for ampicillin, ceftazidime, imipenem, and meropenem; 81.8% for cefuroxime, and 63.6% for ampicillin/sulbactam, respectively. For *P. aeruginosa*, 100% CA was reached with piperacillin, piperacillin/tazobactam, cefepime, ceftazidime, ceftazidime/avibactam, meropenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin and amikacin. CA was 91.7% for ceftolozane/tazobactam, imipenem, fosfomycin; 66.7% for colistin, and 58.3% for aztreonam, respectively.

Conclusions: MALDI-TOF MS-based DOT-MGA was proved to be a universal AST method suitable for rapid simultaneous testing of multiple antibiotics. Automated processing and improved software analysis would enable comfortable workflow and increased test performance.