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Rapid detection of carbapenem non-susceptibility by MALDI-TOF mass spectrometry using a novel direct-on-target microdroplet growth assay

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Background: Various phenotypic methods for rapid detection of carbapenemase activity in gram-negative bacteria have been suggested, but they do not detect carbapenem resistance due to other mechanisms. A recently described quantitative assay using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) lacks this disadvantage, however, it requires considerable time for processing including protein extraction. In this proof-of-principle study, we investigated rapid detection of carbapenem resistance as an exemplar of a novel easy-to-perform variant of antimicrobial susceptibility testing (AST), which is independent of the underlying resistance mechanism.

Material/methods: Seven meropenem-susceptible *Klebsiella pneumoniae* and seven meropenem-non-susceptible *K. pneumoniae* isolates were included. Minimum inhibitory concentrations (MICs) of meropenem were determined by broth microdilution reference method. For rapid test, bacterial suspension in cation-adjusted Mueller-Hinton broth (CA-MHB) was added to the same volume of meropenem-containing CA-MHB directly on a hydrophilic spot of a disposable MALDI-TOF MS target (MBT Biotargets-96, Bruker Daltonics, Germany) resulting in microdroplets. The final inoculum was approximately $5x10^5$ cfu/ml and the final breakpoint concentration of meropenem was 2 mg/L. Growth controls without antibiotic were similarly applied. For each isolate, different volumes of microdroplets were tested: 2, 4, 6, 8 and 10 µl. Each condition was tested on three different spots, and median was used for analysis. The microdroplets were incubated directly on target in a plastic transport box (Bruker) using it as humidity chamber to avoid evaporation of microdroplets. Three targets with microdroplets were inoculated from the same suspension and incubated in parallel for 3, 4 and 18

hours at 36°C allowing attachment of bacterial cells onto the targets. After incubation, medium was removed and MALDI-TOF MS was performed directly from dried spots. For samples with antibiotic, isolates were interpreted as non-susceptible if MALDI Biotyper software (Bruker) provided successful species identification (score ≥1.7) and as susceptible, respectively, if no identification (score <1.7) was achieved.

Results: While MICs of all susceptible isolates were 0.016 mg/L, MICs of non-susceptible isolates ranged from 8 to 16 mg/L, as determined by the reference method. The best performance of rapid assay was achieved using 8 µl droplets. Applying this volume, all susceptible and non-susceptible isolates were correctly categorised at 3 hours, except of one resistant isolate, which was identified neither in the meropenem-containing sample nor in its growth control at this time point. At 4 hours, all isolates were correctly categorised, thus providing 100% agreement with the reference method. At 18 hours, growth control of one susceptible isolate failed; however, all results were otherwise correct in this testing approach.

Conclusions: Our novel MALDI-TOF MS-based direct-on-target microdroplet growth assay is promising for easy, fast and mechanism-independent determination of carbapenem-non-susceptibility, and holds potential for a universal application in rapid AST. Software-based quantitative evaluation might further increase the test performance.