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REVIEW

Direct-on-Target Microdroplet Growth Assay Applications for Clinical Antimicrobial Susceptibility Testing

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Abstract: Direct-on-target microdroplet growth assay is a new technique for analysing bacterial sensitivity and mechanisms of resistance. It is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and allows for easy and rapid testing. Here, we describe the development and procedure of the direct-on-target microdroplet growth assay and summarise the latest clinical applications.

Keywords: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF MS, direct-on-target microdroplet growth assay, DOT-MGA, antimicrobial susceptibility testing

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was a newly developed laboratory method for the identification of microorganisms in the clinic. More recently, researchers have focused on the application of MALDI-TOF MS in the detection of microbial resistance. MALDI-TOF MS detected drug sensitivity in microorganisms by measuring the enzyme activity and analyses drug resistance using a self-built library and the characteristics of the mass spectrum peak as well as the composite correlation index (CCI).[1–3] Recently, researchers published a method for analysing bacterial sensitivity by direct-on-target microdroplet growth assay (DOT-MGA),[4,5] which was simple, practical, and could be rapidly executed.[6] It could be used to provide valuable information on potential resistance mechanisms.

The principle of this methodology was as follows: to perform DOT-MGA, microorganisms were incubated with and without the index antibiotic in nutrient broth as microdroplets directly on the target spots of MALDI-TOF MS. To avoid droplet evaporation, the target plate was cultured inside a plastic container and water was added to the bottom. At the end of the culture period, the broth was separated from the microbial cells by direct absorption of the microdroplets using absorptive material. The microbial growth in each target plate was analysed to validate whether MALDI-TOF MS successfully identified each microbial spot at different antibiotic concentrations. Since the exact concentration of antibiotics in each droplet was known, the minimum inhibitory concentration (MIC) could be calculated and microorganisms could be identified as sensitive or insensitive to certain antibiotics.[6,7] By evaluating the growth in the presence of different antibiotics, it was possible to not only determine the sensitivity of an isolate but also analyse the potential mechanisms of drug resistance.

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In the initial experiments, DOT-MGA was mainly performed on gram-negative bacteria such as *Enterobacteriaceae*. In the study of Idelevich et al, 24 strains of *Klebsiella pneumoniae* and 24 strains of *Pseudomonas aeruginosa* were co-cultured with meropenem to rapidly detect carbapenemase resistance. The authors used microdroplets of 2, 4, 6, 8, and 10 μL and incubated the samples for 3, 4, 5, and 18 hours and found that 6 μL microdroplets combined with an incubation time of 4 hours resulted in the best validity (100%), sensitivity (100% and 83.3%), and specificity (100%) for *K. pneumoniae* and *P. aeruginosa*, respectively.[6] In 2020, Shen et al extended the application of this technique to imipenem, meropenem, and ertapenem.[8] The DOT-MGA outcomes in *K. pneumoniae* were consistent with that of the broth microdilution method and showed superior performance in *Escherichia coli* for ertapenem and imipenem. Moreover, Horseman et al used the same method to analyse methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus*, extended-spectrum β-lactamase (ESBL) *E. coli*, and carbapenem-resistant *K. pneumoniae*.[9] The authors demonstrated that while a droplet size of 4, 6, or 8 μL did not affect the identification and detection of drug resistance, larger than 6 μL droplets resulted in frequent overflow and difficulties in completely separating the broth from the microorganism. Therefore, 4 μL was determined to be optimal. Furthermore, the assay showed higher validity in determining drug resistance in *S. aureus* and *K. pneumoniae* (97.5%) than in *E. coli* (82.5%) and *Enterococcus* (65%). These data were consistent with those using the broth extraction method.

In the clinic, sensitivity analyses of microorganisms present in a patient's bloodstream were critical for determining treatment strategy. Nix et al examined DOT-MGA for the rapid detection of microbial resistance in patients using positive blood culture bottles and confirmed its applicability in MRSA using cefoxitin and determined an optimal microdroplet size of 6 μL.[10] By investigating the effects of three pre-treatment methods including continuous dilution broth, lysis/centrifugation, and differential centrifugation, the authors found that lysis/centrifugation combined with an incubation time for 4 hours results in the best validity (96.4%), sensitivity (100%), and specificity (100%). Importantly, it was necessary to destroy the MRSA cell membrane with formic acid before adding the matrix in contrast to performing DOT-MGA on gram-negative bacteria. Similar analyses were executed by Idelevich et al in *K. pneumoniae, Enterobacter cloacae, Enterobacter aerogenes, Proteus mirabilis*, and *Klebsiella aerogenes* for determining carbapenemase resistance.[11] In addition to the three pre-treatment methods mentioned above, the authors also examined a filtration/dilution method and observed that lysis/centrifugation and an incubation time of 4 hours showed the best performance with a validity, sensitivity, and specificity of 96.3%, 91.7%, and 100%, respectively.

In addition to its applicability as a rapid detection method of bacterial resistance in the clinic, DOT-MGA could also be used to explore the mechanism of bacterial resistance and synergistic inhibition by phenotypic screening. In the study of Correa-Martínez et al, *Enterobacteriaceae* were exposed to cephalosporins and inhibitors in the microdroplets at 12 different concentrations to examine the following eight conditions: bacteria with (1) cefpodoxime, (2) ceftazidime, (3) ceftazidime/clavulanic acid, (4) cefotaxime, (5) cefotaxime/clavulanic acid, (6) cefepime, (7) cefepime/clavulanic acid, and (8) cefotaxime/clavulanic acid/cloxacillin.[12] The authors reported an 8-fold decrease in MIC when clavulanic acid and cloxacillin were added, indicating a synergistic inhibition effect and analysing potential resistance mechanisms. The standards and algorithms were proposed by EUCAST.[13] Compared with PCR analyses, the positive and negative coincidence rates of ESBL, AmpC, and ESBL + AmpC were 94.4% and 100%, 94.4% and 93.8%, 100% and 100%, respectively. Additionally, the authors also screened carbapenemase-resistant strains by co-culture with meropenem, in which the microorganisms were exposed to meropenem mixed with a variety of antibiotics in the microdroplets. In these experiments, DOT-MGA showed comparable results with PCR, broth dilution, and disk diffusion for KPC, MBL, and OXA. The results of AmpC were consistent with those of broth dilution and disk diffusion, although there were discrepancies with the results obtained by PCR.[14] It was not ruled out that PCR results were false negative.

MALDI-TOF MS-based DOT-MGA is a promising method for identifying microorganisms as well as for analysing drug sensitivity and mechanisms of microbial resistance. It is simple to execute with rapid and accurate results and can be used to analyse both gram-positive and -negative microorganisms. Furthermore, it can be directly applied to culture plates and positive blood culture bottles, which accelerates the antibiotic resistance analyses and allows for faster intervention by clinicians in patients with bacteraemia. However, it is worth noting that the standardisation of this method still needs further optimization.

Code Availability (Software Application or Custom Code)

The authors declare the software application and custom code support their published claims and comply with field standards.

Data Sharing Statement

The authors declare that all data and materials support their published claims and comply with field standards.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest.

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