COMMENT

MALDI-TOF MS-based direct-on-target microdroplet growth assay: Latest developments

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Received June 19, 2020; Accepted July 8, 2020

DOI: 10.3892/etm.2020.8976

Abstract. The matrix-assisted laser desorption-ionization time-of-flight mass spectrometry direct-on-target microdroplet growth assay for the rapid susceptibility testing and the detection of the underlying antibiotic resistance mechanisms of microbia has been recently introduced. In the present study, we review the latest developments in the field.

Recently, the matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) direct-on-target microdroplet growth assay (DOT-MGA) for the rapid susceptibility testing and the detection of the underlying antibiotic resistance mechanisms of microbia has been introduced (1-3). The assay has previously been reviewed (4). In the present study, we review the latest developments in the field.

The principle of this methodology is as follows (5): the microorganisms are incubated with and without (growth controls) the index antibiotic in nutrient broth as microdroplets directly on MALDI-TOF MS target spots. An antibiotic concentration gradient can be achieved in a series of consecutive spots. In order to avoid evaporation of the microdroplets, the target is incubated in a simple plastic transport box (Bruker Daltonik, Germany), used as a humidity chamber with the addition of 4 ml water onto the bottom of the plastic box. After the incubation period, the broth is separated from microbial cells by just contacting the microdroplets with an absorptive material. MALDI-TOF can detect the presence or absence of the strain growth in each spot and identify the microorganism. Since the exact concentration of the antibiotic in each droplet spot is known, the MIC can be evaluated and the microorganism can be characterized as susceptible or not. With this assay and using a panel of different antibiotics, not only can the susceptibility status of an isolate be determined but also the underlining resistance mechanisms be revealed.

In the initial reports, the assay was used for the detection of carbapenem nonsusceptibility in Gram-negative pathogens both from colonies (1) and directly from positive blood cultures (BCs) (2). The assay was also used for the detection of two additional resistance mechanisms among *Enterobacterales*, namely the extended-spectrum β-lactamase (ESBL) production and the AmpC production (3).

The most significant resistance phenotype in Gram-negative bacteria is the carbapenem non-susceptibility. In order to rapidly detect this phenotype, to accurately determine the MIC and to differentiate the class of the carbapenamase in one step Correa-Martínez *et al* proposed a phenotypic screening panel based on DOT-MGA (6). They used two MALDI-TOF targets (Bruker Daltonik): the first one for sterility and growth controls and the second one, a 96-spot target that was divided into eight zones for screening. According to the authors, carbapenem non-susceptibility was detected by zone 1, which contained a two-fold dilution series of meropenem alone. Each of the next six zones contained a dilution series of the combination of meropenem with an individual inhibitor. In particular, zone 2 a combination with phenylboronic acid (PBA), zone 3 with aminophenylboronic acid (APBA), zone 4 with cloxacillin (CLX), zone 5 with ethylenediaminetetraacetic acid (EDTA), zone 6 with dipicolinic acid (DPA) and zone 7 with avibactam (AVI). Finally, zone 8 contained a two-fold dilution series of temocillin (TEM) alone. The latter was used since the high-level TEM resistance (>128 μg/ml) is frequently observed in OXA-producing strains.

Using this panel it is very simple with a standard, single MALDI-TOF run not only meropenem non-susceptibility to be revealed but simultaneously the type of the carbapenemase produced to be determined based on the decrease (8-fold or more) of the MIC of the combination of meropenem with a particular carbapenamase inhibitor due to the synergistic

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Key words: MALDI-TOF MS, direct-on-target microdroplet growth assay, DOT-MGA, carbapenamases, MRSA, resistance mechanisms, *Streptococcus pneumonia*, blood cultures, susceptibility testing

effect. Three carbapenemase classes could be detected, namely KPC (using PBA, APBA), MBL (using EDTA, DPA) and OXA (using AVI and high-level TEM resistance). The combination of meropenem with CLX was used in the panel in order to reveal a possible underlying AmpC production and porine loss resistance mechanism.

The assay was evaluated using seven reference strains recommended by the EUCAST (7) and 20 meropenem non-susceptible *Enterobacterales* clinical isolates. PCR was used as reference method, and broth microdilution (BMD) and combination disk test (CDT) were also performed. Best results were obtained with a 4 h incubation protocol. All foreknown resistance mechanisms of the reference strains (also confirmed by PCR) were correctly identified by DOT-MGA. Furthermore, the assay successfully identified the carbapenemase activity in 10 of 10 isolates with carbapenamase production confirmed by PCR. As the authors reported, DOT-MGA correctly identified KPC, MBL and OXA (100% agreement with PCR), whereas detection of AmpC coincided with BMD and CDT but agreement with PCR was low, not ruling out false negative PCR results. Since the assay is based on a phenotypic approach it has the advantage of detecting unknown or uncommon carbapenemases. Despite the low number of strains tested for the evaluation of the method, this innovated, rapid, easy-to-perform, one-step assay contributes significantly in detection of carbapenem non-susceptibility and revealing the class of the carbapenemase produced.

The first implementation of DOT-MGA in Gram-positive strains was reported in 2020 by Nix *et al* (8) in detecting methicillin resistance in *Staphylococcus aureus* from both agar cultures and directly from positive BCs. Fourteen consecutive methicillin-resistant *S. aureus* (MRSA) and 14 methicillin-susceptible *S. aureus* (MSSA) clinical isolates along with a challenge collection comprised of 16 highly genetically diverse MRSA strains were analyzed. Furthermore, human blood along with MRSA or MSSA bacterial suspension was spiked in BC vials to simulate bacteremias. Each positive BC broth was processed by three different methods, serial dilution of BC broth, lysis/centrifugation, and differential centrifugation and furthermore, three different incubation periods were evaluated (4, 5 and 6 h). The lysis/centrifugation method with a final dilution step 10^{-1} of the 0.5 McFarland suspension provided the best results in combination with a 4 h incubation period. Both for agar cultures and positive BCs the standard DOT-MGA protocols were applied with the addition of a step using formic acid for cell membrane disruption before adding matrix. The standard 6 μl droplets with and without cefoxitin as the index antibiotic at the breakpoint concentration for MRSA detection were spotted on the MALDI target. If the tests were valid (growth controls with identification score ≥1.7), the examined strains incubated with cefoxitin were characterized either as MRSA (identification score ≥1.7) or MSSA (identification score <1.7). As the authors report, 96.4% test validity, 100% sensitivity, and 100% specificity were achieved in detecting methicillin resistance in clinical isolates and furthermore all MRSA strains of the challenge collection were successfully identified as methicillin-resistant.

Idelevich *et al* (9) managed to differentiate *Streptococcus pneumoniae* from viridans group streptococci applying the DOT-MGA methodology using optochin as the index substance at a concentration of 32 mg/l. In the cases of *S. pneumoniae* the growth controls (without optochin) produced a specific spectrum and in parallel the samples treated with optochin revealed no spectrum. On the other hand, in cases of viridians group streptococci characteristic MS spectra were obtained both in growth controls and in the samples with optochin. Since the incubation time required was 20 h further development and improvement of the protocol is necessary.

MALDI-TOF MS-based DOT-MGA is a very promising novel methodology. It is easy, rapid, and practical and can provide simultaneously both identification and antibiotic susceptibility testing for either Gram-negative or Gram-positive pathogens. Furthermore, it can be applied both in cultures from solid media and directly in positive BCs. Although further standardization and optimization is required its potential and its significance especially in the sepsis management is great.

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