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Rapid antimicrobial susceptibility testing by matrix-assisted laser desorption ionization–time of flight mass spectrometry using a qualitative method in *Acinetobacter baumannii* complex

Mengjiao Li, Minxue Liu, Qifei Song, Li Xiong, Zhixing Chen, Mei Kang, Yi Xie*

West China Hospital of Sichuan University, Department of Laboratory Medicine, Chengdu, China

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ABSTRACT

The transmission and infections of multidrug-resistant bacteria can be prevented by rapid identification and antibiotic susceptibility testing (AST) for pathogenic bacteria in a clinical microbiology laboratory. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been routinely used as a tool for the identification of pathogens; however, a simple and accurate method for a rapid determination of the antimicrobial susceptibility profile is an urgent requisite. The present study established a method based on mass spectrometry to determine the drug resistance. *Acinetobacter baumannii* complex isolates were tested as an example. After short-term culture, the isolates were incubated with meropenem of different concentrations to determine the growth or the inhibition of the growth by MALDI-TOF MS. The agreement of minimum inhibitory concentration (MIC) values between MALDI-TOF MS-based rapid AST and broth microdilution method in susceptible and resistant strains was 77.1% and 70.1%, respectively. The susceptibility-breakpoint concentration (2 μg/mL) achieved a 98.9% sensitivity and 100% specificity with respect to resistance detection. Similarly, 96.9% sensitivity and 100% specificity were obtained for resistance detection with meropenem concentration at 8 μg/mL. MALDI-TOF MS-based rapid AST was applied to determine the drug resistance at breakpoint concentration, although MS-MICs might shift to a low dilution. Thus, it is critical for patients to accelerate the AST result from two days to several hours.

1. Introduction

The dramatic increase and spread of antimicrobial resistant bacteria is an urgent public health threat. Due to the limited treatment alternatives, multidrug-resistant bacterial infections are associated with prolonged length of stay and an increased risk of mortality in hospital settings (Cai et al., 2017; Kritsotakis et al., 2017). Consequently, the economic burden rises proportionally with the increasing incidence of multidrug-resistant bacterial infections. Thus, reducing the number of drug-resistant bacteria carriers is beneficial for preventing the transmissions and infections; also, it can reduce the health and economic burdens (Bartsch et al., 2017). Rapid identification and antibiotic susceptibility testing of the pathogen plays a vital role in discovering the carriers and preventing the transmission events.

Recent advances in laboratory techniques, such as mass spectrometry (MS) and polymerase chain reaction (PCR) can shorten the turnaround time. However, the DNA-based methods are neither cost-effective not generalizable for different pathogens or mechanisms underlying resistance, thereby necessitating the need for specific probes for detection (Mezger et al., 2015; Schoepp et al., 2017). In clinical microbiology laboratories, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is routinely used for pathogen identification. Furthermore, it has been developed as a novel method for rapid antibiotic susceptibility testing (AST) in recent years (Ceyssens et al., 2017; De Carolis et al., 2017; Hrabak et al., 2012; Idelevich et al., 2018; Jung et al., 2016; Oviano et al., 2017; Sparbier et al., 2013; Sparbier et al., 2016). The common approaches using mass spectrometry for rapid AST include MALDI Biotyper selective testing of antibiotic resistance β-lactamase (MBT-STAR-BL) assay, MALDI Biotyper resistance testing with stable isotopes (MBT-RESIST) assay, MALDI Biotyper antibiotic susceptibility test rapid (MBT-ASTRA) assay, and direct-on-target microdroplet growth assay. The MBT-STAR-BL

Abbreviations: MALDI-TOF MS, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MS-MIC, MALDI-TOF mass spectrometry based minimum inhibitory concentration; MS-based RAST, MALDI-TOF mass spectrometry based rapid antimicrobial susceptibility test

* Corresponding author at: Number 37 Guoxue Alley in the Wuhou District, Chengdu, Sichuan, China.
E-mail address: xieyi@scu.edu.cn (Y. Xie).

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assay detects β-lactamase activities in Gram-negative bacteria by evaluating their hydrolysis products (De Carolis et al., 2017; Hrabak et al., 2012; Oviano et al., 2017). However, the method is limited to the detection of β-lactamase-mediated resistance and not generalizable to other mechanism-mediated resistance. The MBT-RESIST assay is based on the detection of new proteins synthesized by isotope-labeled (non-radioactive) amino acid (Sparbier et al., 2013). The major limitation of this method is the need for a specific medium labeled with isotope. The MBT-ASTRA allows the detection of resistance independent of the underlying mechanism (Ceyssens et al., 2017; Jung et al., 2016; Sparbier et al., 2016). However, the protocol for data analysis of this method is sophisticated which might impede its application. The direct-on-target microdroplet growth assay allows easy and rapid AST; however, the estimation might be disturbed by the remaining broth (Idelevich et al., 2018). Herein, we applied a method based on mass spectrometry to detect drug resistance, which was a combination of MBT-ASTRA and direct-on-target microdroplet growth assay. This approach allows a rapid and universal AST with a short incubation for 4 h. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) was tested to demonstrate the applicability of the method. CRAB is defined as critical-priority bacterium by World Health Organization last year (Tacconelli et al., 2017).

2. Materials and methods

2.1. Bacterial strains

A total of 132 nonduplicate *A. baumannii* complex clinical isolates were included in the study: 35 meropenem-susceptible and 97 meropenem-resistant isolates (intermediate strains were recorded as resistant in this study). All isolates were collected from the West China Hospital of Sichuan University in Chengdu, China. These isolates were identified using the Vitek2 Compact system (bioMérieux, France). The reference strains, *A. baumannii* ATCC 19606 and *Pseudomonas aeruginosa* ATCC 27853, were used as control strains. Freshly cultured bacteria were incubated on Columbia blood agar plates (Autobio, China) overnight at 37 °C.

2.2. MALDI-TOF MS-based rapid AST

A bacterial suspension was prepared with standard turbidity of 1 McFarland for each strain then diluted at 1:200 with cation-adjusted Mueller-Hinton broth (Ca^{2+}: 20-25 mg/L, Mg^{2+}: 10–12.5 mg/L). The diluted suspension (1×10^6 CFU/mL) was seeded into 96-well plates (Mindray, China) precoated with a series of concentration gradient meropenem. Twelve different concentrations were tested for each isolate: 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 μg/mL in 200 μL volume. The inoculum was incubated at 37 °C for 4 h. Subsequently, the suspension was transferred in 1.5-mL Eppendorf tube and centrifuged at 12000 ×*g* for 3 min to isolate the cells. Growth controls without antibiotics were applied similarly. Next, the supernatant was discarded and the bacteria were lysed with 5 μL of 70% formic acid and 5 μL of 100% acetonitrile. MALDI-TOF MS analysis was performed according to the standard protocol provided by the Bruker Daltonics (Germany). 1 μL of the lysate was spotted onto a polished steel MALDI target plate. Then, dried spots were overlaid with 1 μL MALDI matrix (10 mg/mL of α-cyano-4-hydroxycinnamic acid [HCCA] in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (Bruker). After matrix drying, MALDI-TOF MS estimations were performed in manual mode using the routine identification settings (parameter settings: linear positive mode; laser frequency 60 Hz; laser range 40–50%; ion source 1, 20 kV; ion source 2, 18.8 kV; pulsed ion extraction, 150 ns; lens, 6.0 kV; detector gain, 3.5 V). Each spectrum was obtained after 240 shots. The 240 shots were applied to more positions by random walk. Each concentration was tested on three different spots. The reference strains, *A. baumannii* ATCC 19606 and *P. aeruginosa* ATCC 27853, were tested similarly. A calibration standard (bacterial test standard; Bruker Daltonics) was used for instrument calibration.

2.3. Reference method

According to the guidelines of the Clinical Laboratory Standards Institute (CLSI; Clinical and Laboratory Standards Institute, 2017), the broth microdilution method was applied to determine the minimum inhibitory concentration (MIC) of the strains. Briefly, meropenem was tested in the concentration range from 0.06–128 μg/mL. The growth control and sterile control were also included. The bacterial suspension with standard turbidity 0.5 McFarland was applied in the test; it was diluted to 1:200 with cation-adjusted Mueller-Hinton broth. Then, the inoculum (5×10^5 CFU/mL) was seeded into 96-well plates and incubated for 18–20 h at 37 °C with a series of meropenem concentrations. After incubation, the complete inhibition of the visible growth was assessed as the result of MIC. The reference strains, *A. baumannii* ATCC 19606 and *P. aeruginosa* ATCC 27853, were used as control strains.

2.4. Data analysis

The mass spectra were interpreted by MALDI Biotyper 3.1 software (Bruker Daltonics). For samples with different concentrations of meropenem, the isolates were interpreted to exhibit growth if MALDI Biotyper software achieved correct identification with scores ≥1.7 and as growth inhibited if the identification failed with scores < 1.7. The lowest concentration of meropenem, which was recorded as growth inhibited by MALDI-TOF MS, was defined as the MIC based on mass spectrometry (MS-MIC). Each concentration was tested in triplicate and the median used for analysis. The test was recorded as valid if the growth of the control was detected successfully. Moreover, the isolates were classified as resistant if identification (scores ≥1.7) was achieved at breakpoint concentration of 2 or 8 μg/mL which is the CLSI susceptibility-breakpoint/resistance-breakpoint for *A. baumannii* and as susceptible if identification (scores < 1.7) failed.

3. Results

For all the isolates, valid identification was achieved in growth controls without antibiotics. The data revealed that the agreement of MIC values between MALDI-TOF MS-based rapid AST (RAST) and the reference method was 71.9% in all the isolates. The agreement of MIC values between the two methods in susceptible and resistant strains was 77.1% and 70.1%, respectively (Table 1). The comparison of the disagreement between the MS-MIC and MIC values revealed that the MS-MIC values were mainly shifted to a low concentration (Fig. 1). In the case of resistant strains, the MS-MIC values of 29.9% (29/97) isolates were at least one dilution lower than the corresponding MIC values; of these, the MS-MIC value (16 μg/mL) of one isolate was two dilutions lower than its MIC value (64 μg/mL) (Fig. 1B). Moreover, one isolate was misclassified as susceptible by MALDI-TOF MS as its MS-MIC value was equivalent to 2 μg/mL that was lower than its MIC value (4 μg/mL). Furthermore, the MS-MIC values of 14.3% (5/35) susceptible strains were one concentration gradient lower than its corresponding MIC values (Table 1). This phenomenon might be attributed to the incubation of the suspensions for 4 h with antibiotics at MS-MIC, which did not reach the MALDI-TOF MS detection limit. In addition, three isolates presented MS-MIC values (0.25, 1, 1 μg/mL, respectively) higher than the corresponding MIC values (0.125, 0.5, 0.5 μg/mL, respectively); however, they were still deemed as susceptible by MALDI-TOF MS (Fig. 1A). To confirm the disagreement between the two methods in the three isolates, the MS-based RAST and reference method-derived MIC testing were repeated, and the same results were obtained. For reference strains *A. baumannii* ATCC 19606 and *P. aeruginosa* ATCC 27853, the MS-based MIC value was equivalent to the reference method-derived MIC (0.25 and 0.5 μg/mL, respectively).