**ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR Burkholderia cepacia complex (BCC) CLINICAL ISOLATES: COMPARISON OF BROTH MICRODILUTION, AGAR DILUTION, E-TEST* AND DISK DIFFUSION METHODS**


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**INTRODUCTION AND PURPOSE**

Burkholderia cepacia complex (BCC) is a group of 16 genetically related bacteria. They are important opportunistic pathogens that cause severe infections in patients with cystic fibrosis and as well as other ventilator patients. The treatment of BCC infections is often problematic due to the development of resistance to many antimicrobial agents, including aminoglycosides, ampicillin, and most β-lactams, as well as emergence of antibiotic resistance during the therapy that has also been reported (5, 7, 8).

A variety of laboratory methods can be used to measure the in vitro susceptibility of bacteria to antimicrobial agents. In clinical microbiology laboratories, disk diffusion method is routinely used for susceptibility testing of antimicrobial agents (9). Though easy to perform, this method is known to have limitations, such as the fact that broth microdilution is difficult to be automated, and thus does not allow the evaluation of the performance of distinct methodologies to accurately predict the antimicrobial susceptibility of a bacterial strain. The objective of this study was to comparatively evaluate the performance of different methodologies, broth microdilution, agar dilution, E-Test and disk diffusion for the susceptibility testing of strain of the BCC complex species mentioned by the Clinical and Laboratory Standards Institute (CLSI) in the 11th edition susceptibility testing against BCC.

**MATERIALS AND METHODS**

Clinical isolates. A total of 82 clinically isolated BCC clinical isolates were received from four different clinical isolates of patients admitted at two tertiary hospitals between 2010 and 2015. Only one sample per patient was included for testing. These samples were collected from patients with chronic lung disease and were subcultured on Brucella chocolate agar, supplemented with polyclonal 75 µg/mL of gentamicin, 25 µg/mL of streptomycin and 50 µg/mL of vancomycin (BioMérieux, France). They were identified by 16S rRNA sequencing (4, 10, 11), and were confirmed by Biolog. All others were identified as BCC by Biolog. All BCC strains, hereafter referred to as BC, were identified as BCC by Biolog. All BC strains, hereafter referred to as BC, were compared to 12 reference strains of the BCC complex species from human and environmental isolates by MALDI-TOF (Table 1).

Antimicrobial susceptibility testing (AST). AST was carried out for seven antimicrobial agents established for testing by CLSI M02-ED9 (ciprofloxacin, ceftazidime, imipenem, meropenem, trimethoprim/sulfamethoxazole, tetracycline and amikacin) against BC. The MICs were determined according to the Clinical and Laboratory Standards Institute (CLSI, 2013). The disk diffusion and E-Test were interpreted according to the breakpoints established by CLSI M100-S17 (2013). The broth microdilution (BMD) was interpreted according to the breakpoints established by CLSI M100-S17 (2013). The broth microdilution was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013)

**RESULTS**

**MICRODILUTION VS. DISK DIFFUSION**

**BreakMIC vs. age at death**

- Essential agreement (96.8%) between both broth microdilution and disk micromethods was greater than 90% for all antimicrobials evaluated, except for chloramphenicol 90%.
- An excellent essential and categorical agreement rates were observed for TMPSMX (96.9% and 100%, respectively) (Table 1).
- Unacceptable minor errors rates were observed only for ceftriaxone (19%), meropenem (10%) and imipenem (12%) (Table 2).
- Break MIC vs. age at death (P = 0.05)
- A good essential agreement (94.5%) between both broth microdilution and E-Test were observed for meropenem (94.5%), chloramphenicol (88.7%), tetracycline (92.7%) and ceftriaxone (91.4%).
- Despite showing an excellent categorical agreement rate (100%), the essential agreement rate between the reference methodolgy and E-Test for TMPSMX was seen that of that of E-Test at age at death (94.5%) (Figure 3 and Table 2).
- When comparing E-Test results with those of broth microdilution, the perfect categorical agreement was observed for chloramphenicol followed by ceftriaxone (94.5%). High unacceptable minor errors rates were also observed for chloramphenicol (46.5%) (Figure 3 and Table 2).

**BreakMIC vs. disk diffusion**

- Categorical agreement between the disk diffusion and broth microdilution were greater than 95% for ceftriaxone (95.4%), imipenem (91.5%), meropenem (88.7%) and tetracycline (88.7%).
- Species showing a low percentage of categorical agreement was observed for TMPSMX (16.7%) (Figure 4). High rates of major (23.5%) and minor (2.4%) errors were detected for this antimicrobial (Table 2).

In general, the age at death results were more consistent with those of the broth microdilution, reference methodology, than with those of the E-Test.

The emergence of multidrug-resistant Gram-negative bacilli has resuscitated the use of older antimicrobial compounds like chloramphenicol. In our study, the worst essential and categorical agreement rates were observed for this agent, independently of the antimicrobial susceptibility technique applied. Thus, our results suggest that the breakpoints applied by the CLSI for chloramphenicol are not appropriate for BCC and that alternative breakpoints should be evaluated.

**Antibiogram**

Table 1 shows the essential and categorical agreement rates between both broth microdilution, disk diffusion and E-Test. Unacceptable minor errors rates were observed only for TMPSMX. Overall, the break MIC method seems to be the most suitable for the susceptibility testing of BCC clinical isolates.

**DISCUSSION**

BCC infections are often problematic due to the development of resistance to many antimicrobial agents, including β-lactams. In addition, the successful therapy of BCC infections is difficult to be achieved (5). To date, no study comparing the performance of distinct methodologies to accurately predict the antimicrobial susceptibility of BCC strains has been reported.