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Antimicrobial activity evaluation and comparison of methods of susceptibility for *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacter* spp. isolates

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ABSTRACT

The production of KPC (*Klebsiella pneumoniae* carbapenemase) is the major mechanism of resistance to carbapenem agents in enterobacterias. In this context, forty KPC-producing *Enterobacter* spp. clinical isolates were studied. It was evaluated the activity of antimicrobial agents: polymyxin B, tigecycline, ertapenem, imipenem and meropenem, and was performed a comparison of the methodologies used to determine the susceptibility: broth microdilution, Etest® (bioMérieux), Vitek 2® automated system (bioMérieux) and disc diffusion. It was calculated the minimum inhibitory concentration (MIC) for each antimicrobial and polymyxin B showed the lowest concentrations for broth microdilution. Errors also were calculated among the techniques, tigecycline and ertapenem were the antibiotics with the largest and the lower number of discrepancies, respectively. Moreover, Vitek 2® automated system was the method most similar compared to the broth microdilution. Therefore, is important to evaluate the performance of new methods in comparison to the reference method, broth microdilution.

Introduction

Klebsiella pneumoniae carbapenemase (KPC) – producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality.[1,2] These infections also represent a challenge in clinical practice involving hospitalized patients, requiring multidisciplinary efforts for infection prevention. Found primarily in *K. pneumoniae*, KPC is an enzyme capable of hydrolysing a broad spectrum of β-lactams including the penicillins, cephalosporins, carbapenems and monobactams. In addition, this resistance mechanism has high potential for dissemination due to its plasmid location, which facilitates transfer to the interspecies gene. Therefore, KPC has

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been identified in several other *Enterobacteriaceae* and non-fermenting Gram-negative bacilli (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*).[3,4]

Carbapenems are first-line agents for the treatment of serious nosocomial infections caused by multidrug-resistant *Enterobacteriaceae*. However, the increasing incidence of carbapenemase-producing *Enterobacteriaceae* (CPE) infections hinders the use of this class of antibiotics.[2,5] Therefore, tigecycline and polymyxins are commonly required to treat infections caused by CPE.[6]

Due the few remaining available treatment options, optimization of dosing regimens and combination therapy may be the most appropriate treatment strategies. The benefits of combination therapy include potential synergistic effects and suppression of emerging resistance.[7] In addition, the selection of a dosing regimen depends on the ability to determine the minimum inhibitory concentration (MIC) to an antibiotic.[8] Few studies about methods of evaluation of multidrug-resistant *Enterobacter* spp. have been found. Therefore, the aim of this study was to compare four methods used for detect antimicrobial susceptibility: broth microdilution (BMD), Etest® – plastic strip with gradient of antibiotic concentrations to determine the minimum inhibitory concentration of antibiotics, Vitek 2® automated system for microbial identification and antimicrobial susceptibility testing and disc diffusion (Kirby Bauer) for five antimicrobials agents (polymyxin B, tigecycline, ertapenem, imipenem and meropenem) among KPC-producing *Enterobacter* spp. clinical isolates.

Materials and methods

Bacterial isolates

Forty samples of KPC-producing *Enterobacter* spp. (thirty *E. cloacae*, nine *E. aerogenes* and one *E. gergoviae*), isolated from patients hospitalized in University Hospital of Londrina, between July of 2010 and December of 2013, that showed carbapenem resistance, according to CLSI (*Clinical and Laboratory Standards Institute*),[9] were evaluated. The isolates were stored in TSB (Oxoid-England) glycerin at −20 °C until use for the study. The samples were previously identified by the BD Phoenix™ automated system.

Characterization of clinical isolates

The isolates were subjected to modified Hodge test (MHT), according to Lee et al.[10] to phenotypically identify the presence of carbapenemases. Class A carbapenemase production was confirmed by using of boronic acid, as described by Tsakris et al.[11] The polymerase chain reaction (PCR) was performed with specifics primers, as described by Bradford et al.[12] for research of bla_{KPC-2} gene, previously identified. The performance of the tests for the detection of carbapenemases was determined using PCR as the reference standard. Sensitivity was calculated from the number of true-positive isolates, whereas specificity was calculated from the number of true-negative isolates.

Antimicrobial susceptibility testing

Disc diffusion

The disc diffusion test was performed with Mueller Hinton Agar and discs from Oxoid®-England by Kirby-Bauer technique and according to CLSI document M2-A10.[9] The test discs were: tigecycline 15 μg, imipenem 10 μg, meropenem 10 μg, ertapenem 10 μg and polymyxin B 300 U. There is no standardization, by CLSI, for polymyxin by disc diffusion test, only for broth microdilution. Therefore, standard breakpoints for *P. aeruginosa* were used for polymyxin B.

MIC testing

Susceptibility to antibiotics was also determined by broth microdilution (BMD), the reference method, Vitek 2® automated system (bioMérieux) and Etest® (bioMérieux), but the Etest® was performed only to tigecycline. The bacterial suspensions were adjusted according to CLSI recommendations. As described in CLSI, for meropenem, imipenem e ertapenem, *P. aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were used for quality control to broth microdilution test. For tigecycline, the bacteria used were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 and for polymyxin B, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. The microplates were incubated at 37 ± 2 °C for 21 to 24 hours and visually evaluated. The MIC is defined as the lowest concentration of antibiotic capable of inhibiting the growth of the microorganism. Therefore, the concentrations required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively), were calculated for all tested antibiotics.

MIC breakpoints

According to the recommendation of the ANVISA (*Agência Nacional de Vigilância Sanitária*), in Technical Note N° 01/2010,[13] the following breakpoints were used to evaluate polymyxin B susceptibility (susceptible ≤ 2 μg/mL and resistant ≥ 4 μg/mL), tigecycline (susceptible ≤ 1 μg/mL, intermediate 2 μg/mL and resistant ≥ 4 μg/mL), ertapenem (susceptible ≤ 0.5 μg/mL, intermediate 1 μg/mL and resistant ≥ 2 μg/mL), imipenem (susceptible ≤ 1 μg/mL, intermediate 2 μg/mL and resistant ≥ 4 μg/mL) and meropenem (susceptible ≤ 1 μg/mL, intermediate 2 μg/mL and resistant ≥ 4 μg/mL).

Incidence of errors

Results obtained from BMD testing were considered the reference standard to which results from Vitek 2® automated system, Etest® and disc diffusion were compared. Error classification was assessed using interpretive criteria for susceptibility. Very major errors (VMEs) were identified when an isolate was determined to be susceptible to a given agent by Vitek® automated system, Etest® and disc diffusion, but resistant by BMD. Major errors (MEs) were identified when an isolate was determined to be resistant to a given agent by Vitek 2® automated system, Etest® and disc diffusion, but susceptible by BMD. A result was deemed to be a minor error (MiE) when the same for a given agent was intermediate by any of the testing methods studied but was determined to be either susceptible or resistant by the other comparative method, as recommended in CLSI.

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