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Evaluation of Different Cabapenems in Detection of Carbapenemase Producing Gram Negative Bacilli.

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ABSTRACT

Carbapenems are beta lactam antibiotics presently considered as most potent agents for treating multidrug resistant gram negative bacilli infections. Detection of carbapenem resistance in clinical laboratory, as the isolates do not always show the accurate MIC range, may go undetected. CLSI recommends initial screening with carbapenems by disc diffusion method and confirmation by MHT for the production of carbapenemases. The present work is undertaken to investigate the accuracy of four commercially available carbapenems viz imipenem, meropenem, doripenem, ertapenem discs in detection of resistance to carbapenems. A total of 24 genetically confirmed carbapenem resistant gram negative isolate were included in the study. 23 isolates were resistant to meropenem and doripenem each. 19 were resistant to ertapenem and 12 to imipenem. 7 isolates were medially sensitive to imipenem, 1 to meropenem and doripenem each and 2 to ertapenem. In conclusion, meropenem and doripenem are equally effective for the detection of carbapenemase producing gram negative strains by Kirby Bauer disc diffusion method.

Key words: imipenem, meropenem, ertapenem, doripenem, carbapenemases

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INTRODUCTION

Carbapenems are beta lactam antibiotics presently considered as most potent agents for treating multidrug resistant gram negative bacilli infections [1]. The emergence of carbapenem resistant gram negative bacilli is of great concern as there are limited options to treat the infections of these strains. Carbapenem resistance is due to the production of carbapenem hydrolyzing enzymes or production of Amp C beta lactamase with or without porin loss. Detection of carbapenem resistance in clinical laboratory, as the isolates not always show the accurate MIC range, may go undetected. This may lead to inadequate infection prevention and control practices causing a widespread resistance. The reference MIC determination methods such as broth microdilution and agar dilution methods are more sensitive than disc diffusion, E test or automated systems [2].

Phenotypic methods using phenylboronic acid and EDTA were recently demonstrated to be very effective in detecting class A and B carbapenemases. However, this method is cumbersome and not commercially available [3-5]. In low income countries, most of the laboratories still depend on the disc diffusion as the primary method of determining antibiotic resistance [2].

CLSI recommends initial screening with carbapenems by disc diffusion method and confirmation by MHT for the production of carbapenemases [6]. Performing MHT also delays the availability of results for a day [3]. The final confirmation of presence of carbapenem genes is, however, done by molecular methods. The present work is undertaken to investigate the accuracy of four commercially available carbapenems viz imipenem, meropenem, doripenem, ertapenem discs in detection of resistance to carbapenems.

MATERIALS AND METHODS

The genetically confirmed carbapenem resistant gram negative bacilli isolated from various clinical samples for a period of one year were included in the study. Multiplex PCR was performed with the strains which were resistant and with decreased susceptibility to meropenem to detect class A, class B and class D carbapenemase genes. All the isolates were tested for the susceptibility to carbapenems namely imipenem (10µg), meropenem (10µg), ertapenem (10µg) and doripenem (10µg) discs by Kirby Bauer's disc diffusion method according to CLSI guidelines. The zone interpretive criteria for carbapenems used are mentioned in table no 1 [6].

Table 1: zone interpretive criteria for carbapenems by disc diffusion method

Disc	Disc Strength	Sensitive	Intermediate	Resistant
Doripenem	10µg	≥23	20-22	≤19
Ertapenem	10 µg	≥22	19-21	≤18
Imipenem	10 µg	≥23	20-22	≤19
Meropenem	10 µg	≥23	20-22	≤19

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RESULTS

A total of 24 genetically confirmed carbapenem resistant gram negative isolate were included in the study over a period of 12 months. Among 24 gram negative bacilli, NDM were detected in 16 strains, IMP in 1, OXA-48 in 2 strains. Simultaneous multiple gene detection were in 5 strains. 3 strains detected NDM and OXA-48, 1 in NDM and VIM and 1 in OXA-48, VIM and IMP. Carbapenem resistant gram negative bacilli includes 7 strains of *K.pneumoniae*, 4 strains of *P.aeruginosa*, 5 *E.coli*, 4 *P.mirabilis* and 3 *C.koserii*.

The susceptibility pattern of the isolates to different carbapenems are shown in the table 2. 23 isolates were resistant to meropenem and doripenem each. 19 were resistant to ertapenem and 12 to imipenem. 7 isolates were medially sensitive to imipenem, 1 to meropenem and doripenem each and 2 to ertapenem. 5 isolates were susceptible to imipenem and 3 to ertapenem.

Table 2: The susceptibility pattern of the isolates to different carbapenems

	Sensitive	Intermediate	Resistant
Imipenem	5(20.8%)	7(29.2%)	12(50%)
Meropenem	0	1(4.2%)	23(95.8%)
Doripenem	0	1(4.2%)	23(95.8%)
Ertapenem	3(12.5%)	2(8.3%)	19(79.2%)

DISCUSSION

Carbapenems are used to treat severe infections caused by multidrug resistant organisms, especially extended spectrum beta – lactamase producing pathogens. However the emergence of carbapenem resistant strains is an increasing therapeutic challenge because this enzyme hydrolyze not only carbapenems but also penicillins, cephalosporins and monobactams [7]. Reliable detection of carbapenemases is necessary to implement contact precautions and for outbreak detection [8].

According to CLSI document, carbapenemase-producing isolates usually test intermediate or resistant to one or more carbapenems and ertapenem nonsusceptibility is the most sensitive indicator of carbapenemase production. MHT is limited to the strains for infection control or epidemiological investigation purpose only. This is recommended for Enterobacteriaceae family [6]. In our study both meropenem and doripenem could detect 95.8% of the carbapenemase procucing strains each. 4.2% strains showed intermediate sensitivity pattern to both of them. But Ertapenem detected 79.2% of the resistant and 8.3% intermediate strains. Gupta V from Chandigarh, used meropenem and imipenem discs for the detection of CRE as ertapenem has lower specificity which could be due to porin loss associated with ESBL or AmpC production. Use of ertapenem can cause the prevalence of carbapenemases to be potentially high. Imipenem could detect only half of the carbapenemase producing strains [2,9]. Endimian A et al. calculated the statistical analysis of old and newer interpretive

criteria of carbapenems (imipenem, meropenem, etrapenem and doripenem) by CLSI guidelines and showed the sensitivity to 100% for newer criteria [3].

Detection of the presence of carbapenemases among GNB is an infection control emergency and it is a critical step required in the clinical laboratory for appropriate management of patients and infection prevention and control efforts. In conclusion, meropenem and doripenem are equally effective for the detection of carbapenemase producing strains by disc diffusion method.

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