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Original Research Article

Detection of Carbapenem Resistance in Clinical Isolates in a Tertiary Care Hospital in Central India

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Abstract:

Background: The increase in the rates of antibiotic resistance is a cause of concern worldwide. The aim of this study is to detect the prevalence of carbapenem resistance, production of carbapenemase and the various mechanisms of carbapenem resistance other than carbapenemase production.

Methods: This descriptive laboratory based study was conducted over a period of 3 months, from December 2014 to February 2015. A total of 600 non-duplicate Gram Negative Bacterial (GNB) isolates from clinical samples from admitted patients were included. Antimicrobial susceptibility was performed by disc diffusion method. The isolates that showed resistance or intermediate sensitivity to carbapenems were considered as screening test positive. The minimum inhibitory concentration (MIC) of these isolates was determined by using E-test of Ertapenem and Meropenem. Modified Hodge Test (MHT) was performed for carbapenemase detection. MBL was detected by combined Disk Test (CDT) using meropenem with and without EDTA and by MBL E-Strip. AmpC detection was done by E-test.

Statistics: P value was calculated by applying Pearson Chi- Square, and Fisher's Exact Test.

Results: A total of 35 out of 600 (6 %) GNB isolates were found to be carbapenem resistant. MHT detected carbapenemase production in 17 out of 35 (49 %) isolates. Remaining 18 (51 %) isolates were negative for carbapenemase production. Carbapenem resistance due to Amp C overproduction was seen in 4 isolates by AmpC E test. MBL production was detected by CDT in 21 isolates (60 %) and by E test in 25 isolates (71 %).

Conclusions: MBL production is the most common mechanism of carbapenem resistance besides porin loss and AmpC over-production. Simple tests like CDT or E test can be used routinely to detect MBL in microbiology laboratories.

Keywords: Carbapenem, Carbapenemase, Modified Hodge Test (MHT).

Introduction

The increase in the rates of antibiotic resistance is a cause of concern worldwide especially in low middle income countries including India. Third generation cephalosporins and carbapenems have been the mainstay of treatment for life-threatening infections. [1]

However, the irrational use of these antibiotics is the major cause of resistance in bacteria and has been the subject of extensive microbiological and genetic investigations. [2] Numerous β-lactamases exist, encoded either by chromosomal or transferable genes located on plasmids or transposons. As per Ambler classification, based on amino acid and nucleotide sequence studies, four distinct classes of β-lactamases have been defined namely, Class A and C using serine as an active site residue, Class B (metallo-β-lactamase) using Zinc and Class D or OXA-enzymes which are also serine based but quite distinct from class A or C. [3]

Due to the broad spectrum activity of carbapenems and their stability to hydrolysis by most β-lactamases, they have been the drug of choice for treatment of infections caused by cephalosporin-resistant Gram-negative bacilli (GNB), particularly Escherichia coli and Klebsiella pneumoniae, and non-fermenters including Pseudomonas spp. and Acinetobacter spp. On the other hand, there are increasing reports of carbapenem resistance amongst them due to carbapenemase production. [4] The majority of acquired carbapenemases belong to three of the four known classes of β-lactamases, namely Class A, B, and D. The bacterial host range is wide producing these three

classes of enzymes, which confer clinically significant resistance to carbapenems. [5,6]

The production of a given carbapenemase may confer a particular β-lactam resistance phenotype, depending on the bacterial species, the expression level, the enzyme type or variant, and the presence of additional resistance mechanisms such as permeability reduction and/or efflux and/or activity of other β-lactamase. [5,7]

Increased carbapenem minimum inhibitory concentrations (MICs) in Enterobacteriaceae may also result from high expression of AmpC or CTX-M Extended Spectrum β- lactamases (ESBLs) in combination with porin alterations. [8] This limits our treatment options leading to increased morbidity and mortality rates. Colistin and tigecycline are the only available antibiotics for treatment and both have limitations.

Detection of carbapenemase-producing organisms in the clinical microbiology laboratory is crucial for the choice of appropriate therapeutic options and the implementation of infection control measures. Nonetheless, it poses a number of difficulties, as it cannot be based simply on the resistance profile and the relevant methodology of specific tests for detection has not yet been well standardized. [8]

The aim of the present study is to detect the prevalence of carbapenem resistance, production of carbapenemase and the various mechanisms of carbapenem resistance other than carbapenemase production contributing to carbapenem resistance in clinical isolates from our tertiary care teaching hospital.

Material and methods

This descriptive laboratory based study was conducted over a period of 3 months, from December 2014 to February 2015. A total of 600 GNB isolates from various clinical samples from admitted patients were included in the study.

All isolates were non-duplicate and they were initially identified by standard laboratory methods [9] and further confirmed by NEFERM-24 Entero identification system for Enterobacteriaceae and NF API identification system for Non-fermenters (Erba Lachema, MIKRO-LA-TEST, Scotland).

Bacterial Strains

Quality control strains used were Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 700603, Klebsiella pneumoniae ATCC BAA-1705-MHT positive control (PC) (Carbapenemase producer) and Klebsiella pneumoniae ATCC BAA-1706-MHT negative control (NC) (Carbapenemase non-producer).

Antimicrobial susceptibility test

Antimicrobial susceptibility was routinely performed by disc diffusion method according to CLSI guidelines.10 The following antibiotics from Hi-Media Laboratories (Mumbai, India) were tested for Enterobacteriaceae: Ampicillin (10ug), cefoxitin (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), ertapenem (10µg), meropenem (10µg), ceftazidime/clavulanic acid (30/10µg), gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg) and piperacillin-tazobactam (100/10µg). Piperacillin (100µg) was additionally tested for Pseudomonas spp. isolates whereas, Acinetobacter spp. were tested for piperacillin (100µg) and ampicillin-sulbactam (10/10µg). Ertapenem was not tested for non-fermenters. For urinary isolates, norfloxacin (10µg), ofloxacin (5µg), nitrofurantoin (300µg) and cotrimoxazole (1.25/23.75µg) were tested.

The isolates that showed resistance or intermediate sensitivity to carbapenems (either meropenem or ertapenem) were considered as screening test positive isolates for carbapenem resistance and subjected to further testing.

Minimum inhibitory concentration (MIC) using E-test

The minimum inhibitory concentration (MIC) of the screening test positive isolates was determined by using E-test (Ezy MIC strip, HiMedia Laboratories, Mumbai, India). MIC was detected for Ertapenem (EM085, Ertapenem Ezy MIC Strip 0.002-32 mcg/ml) and Meropenem (EM092, Meropenem with and without EDTA Ezy MIC Strip with Meropenem 4-256 mcg/ml). Etest assay was performed as per manufacturer's recommendations.

Carbapenemase Production

Modified Hodge Test (MHT) was performed according to CLSI guidelines for carbapenemase detection. [11]

MBL detection by using Combined Disk Test (CDT)

MBL was detected in the test isolates by combined Disk Test using meropenem with and without EDTA according to Yong et al. [12] Test organisms were inoculated on to plates of Mueller Hinton agar. Two 10- µg meropenem disks were placed on the plate and 10 µL of EDTA solution was added to one of them to obtain the desired concentration (750 µg) of EDTA. The inhibition zones of meropenem and meropenem-EDTA disks were compared after 16–18 h of incubation at 35°C. If the increase in inhibition zone with the meropenem and EDTA disc was ≥ 7 mm than the meropenem disc alone, it was considered as MBL positive.

MBL detection by MBL E-Strip

MBL was also detected by MBL E-Strips (HiMedia Laboratories, Mumbai, Ezy MIC strip, EM092, Meropenem 4-256 mcg/ml and Meropenem + EDTA 1-64 mcg/ml). Both halves of the Ezy MIC strip are impregnated with meropenem giving varying concentration gradient along the strip. One of the half is overlaid with a fixed concentration of EDTA. The intersection of the ellipses at the strip is read from two halves, i.e., at the section with meropenem alone and meropenem with EDTA. A reduction in the MIC of meropenem by more than or equal to 3 doubling dilutions in the presence of EDTA was interpreted as a positive test for MBL detection. Internal controls used were: MBL positive E.coli for Enterobacteriaceae and Pseudomonas aeruginosa for non-fermenters.

AmpC detection with E-strip

AmpC detection was done by Etest for isolates which were MBL negative by CDT or Etest. AmpC test strips were procured from Himedia labs, Mumbai. (Ezy MIC strip, EM081A, Ceftazidime, Cefotaxime, Cefepime and Cloxacillin 0.125-16 mcg/ml (Mix) and Ceftazidime, Cefotaxime, Cefepime, Cloxacillin + Clavulanic acid and Tazobactam 0.032-4 mcg/ml (Mix+). A reduction in the MIC of Mix+ by more than or equal to 3 doubling dilutions in the presence of AmpC inhibitor i.e. Cloxacillin is interpreted as a positive test for AmpC detection.

Statistics

Data was analysed by using SPSS 16.0 software. P value was calculated by applying Pearson Chi-Square, and Fisher's Exact Test.

Results

A total of 35 out of 600 (6 %) GNB isolates from various clinical samples were found to be carbapenem resistant. Their antibiogram is depicted in Fig.1. MHT detected carbapenemase production in 17 out of 35 (49%) screening positive isolates. Remaining 18 (51%) isolates were negative for carbapenemase production. Different types of results of MHT are depicted in Fig.2. Carbapenem resistance due to AmpC overproduction was seen in 4 isolates by AmpC E test. A total of 31 isolates were found to show one or more of the above described mechanisms. Four isolates which were screening positive did not reveal any of the above tested mechanisms. All results are depicted in Table.1.

ORIGINAL

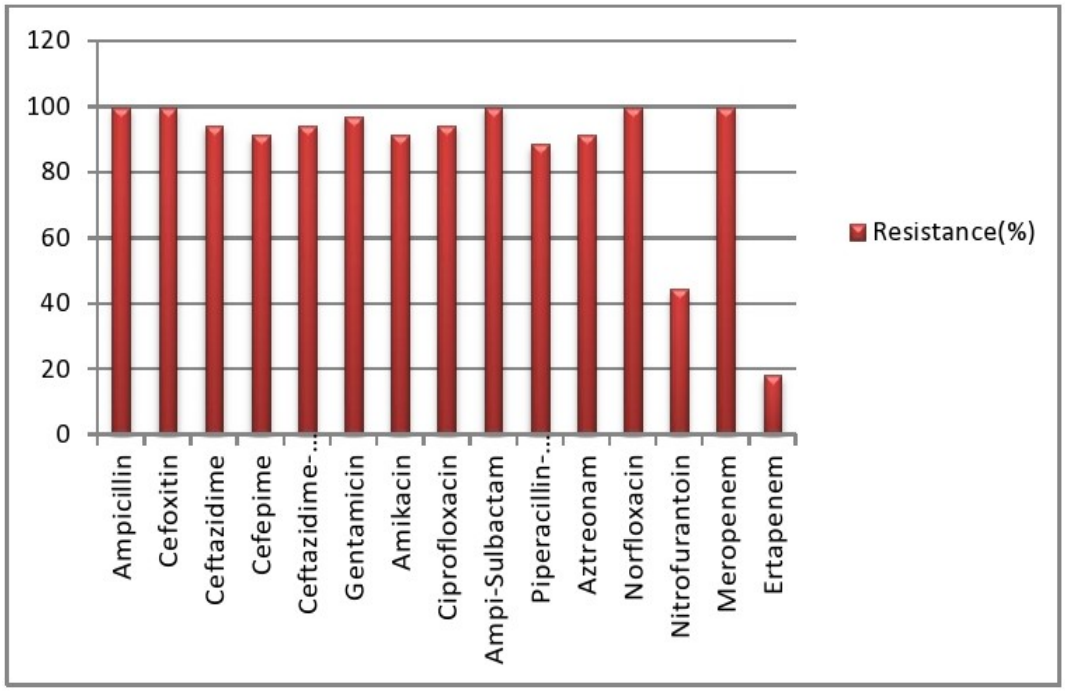

Figure.1. Antibiogram of isolates positive for carbapenemase production by screening test (n=35)

ORIGINAL

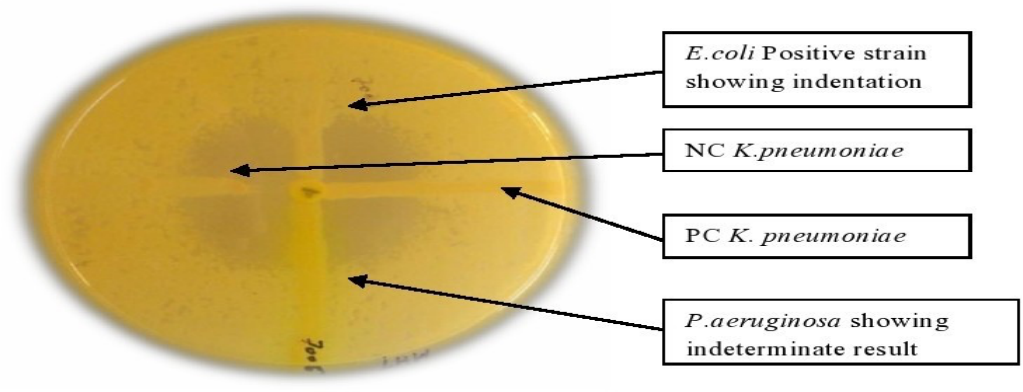

Figure.2. Modified Hodge Test

Figure 2:

ORIGINAL

Table.1. Different mechanisms of carbapenem resistance in screening test positive clinical isolates from CRGH, Ujjain (n=35)

	Screening positive isolates n (%)	MHT (+)	MHT (-)	CDT (+)	CDT (-)	Etest (+)	Etest (-)	AmpC Etest (+)	(-) by mechanisms tested
E.coli	7 (20)	3	4	4	3	4	3	-	3
K.pneumoniae	8 (23)	7	1	7	1	5	3	-	1
K.oxytoca	1 (3)	1	0	1	0	1	0	-	-
C.freundii	1 (3)	0	1	0	1	0	1	1	-
E.cloacae	1 (3)	1	0	0	1	0	1	1	-
E.aerogenes	1 (3)	1	0	0	1	1	0	-	-
P. vulgaris	1 (3)	0	1	0	1	0	1	1	-
P.mirabilis	1 (3)	0	1	0	1	0	1	1	-
P.rettgeri	1 (3)	0	1	0	1	1	0	-	-
P.aeruginosa	9 (26)	0	9	8	1	9	0	-	-
A.baumanii	4 (11)	4	0	1	3	4	0	-	-
Total No. (%)	**35 (100)**	**17 (49)**	**18 (51)**	**21 (60)**	**14 (40)**	**25 (71)**	**10 (29)**	**4 (11)**	**4 (11)**

MBL production was detected by CDT in 21 isolates (60%) and by E test in 25 isolates (71%). By combination of both methods, MBL production was detected in 27 isolates (77%). Out of 27 MBL positive isolates, 19 isolates (70%) were detected by both, CDT and E test. Two isolates (A.baumanii and P.aeruginosa) were meropenem resistant but found to be sensitive to ceftazidime. (Fig.3.)

Discussion

Carbapenem resistance due to AmpC overproduction was seen in 4 isolates by AmpC E test. MBL production was detected by CDT in 21 isolates (60%) and by E test in 25 isolates (71%). By combination of both methods, MBL production was detected in 27 isolates (77%). Out of 27 MBL positive isolates, 19 isolates (70%) were detected by both, CDT and E test. Two isolates (A. baumanii and P. aeruginosa) were meropenem resistant but found to be sensitive to ceftazidime.

Carbapenemase-producing organisms in the clinical settings are a cause of concern for the clinicians to choose appropriate therapeutic regimen. Nevertheless, it poses a number of difficulties, as it cannot be based simply on the resistance profile and the relevant methodology of specific tests for detection has not yet been well standardized. Microbiologists act as a bridge between clinician and hospital infection control committee.

During the 3 months study period, 35 out of 600 (6%) GNB isolates were found to be carbapenem-resistant. This was similar to few other studies viz; Pandya et al, 2011 [13] and Deshpande et al, 2010 [14] who reported 6% and 8%, respectively.

Ertapenem nonsusceptibility is the most sensitive indicator of carbapenemase production. [11] We found that 14 (64%) isolates which were sensitive to ertapenem were resistant to meropenem. These meropenem resistant isolates may carry different carbapenem resistant mechanisms and will be missed if ertapenem non- susceptibility is considered as a surrogate marker for carbapenem resistance. To improve the sensitivity of detection of carbapenemases, we used both ertapenem and meropenem for screening. Imipenem was not used as it performs poorly. [11] Also, further revisions may be required in the criteria for screening test for carbapenemase production.

CLSI guidelines do not advocate the use of the MHT for the detection of carbapenemase production in nonfermenting GNB. In spite of this, several authors have found the MHT as a useful screening test for carbapenemase production. [15-17] We have also used MHT in non-fermenters. MHT detected carbapenemase production in 17 out of 35 (49%) screening positive isolates. Remaining 18 (51%) isolates were negative including 9 Pseudomonas and 2 Proteus spp. MHT could not be interpreted in Pseudomonas spp. and in Proteus spp. due to spreading and swarming growth, respectively. (Fig.2.) since the value of MHT for detecting the currently widespread carbapenemase producers such as KPC, NDM-1, OXA-48 strains has been poorly documented [18], we performed various phenotypic tests to ascertain different mechanisms of carbapenem resistance in our test isolates.

In the present study, we detected MBL production in 21 (60%) and 25 (71%) isolates by using CDT and E test, respectively. When used in combination, MBL production was detected in 27 isolates (77%). A total of 19 isolates were detected by both CDT and E test. Two isolates were detected by CDT which was not detected by E test whereas; E test detected 6 isolates which remained undetected by CDT. Picao et al [19] found 80% MBL by CDT amongst PCR confirmed MBL isolates. Chakraborty et al [20] used E test for MBL production in isolates from ICU patients and found 90% positivity. Our results showed better detection by E test for MBL production as compared to CDT. A single isolate of P. aeruginosa and A. baumannii each showed carbapenem resistance but sensitivity to ceftazidime. This may be attributed to loss of porin mechanism. These isolates were also showing MBL production by E test. (Fig.3.) This shows that multiple mechanisms can be present in same isolate.8 Carbapenem resistance can also be caused due to AmpC overproduction8 which was seen in 4 isolates (11%) in our study. A total of 31 isolates were found to show one or more of the above described mechanisms. A total of 4 isolates which were screening positive were not showing any of the above described mechanisms. These might be causing carbapenem resistance due to Klebsiella pneumoniae Carbapenemase (KPC) or Oxacillinases (OXA) for which no phenotypic tests are currently available and hence could not be detected. KPC or OXA can also be present in isolates showing other mechanisms because multiple mechanisms can be present in same isolate. [8]

In our study, 27 (77%) out of 35 screening positive isolates were MBL producers. MBL production was the most common mechanism of carbapenem resistance. Deshmukh et al, 2011 [21] found 90% and Deshpande et al, 2010 [14] found 92% MBL production amongst imipenem resistant isolates.

When MHT was compared with CDT in the present study, we found insignificant correlation (p=0.733) between the two. Similar results were found on comparison of MHT with E test for MBL detection (p=0.711). This is because of the reason that MHT is a test to detect carbapenemase production, but it performs poorly in case of non-fermenters and Proteus isolates. The sensitivity of the test for detecting New Delhi Metallo-beta lactamases

(NDM)-type carbapenemases is low (11%). [11] We found significant correlation (p=0.006) between both tests, CDT and E test used for MBL detection, suggesting that both tests are equally effective to detect MBL in clinical isolates.

Conclusions

In the present study, MBL production is the most common mechanism of carbapenem resistance in clinical isolates besides porin loss and AmpC over-production. Although our data refers to phenotypic detection of resistance mechanisms in a small number of clinical isolates, routine screening can be recommended with less stringent criteria because carbapenemases in Enterobacteriaceae may not be detected as their MICs can sometimes be below the current breakpoints. Simple tests like CDT or E test can be used routinely to detect MBL in microbiology laboratories, both being equally effective. Use of carbapenems in clinical practice by unwary clinicians without prior testing for its resistance mechanisms will not only result into treatment failure but may also contribute to spread of resistance.

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