Research Article

Detection of Carbapenem resistance in clinical isolates in a tertiary care hospital, Yavatmal India

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Abstract

Detection of carbapenemase producers in clinical isolates 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. The production of a given carbapenemase may confer a particular β-lactam resistance phenotype, depending on the bacterial species and may be associated with additional resistance mechanisms such as permeability reduction and/or efflux. In our study, MBL production was the most common mechanism of carbapenem resistance. AmpC over-production was also found to cause carbapenem resistance in clinical isolates. Both, CDT and Etest were found equally effective for detecting MBL in clinical isolates and hence, these simple tests can be routinely used in microbiology laboratories for choice of appropriate options to clinicians and to avoid treatment failure.
Keywords: Carbapenemase, metallo-β-lactamase, Enterobacteriaceae, non-fermenters.

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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 (the 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] 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, particularly *Escherichia coli* and *Klebsiella pneumoniae*, and non-fermenters including *Pseudomonas* 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 ESBLs in combination with porin alterations.[8] This limits our treatment options leading to increased

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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 in the department of Microbiology of R.D.Gardi medical college,Ujjain and its 570-bedded teaching hospital. A total of 600 gram negative bacterial isolates from various clinical samples from admitted patients were included in the study. All isolates were non-duplicate. The isolates 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). 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.

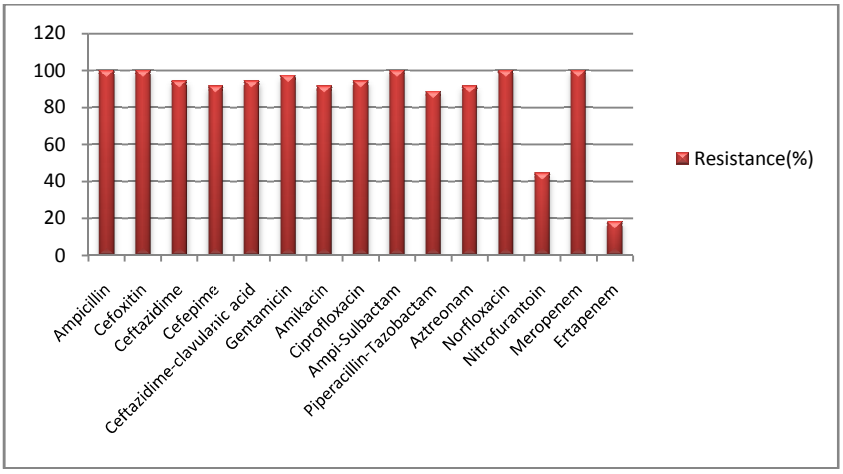

Figure 1: Antibiogram of isolates positive for carbapenemase production by screening test (n=35)

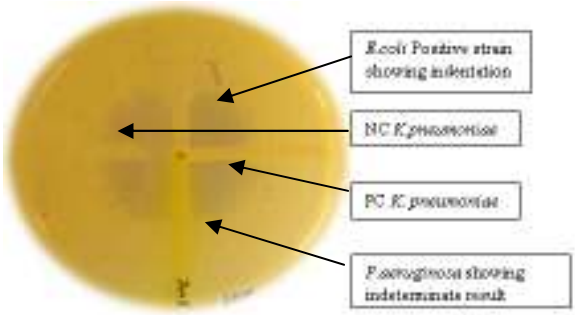

Figure 2: Modified Hodge Test

MBL production was detected by CDT in 21 isolates (60%) and by Etest 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 Etest. Two isolates(A.*baumanii* and *P.aeruginosa*) were meropenem resistant but found to be sensitive to ceftazidime.(Fig.3.)

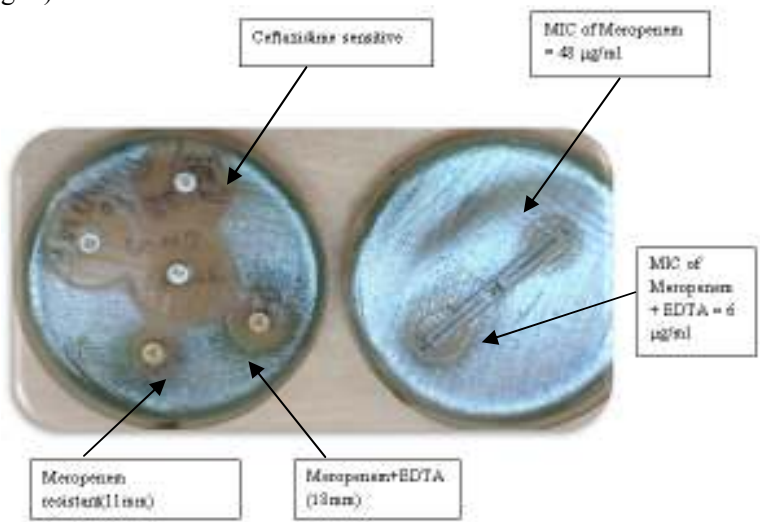

Figure 3: Ceftazidime sensitive and Meropenem resistant, MBL positive isolate of *P.aeruginosa*

Carbapenem resistance due to AmpC overproduction was seen in 4 isolates by AmpC Etest. 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.

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

	Screening positive isolates n (%)	MHT (+) n	MHT (-) n	CDT (+) n	CDT (-) N	Etest (+) n	Etest (-) n	AmpC Etest (+) n	(-) by mechanisms tested n
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)**

DISCUSSION

Carbapenemase-producing organisms in the clinical settings is a cause of concern for the clinicians to choose appropriate therapeutic regimens. 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 modified Hodge test for the detection of carbapenamase production in nonfermenting gram negative bacilli. In spite of this, several authors have found the modified Hodge test as a useful screening test for carbapenamase 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 Etest, respectively. When used in combination, MBL production was detected in 27 isolates (77%). A total of 19 isolates were detected by both CDT and Etest. Two isolates were detected by CDT which were not detected by Etest whereas, Etest detected 6 isolates which remained undetected by CDT. Picao *et al.* found 80% MBL by CDT amongst PCR confirmed MBL isolates.[19] Chakraborty *et al.* used Etest for MBL production in isolates from ICU patients and found 90% positivity.[20] Our results showed better detection by Etest 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 CDT and Etest.(Fig.3.) This shows that multiple mechanisms can be present in same isolate.[8] Carbapenem resistance can also be caused due to AmpC overproduction[8] 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 by using imipenem discs for screening. When MHT was compared with CDT in the present study, we found insignificant correlation(p=0.724) between the two. Similar results were found on comparison of MHT with Etest 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-betalactamases(NDM)-type carbapenemases is low (ie, 11%).[11] We found significant correlation(p=0.006) between both tests, CDT and Etest used for MBL detection, suggesting that both tests are equally effective to detect MBL in clinical isolates.

CONCLUSIONS

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 Etest 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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