International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Infections by Carbapenemase Producing Enterobacteriaceae in a Rural Tertiary Care Hospital, Karnataka: Prevalence, Treatment Options and Outcome

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Received: 23/02/2015 *Revised: 23/03/2015* *Accepted: 30/03/2015*

ABSTRACT

Background: The development of bacterial resistance to carbapenems hampers effective treatment.
Objectives: To find out the prevalence of carbapenemase producing Enterobacteriaceae (CPE) and the treatment options available and outcome in patients infected with CPE.
Methods: Total of 1,245 isolates of Enterobacteriaceae was screened for carbapenem resistance by disc diffusion method. Minimal Inhibitory Concentration (MIC) of ertapenem was determined for resistant strains. The CPE were confirmed by Modified Hodge Test (MHT). Sensitivity of CPE to other antibiotics was determined. Presence of bla_{NDM}, bla_{IMP}, bla_{VIM} and bla_{KPC} was determined by PCR for 60 isolates. Patients infected with CPE were followed up for outcome.
Results: Among 1,245 isolates, 108(8.67%) were confirmed to be carbapenamase producer both by MIC estimation and MHT. bla_{NDM} alone or in combination with bla_{IMP}/bla_{VIM} genes were detected among 55(96%) of isolates. None of the CPE harboured bla_{KPC}. The susceptibility pattern of the CPE showed moderate sensitivity to chloramphenicol (76%), tetracycline (42%), aminoglycosides (32%) and cotrimoxazole (30%). Eighty one (88%) of the 92 patients, who were followed, recovered completely with antibiotic therapy based on sensitivity pattern or with local wound care. A mortality rate of 11.3% was observed. Many of the patients who succumbed had severe co morbid conditions.
Conclusion: In our study the prevalence of CPE was 8.67%. Most of CPE carried bla_{NDM}. Treatment with antibiotics based on sensitivity pattern was beneficial in patients infected with CPE.

Key words: Carbapenem resistance, Carbapenemase producing Enterobacteriaceae, New Delhi Metallo beta lactamase, Carbapenemases

INTRODUCTION

Carbapenems are the mainstay in treating infections caused by extended spectrum beta-lactamase producing members of Enterobacteriaceae. [1] Carbapenem resistance among members of Enterobacteriaceae varies in geographical regions, it is to the tune of 0.6% in a study reported from China and as high as 7.8% in a study from India. [2,3] Among the several mechanisms responsible for carbapenem resistance, production of carbapenem-hydrolysing beta-lactamases, the

carbapenemases, is the major mechanism conferring resistance. [4]

A variety of carbapenemases are known to be produced by the members of Enterobacteriaceae, which have been assigned different groups in the two prominent methods of classification: Ambler molecular classification and Bush-Jacoby's classification based on functional group. In the Amblers molecular classification, carbapenemases fall under groups A, B and D. [4] The genes of carbapenemases being plasmid mediated can be easily transferred to other organisms. [5] As development of resistance to carbapenems, excludes the use of large number of antibiotics and narrows the treatment options an early detection of CPE is of utmost importance in clinical practice.

Here we report the prevalence of carbapenemase production among clinical isolates of Enterobacteriaceae, the genotypes of carbapenemases involved and outcome of infections in patients admitted to a rural tertiary health care center in Karnataka.

MATERIALS AND METHODS

Study settings:

This study was conducted at Department of Microbiology, Sri Devaraj Urs Medical College, Kolar a tertiary care hospital in rural Karnataka, Southern India. Ethical approval was obtained from the institutional ethical committee. This study did not require any human participation and was performed on clinical isolates obtained from the different clinical samples of patients admitted in the associated hospital.

Bacterial Clinical Isolates:

Between January 2012 and May 2013, a total of 1,245 consecutive non-duplicate clinical isolates of Enterobacteriaceae recovered from pus (628), urine (323), sputum (97), endotracheal secretion (82), pleural fluid (56), blood (34) and others (25) were included in the study.

Bacterial identification and antimicrobial susceptibility testing:

Identification of the Enterobacteriaceae strains up to the species level was performed using biochemical tests as per standard techniques. Antibiotic susceptibility of these isolates was determined by Kirby-Bauer disc diffusion method according to CLSI, guidelines [6] using commercial discs (Himedia Laboratories Pvt. Ltd., Mumbai) on Muller-Hinton Agar. The antibiotics tested were ampicillin (10µg), piperacillin (100µg), amoxicillin-clavulanic acid (20/10µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), ceftriaxone (30µg), cefoxitin (30µg), gentamicin (10µg), tobramycin (10µg), amikacin (30µg), tetracycline (30µg), chloramphenicol (30µg) ciprofloxacin (5µg), levofloxacin (5µg), and cotrimoxazole (1.25/23.75µg).

Phenotypic screening for carbapenemase production:

All the isolates were screened using ertapenem (10µg), imipenem (10µg) and meropenem (10µg) discs (Himedia Laboratories Pvt. Ltd., Mumbai, India) for evidence of carbapenem resistance. For confirmation of carbapenem resistance, Minimal Inhibitory Concentration (MIC) levels for ertapenem was determined using E-strip (BioMe´rieux,France) for isolates showing resistance to any of three tested carbapenems by disc-diffusion. Those strains confirmed as carbapenem resistant as above were tested for carbapenemase production by Modified Hodge Test (MHT) as per CLSI, guidelines. [6]

Molecular Detection of carbapenemase genes:

Sixty of the isolates detected to produce carbapenemase by MHT were subjected to multiplex PCR for the detection of carbapenemase genes bla_{NDM}, bla_{VIM},

bla$_{IMP}$ *and bla*$_{KPC}$. DNA extraction was performed from the bacterial isolates using alkaline lysis method. [7] Amplification and identification of carbapenemase producing genes was done using previously described primers with modified cycling conditions. [8]

Clinical Outcome:

The clinical findings of patients infected with carbapenemase producing organisms were retrospectively studied from medical records of the patients to know the clinical outcome.

RESULTS

Of the 1,245 Enterobacteriaceae isolates, 174 (14%) isolates were found to be resistant to one or more of the carbapenems (meropenem 89%, imipenem 68% and ertapenem 64%) by disc diffusion assay. Further confirmation by MIC levels for ertapenem revealed 108 (8.7%) isolates to be carbapenem resistant (Table 1).All the 108 isolates confirmed carbapenem resistant strains were found to be producing carbapenemase by MHT. These 108 isolates were obtained from 97 patients (11 patients yielded two different species of Enterobacteriaceae)

Table 1: MIC levels against ertapenem of different organisms of Enterobacteriaceae showing resistance to carbapenems in screening test

Ertapenem MIC range (µg/ml)						
	Sensitive	Intermediate	Resistant			
Organisms	≤0.25	0.5-0.95	1-1.5	2-6	8-24	≥32
Klebsiella pneumoniae	12	5	2	21	13	5
E. coli	26	9	3	27	9	2
Enterobacterspp.	8	5	1	12	2	3
K.oxytoca	-	1	-	3	-	-
Proteaegroup	-	-	1	-	2	-
Citrobacterspp	-	-	-	1	1	-
TOTAL	46	20	7	64	27	10

Among the carbapenemase producing isolates *K. pneumoniae* was the most frequently isolated, followed by *Enterobacter* spp. (10.7%) and *E. coli* (6.87%) as depicted in table 2. These carbapenemase producing Enterobacteriaceae were majorly isolated from endotracheal secretion (15.9%), followed by pus (11.3%), blood (8.8%), urine (5.9%), pleural fluid (1.8%) and sputum (1%).

Table 2: Carbapenemase producing strains among the different species of Enterobacteriaceae

Isolates	Total no of isolates screened	Carbapenem resistant by disc-diffusion method	Confirmed carbapenemase producing isolates*
E. coli	596	76 (12.8%)	41 (6.87%)
K.pneumoniae	258	58 (22.5%)	41 (15.9%)
Enterobacter spp.	168	31 (18.5)	18 (10.7%)
Proteaegroup	115	3 (2.6%)	3 (2.6%)
Citrobacterspp.	56	2 (3.5%)	2 (3.5%)
K.oxytoca	52	4 (7.7%)	3 (5.8%)
Total	1245	174 (14%)	108 (8.7%)

* Confirmed by Ertapenem MIC and Modified Hodge test.

Molecular characterization of carbapenemase producing genes performed on 60 carbapenemase producing isolates of *K. pneumoniae (n=30), E. coli (n=23) and Enterobacter spp*.(n=7) is presented in table 3. Of the 60 carbapenemase producing strains, 57(95%) were found to be harbouring one or more of carbapenemase producing genes (*bla*$_{NDM,}$ *bla*$_{VIM,}$ *bla*$_{IMP}$ and *bla*$_{KPC}$). Of these, *bla*$_{NDM}$ gene alone was carried by 70.2% (40/57) of the isolates and *bla*$_{NDM}$ in combination with *bla*$_{IMP}$ and *bla*$_{VIM}$ genes was carried by another26.3% (15/57). There were only 2 (3.5%) isolates

that carried *bla*$_{IMP}$ alone. In our study none of the isolates harboured *bla*$_{KPC}$.

Table 3: Carbapenemase producing genes carried by the isolates of Enterobacteriaceae found to be carbapenemase producing by MHT

Organisms	Only NDM	Only IMP	NDM+IMP	NDM+VIM	NDM+IMP+VIM
K.pneumoniae(N=30)	26	-	1	1	-
E.coli (N=23)	7	2	9	2	2
Enterobacterspp (N=7)	7	-	-	-	-
TOTAL	40	2	10	3	2

The susceptibility to the antibiotics is presented in fig 1. About 3/4th (76%) of the isolates were sensitive to chloramphenicol and 42% were sensitive to tetracycline. The sensitivity to aminoglycosides and cotrimoxazole was 32% and 30%, respectively. None of the carbapenemase producing isolates was found sensitive to ampicillin, piperacillin-tazobactam, and 3rd generation cephalosporins.

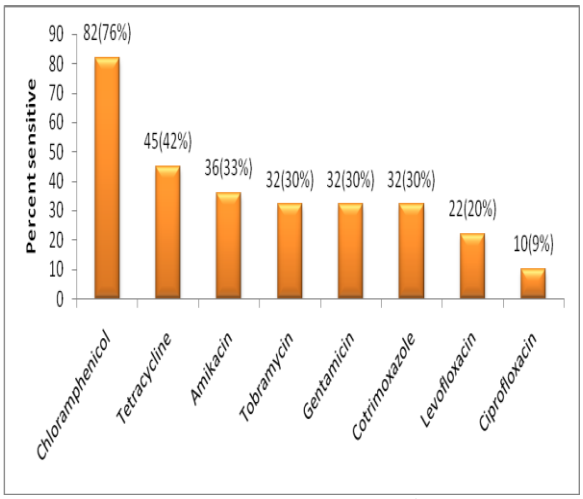

Fig 1: Antibiotic sensitivity pattern of carbapenemase producing strains

Majority of the carbapenemase producing organisms were isolated from patients admitted to ICU (31%) or general surgery wards (28%).Ninety-two of the 97 patients infected with carbapenemase producing strains could be followed up till outcome. Among them 81 (88%) patients recovered. Alternative antibiotics according to the sensitivity pattern were administered to 59 (73%) of these patients. No systemic antibiotics were administered to 22 (27%) patients who had wound infections with carbapenemase producing organisms. Dressing the wounds with locally active antimicrobial agents brought about healing in them. Eleven (11.3%) patients expired.

DISCUSSION

In a rural tertiary care setting of Southern India, we observed an 8.67% prevalence of carbapenemase producing Enterobacteriaceae, majority of which was harboring blaNDM gene. This is consistent with finding from other parts of India.

The carbapenem resistant Enterobacteriaceae species have been detected increasingly in the last few years. [9] A multicentric study involving clinical samples from patients from three large cities in India: Chennai, Bangalore, and Mumbai reported an overall prevalence rate of 5.2%. [10] Studies from Guwahati and Chandigarh have reported prevalence rates of 5.2% and 7.8%, respectively. [3,11] Our findings are also comparable with other studies conducted across the country.

We observed that carbapenemase producing Enterobacteriaceae were mainly found among the species of *Klebsiella, E. coli, and Enterobacter*. *K. pneumoniae* accounted for the highest number (15.89%). Most of carbapenemase producing isolates were isolated from patients admitted to ICU (31%) and surgical wards (28%). These findings are similar to that reported in a study by Wattal *et al.* [12] Isolation of carbapenemase producing Entero-bacteriaceae from ICU patients is thought to be associated with the use of different invasive devices (intubation tubes, surgical

drains, intravascular devices) in patients with co-morbidities. [13] *K. pneumoniae* appears to be a nosocomial pathogen easily transmitted from patient to patient through health care givers. [14]

Among the 57 isolates of Enterobacteriaceae found to harbor one or more of carbapenemase genes in our study, 55 (96%) were found to carry bla_{NDM} alone or in combination with other carbapenemase genes, bla_{VIM} and bla_{IMP}. The predominance of bla_{NDM} in our isolates is similar to the observations made in studies from India and elsewhere. [10,15,16] It is known that bla_{NDM} genes located in plasmids easily get transferred between strains and species of Enterobacteriaceae and get widely disseminated. [5] None carried bla_{KPC} which is similar to findings of an earlier study from Bangalore. [15] Thus, to date bla_{KPC} carrying strains seem to be rare in southern Karnataka in and around Bangalore. We could not however test for bla_{OXA} carbapenemase producing genes.

The carbapenemase producing isolates in our study exhibited complete resistance to cell wall acting agents such as ampicillin, piperacillin-tazobactam, and 3rd generation cephalosporins. However, antibiotics like cotrimoxazole (30%), aminoglycosides (32%), tetracycline (42%) and chloramphenicol (76%) showed considerable susceptibility. Thus, there exists an opportunity to treat infections by carbapenemase producing organisms with some of these antibiotics to which organisms are sensitive, provided there are no contraindications. [17] In line with this observation many patients with post operative infections in our hospital admitted to departments of orthopedics and OBG were treated successfully with chloramphenicol and tetracycline. No systemic antibiotics were administered to 22 (27%) patients who had wound infection with carbapenemase producing organisms. Dressing the wounds with locally active antimicrobial agents brought about healing in them. In our study a mortality rate of 11.3% was encountered in patients infected with carbapenemase producing organisms; the mortality rate observed was comparatively lower. In contrast to our experience, a study from Taiwan, in 2010 reported a mortality rate of 37.3% in patients infected with carbapenem resistant organisms. [18]

CONCLUSION

To conclude, our study observes that carbapenemase producing Entero-bacteriaceae poses a therapeutic challenge in rural tertiary care settings also. The 8.67%prevalence of CPE, majority of which was carrying bla_{NDM} gene in our setting was comparable to that of reports from urban settings of India. Individualized treatment strategies based on the susceptibility pattern and clinical status can be beneficial and reduce mortality. Some of the old antibiotics like tetracycline and chloramphenicol might be useful in treating CPE infections. However more studies are required to study the clinical outcome of these antibiotics.

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How to cite this article: Gowda T, Prasad SR, Gupta S et. al. Infections by carbapenemase producing enterobacteriaceae in a rural tertiary care hospital, Karnataka: prevalence, treatment options and outcome. Int J Health Sci Res. 2015; 5(4):90-96.

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