



Emerging resistance to carbapenem among Gram-negative bacteria in a tertiary care hospital

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Article History:

Received on: 04 Dec 2019

Revised on: 06 Jan 2020

Accepted on: 10 Jan 2020

Keywords:

Carbapenem,
E Test,
Gram-negative bacilli,
Modified Hodge Test,
Resistance

ABSTRACT

The emergence of bacterial antibiotic resistance is a cardinal concern in the health care system. The spread of resistance in Enterobacteriaceae and non-fermenters to the currently available drugs make the treatment of serious nosocomial infections troublesome. The purpose of the study is to find out the carbapenem resistance among Gram-negative bacilli in a tertiary care hospital. Antibiotic susceptibility pattern of 1913 aerobic Gram-negative bacilli isolated from clinical samples was made for a period of 6 months. All the isolates were tested for susceptibility to antibiotics by the Kirby-Bauer disc diffusion technique according to CLSI guidelines. Carbapenemase production was confirmed by the Modified Hodge Test (MHT). Minimum Inhibitory Concentration (MIC) by Epsilometer (E) test was performed (for Imipenem and Meropenem) for carbapenem-resistant strains. A total of 1731 clinical samples, 1913 Gram-negative bacilli were isolated. 1476 (77.1%) were Enterobacteriaceae and 433 (22.6%) were non-fermenters. 54 were carbapenemase-producing Gram-negative bacilli. Meropenem E test was done for carbapenemase-producing Gram-negative bacilli. The minimum inhibitory concentration for Meropenem ranged from 0.002 µg/ml to 32 µg/ml. To overcome the problem of emerging resistance, combined interaction and cooperation of microbiologists, clinicians and the infection control team is needed.



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11iSPL2.2177>

Production and Hosted by

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INTRODUCTION

One of the major burdens in the health care system is the emergence of antibiotic resistance. The spread of resistance among Enterobacteriaceae and non-fermenters to the currently available drugs

makes the treatment of severe nosocomial infections complicated (Paterson, 2006). Carbapenemase has high hydrolytic activity against penicillins, cephalosporins and carbapenems (John and Balagurunathan, 2011). Acquired resistance has been reported in *E.coli*, *Klebsiella pneumoniae*, *Pseudomonas* spp, *Acinetobacter* spp and various other non-fermenter Gram-negative bacilli (Zavascki et al., 2013).

Carbapenemase gene detection by a molecular method is the gold standard but is available in only a few reference laboratories and phenotypic tests have therefore been developed. The Modified Hodge Test (MHT) is the phenotypic test recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013) for carbapenemase screening method (Birgy et al., 2012). The purpose of this study was to determine the carbapenem resistance among Gram-negative bacilli isolated from clinical

samples and to confirm the carbapenemase production by Modified Hodge Test (MHT) and Minimum Inhibitory Concentration (MIC) by E test for carbapenem-resistant strains.

MATERIALS AND METHODS

The study was performed in a tertiary care hospital in Tamil Nadu for a period of 6 months. 1913 aerobic gram-negative bacilli were isolated in the clinical microbiology laboratory from various samples. The study was reviewed and approved by the Institutional Ethical Committee.

All the isolates have been tested for susceptibility to antibiotics by the Kirby-Bauer disc diffusion technique according to CLSI guidelines (Wayne). Antibiotics (1st and 2nd line) used for the susceptibility testing in gram-negative bacilli other than *Pseudomonas* species were Ampicillin(A), Gentamicin(G), Cephalexin(Cn), Ciprofloxacin(Cip), Ceftazidime(Caz), Cefotaxime(Ctx), Ceftazidime-Clavulanic acid(Cac), Cotrimoxazole(Cot), Cefuroxime(Cxm), Amikacin(Ak), Norfloxacin(Nx), Nitrofurantoin(Nit), Cefaperazone-sulbactam(Cfs), Ofloxacin(Of), Piperacillin-tazobactam(Pit), Cefepime(Cpm) and Imipenem(Imp).

Antibiotics used for susceptibility testing for *Pseudomonas* species were Gentamicin(G), Ciprofloxacin(Cip), Ceftazidime(Caz), Amikacin(Ak), Ofloxacin(Of), Cefaperazone-sulbactam(Cfs), Piperacillin-tazobactam(Pit), Imipenem(Imp) and Cefepime(Cpm).

Detection of Carbapenemase Production

Modified Hodge Test (MHT)

The imipenem resistant strains were subjected to the Modified Hodge test for the detection of carbapenemases. A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 was inoculated by lawn culture on the surface of Mueller-Hinton agar (MHA). After drying, 10 µg imipenem disc was placed at the center of the plate and the 0.5 McFarland dilution of the test strain was streaked from the edge of the disc to the periphery of the plate in four different directions. The plates were incubated overnight at 37°C. The presence of a 'cloverleaf shaped' zone of inhibition due to the production of carbapenemase production by the test strain was considered as positive (Chande *et al.*, 2013).

Minimum Inhibitory Concentration (MIC) by Epsilometer test

MIC to Imipenem and Meropenem of isolates which are positive for carbapenemase activity were tested by using E strips and inhibition ellipses around the

strip were measured (Nair, 2013).

RESULTS AND DISCUSSION

In the present study, an attempt was made to know the rate of carbapenemase-producing aerobic Gram-negative bacilli and to know their antibiogram in a tertiary care hospital. A total number of Gram-negative bacilli isolated from 1731 clinical samples during the study period of 6 months was 1913. Out of the 1731 clinical samples, 1088 (62.8%) isolates from urine samples, 517 (29.9%) isolates from exudates samples, 92 (5.3%) isolates from respiratory samples and 34 (2%) isolates were from blood samples. Among these 1913, *Escherichia coli* was the commonest followed by *Klebsiella* spp and *Pseudomonas* spp (Figure 1). In this study, it was found that about 77.1% of Gram-negative bacterial isolates belonged to Enterobacteriaceae, which was almost similar to that of the study done in Pakistan (Saghir *et al.*, 2009) with 63%.

Table 1: Distribution of carbapenem-resistant organisms

Organisms	Percentage
<i>Pseudomonas</i> spp	20 (37%)
<i>Acinetobacter</i> spp	18 (33.3%)
<i>Klebsiella</i> spp	9 (16.7%)
<i>Escherichia coli</i>	5 (9.3%)
<i>Proteus</i> spp	2 (3.7%)
Total	54

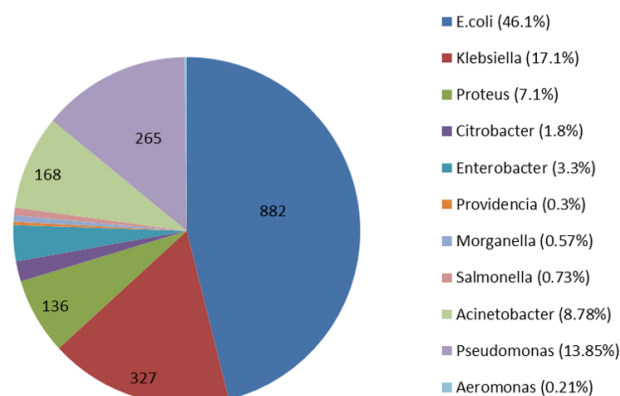


Figure 1: Percentage of different GNB isolated

Out of 1913 isolates, 54 (2.82%) were found to be carbapenem-resistant by Kirby-Bauer disc diffusion method for 30 µg of imipenem and their distribution in various clinical samples are elucidated in Figure 2. Carbapenemase production was confirmed for all the 54 isolates by the Modified Hodge test (Figure 3).

Table 2: MIC of meropenem for carbapenemase-producing Gram-negative bacilli

Organism	No.	MIC value ($\mu\text{g/ml}$)	Interpretation
Acinetobacter spp	4	0.75	Sensitive
Proteus spp	2	2	Intermediate
Escherichia coli	10	>32	Resistant
Acinetobacter spp	15	>32	Resistant
Pseudomonas spp	19	>32	Resistant
Klebsiella spp	4	>32	Resistant

Table 3: Percentage of carbapenem resistance among different organisms from various literature

Organisms	Percentage of carbapenem resistance	Place	Author
Non-fermenters	28/50 (56%)	Vellore	(Jesudason <i>et al.</i> , 2005)
Acinetobacter spp	21/150 (14%)	Bangalore	(Sinha and Srinivasa, 2007)
Pseudomonas spp	39/140 (27.9%)	Pondicherry	(Noyal <i>et al.</i> , 2009)
Acinetobacter spp	53/100 (53%)		
Acinetobacter baumannii	43/130 (33.1%)	Vellore	(Mendiratta <i>et al.</i> , 2012)
Acinetobacter spp	28/140 (20%)	Uttar Pradesh	(Agarwal <i>et al.</i> , 2013)
Enterobacteriaceae	57/465 (12.3%)	Mumbai	(Nair, 2013)

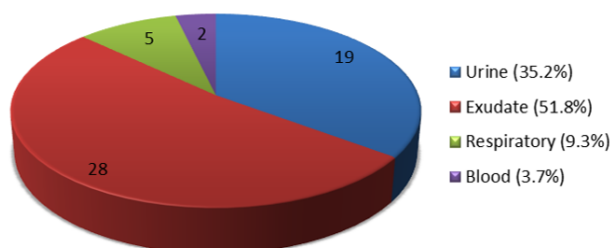


Figure 2: Distribution of Carbapenemase producers in various clinical samples



Figure 4: Minimum Inhibitory Concentration (MIC) of Meropenem by E test

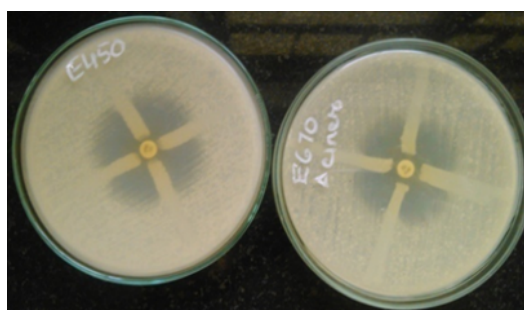


Figure 3: Modified Hodge Test showing indentation towards Imipenem disc for the confirmation of Carbapenemase production

In our study, among the carbapenemase producers, Pseudomonas spp (37%) was the predominant one followed by Acinetobacter spp (33.3%), as shown in Table 1. But in a study for simple screening for carbapenemase, 25.64% of Pseudomonas spp were found to be carbapenemase producers (Noyal *et al.*, 2009). The percentage of carbapenem resistance from different literatures is outlined below in Table 3.

Minimum inhibitory concentration for Meropenem ranged from 0.002 $\mu\text{g/ml}$ to 32 $\mu\text{g/ml}$ (Figure 4). Meropenem E test was done for all the 54 carbapenemases producing Gram-negative bacilli [Table 2]. The MIC of Meropenem for resistant strains of Pseudomonas spp and Acinetobacter spp is > 16 $\mu\text{g/ml}$. The MIC value of meropenem for resistant strains of members of the Enterobacteriaceae is >4 $\mu\text{g/ml}$. The

Among the 54 clinical samples from which carbapenem-resistant organisms were isolated, 13 were from the age group 41-50 years. The least number of samples collected were 1, from each of both the age group 0-10 years and 11-20 years.

strains of *Acinetobacter* spp with MIC of 0.75 µg/ml for meropenem were sensitive to aminoglycosides, nitrofurantoin and combination of beta-lactamase inhibitors with cephalosporins. These strains were resistant to third-generation cephalosporins and imipenem only.

All Meropenem resistant isolates were resistant to Imipenem, but not all Imipenem resistant organisms were resistant to Meropenem. Some of the imipenem resistant strains of *Acinetobacter* spp showed MIC < 4 µg/ml (sensitive). This could be because the mechanisms of resistance to these two drugs are different (Quale *et al.*, 2003). In another study, among the 21 carbapenemases producing *Klebsiella pneumoniae*, 7 strains were found to have 0.5 µg/ml MIC for meropenem, which is sensitive (Chande *et al.*, 2013). In a similar study, out of 36 multidrug-resistant *Acinetobacter* spp, 5 strains that were resistant to meropenem by the disk diffusion method were found to have MICs to meropenem in the sensitive zone (John and Balagurunathan, 2011).

CONCLUSION

In this study, the Modified Hodge Test and test for Minimum Inhibitory Concentration were used for detecting carbapenemase production and confirming resistance to carbapenem. Modified Hodge Test proved to be a good screening test for the detection of carbapenemase production as the results obtained with this study correlates with other studies. To overcome the problem of emerging resistance, combined interaction and cooperation of microbiologists, clinicians and infection control teams are needed.

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