

Prevalence of Carbapenem Resistance among Gram-Negative Bacteria in a Tertiary Care Hospital in North-East India

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Summary:

Introduction: Carbapenems are considered to be the last choice for drug resistant Gram-negative bacteria but emergence of carbapenem resistant Gram-negative bacteria worldwide due to the production of carbapenemase has jeopardize their use.

Aims and objectives: To detect the prevalence of carbapenem resistance in Gram-negative bacteria and carbapenemase production by Modified Hodge Test.

Materials and Methods: A total of 160 isolates of Gram-negative bacteria from November 2012 to October 2014, from different clinical samples were included in the study. Those isolates with intermediate or resistant zone to meropenem and doripenem by disc diffusion methods according to CLSI guidelines were confirmed by E-test and were again tested for carbapenemase production by Modified Hodge Test (MHT).

Results: Out of 160 isolates, 48 (30%) showed resistance to carbapenem by disc diffusion and E-test. Carbapenem resistance was higher in female patients 41.6% than in male patients 15.5% and among ICU patients 57.1%, ward patients 33.3% and among OPD patients 13.8%. Maximum number of carbapenem resistant isolate was obtained from urine sample n= 20, followed by pus n=13 and wound swab n=7. MHT for carbapenemase production was positive in 29 (60.4%) of the isolates. Carbapenemase production by MHT was highest with *Acinetobacter* spp. with n=5 (83.3%), followed by *Pseudomonas* spp. n= 6 (75%) and *E.coli* spp. n=16 (65.5%) and nil among *Citrobacter* spp.

Conclusions: Carbapenem resistance due to production of carbapenemase is prevalent in our hospital. MHT is a simple test in the routine lab for detection of carbapenemases.

Key words : Carbapenem, resistance, Gram-negative bacteria, Modified Hodge Test.

I. Introduction

Carbapenems are often used as antibiotics of last resort for treating infections due to multidrug-resistant gram-negative bacilli, because they are stable even in response to extended-spectrum and AmpC β -lactamases.¹ However, the emergence and proliferation of bacteria resistant to this important group of drug is jeopardizing the use of carbapenems. Resistance to carbapenem mostly is due to production of enzymes-Carbapenemases that hydrolyse carbapenems and other β -lactams.² Carbapenemase enzymes fall into Ambler classification - A, B and D.³ Carbapenemase gene detection by molecular methods is the gold standard but is available in only a few reference laboratories, and phenotypic tests have therefore been developed.⁴ Clinical and Laboratory Standards Institute (CLSI) incorporated the modified Hodge test (MHT) for the detection of carbapenemases.⁵

II. Materials And Methods

The study was done in the Department of Microbiology, Regional Institute of Medical Sciences, Imphal from November 2012 to March 2014. A total of 160 non-consecutive and non-duplicative isolates of Gram-negative bacteria from different clinical samples were included in the study. The clinical samples included in the study were pus, wound swabs, body fluids, sputum, stool, urine, throat swab, CSF, etc. collected from various patients admitted in wards and those coming to OPDs of the hospital. The bacterial isolates were identified according to standard microbiological procedure.⁶

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of Gram-negative isolates was done by Kirby Bauer's disc diffusion method using the following antibiotics: doripenem, meropenem, ciprofloxacin, amikacin, piperacillin/tazobactam, ceftazidime, nitrofurantoin, norfloxacin, gentamicin, ampicillin, ceftriaxone, amoxycylav, colistin and cotrimoxazole in Mueller Hinton Agar (MHA) according to CLSI guidelines. *E.coli* strain ATCC 25922 was used as control.⁷

Minimum Inhibitory Concentration (MIC)

MIC of carbapenems was determined by E-test. Those strains which showed reduced susceptibility to doripenem/meropenem in disc diffusion test were confirmed to be carbapenem resistant by E-test (HIMEDIA, INDIA). Resistance of Gram-negative strains to carbapenem was reported if MIC to meropenem and doripenem was ≥ 4 $\mu\text{g/ml}$.⁸

Modified Hodge Test

The detection of carbapenamase was done by the Modified Hodge Test as suggested by Lee et al¹³. An overnight culture suspension of *E. coli* ATCC 25922 which was adjusted to one-tenth turbidity of the McFarland 0.5 tube was inoculated evenly on the surface of a Muller-Hinton agar plate using a sterile cotton swab. After a brief drying at room temperature, a meropenem disk (10 μg) was placed in the center of the plate. Carbapenem resistant test strains from an overnight culture were streaked heavily from the edge of the disk to the periphery of the plate. The presence of a distorted or clover leaf shaped inhibition zone was interpreted as positive for carbapenamase producing isolates. *Klebsiella pneumoniae* ATCC BAA-1705 was used as control.⁹

III. Results And Observation

The study was conducted in the Department of Microbiology, RIMS, Imphal, Manipur from November 2012 to October 2014. A total of 160 Gram-negative bacilli isolated from various clinical samples were taken in the study.

The prevalence of carbapenem resistance among Gram-negative bacterial isolates was 30.0% (48/160) and sensitivity rate was 70.0% (111/160). Fig.1. Female patients 55.6% outnumbered male patients 44.4%. Carbapenem resistance was also higher in female patients 41.6% than in male patients 15.5%. ($p=0.001$) as shown in table 1. Majority of the patients were from different wards of the hospital (75%), followed by OPDs (20.6%) and minimum were from ICUs (4.4%). But carbapenem resistance rate was highest among ICU patients (57.1%), followed by ward patients (33.3%) and lowest among OPD patients (13.8%) ($p=0.01$). Table 2. Maximum number of sample was urine 83 (51.9%) followed by pus (23.1%), sputum (7.5%), wound swab (6.2%) and others (fig 3). Maximum number of carbapenem resistant isolate was obtained from urine sample $n=20$, followed by pus $n=13$ and wound swab $n=7$. Fig 2.

In this study, it is found that out of 48 carbapenem resistant isolates, 29 (60.4%) was positive for carbapenamase production by Modified Hodge Test. Carbapenamase production by MHT was highest with *Acinetobacter* spp. with 5/6 (83.3%), followed by *Pseudomonas* spp. 6/8 (75%) and *E. coli* spp. 16/26 (65.5%) and nil among *Citrobacter* spp. (table 3).

IV. Discussion

The prevalence of carbapenem resistance among Gram-negative isolates was 30.0% in this study. Similarly, overall meropenem resistance was about 30% in a study by Mulla S et al¹⁰ and 31.81% by Mahajan G et al¹¹ by disk diffusion method. Some studies recorded lower level of carbapenem resistance. Shivesh P et al¹² found 15% and Shashikala et al¹³ got 10.9% carbapenem resistance in their respective studies. In a study by Sachinkumar Wankhede et al¹⁴ out of 1546 samples screened, 19.40% of them were carbapenem resistant and Datta P et al¹⁵ found that the prevalence of CRE in their Institute is 7.87%. Resistance to carbapenem in this study is low compared to studies by various authors from India. In a study by Bijayini Behera et al¹⁶ at the All India Institute of Medical Sciences, New Delhi, carbapenem resistance was found to be 69%, which is much on the higher side.

There was no discordance between two phenotypic methods by Kirby bauer disc diffusion and E-test on carbapenem resistance in this study. In a comparable study by Datta P et al¹⁵ twenty-six strains were resistant to carbapenem by both disc-diffusion and E-test. There was no discordance between these two phenotypic methods.

Isolates resistant to meropenem was 30% (48), while to that of doripenem alone was 26% (40). This may be due to increased use of meropenem in this area. A study by Mohamudha R.P et al¹⁷ also reported that among the 45 meropenem resistant isolates, 33 and 21 were resistant to imipenem and ertapenem respectively and Gupta E et al¹⁸ also found that overall, resistance to meropenem was 22.16 per cent as compared to imipenem 17.32 per cent ($P<0.001$).

In this study maximum sample was from patients of the wards 75.0% followed by OPDs 20.6% and ICUs 4.4% but carbapenem resistance among the isolates is highest in ICUs 57.1%, ward 33.3.6% and lowest in OPDs 13.8%. ($p=0.01$) Our study is comparable with the study of Dr. Sachinkumar Wankhede et al¹⁴ where a higher number of MBL producers were from the ICUs (57.63%) as compared to wards (42.37%). Similar findings were made by Braykov NP et al¹⁹ where isolates obtained at ICUs had the highest carbapenem resistant *Klebsiella pneumoniae* (CRKP) prevalence (aOR, 3.27 [95% CI, 2.87–3.7] and for 3rd generation cephalosporin and CRKP (G3CRKP), the prevalence of resistance was greatest among isolates obtained at

nursing homes, followed by isolates obtained at ICUs and then isolates obtained from inpatients and cultures obtained at nursing homes were associated with the highest G3CRKP prevalence, nearly 4 times as high as the prevalence among cultures from outpatient (aOR, 3.9 [95% CI, 3.71–4.1]).

In another study by Pravin K et al²⁰ most of the CRE isolates were detected in patient samples from the wards (42%), then ICU (26%) and a significant number of isolates was also detected from the OPD patients (19%) and Kumar et al²¹ also found that out of 57 carbapenem resistant cases, 30 (52.6%) from IPDs, 24 (42.1%) from ICUs and 3 (5.3%) from others (triage and OPD).

Maximum number of sample was urine 51.9% (83/160) followed by pus 23.1%, sputum 7.5%, wound swab 6.2% and others. In a comparable study Mohamudha R.P et al¹⁷ also found that the distributions of the sources of the isolates were: urine 37% (n=39), blood 22.3% (n=23), wound discharge 11.7% (n=12), peritoneal fluid 5.8% (n=6), ascitic fluid 10.7% (n=11), tracheal aspirate 6.8% (n=7), and sputum 4.9% (n=5). In the present study, carbapenem resistant organism was isolated mainly from urine 47.1% (n=20) followed by pus 27.1% (n=13), wound swabs 14.6% (n=7), sputum 8.3% (n=4) and stool 6.3% (3). Nagaraj S et al²² had comparable findings where they observed that the carbapenem-resistant organisms were isolated mainly from urine samples up to 42% (n = 21), followed by wound discharge 18% and respiratory secretions 16%.

Another study by Yilmaz N O et al²³ had discordant findings when a total of 38 IMP resistant *P. aeruginosa* strains were analysed, strains were dominantly isolated from pus 42.1% (n=16), blood cultures 26.3% (n=10), urine 15.8% (n=6) and tracheal aspirate specimens 15.8% (n=6). Urine was the most frequent sample received during our study and in most of the studies analysed. The reason for this could be urinary tract infection (UTI), being the most common hospital-acquired infection, which accounts for almost 40% of all nosocomial infections.

In this study, it is found that out of 48 carbapenem resistant isolates, 60.4% was positive for carbapenemase production by Modified Hodge Test. Carbapenemase production is highest with *Acinetobacter* spp. with 83.3%, followed by *Pseudomonas* spp. 75% and *E.coli* spp. 65.5%. None of the 2 *Citrobacter* spp. was positive for carbapenemase production by MHT or MBL Etest. Carbapenem resistance in these 2 isolates could be due to over production of ESBL or AMPc. In a study by Amjad A et al.²⁴ out of 100 isolates which were carbapenem intermediate or sensitive, 69% showed the presence of carbapenemase by MHT which is similar with this study. In the study by Mahajan G et al¹⁶ 47.6% of the isolates were found to produce carbapenemase enzyme by MHT and the remaining 22 strains were found to be carbapenemase negative. In a study by Rajkumar MS et al²⁵ among the thirty seven meropenem resistant isolates, MHT was positive for 91.89% of the isolates.

V. Conclusion

This report showed that prevalence rate of carbapenem resistance is high in our hospital. Majority of carbapenem resistance here is due to Carbapenemase production. Hence rapid and accurate identification of carbapenem resistance is required for therapeutic and epidemiological reasons so that timely intervention, such as good infection control practices and prudent use of antibiotics will ensure that the spread of carbapenem resistance among organisms is kept under control. The different types of carbapenemases could not be ascertained due to unavailability of PCR in our hospital at the time of this study and perhaps, this is the major limitation in our studies.

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Figures And Tables

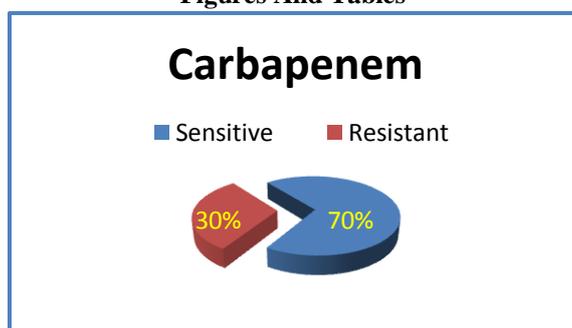


Figure 1: Prevalence of carbapenem resistance in Gram-negative clinical isolates.

Table 1: Distribution of gender.

Sex	Carbapenem resistant No. Percentage	Carbapenem sensitive No. Percentage	Total No. Percentage
Male	11 (15.5)	60 (84.5)	71 (44.0%)
Female	37 (41.6)	52 (58.4)	89 (55.6)
Total	48 (30)	112 (70)	160 (100.0)

$\chi^2 = 11.315, P = 0.001$

Table 2: Distribution of carbapenem resistant clinical isolates in different locations of the hospital

Location	Carbapenem		Total	CHI SQUARE TEST
	Resistant	Sensitive		
Wards	40 (33.3)	80 (66.7)	120 (75.0)	6.33 0.01
OPDs	4 (13.8)	29 (86.2)	33 (20.6)	
ICUs	4 (57.1)	3 (42.9)	7 (4.4)	
Total	48 (30.0)	112 (70.0)	160 (100.0)	

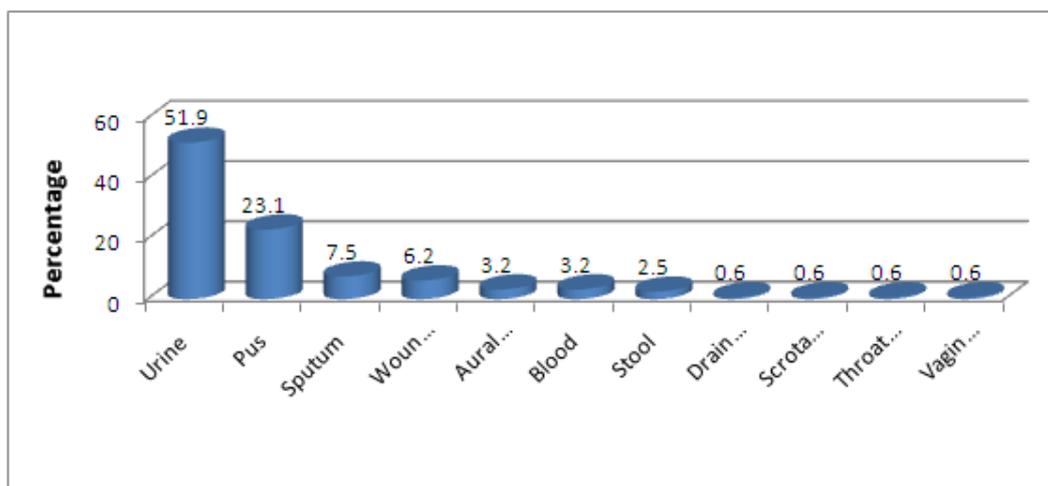


Figure 2: Distribution of the sample

Table 3: Distribution of carbapenemases among the isolates

Microorganisms (No. of carbapenemresistant isolates)	Carbapenemases production by MHT (%)
E.coli spp. (26)	16 (61.5)
Pseudomonas spp. (8)	6 (75)
Acinetobacter spp. (6)	5 (83.3)
Klebsiella spp. (3)	1 (33.3)
Citrobacter spp. (2)	0 (0.0)
Proteus spp. (2)	1 (50)
Stenotrophomonas maltophilia (1)	0 (0.0)
Total	29 (60.4)