Prevalence of Carbapenem-Resistant Hospital Acquired Infections and Their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: The emergence of antimicrobial-resistant bacteria has become a public threat, creating a burden on medical care in hospitals. Carbapenem-resistant organisms are a source of both community-acquired and healthcare-acquired infection that poses a substantial hazard to public health. This study aimed to conclude the prevalence of carbapenem resistance gram-negative bacteria from a clinical specimen at Index Medical College -Indore.
Methodology: This study was conducted in the Department of Microbiology, at Index Medical College, Indore, between January 2020 and January 2022.The isolates were subjected to antimicrobial susceptibility tests by Kirby Bauer's disk diffusion test. Most of the isolates were

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resistant to beta-lactam drugs, cephalosporin's and aminoglycosides. These isolates were further confirmedby phenotypic detection usingthe Modified Hodge test, Modified carbapenem Inactivation, Combined disc diffusion test and Double Disk Synergy.

Results: The percentage distribution of health-associated infection show highest resistance in both urinary tract and respiratory tract infection, followed by skin & soft tissue infection and least in septicemia and other health associated infection. Highest percentage of resistance was seen in the age group between 21-30 and the least in less than 10 years with a statistical significance of *p*-value=.00001. The most common isolates recover was E.coli in *Enterobacterals* and in Non fermenter it was *Pseudomonas aeruginosa* and *Acientobacter.* The sensitivity of MHT, mCIM, CDDT, and DDST within CI 95% were 74%, 95%, 84%, and 95% respectively. The overall prevalence of carbapenem resistance is 17.75%.

Conclusion: The production of carbapenemase is the major mechanism underlying carbapenem resistance around the world and represents a great health concern. More knowledge is needed to control resistant genes and resistant organisms and their dissemination. There is an urgent need for global collaboration to plan valid strategies to prevent the spread of carbapenemase and the development of new antimicrobial molecules.

1. INTRODUCTION

The most versatile family of beta-lactamase, are carbapenemase [1] Carbapenemase, enzymes hydrolyze almost all beta-lactamase and does not work against inhibitor [2,3]. Carbapenem-resistant gram-negative bacteria are difficult to treat infections in hospitalized patients. It leads to high mortality [4] and is the last resort for salvage treatment of multidrug-resistant Gram-negative bacteria. Carbapenem becomes a life threateningto the survival of critically iill patients, with 50% mortality [5]. There is an increasedalert of prevalence in multidrug-resistant organism which causes serious nosocomial infections. Globally Carbapenem-resistant gram-negative organisms are the main cause of nosocomial infection [6].This is a cross-sectional study performed to determine the prevalence of carbapenem-resistant Gram-negative bacilli isolated from patients admitted in wards tertiary care hospitals in Central India. We compared different methods for the detection of carbapenemase and Metallo beta-lactamase (MBL).

2. MATERIALS AND METHODS

Thisstudy is a cross-sectional study, conducted in the Bacteriology section of the microbiology laboratory of Index Medical College ,Hospital and Research Center, Indore (M.P.) from January 2020 to January 2022 A total of 246 clinical samples from patient admitted for more than 48 hours were collected from different clinical department.

2.1 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of Gram-negative isolates was done by Kirby Bauer`s disc diffusion method using the following antibiotics Imipenem, Meropenem, Ertapenem Ciprofloxacin, Amikacin, Piperacillin/tazobactam, Ceftazidime, Gentamicin, Ampicillin, Cefazolin, Tobramycin, Tigecycline Cefepime, Ceftriaxone, Amoxiclav, Cefotaxime, Colistin in Mueller Hinton Agar according to CLSI guidelines [7].

2.2 Phenotypic Detection of Carbapenem and Metallo β Lactamase Production

The detection was done by the Modified Hodge test [8], Modified carbapenem Inactivation Method [8], for phenotypic detection of MBL, Combined disc diffusion test [9] and Double Disk Synergy test [10] was done.

3. RESULTS

A total of 246 clinical samples were collected during the study period from Jan 2020 to Jan 2022 from various clinical departments from which 129 (52.43%) were positive for different isolates. From the 129 positive isolates, 22 (8.94%) were gram-positive bacteria and 107 (43.49%) were gram negative bacilli. From 107 gram-negative isolates 80.37% were *Enterobacterales* and 19.62% were non-fermenter. *E.coli* (32.55%) followed by, *Klebseilla pneumoniae* (17.5%), *Citrobacter species* and *Pseudomonas aeruginosa* (8.52), *Acientobacter*

Species 7.55% , *Proteus mirabilis* 4.56% and *Enterobacter species* 3.87%.

On performing Kirby Bauer disc diffusion method 59.09% of male patients were sensitive and 63.15% were resistant to carbapenem where as 40.90% were sensitive and 36.84% were resistant to carbapenem. The statistical analysis of p-value = 0.00001 for both carbapenem-resistant and carbapenem sensitive as shown in Table 1.

Percentage distribution of carbapenem-resistant drug show 21% resistance by Imipenem and Meropenem followed by Ertapenem 11%. In coexisting resistance was seen as Meropenem plus Ertapenem, Imipenem plus Ertapenem 5% each, Imipenem plus Meropenem 11% and 26% were resistant to all three drugs shown in Fig. 1. The prevalence of carbapenem resistance was 17.75%. The percentage of sensitivity of Imipenem was 79%, Meropenem 79%, and Ertapenem 89% respectively.

The antimicrobial sensitivity pattern highest sensitivity of Colistin (89%) followed by Tigecycline (58%), Amikacin (21%), Ciprofloxacin and Amoxiclav (16%) Ampicillin, Cefazolin, Tobramycin, Piperacillin/Tazobactam, Levofloxacin (11%), Cefepime and Gentamicin (5.2%), Cefotaxime, Ceftriaxone and Ceftazidium with no sensitivity. Ceftriaxone, Cefotaxime and Ceftazidium show 100% resistance followed by Gentamicin and Cefepime (95%), Ampicillin, Cefazolin, Levofloxacin, Tobramycin, Piperacillin /tazobactam (89%), Ciprofloxacin and Amoxiclav (84%), and least resistance was observed in Tigecycline (42%) Fig. 2.

Highest percentage of nosocomial infection was seen in urinary tract infection (65.4%) followed by skin & soft tissue infection (15.88%), respiratory tract infection (10.28%), (4.67%) septicemia and least infection was seen in other health associated infection (3.73%). The percentage distribution of health associated infection has highest carbapenem resistance in urinary tract and respiratory tract infection account of (31.57%) followed by skin & soft tissue infection (15.78%), and least about (10.52%) was seen in septicemia and other health associated infection Table 2.

Age wise distribution accounts for sample collected from patients have highest percentage of 23.17% age group 41-50yrs, followed by 21.95% , 31-40 yrs 19.1%, 21-30 year ,15.44% more than 50years, 32 (13%) and 7.31% age less than 10year. In overall age group positive isolates were observed in age group 21-30 year , 28.68%, followed by 18.6%% (31-40 yrs), 17.05%(10-20 yrs), 15.5% more than 50 yrs, 12.43%(41-50 yrs) and 7.75% less than 10 yrs.

Table 1. Gender-wise distribution of Gram-Negative carbapenem-resistant and Gram-Negative carbapenem-sensitive isolates

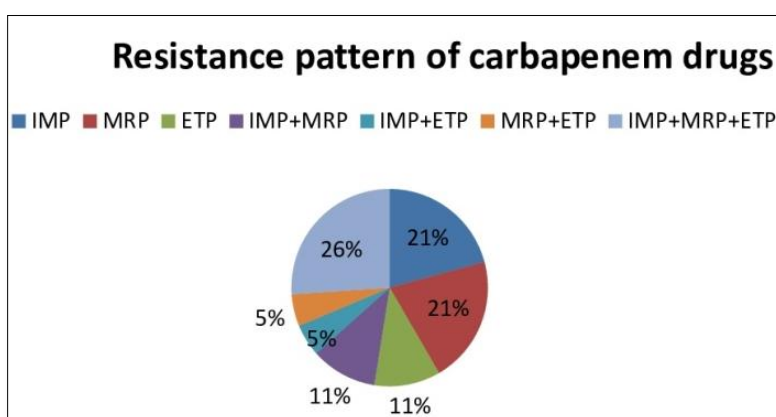| Gender	Carbapenem resistant	Carbapenem sensitive	Total	p-value
Male	12(63.15)	52(59.09)	64(59.81)	0.00001
Female	7(36.84)	36(40.90)	43(40.18)	0.00001
	19	88	107	

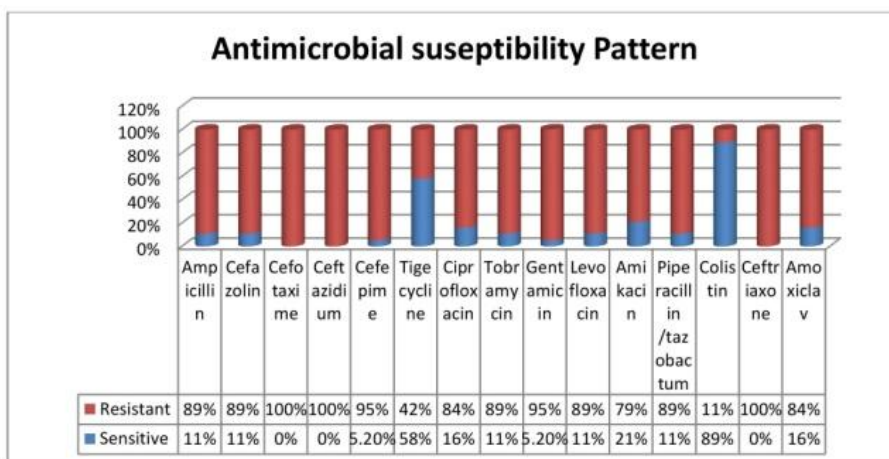**Fig. 1. Resistance pattern of carbapenem drugs**

Fig. 2. Antimicrobial susceptibility test

Table 2. Types of hospital acquired infection in relation to gram-negative carbapenem-resistance and gram-negative carbapenem-sensitive

Type of HAI	Positive Sample N (129)	GNR N(109)	CRGNR N(19)	p-value
Urinary tract infection	75(58.10)	70(65.4)	6(31.57)	.000001
Skin & Soft tissue infection	26(20.10)	17(15.88)	3(15.78)	. 000943
Septicemia	8(6.20)	5(4.67)	2(10.52)	.004893
Respiratory tract infection	12(9.30)	11(10.28)	6(31.57)	.001058
Other HAI's	8(6.20)	4(3.73)	2(10.52)	.024271

GNR-Gram-Negative, CRGNR-Carbapenem Resistant Gram-Negative

The overall gram negative bacilli were highest in age group between 21-30 (28.9%) followed by 31-40 (20.5%), 10-20 (15.8%), more than 50 (15.3%), 41-50 (13.0%), less than 10 years (6.5%) respectively. Overall carbapenem resistance is highest in age group 21-30 (42.1%) followed by 10-20 age group (21.05), more than 31 (10.52%) and less than 10 yrs (5.26%) Overall p-value= .000857 Table 3.

Gram negative bacteria were isolated from the different department; the maximum isolates were medicine 51% followed by surgery 21%, intensive care unit 14%, obstetrics & gynaecology 7% and pediatric 6%. Among the different departments the maximum carbapenem-resistantgram-negative bacteria was present in medicine 31.57% which was followed by surgery 26.31% , intensive care unit 21.05% and obstetrics & gynecology and Pediatric showing same percentage of resistance 10.52%. Among the gram-negative bacteria the maximum bacteria were isolated from Urine 65.4% followed by Pus 15.8%, Endotracheal

aspirate 6.5%, Blood 4.6%, Sputum and other health associate infection [HAI's] 3.7%. Among them maximum carbapenem resistant GNR were isolated from Urine 31.5% followed by Endotracheal aspirate 21%, Pus 15.7% and 10.5% Blood, Sputumand other health associate infection (Table 4) The statistical significant difference of p-value=.00001.

Among the nosocomial infection, the maximum number of organisms were isolated from urinary tract infection which include *E.coli* 423% followed by *Klebseilla pneumoniae* 20%, *Citrobacter species* 13%, *Pseudomonas aeruginosa* 10%, *Enterobacter species* 8%, *Proteus species* 6 % and *Acientobacter species* 1%. The secondhighest infection is caused by skin soft tissue infection *E.coli* contribute the highest prevalence in causing soft & skin tissue infection species and *Pseudomonas aeruginosa* 6% with no isolates from *Enterobacter species* and *Acientobacter species* isolated. Among the respiratory tract infection accounts for the third highest infection, responsible organism include

Table 3. Age wise distribution of isolates

Age group (in Years)	Total sample N(%)	Positive isolates N(%)	GNR N(%)	CRGNR N(%)	p-value
Less than 10	18(7.31)	10(7.75)	7 (6.5)	1(5.26)	.016157
10_20	32(13)	22(17.05)	17(15.8)	4(21.05)	.000538
21-30	47(19.1)	37(28.68)	31(28.9)	8(42.1)	.00001
31-40	54(21.95)	24(18.6)	22(20.5)	2(10.52)	.00001
41-50	57(23.17)	16(12.43)	14(13.0)	2(10.52)	.0001214
More than 50	38(15.44)	20(15.5)	16(15.3)	2(10.52)	.000159

Table 4. Department wise distribution of GNR and CRGNR

Department	Blood	Urine	Pus	Sputum	ET	Other HAI's	GNR
Medicine	0	53	0	2	0	0	55
Surgery	0	8	12	1	0	2	23
Obstetrics & Gynaecology	0	4	3	0	0	1	8
Pediatric	4	0	1	0	0	1	6
Intensive care unit	1	5	1	1	7	0	15
Total GNR	5(4.6)	70(65.4)	17(15.8)	4(3.7)	7(6.5)	4(3.7)	107
CRGNR	2(10.5)	6(31.5)	3(15.7)	2(10.5)	4(21.0)	2(10.5)	19

ET- Endotracheal Aspirate, HAI- Hospital associated infection, GNR-Gram negative rod,
CRGNR- Carbapenem resistant gram negative Rods

Acientobacter species 63% followed by *Klebseilla pneumoniae* 18%, *E.coli* and *Proteus species* each 9%. Fourth highest nosocomial infection was septicemia and other hospital-associated infection, which was caused by 60%, *Klebseilla pneumoniae* followed by *E.coli* and *Pseudomonas aeruginosa* 20%, respectively. Among the least nosocomial findings were from other hospital-associated infections in which 50% of isolates were from *Pseudomonas aeruginosa* and *Acientobacter Species*. Carbapenem resistance in E.coli and Klebseilla pneumoniae 21.5% wasthe highest among the *Enterobacterals*. The least carbapenem resistance was seen among *Enterobacter species* and *Proteus species* 5.26%. Carbapenem resistance among non-fermenter*Pseudomonas aeruginosa* and *Acientobacter species* were the same about 15.7%.

Among the phenotypic methods for the detection of Carbapenem were done by MHT and mCIM. For Metallo beta lactamase detection was done by CDDT and DDST. The sensitivity of MHT, mCIM, CDDT, and DDST within CI 95% were 74%, 95%, 84%, 95% respectively. The overall prevalence of carbapenem resistance is 17.75%.

4. DISCUSSION

The overall prevalence of carbapenem-resistant in this study was 17.75%. Similarly, the study was found with the prevalence of 18%, 17%, 17.32% respectively [11,12,13]. Incontrast Mulla S et al. [14] and Mate et al. [15] showa prevalence of 30%. The prevalence of carbapenem-resistance in different parts of India varies from 14-69%. The prevalence of carbapenem-resistant from various countries was found to be around 36% in Egypt [16], 13.6% and 37.9% in Iran [17,18], 56% in Pakistan [19], 24.6% in China [20], 19% in Algeria [21], 2.82% in Turkey [22], 86.3% in Tunis [23], 5.99% in Morocco [24] and 2.9% in Ghana [25], 14.6%, 65%, 30%, in India [26] 27.1% in Ethiopia [27], 0.22% in Germany [28].

On performing Kirby Bauer disc diffusion method 59.09% of male patient were sensitive and 63.15% were resistant to carbapenem where as 40.90% were sensitive and 36.84% were resistant to carbapenem with the statistical significance of *p*-value 0.00001 ; which was similar to T.V. Parimala 50.90% [29] Satyajeet K. Pawar et al., 65.3% [30] and Namitha Thomas [31]

In this study overall carbapenem resistance is highest in age group 21-30 (42.1%) similar to Namitha Thomas (31) reported highest carbapenem resistance in age group between 21-40 years (36.25%).Overall p-value= .000857.Incontrast the study by Pawar SK et al. [32] and Monika Saini et al [33] found highest percentage in age 41-50 years.

In our study the maximum organisms were isolated from urinary tract infection, followed by skin soft tissue infection, respiratory tract infection, septicemia and least nosocomial findings were from other hospital associated infections. Similar findings were from Urinary tract infection from [34,35].

Carbapenem resistance in *E. coli* and *Klebseilla pneumoniae* (21.5%) were highest among the *Enterobacterals*. Least carbapenem resistance was seen among *Enterobacter species* and *Proteus species* (5.26%). Carbapenem resistance among non- fermenter *Pseudomonas aeruginosa* and *Acientobacter Species* were same about 15.7%. A similar study was found which show *E.coli* and *Klebseilla pneumoniae* to be the commonest cause of infection [36,37]. Incontrast the most prevalent bacteria reported were Klebsiella species, *A. baumanii*, followed by *E. coli* and *P. aeruginosa* [17].There is a statistical significant difference of $p < 0.05$ in the prevalence of gram negative bacteria and carbapenemase- resistant isolates recovered from different types of specimens.

Among the different departments the maximum carbapenem-resistance werepresent is medicine 31.57% which was followed by surgery 26.31% , intensive care unit 21.05% and obstetrics & gynecology and pediatric showing the same percentage of resistance 10.52%. In contrast to our study it show the maximum number of isolates from the Surgery ward 22% followed by 19% medicine ward, 18% orthopedic ward, 15% MICU and 12% Pediatric ward , 14% isolates obtained from other wards and ICUs [38,39].

Among maximum carbapenem-resistant GNR were isolated from Urine 31.5% followed by Endotracheal aspirate 21%, Pus 15.7% and 10.5% Blood, Sputum and other health associate infection. A similar study by Lim et al. [40] it show high percentage of resistance in urine 25.9%, lower respiratory tract 14.3% and blood 17%. In the study of Pano Pardo et al. [41] and Seibert et al. [42] show these bacteria are commonly isolated from bronchial alveolar lavage (BAL), urine, and blood.

Our study it showsthe highest sensitivity of Colistin 89% and 3rd generation cephalosporine show 100% resistance. A study showed that many carbapenemase producers are susceptible in vitro to the glycylcyclline group (Tigecycline), but there is rapid increase in resistance to this drug [43]. Morrill et al. [44], reported monotherapy is not effective against infection caused by carbapenem-resistant bacteria.

In our study the highest sensitivity was found in DDST 95% followed by mCIM 95%, CDDT 84%, and MHT 74%, within CI 95% according to Naim H et al. [45] also found CDDT 84.81%. In contrast the study by Naim H,et al [45] found sensitivity of MHT, and DDST as 97.41% and 84.81%. In a study by Cury et al. [46], it shows least percentage of 35.5% MHT.

5. CONCLUSION

Our study illustrates the emergence of carbapenem-resistant Gram-negative hospital acquired infection from patients. To identify the responsible agent that leads to infection in healthcare settings. The common agents were *E. coli, Klebseilla pneumoniae, Citrobacter species, Pseudomonas aeruginosa, Enterobacter species, Proteus species* and *Acientobacter species*. The recovered isolates show the prevalence of 17.75% carbapenem resistance to Imipenem, Ertapenem and Meropenem drugs. , the carbapenem resistance is considered as the global alarm for pandemic resistance. As these carbapenem were the last resorts to combat from multidrug-resistant organism. Warning signal of MDR, XDR and PDR left with few drugs like Colistin and Tigecycline. In few studies we can see these drugs are also showing resistance.

ETHICAL APPROVAL

The study was approved by Ethics committee MU/MM/BNS/2020/51(a).

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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