



Original Research Article

Clinical, phenotypic and genotypic profile of carbapenem resistant gram negative infections in intensive care units

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ARTICLE INFO

Article history:

Received 15-12-2020

Accepted 07-01-2021

Available online 02-04-2021

Keywords:

Carbapenem resistant gram

Antimicrobial Resistance

Carbapenems

NDM-1

KPC

Mortality

Disc-diffusion

Multiplex-PCR

ABSTRACT

Background: Carbapenems are currently the only active beta-lactams effective against the ESBL producing pathogens; this has led to an increase in their use not only for documented infections but also for empirical treatment of acquired infections in ICU patients. Thus, there is a selective pressure for carbapenem resistance in intensive care units of tertiary care hospitals. Carbapenem resistant infections are associated with high morbidity & mortality.

Objectives: 1. To screen for carbapenem resistance among gram negative infections in ICU patients by disc diffusion & confirmation by Vitek 2 system; 2. To detect the carbapenemase production by phenotypic methods; 3. To confirm the presence of resistant gene by PCR; 4. To find out the attributable risk factors from the clinical history of such patients; 5. To follow up & estimate mortality rate in such infections and its statistical correlation

Materials and Methods: A total of 160 gram negative isolates [E.coli(71), Klebsiella(63), Acinetobacter(10), Pseudomonas(9) and Enterobacter(7)] from patients of various intensive care units were screened by disc diffusion out of which 53(33.12%) were carbapenem resistant. Resistant isolates were further tested by Vitek 2 system for determination of MICs, Modified Hodge test(MHT) and Combined Disc test(CDT) for carbapenemase production and subjected to multiplex PCR for detection of resistant gene i.e., bla NDM-1, bla VIM, bla IMP and bla KPC. Follow up of all the cases till the end of hospital stay was done and mortality rate was calculated.

Results: 53 isolates(33.12%) were carbapenem resistant, 17 isolates were MHT positive while 39 were CDT positive. 24 isolates [E.coli(12), Klebsiella(7), Acinetobacter(3), Pseudomonas(1) and Enterobacter(1)] showed the presence of NDM-1 gene on PCR. Gentamicin(35%), Amikacin (45%), Tigecycline(88%) & Colistin(100%) were sensitive for carbapenem resistant GNBs. Mortality in carbapenem sensitive infections in ICUs was 21% whereas resistant infections showed 37% (p value <0.05).

Conclusion: 45% of carbapenem resistance is due to presence of NDM-1 gene, E.coli was the commonest isolate. Other mechanisms of resistance such as excessive ESBL production, porin loss may be responsible for resistance in remaining isolates. Effective infection control measures should be taken to prevent the spread of these super bugs in a ICU.

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1. Introduction

Carbapenems are beta-lactam antibiotics with a broad spectrum and high efficacy, they are often used as “last-line agents” or “antibiotics of last resort” when patients with infections become gravely ill or are suspected of harboring

resistant bacteria and used as drug of choice for infections caused by Gram-negative bacteria that produce extended spectrum beta-lactamases (ESBL).¹ The intensive care unit (ICU) often is called the epicenter of infections, due to its extremely vulnerable population and there is an increase in the use of carbapenems not only for documented infections but also for empirical treatment of acquired infections.² Thus, there is a selective pressure for carbapenem resistance

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in intensive care units of tertiary care hospitals.

Antimicrobial resistance against carbapenems in Gram-negative bacteria can be caused by high level expression of ESBLs accompanied by changed porin expression or by specific enzymes named carbapenemases. Although known as “carbapenemases,” many of these enzymes recognize almost all hydrolyzable β -lactams and most are resilient against inhibition by all commercially available β -lactamase inhibitors.

The present study was done for screening the patients of Intensive care units of a tertiary care hospital for carbapenem resistant gram negative infections, phenotypic detection of the carbapenemases by Modified Hodge test, combined disc tests and genotypic confirmation of resistant gene by PCR as most of the genes conferring resistance are plasmid mediated and bacteria may be harboring more than one such resistant genes, finding out any attributable risk factors for carbapenem resistance & associated mortality in such infections.

2. Objectives

1. To screen for carbapenem resistance among gram negative infections in ICU patients by disc diffusion & confirmation by Vitek2 system.
2. To detect the carbapenemase production by phenotypic methods.
3. To confirm the presence of resistant gene by PCR.
4. To find out the attributable risk factors from the clinical history of such patients.
5. To follow up all the cases till the end of hospital stay & estimate mortality rate in such infections and its statistical correlation.

3. Materials and Methods

3.1. Source of data

Patients admitted in various Intensive care units of a tertiary care hospital, suspected of having Carbapenem resistant gram negative infections.

3.2. Study period

Study conducted over a period of one and half year from Jan- 2015 to July2016.

3.3. Study design

Cross sectional study.

3.4. Inclusion criteria

1. Patients of any age admitted in Intensive care units of a tertiary care hospital, suspected of having carbapenem resistant infections.

2. Patients from various Intensive care units whose samples yielded only gram negative bacteria.

3.5. Exclusion criteria

1. Patients not admitted in Intensive care units i.e admitted in general wards or treated in out patient department.
2. Patients from Intensive care units whose samples yielded gram positive bacteria.
3. Patients from Intensive care units whose samples yielded anaerobic bacteria.
4. Patients from Intensive care units whose samples were negative for bacterial culture.

All the relevant details of the patients included in the study, i.e name, age, sex, occupation, duration of illness, whether any invasive procedures or surgical intervention done, history of antibiotic usage, site of infection, past history, family history, were taken in a structured proforma.

3.6. Quality control

When performing phenotypic and genotypic tests, both positive and negative controls were used.

3.7. Positive control

Carbapenemase producing organism (Klebsiella pneumoniae ATCCBAA 1705).

3.8. Negative control

Non-carbapenemase-producing organism (Escherichia coli ATCC 25922).

3.9. Sample collection

A total of 160 non repetitive, clinical isolates of Gram negative bacteria from the patients admitted in various Intensive Care Units which were screened in the microbiology laboratory over a period of one and half year (Jan 2015 to Aug 2016), were identified, based on the colony morphology³ and the biochemical reactions⁴ from a variety of clinical samples like urine, stool, sputum, blood, pus were included in the study.

Screening test (Kirby bauer disk diffusion test): Isolates were screened for possible Carbapenemase production using Meropenem(10 μ g) disc. All the isolates showing meropenem disc diffusion zone diameter less than 21mm were considered to be screen positive.⁵

3.10. Phenotypic confirmatory methods

All the meropenem screen positive isolates were tested by the following phenotypic methods:

1. Vitek 2 system: All the resistant isolates were tested in Vitek 2 automated system (Biomeriux) by both ID and AST cards for accurate identification of the isolate and determining the MICs.
2. Modified Hodge test (MHT): Test isolates were subjected to Modified hodge test using E.coli ATCC 25922 as an indicator organism to detect the clover leaf zone of inhibition which signifies carbapenemase production.
3. Combined disc test (CDT):⁶ or detection of metallo-beta-lactamase (MBL) production. Combined disc test was done using Muller Hinton agar with 10 µg plain meropenem disc and a meropenem disc with added inhibitors i.e, 20 micro litre of Phenyl boronic acid (PBA) (20mg/ml) for KPCs and 10 micro litre of 0.5M EDTA for metallo-beta-lactamase production. The isolate was considered KPC producing when the growth-inhibitory zone diameter around the meropenem disc with PBA increased ≥ 5 mm when compared with the growth-inhibitory zone diameter around the disc containing meropenem alone. The isolate was considered MBL producing when the growth-inhibitory zone diameter around the meropenem disc with EDTA and the meropenem disc increased ≥ 5 mm when compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

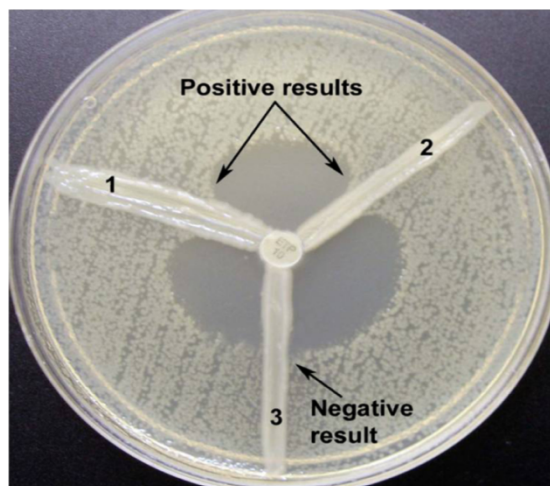


Fig. 1: Modified Hodge test

3.11. Genotypic detection of carbapenemase genes

Multiplex PCR for detecting NDM, VIM, IMP and KPC genes:

The multiplex PCR was done according to the methodology described in the Sambrook and Russell editors. In Molecular Cloning: A Laboratory Manual.⁷ Previously

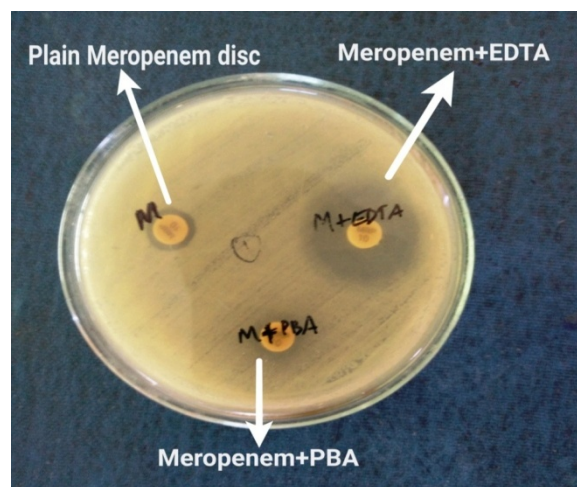


Fig. 2: Combined disc test

published primers were used by referring an article by Rachna Solanki et al., (2014)⁸

Primer sequences of 4 target genes (*bla_{NDM-1}*, *bla_{VIM}*, *bla_{IMP}* and *bla_{KPC}* genes) for Multiplex PCR: (Rachna Solanki et al., 2014) Table 1.

4. Results

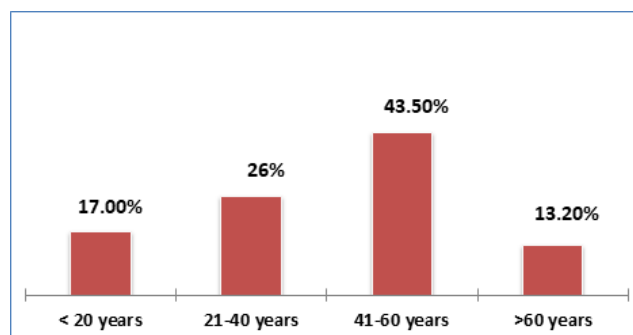


Fig. 3: Age wise distribution of patients admitted in ICUs with carbapenem resistant gram negative infections

Patients within the age group of 41-60yrs showed highest percentage of resistance.

Skin & soft tissue infections account for the highest resistance among various types of infection.

Surgical ICU showed the highest prevalence of carbapenem resistant among GNBs shown in Figure 6.

Gentamicin(35%), Amikacin (45%), Tigecycline (88%) & Colistin(100%) were sensitive for carbapenem resistant GNBs. All the 3rd generation cephalosporins, Amoxicillin+clavulanic acid were 100% resistant to all carbapenem resistant gram negative bacilli shown in Figure 7.

Table 1:

Primer	Orientation	Oligonucleotide sequence(5'-3')	Size(bp)
NDM-1	Forward	GCATAAGTCGCAATCCCCG	237
	Reverse	CTTCCTATCTCGACATGCCG	
VIM	Forward	GTTTGGTCGCATATCGCAAC	382
	Reverse	AATGCGCAGCACCAGGATAG	
IMP	Forward	GAAGGCGTTTATGTTTCATAC	587
	Reverse	GTAAGTTTCAAGAGTGATGC	
KPC	Forward	TCGAACAGGACTTTGGCG	201
	Reverse	GGAACCAGCGCATTTTGC	

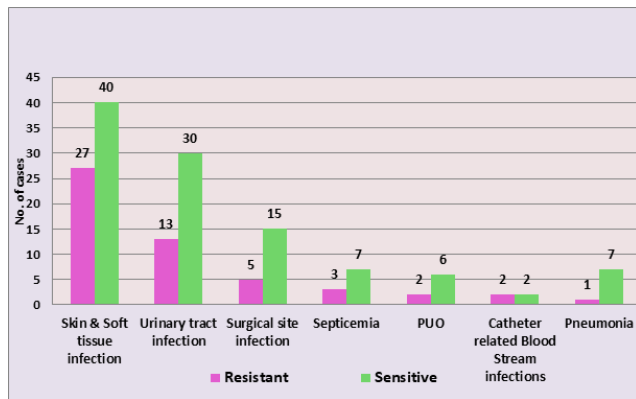


Fig. 4: Distribution of ICU patients according to the type of gram negative infection

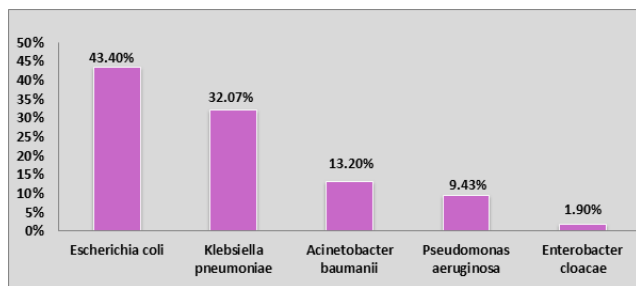


Fig. 5: Distribution of carbapenem resistant organisms among the various gram negative bacilli isolated from ICU patients

Significantly higher mortality was seen in carbapenem resistant infections.(p value 0.029) shown in Figure 8.

Correlation between presence of co-morbidities as a risk factor for carbapenem resistance was highly significant.(p value 0.01) shown in Figure 9.

Out of 20 deaths that occurred in ICU patients with carbapenem resistant infections, 13 cases (54%) were NDM-1 gene positive and 7 cases (24%) were genotype negative (p value is 0.026) i.e < 0.05 and hence presence of NDM-1 gene and mortality is statistically significant shown in Figure 10.

Of the total 160 gram negative isolates from ICU patients, 53 were resistant to carbapenems, Out of 53

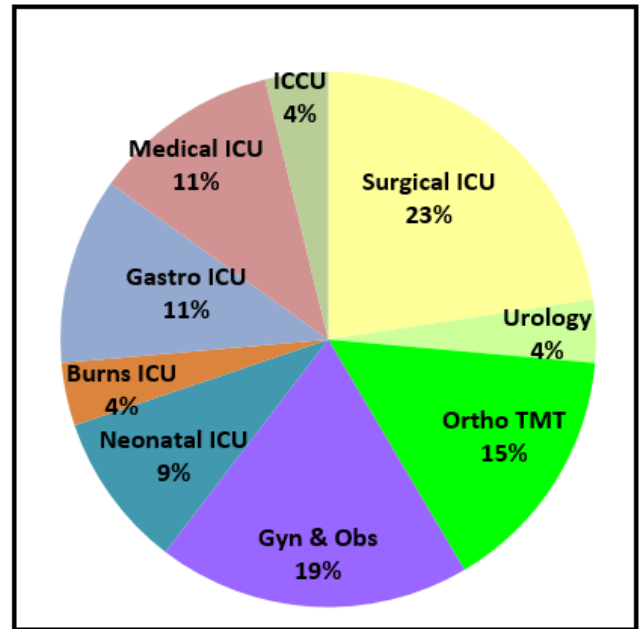


Fig. 6: Department wise distribution of patients infected carbapenem Resistant gram negative bacilli

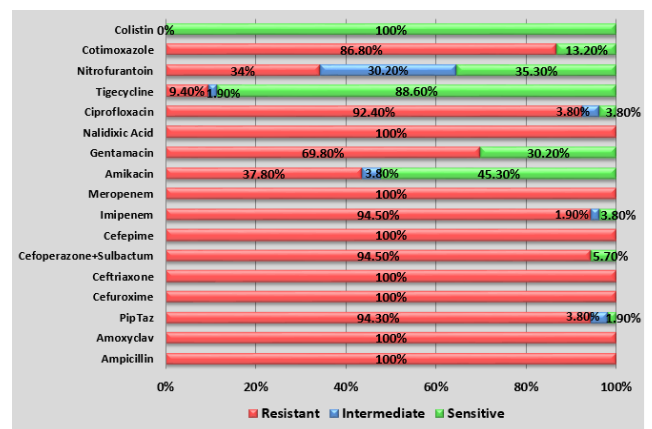


Fig. 7: Antimicrobial resistance pattern among carbapenem resistant GNBS isolated from ICU patients

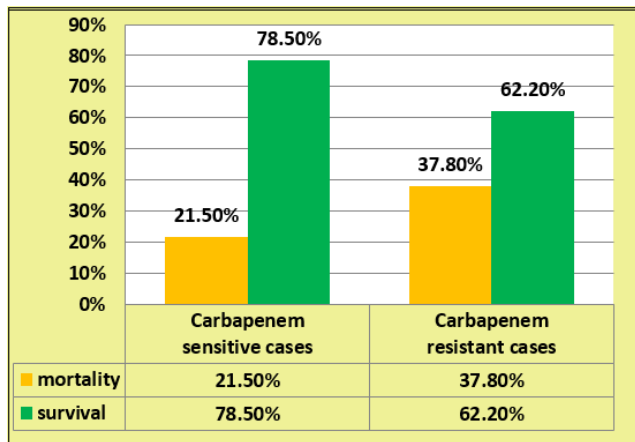


Fig. 8: Correlation of mortality & carbapenem resistance in patients of Intensive care units

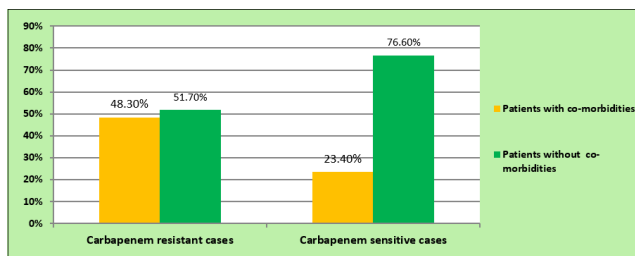


Fig. 9: Correlation of co-morbidities & Carbapenem resistance in patients of Intensive care units

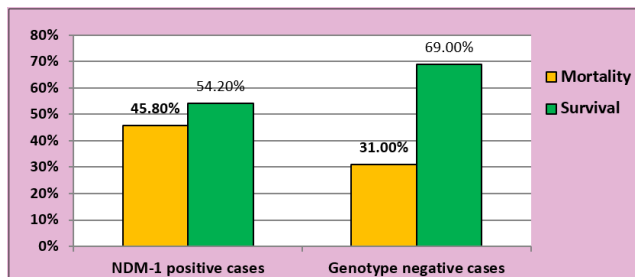


Fig. 10: Correlation of mortality in NDM-1 genotype positive Carbapenem resistant infections and genotype negative Resistant infections

resistant isolates, only 17 were MHT positive whereas 39 were CDT positive using EDTA as inhibitor and none of the isolate was positive using PBA as inhibitor. Multiplex PCR showed the presence of NDM-1 gene in 24 isolates whereas 29 isolates were negative for all the four genes.

From the Table 3, significant risk factors for development of carbapenem resistance in the present study are prior hospitalization, previous exposure to antibiotics, invasive procedures done in last 3 months, presence of co-morbidities and longer duration of ICU stay. Significantly higher mortality was seen in Carbapenem resistant

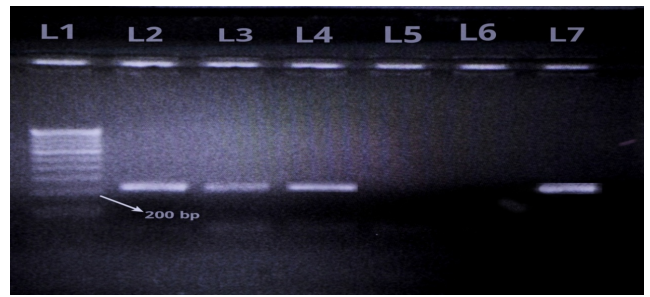


Fig. 11: Gel documentation of multiplex PCR analysis showing the presence of NDM-1 gene detected at 237 bp
Lane-1: 100bp DNA ladder Lane-2, 3 &4: NDM-1 positive samples
Lane-5: negative sample
Lane-6: Negative control
Lane-7: Positive control for NDM-1 gene at 237bp

cases(37%) compared to sensitive cases (21%).

5. Discussion

Carbapenem resistance can be due to presence of Carbapenemase enzymes, excessive production of ESBL or porin loss or efflux pumps or combination of more than one mechanism.

1. The prevalence of Carbapenem resistant gram negative bacilli among the patients admitted in ICUs in this study was found to be 33.12% which was closely correlating with Jamal Wafaa et al., 2016 (34.4%) from Kuwait and Kumari Seema et al.,2011(35%) from Varanasi. Studies from various parts of India had reported a varying prevalence ranging from as low as 14% by Chakrapani et al. from Chennai in 2014 to as high as 67% by Tempe DK et al. from New Delhi in 2015. Such a wide variation is probably due to variation in risk factors, extent of antibiotic use, variation in adherence to proper infection control practices, variation in knowledge of nursing staff regarding hospital infection control and lastly being a public sector or a private sector hospital to have proper infrastructural facilities for adequate patient care.
2. The highest prevalence of Carbapenem resistant gram negative infections in ICUs in the present study was observed in the patients of age group 41to 60 years which closely correlates with the findings of Vasundhara Devi et al., 2014 from Nellore and Humaira B et al.,2013 from Srinagar. Probably because this age group is more exposed to antibiotics, has more co-morbidities like diabetes mellitus, hypertension, coronary artery disease, etc for which they are more often hospitalized than the population of the other age groups.

Table 2: Results of phenotypic & genotypic detection of carbapenem resistance at a glance

Organism (n=160)	Meropenem screen Positive by disc diffusion (n=53)	MHT positive (n=17)	Combined disc test positive (n=39)		PCR positive (n=24)			
			with PBA for KPC	with EDTA for MBL	NDM	VIM	IMP	KPC
E.coli (n=71)		9	0	16	12	0	0	0
Klebsiella (n=63)	17	5	0	13	7	0	0	0
Acinetobacter (n=10)	7	2	0	6	3	0	0	0
Pseudomonas(n=9)	5	0	0	3	1	0	0	0
Enterobacter (n=7)	1	1	0	1	1	0	0	0

Table 3: Clinical characteristics of study population at a glance

S.No.	Clinical characteristics	Carabpenem sensitive Infections, (n=107)	Carbapenem resistant Infections, (n=53)	p value	
1	Mean Age	45.4± 15 years	41.49± 14 years		
2	Gender	Male Female	34 19	0.028 0.23	
3	Prior Hospitalization (last 3 months)	16	15	0.045	
4	Fever on admission	13	3	0.19	
5	ICU stay	17.5 ± 2 days	25.2 ± 7 days	0.00016	
6	Antibiotic exposure (last 3 months)	23	31	0.000003	
7	Invasive/surgical procedures in last 3 months	25	22	0.018	
8		Diabetes mellitus	18	8	0.78
9		Coronary Artery disease	9	6	0.55
10	Risk Factor	Liver failure	0	3	0.013
11		Respiratory illness	4	3	0.5
12		Neurological illness	2	1	0.4
13		Malignancies	0	2	0.04
14	Mortality	23(21%)	20(37%)	0.029	

3. In the present study, highest prevalence of carbapenem resistance was seen in patients of surgical ICU(23%) which correlated to the findings of Elandevi et al., 2016 (Chennai) who also reported highest prevalence among surgical department(47%).

4. Highest number of Carbapenem resistant Gram negative isolates was obtained from samples of patients suffering with Skin & soft tissue infections (51%), followed by UTI (24.5%). This closely correlates with Elandevi R et al., 2016 from Chennai which reported Carbapenem resistance in 38% surgical wound & 30% in UTI. In contrast, other studies from various places like Tempe DK et al.,2015 from New Delhi, Hengkoneng M et al., 2014 from Manipur, Humaira B et al., 2013 from Srinagar, PK Nair et al.⁹ 2012 from Mumbai and RM Parveen et al.,¹⁰ 2010 from Pondicherry reported highest cases of carbapenem resistant gram negative infection from Urinary tract infection.

5. The highest prevalence of carbapenem resistance in the present study was observed in Escherichia coli (43.4%) which correlates with Hengkoneng et al., 2014 (Manipur) and Sanjeev K et al.2015 (Udaipur).

6. Carbapenem resistant gram negative isolates in the present study showed maximum antimicrobial susceptibility to Colistin (100%), followed by Tigecycline (88%) which correlates with the findings of Chauhan K et al., (Meerut), 2015 who also reported Colistin (100%) & tigecycline (98%) sensitivity. Susceptibility to aminoglycosides in this study i.e., Amikacin (45%) & gentamicin (30%) correlates with Mulla S. et al., 2016 from Surat, with amikacin (41%) & gentamicin (23%) sensitivity. Other studies from various parts of India, i.e Mulla S. et al.(Surat), Chauhan et al., (Meerut), Shrivastava et al., (Indore) and Kumaraswamy et al., (Chennai) reported least susceptibility to Meropenem & Imipenem which was consistent with the findings of this study.

7. Present study showed that among phenotypic detection tests for carbapenemase production, Combined disc test is more sensitive than Modified Hodge test which is consistent with the findings of Sanjeev K et al., 2016 (Udaipur), Swetha et al., 2016 (Mysuru) & Chauhan K et al., 2015 (Meerut). In contrast to the findings of this study, R Solanki et al., 2014 (Hyderabad) and Aparna S et al., 2014 (Mangalore) reported a higher detection rate of carbapenemase production by Modified Hodge test when compared to Combined disc tests.
8. NDM-1 gene was detected in 45% of carbapenem resistant isolates. Other genes like KPC, VIM, IMP were not detected in any isolate. Other studies from various parts of India also reported high detection of NDM-1 gene, highest being reported by Balvinder M et al., 2015 (Chandigarh). Most of the south east asian countries reported a high prevalence of NDM-1 gene correlating to the findings of the present study signifying the endemicity of the NDM-1 gene.
9. Contrary to this, studies from most of the European countries, USA & Canada reported a higher incidence of KPC gene responsible for carbapenem resistance Munoz-Price et al. in 2013 as reported by S Bratau et al., 2005 (USA) and Munoz-Price et al. in 2013 signifying endemicity of KPC
10. Mortality in Carbapenem resistant gram negative infections, in the present study was found to be 37.6% (p value < 0.05) which correlated with Tian L et al., 2015 (China), Yang et al., 2015 (China) and Porwal et al., 2014 (Chennai)
11. 58% of the patients who had carbapenem resistant infections were previously exposed to three or more antibiotics in the last three months (p value < 0.05) which correlated with the findings of Ling M et al., 2015 (Singapore) 60%, K Kalam et al.,¹¹ 2014 (Pakistan) 62% and Chang HJ et al., 2010 (Taiwan) 52%.

6. Conclusion

Prevalence of carbapenem resistance is very high in Intensive care units, history of prior hospitalization should be considered before starting any antimicrobial therapy. Rapid detection tests like Combined disc tests can be used to detect metallo-beta-lactamase production. Tigecycline, Colistin and to some extent Aminoglycosides can be used as therapeutic options for such infections and hence reduce morbidity & mortality.

Presence of co-morbidities, prior hospitalizations and previous exposure to antibiotics were significant risk factors for carbapenem resistant gram negative infections. NDM-1 genotype was associated with significant mortality among carbapenem resistant cases. Effective infection control

measures should be taken to prevent nosocomial spread of these multidrug resistant pathogens.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

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Cite this article: Mohammed MU, Manisha D R, Nagamani K. Clinical, phenotypic and genotypic profile of carbapenem resistant gram negative infections in intensive care units. *Indian J Microbiol Res* 2021;8(1):28-34.