

# Prevalence of Carbapenem Resistance in Gram Negative Bacilli Isolates and Their Antimicrobial Susceptibility Pattern

Monika Saini<sup>1</sup>, Aditya Mishra<sup>2\*</sup>, Sweta Gupta<sup>3</sup>

<sup>1</sup>M.Sc Medical Microbiology, <sup>3</sup>Professor & Head, Department of Microbiology, Mahatma Gandhi Medical College & Hospital, Jaipur, Rajasthan, INDIA.

<sup>2</sup>Ph.D Scholar SMS Medical College, Jaipur, Rajasthan, INDIA.

## ABSTRACT

**Aim:** The objective of study is to evaluate the prevalence of carbapenem resistance in gram negative bacilli isolates and their antimicrobial susceptibility pattern.

**Method:** All the samples received in the Microbiology laboratory from the various departments were cultured on Blood and MacConkey's agar and blood was on Brain heart infusion broth. Antibigram was done by Modified Kirby Bauer disk diffusion method. Detection of Metallo beta lactamase (MBL) cases were done by double disc synergy test and confirmed by E-test of Meropenem.

**Result:** Total 500 samples were tested in this study. Out of 500, 350 gram negative bacilli were reported. Out of 350, 100 (29%) were carbapenem resistant. 97% Carbapenem resistant organism were isolated from IPD patients. Maximum 65% Carbapenem resistant gram negative bacilli (CRGNB) isolated from respiratory tract infection cases. Total 44% *Acinetobacter*, 25% *E.coli*, 17%, *Klebsiella*, 12% *Pseudomonas* and 2 % others CRGNB were isolated. Out of 100 CRGNB cases, 83 were found MBL. *Acinetobacter* were the most common MBL producer. *Acinetobacter* were 100% MBL while *Klebsiella* were 76.47%, *Pseudomonas* 66.66% and *E.coli* 64%. All CRGNB were sensitive to *Colistin*.

**Conclusion:** Drug resistant microorganisms are increasing rapidly and becoming major problem for society. To control the

MBL producing microorganisms in a hospital/health care setup, strategies such as strict infection control measures, antibiotics resistance surveillance programs & restrict to clinicians, prescribe last resort drugs only where primary & secondary drugs are resistant and antibiotic cycling must be followed. Colistin could be a drug of choice in CRGNB infections.

**Keywords:** Carbapenem resistant gram negative bacilli (CRGNB), Metallo beta lactamase (MBL).


## \*Correspondence to:

Aditya Mishra, Ph.D Scholar, SMS Medical College, Jaipur, Rajasthan, INDIA.  
Email: aditya.mishrajpr@gmail.com

## Article History:

Received: 02-05-2016, Revised: 07-05-2016, Accepted: 09-05-2016

## Access this article online

Website: www.ijmrp.com	Quick Response code 
DOI: 10.21276/ijmrp.2016.2.3.006	

## INTRODUCTION

Antibiotic resistance is now becoming a global threat. The burden is more in developing countries where infectious diseases are building up. In the developing countries, there is rampant use of antibiotics mainly because, due to limited resources, most clinicians choose symptomatic treatment that's why very few microbiological samples are taken for culture and antibiotic sensitivity testing<sup>1,2</sup>. In addition there is an extensive over the counter treatment with widespread self-medication and incomplete course of antibiotics. These are well known factors that facilitate development of antibiotic resistance.

Carbapenem are a group of  $\beta$ -lactam antimicrobial agents with an exceptionally broad spectrum of activity<sup>1</sup>. They are used as a last resort drug against many MDR microorganisms such as Extended spectrum beta-lactamase (ESBL), Metallo beta lactamase (MBL) and *AmpC* enzyme producing gram negative bacilli<sup>3</sup>. The emergence and transmission of carbapenem resistant bacteria in recent time represents a serious threat to public health. Resistance has been observed in the family of enterobacteriaceae, as well as in members of the *Pseudomonas* and *Acinetobacter* genera. These organisms are associated with

high mortality rates and have the potential to spread widely<sup>4</sup>.

Resistance to carbapenems can be brought about by various mechanisms, the most common being the production of carbapenemases, a class of enzymes capable of hydrolyzing Carbapenem and other beta lactam<sup>5</sup>. Resistance to Carbapenem can also be due to the poor binding of Carbapenem to penicillin-binding proteins present in the bacteria, the over-expression of multidrug efflux pumps by the bacteria or lack of porins present in the bacterial cell membrane. However, for significant resistance to emerge, it is the thought that a combination of resistance mechanisms is required<sup>6</sup>.

In 2007, the overall worldwide susceptibility to carbapenems was 98% among the enterobacteriaceae. Presumably this was due to a number of factors, including antibiotic usage, dosing regimens and local hospital practices concerning isolation of patients with MDR microorganisms<sup>7</sup>. With the increasing incidence of Carbapenem resistant gram negative bacilli (CRGNB) in hospitals, a rapid and detection is required. Appropriate detection of CRGNB is vital in patient care and infection control in order to institute correct, targeted treatment and to reduce the escalation of

resistance<sup>5</sup>. The objective of study is to evaluate the magnitude of carbapenem resistance in gram negative bacilli isolates.

## MATERIAL AND METHODS

Samples were collected from various wards, Indoor & outdoor patient departments of Mahatma Gandhi medical college & Hospital, Jaipur from October 2014 to March 2015.

Clinical samples such as blood, CSF, urine, respiratory secretions, swabs from non-healing ulcers, pus/wound swab & other samples from sterile body fluids were collected by taking aseptic precautions. All the samples except blood were cultured on blood agar, MacConkey's agar & Thioglycolate broth. Blood culture was done on Brain heart infusion broth. A culture plates were incubated overnight at 37°C. Isolated gram negative organisms were further identified by standard set of biochemical tests<sup>8</sup>.

Gram Negative bacilli isolates from the various samples; which were having less sensitivity zone size of Imipenem and Meropenem on modified Kirby Bauer disk diffusion method were suspected for carbapenem resistance and tested for MBL detection by Meropenem 'E-test'.

### 1. Antibiotic susceptibility testing (Anti-biogram):

Antimicrobial sensitivity testing was performed on Mueller Hinton agar (Hi-Media, Mumbai) plates by disk diffusion method according to CLSI guidelines<sup>9</sup>. The diameter of the zones of inhibition on MHA was interpreted as sensitive, intermediate and resistant. *Escherichia coli* ATCC 25922 ( $\beta$ -lactamase negative) *Pseudomonas aeruginosa* ATCC 27853 ( $\beta$ -lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) strains were used as control organisms. Organism with intermediate levels of resistance to the antibiotics Meropenem & Imipenem were included in percentage of resistant organisms for final analysis. by Imipenem & EDTA combined disc Imipenem test.

### 2. Double disc synergy test (DDST) using Imipenem and Imipenem plus EDTA:

Test organism was inoculated on to plates with Mueller Hinton agar as recommended by the CLSI<sup>9</sup>. An Imipenem (10  $\mu$ g) disc was placed 20mm centre to centre from a disc containing 10 $\mu$ g Imipenem plus 0.5 M EDTA. After overnight incubation, a zone diameter difference of  $\geq 7$  mm between Imipenem disc & Imipenem plus EDTA disc were interpreted as Metallo- $\beta$ -Lactamase positive<sup>9</sup>. [Img. 1]

### 3. MBL E-Test:

For MIC detection of Meropenem, the E-test strip method is used. The E-test MBL strip (Hi-Media) containing a double sided even dilution range of Meropenem (MRP 4 to 256  $\mu$ g/ml) gradient at one end & Meropenem + EDTA (1 to 64  $\mu$ g/ml) in incubation with a fixed concentration of EDTA at these other end. 100-mm-diameter Mueller-Hinton Agar plates are inoculated with swabs saturated with suspensions of the study isolates equivalent to a 0.5 McFarland standard. The results were read after 24 hrs of the incubation. The ratio of the MIC of Meropenem/(Meropenem+EDTA)  $> 8$  dilution indicate MBL production<sup>10</sup>. [Img. 2]

## RESULTS

Total 500 non-repetitive variable samples from various wards were collected and processed in the Department of Microbiology, Mahatma Gandhi Medical College and Hospital, Jaipur. Out of 500, 350 gram negative bacilli were isolated.

These 350 GNB were further processed for the detection of carbapenemase resistance & other antibiotic sensitivity test by disk diffusion method on Muller Hinton Agar as per CLSI guideline. Out of 350, 100(29%) samples were found CRGNB (carbapenem Resistant Gram Negative Bacilli) rest 71% was sensitive to carbapenems. In 100 CRGNB samples 61% were obtained from males and 39% from females. Maximum 19 cases were in between the age of 41-50 years & mean of the age was  $44.9 \pm 20.53$ .

97% CRGNB were isolated from various wards & ICUs while only 3 % were found in OPD [Fig 1, Fig 2]. Out of 100 CRGNB cases, 65% were reported from respiratory tract infections while 18% from wound infections, 13% of Urinary tract infections and 2% each from meningitis & bacteremia were found. [Fig 3]

Various antibiotics included in the study were sourced from commercial batches belonging to  $\beta$ -lactam, aminoglycoside, quinolone, and tetracycline classes as per the CLSI guideline. Carbapenem resistant organisms were not only resistant to carbapenem group but also resist to most of antibiotics. CRGNB isolated from UTI were sensitive to fosfomycin & nitrofurantion. Colistin is only drug which showed 100% sensitivity in all CRGNB isolates. [Table 1]

Total 83 cases were MBL reported out of 100 CRGNB by Meropenem with & without EDTA Ezy MIC™ Strips. Maximum resistance was found in member of *Acinetobacter*. [Fig. 4]

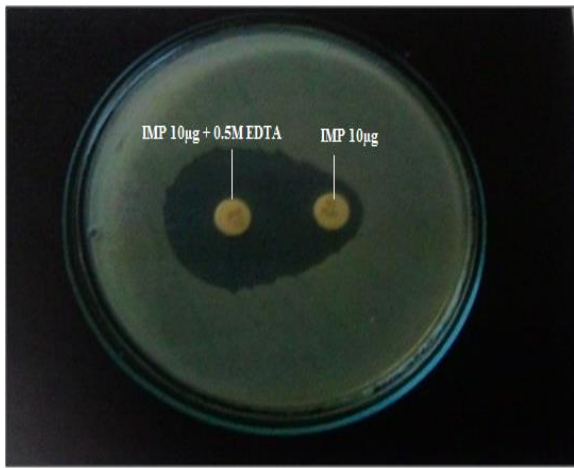
## DISCUSSION

The overall carbapenem resistance in the present study was 29%. The carbapenem resistance rate among GNB varies widely in the literature. Taneja et al. reported it was 36.4%, Gladstone et al. were found 12.2% while Gupta et al mentioned it 17.32% and Datta et al documented it as 7.87%<sup>3,11</sup>. The incidence varies from as low as 1.8% to over 30% in India<sup>12,13</sup>.

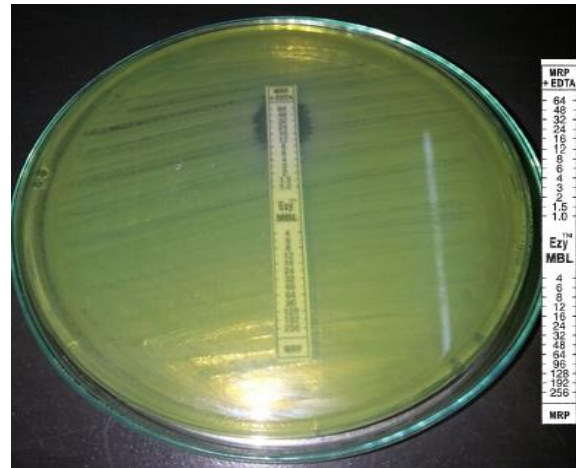
In our study among 237 Enterobacteriaceae, 18.56% (44) were resistant to carbapenems. This resistance rate was compare with several studies done in India. Many authors have used one or more carbapenems as indicator drug for testing resistance to carbapenems by disc diffusion or MIC method. Resistance to carbapenems ranged from 2% to 22% in Indian studies. Gupta et al reported less carbapenem resistance rate 3.61%<sup>12</sup> while Gladstone et al found 12.2%, wattal et al 13-51%, Datta et al 17-22% and Dardi kaur et al reported 8.33%<sup>5,14,15</sup>.

In the present study, out of 113 NFGNB 56 (49.55%) were resistant to carbapenems. Out of 56 CR-NFGNB 44 was *Acinetobacter* & 12 were *P. aeruginosa*. *Pseudomonas* carbapenem resistant was 10.61% (12/113) in the present study. In Indian studies, carbapenem resistance in *P. aeruginosa* has been reported from centres in Pondicherry, Vellore, Bangalore, Chandigarh, Mumbai, New Delhi and Varanasi with the rates of resistance between 10.9% and 69%<sup>16,17</sup>. In 83 *Acinetobacter* isolates out of 113 NFGNB 38.93% (44/113) were carbapenem resistant.

Although molecular techniques are regarded as the most appropriate method for the detection of carbapenem resistance, it becomes impractical in a routine diagnostic laboratory setup up due to cost factors, availability of molecular set up. CRGNB infected patients serve as reservoirs for spreading infection and contaminating the environment. That's why, identified CRGNB colonized patients must be contact isolated.



Img 1: Detection of MBL in *E.coli* by DDST.



Img 2: Detection of MBL *Pseudomonas* by E-Test

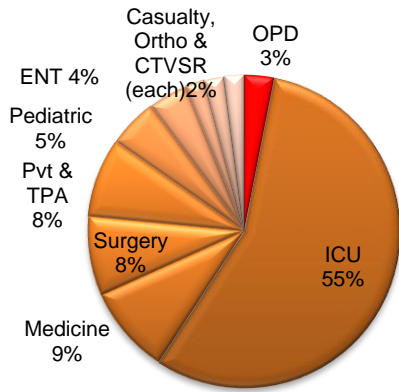


Fig 1: Distribution of the CRGNB isolates from various wards

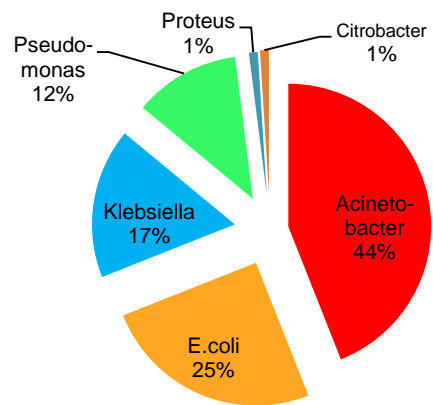


Fig 2: Distribution of CRGNB isolates.

**Distribution of CRGNB isolates from various samples.**

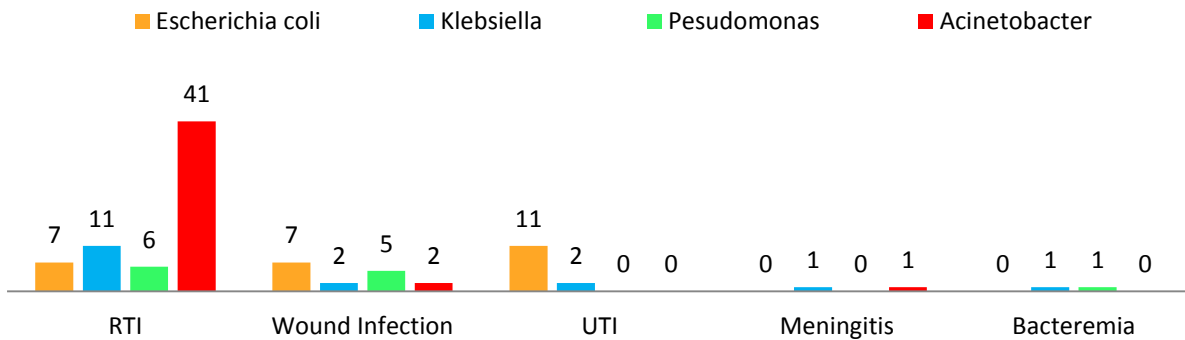


Fig 3: CRGNB isolates from various samples.

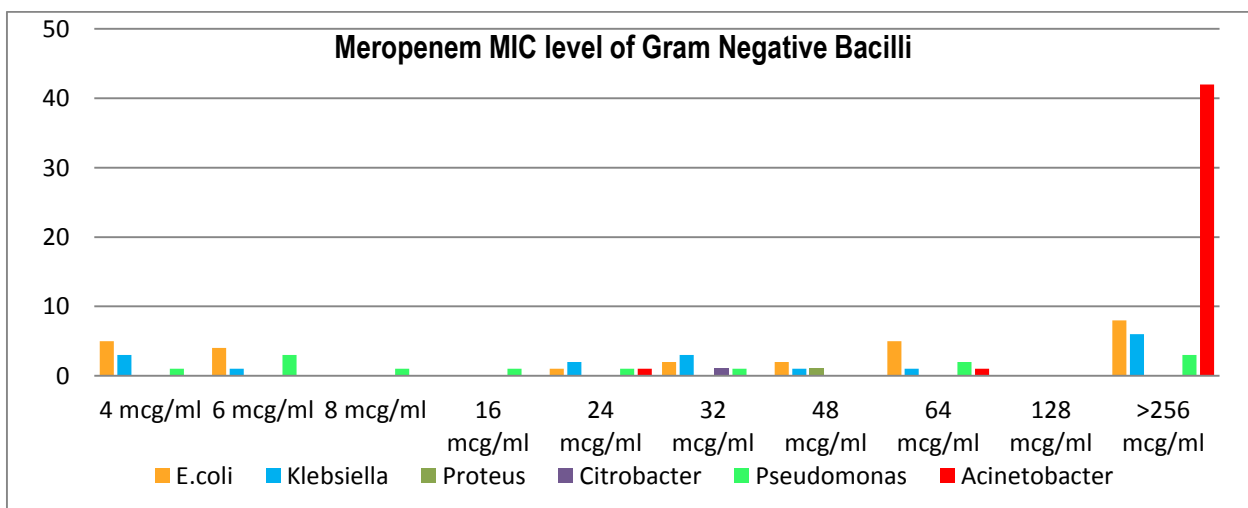


Fig 4: MIC level of Meropenem in Gram Negative bacilli

**Table 1: Antibiotic sensitivity pattern of CRGNB clinical isolates.**

Antibiotic disc	E.coli n= 25			Klebsiella n= 17				Proteus n=1	Citrobacter n=1	Pseudomonas n=12	Acinetobacter n=44			Sensitivity %			
	RTI (n=7)	Wound Infection (n=7)	UTI (n=11)	RTI (n=11)	Wound Infection (n=2)	UTI (n=2)	Meningitis (n=1)	Bacteremia (n=1)	Wound Infection (n=1)	Wound Infection (n=1)	RTI (n=6)	Wound Infection (n=5)	Bacteremia (n=1)		RTI (n=41)	Wound Infection (n=2)	Meningitis (n=1)
CI	7	7	11	11	2	2	1	1	1	1	6	5	1	41	2	1	100%
Pi	2	0	2	2	0	1	0	0	1	0	1	0	1	5	0	0	15%
PiT	2	0	2	2	0	1	0	0	1	1	3	2	1	5	2	1	23%
OF	2	3	2	5	0	2	1	0	0	0	0	0	0	0	0	0	15%
CAZ	1	0	1	2	0	1	1	0	0	0	0	0	0	0	0	0	6%
MRP	2	0	2	1	1	1	1	1	0	0	1	1	1	1	0	0	13%
IMP	4	2	3	2	1	1	1	1	0	0	1	0	0	1	0	0	17%
ETP	1	1	3	0	0	1	1	1	0	1	-	-	-	-	-	-	16.6%
DO	2	2	5	1	1	1	-	-	-	-	0	0	0	1	1	1	15%
GEN	0	1	1	0	0	0	0	0	-	1	0	0	0	1	0	0	04%
COT	1	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0	06%
AT	1	0	0	0	0	0	0	0	-	1	-	-	-	-	-	-	4.65%
A/S	3	0	0	2	0	0	0	0	1	1	1	0	0	22	2	1	33%
CPM	1	0	0	4	0	0	1	1	0	0	1	0	0	0	0	0	8%
TGC	6	5	10	10	2	2	1	1	-	1	-	-	-	16	2	1	65.5%
LE	1	0	-	3	0	-	0	0	1	1	-	-	-	8	1	1	21.3%
AK	1	1	7	3	0	2	0	0	1	1	2	0	0	0	0	0	18%
PB	-	-	-	-	-	-	-	-	-	-	6	5	1	0	0	0	25.3%
CB	-	-	-	-	-	-	-	-	-	-	4	1	1	19	2	1	50%
TOB	-	-	-	-	-	-	-	-	-	-	2	0	0	3	1	0	10.7%
FO	-	-	11	-	-	2	-	-	1	-	-	-	-	-	-	-	100%
NIT	-	-	7	-	-	0	-	-	-	-	-	-	-	-	-	-	53.84%

CI – Colistin, Pi – Piperacillin, PiT- Piperacillin-Tazobactam, OF- Ofloxacin, CAZ- Ceftazidime, MRP- Meropenem, IMP- Imipenem, ETP- Ertapenem, DO-Doxycycline, GEN- Gentamycin, COT- Cotrimoxazole, AT- Aztreonam, A/S- Ampicillin-Sulbactam, CPM- Cefepime, TGC-Tigecycline, LE- Levofloxacin, AK- Amikacin, PB- Polymyxin B, CB- Carbenicillin, TOB-Tobramycin, FO-Fosfomicin, NIT- Nitrofurantoin

**CONCLUSION**

This study shows a clearer spectrum of the current CRGNB scenario in the hospital setup. MDR microorganisms are accelerating and becoming major problem in the area of infectious diseases. In order to control MBL producing microorganisms in a hospital/health care setup, strategies such as strict infection control measures, antibiotics resistance surveillance programs & restrict to clinicians, prescribe last resort drugs only where primary & secondary drugs are resistant and antibiotic cycling must be followed. Regular monitoring and documentation of carbapenem resistance should be done. Colistin could be a drug of choice in carbapenem resistant gram negative bacilli infections. It should be used when no other drug are effective.

**ACKNOWLEDGEMENTS**

I would like to express my heartfelt gratitude to Dr. Neelam Taneja, Sr. Professor Microbiology, PGI Chandigarh. It would not have been possible without your guidance and constant supervision as well as for providing necessary information regarding this project.

**REFERENCES**

- Gould TM. The epidemiology of antibiotic resistance. Int J Antimicrob Agent. 2008; 32(Suppl 1):S2-9.
- Kombe GC, Darrow DM. Revisiting emerging infectious diseases: the unfinished agenda. J Community Heal. 2001;26:113-122

3. Ifan s, Zafar A, guhar D, Ahsan T, Hasan R. metallo b-lactamase producing clinical unit patients of a tertiary care hospital. *Indian j.med.res* 2008;243-45s
4. CRE toolkit, CDC-Guidance of control of carbapenems – resistant, Enterobacteriaceae (CRE),2012. [Accessed 26 Jun 2012]. Available from: <http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html>
5. Datta P, Gupta V, Garg S, Chandra J. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae study from north India. *Indian j pathol microbial* 2012;55:357-360
6. Baldwin CM, Lyseng-Williamson KA, Keam SJ. Meropenem-A review of its use in the treatment of serious bacterial infection, *Drug* 2008;68:803-838
7. Queenam Am, Bush k. carbapenemases: the versatile B - lactamases *clinical microbiology reviews* 2007;20:440-458
8. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, *Practical Medical Microbiology*, 1996, 14th Edition, Churchill Livingstone.
9. CLSI guideline: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, 2014. Available at: [http://ncipd.org/control/images/NCIPD\\_docs/CLSI\\_M100-S24.pdf](http://ncipd.org/control/images/NCIPD_docs/CLSI_M100-S24.pdf)
10. EM092, Hi-Media: Meropenem with & without EDTA Ezy MIC™ Strips manual. Available at: <http://www.himedialabs.com/intl/en/products/Microbiology/Antimicrobial-Susceptibility-Ezy-MIC%E2%84%A2-Strips-Dual-Antibacterial/Meropenem-with-without-EDTA-EM092>
11. Taneja N, Aharwal SM, Sharma M. Imipenem resistance in nonfermentors causing nosocomial urinary tract infections. *Indian J Med Sci.* 2003;57:294-9.
12. Gupta E, Mohanty S, Sood S. Emerging resistance to carbapenems in tertiary care hospital in north India. *Indian J Med Res*, 2006;124:95-98
13. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care units. *Indian J Med Microbiol* 2005;23:189-91.
14. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. *J Assoc Physicians India.* 2010 Mar; 58:147-9.
15. Wattal C, Goel N, Oberoi JK. Surveillance of multidrug resistant organisms in tertiary care hospital in delhi india. *j assoc physicians india* 2010;58:32-36.
16. Varaiya A, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in diabetes and cancer patients. *Indian J. Med. Res.* 2008;243-45
17. Shashikala, Kanungo R, Srinivasan S, Devi S. Emerging resistance to carbapenems in hospital acquired *Pseudomonas* infection: A cause for concern. *Indian J Pharmacol.* 2006;38:287-88
18. Nagaraj S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital in south India. *Indian J Med Microbiol* 2012;30:93-95.

**Source of Support:** Nil. **Conflict of Interest:** None Declared.

**Copyright:** © the author(s) and publisher. IJMPP is an official publication of Ibn Sina Academy of Medieval Medicine & Sciences, registered in 2001 under Indian Trusts Act, 1882. This is an open access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Cite this article as:** Monika Saini, Aditya Mishra, Sweta Gupta. Prevalence of Carbapenem Resistance in Gram Negative Bacilli Isolates and Their Antimicrobial Susceptibility Pattern. *Int J Med Res Prof.* 2016; 2(3):28-32.