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

Monika Saini1, Aditya Mishra2, Sweta Gupta3

1M.Sc Medical Microbiology, 2Professor & Head, Department of Microbiology, Mahatma Gandhi Medical College & Hospital, Jaipur, Rajasthan, INDIA.
2Ph.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. Antibiogram 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 high mortality rates and have the potential to spread widely4. Resistance to carbapenem 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 lactams. 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 required6.

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 testing12. 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 β-lactam antimicrobial agents with an exceptionally broad spectrum of activity7. 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 bacilli8. 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 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
resistance8. 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 & Thiglycolate broth. Blood culture was done on Brain heart infusion broth. A culture plates were reported it was 36.4%, Glads RP 4 to 256 µg /ml) gradient at -tivity zone size of Imipenem and -o 22% in Indian studies -ates equivalent to a -ximum -ems. In 100 CRGNB samples 61% were -tance in -t. 12-16,17 -ntance in -t. That's why, identified CRGNB 500, 350 gram negative bacilli were isolated. Total 500 non-

RESULT
(Imipenem+EDTA) > 8 dilution indicate MBL production

resolved in biological tests8. 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 guidelines8. The diameter of the zones of inhibition on MHA was interpreted as sensitive, intermediate and resistant. Escherichia coli ATCC 25922 (β-lactamase negative) Pseudomonas aeruginosa ATCC 27853 (β-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 CLSI8. An Imipenem (10 µg) disc was placed 20mm centre to centre from a disc containing 10µg Imipenem plus 0.5 M EDTA. After overnight incubation, a zone difference diameter of ≥ 7 mm between Imipenem disc & Imipenem plus EDTA disc were interpreted as Metallo-β-Lactamase positive8. [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 µg /ml) gradient at one end & Meropenem + EDTA (1 to 64 µg/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 production10. [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 ±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 β-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 & nitrofurantoin. 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%-11. The incidence varies from as low as 1.8% to over 30% in India12,13. 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%12 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%5,14,15.

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%16,17. 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 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.
Monika Saini et al. Prevalence of CRGNB Isolates and Their Antimicrobial Susceptibility Pattern

![Image 1: Detection of MBL in E.coli by DDST.](image1)

![Image 2: Detection of MBL Pseudomonas by E-Test.](image2)

**Fig 1: Distribution of the CRGNB isolates from various wards**

**Fig 2: Distribution of CRGNB isolates.**

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

- Escherichia coli
- Klebsiella
- Pseudomonas
- Acinetobacter

**Fig 3: CRGNB isolates from various samples.**

**Fig 4: MIC level of Meropenem in Gram Negative bacilli.**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Escherichia coli</th>
<th>Klebsiella</th>
<th>Pseudomonas</th>
<th>Acinetobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTI</td>
<td>7</td>
<td>11</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Wound Infection</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>UTI</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Meningitis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Meropenem MIC level of Gram Negative Bacilli**

- E.coli
- Klebsiella
- Proteus
- Citrobacter
- Pseudomonas
- Acinetobacter
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 Taneya, 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

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

Copyright: © the author(s) and publisher. IJMRP 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.