

Prevalence of Carbapenem Resistant Enterobacteriaceae in Various Clinical Specimen In A Tertiary Care Hospital, Ahmadabad

Khushbu Vagadia*, N I Shah**, Sumeeta Soni***, M M Vegad****, Ami Mistry*****, Suprabha*****

* Resident, ** Professor, ***Associate Professor, **** Professor, ***** Resident, Department of Microbiology, B.J. Medical college, Ahmadabad -380016, Gujarat.

Abstract: Background and objectives: A study to find out prevalence of carbapenem resistant enterobacteriaceae among various clinical specimen received at Bacteriology Laboratory, Civil hospital, Ahmedabad. Methods: The study was conducted over a period of 5 month from June 2016 to October 2016. Total 16786 samples received. Enterobacteriaceae were isolated by standard microbiological identification techniques. The isolates were subjected to antimicrobial susceptibility testing by Modified Kirby bauer Disc Diffusion method. Imipenem resistant organisms were tested for carbapenemase production by Modified Hodge Test and double disk synergy test. Results: Among 16786 samples tested, 3276 belongs to enterobacteriaceae group. In which 357 screened for imipenem resistance. Carbapenemase production was identified in 268 (8.18%) isolates. Interpretation and Conclusion: This study provides prevalence of carbapenemase production among enterobacteriaceae, which shows rise in resistance against carbapenems. Hence there is a need of awareness among clinicians about the same as limited therapeutic options are available and they should be well versed with the strategies to manage multi drug resistant organism like carbapenem resistant enterobacteriaceae. judicious use of antibiotics and strict infectious control practices needs to be followed. [Khushbu V NJIRM 2017; 8(2):80-83]

Key Words: Carbapenemase, Enterobacteriaceae, Modified Hodge Test

Author for correspondence: Khushbu Vagadia, Department Of Microbiology, B.J. Medical College, Ahmedabad-380016.E- Mail: khushbu29396@gmail.com, M: 9429551037

Introduction: Carbapenem are group of beta – lactam antimicrobial agents with an exceptionally broad spectrum of activity¹. They are used as a last resort against many multi drug resistant gram negative bacteria and in case of infections due to extended spectrum beta-lactamase and AmpC enzyme producing enterobacteriaceae². Carbapenem resistant enterobacteriaceae are gram negative bacteria that are resistant to carbapenem class of antibiotics, considered the drug of last resort. Resistance to carbapenems can brought about by various mechanism. Most common being the production of carbapenemase, class of enzymes capable of hydrolyzing carbapenems.

Carbapenemase: It is an enzyme that is produced by some microorganisms, which leads to resistance against carbapenem group of drugs. It includes following drugs:

- Imipenem
- Meropenem
- Ertapenem
- Doripenem

Carbapenemase enzymes fall into Ambler classification- A, B and D.

Class A(serine carbapenemase) enzymes include enzymes such as KPC, IMI, SME etc and are commonly

present in members of Enterobacteriaceae and rarely in Pseudomonas species and Acinetobacterbaumani.

Class B enzymes are metallo-Beta Lactamase including VIM,IMP, SPM. These occur in various genera of Enterobacteriaceae and non-fermenters.

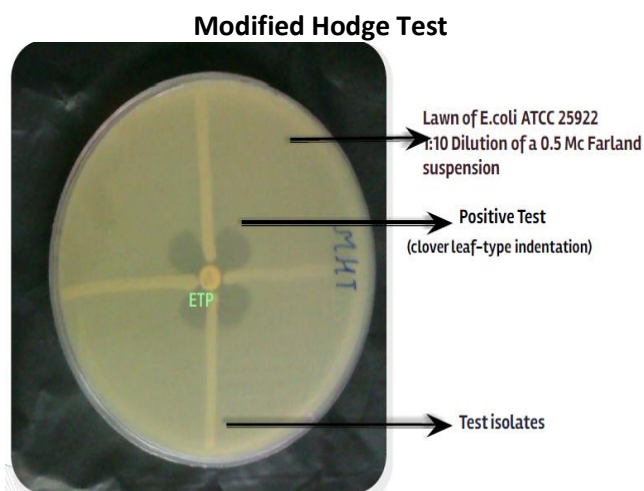
Class D carbapenemase belong to OXA family and are commonly present in Acinetobacter spp. and occasionally in Enterobacteriaceae and Pseudomonas spp.³

Other mechanism is due to poor binding of carbapenems to penicillin binding proteins present in the bacteria, the over expression of multidrug efflux pumps by the bacteria or lack of porins presents in the bacterial cell membrane³. However, for significant resistance to emerge, it is thought that for the resistance various combination is there.⁴They carry a gene that confers resistance to many other antimicrobials. The emergence and dissemination of carbapenem resistance among enterobacteriaceae represent a serious threat to public health with high morbidity and mortality rate and little therapeutic options remains there after. This leads to the financial burden to the family and to the society.

Methods: The study was conducted over a period of 5 month from June-2016 to October-2016. A total 16786 clinical samples were received from wards, ICU and

OPD. Enterobacteriaceae were identified from the specimens by standard microbiological identification techniques. These isolates were then subjected to antimicrobial susceptibility testing by Modified Kirby bauer disc diffusion method. Results were interpreted as per CLSI guidelines in which imipenem resistant organisms were tested for carbapenemase production by Modified Hodge Test and double disc synergy test phenotypically.

Modified Hodge Test: Enterobacteriaceae, those were non-susceptible to one or more carbapenems were tested by Modified Hodge Test. Prepare 0.05% McFarland standard suspension of E. coli ATCC 25922 in broth or saline and dilute 1:10 saline or broth. A lawn culture of 1:10 dilution of ATCC 25922 was streaked to a Muller Hinton agar plate followed by placement of an Ertapenem or Meropenem disc (10 microgram) in the centre of the test area. Using swab pick 2-5 colonies of organism to be tested was streaked in a straight line from the edge of the disc to the edge of the plate. Streaks were 20-25 mm in length. After 16-24 hours of incubation, the plate was examined for a clover leaf type indentation at the insertion of the test organism and the E. coli ATCC 25922 within the zone of inhibition of the carbapenem susceptibility disc⁵.



Double disc synergy test: A lawn culture of test organism was inoculated onto Muller Hinton agar plate as per CLSI guidelines. An imipenem disc (10 microgram) was placed at a distance of 10 mm from an imipenem disc which contain EDTA (750 microgram). The plate was then incubated at 37 degree Centigrade overnight. An enhancement in the zone diameter of Imipenem and EDTA disc is by 4-

5mm or above over Imipenem disc was interpreted as a positive for carbapenemase production⁷.

Result: Zone diameters of carbapenems as per following CLSI guidelines were considered.

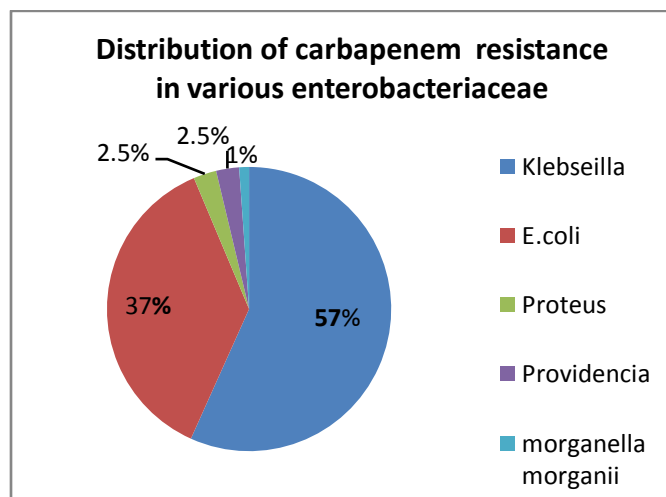
Drug	Dia meter	Sensitive	Inter Mediate	Resistant
Ertapenem	10 ug	>= 22	19-21	<=18
Imipenem	10 ug	>=23	20-22	<=19
Meropenam	10 ug	>= 23	20-22	<=19

Among total 16786 samples tested, in 7375 samples organisms were isolated. In 4634 (62.83%) samples, gram negative bacteria were isolated. Among these 4634 samples, in 3276 (44.42%) samples Enterobacteriaceae was isolated in which 357 (10.89%) isolates were screened for imipenem resistance. Carpenemase production was identified in 268 (8.18%) isolates.

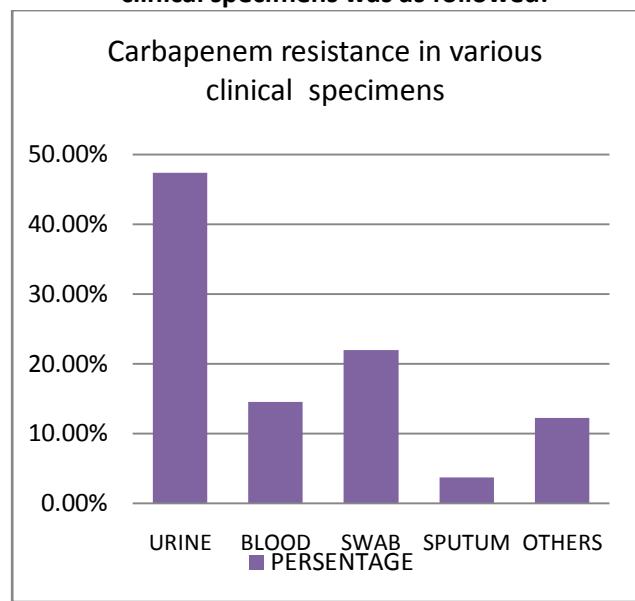
Disribution of carbapnremase producing enterobacteriaceae by modified hodge test and double disc synergy test was as following:

Carbapenemase producing bacteria	Modified Hodge test	Double disc synergy test
Enterobacteriaceae	268 (8.18%)	77(2.35%)
Klebseilla	152 (56.70%)	38 (49.34%)
E. coli	99 (36.90%)	36 (46.70%)
Proteus	7 (2.60%)	1 (1.29%)
Providencia	7 (2.60%)	1 (1.29%)
Morganellamorganii	3 (1.11%)	1 (1.29%)

Disribution of carbapenem resistance in various enterobacteriaceae was as followed:



Distribution of carbapenem resistance in various clinical specimens was as followed:



Discussion: The prevalence of carbapenem resistant enterobacteriaceae in the tertiary care hospital, civil hospital, Ahmedabad was found to be 8.18%.

This is similar to the CRE prevalence rates obtained in similar studies from other parts of India. Pravin K. Nair and Michelle S Vaz reported prevalence of CRE 12.26 % at tertiary care Hospital, Mumbai, India. Datta et al. reported a CRE prevalence rate of 7.87% from a study conducted in a tertiary care hospital in North India⁸.

while Gupta et al reported carbapenem resistance varying from 17 to 22% among Enterobacteriaceae strains⁹.

Wattal et al reported a high CRE prevalence rate ranging from 13 to 51% in a tertiary care hospital in Delhi¹⁰.

Thus, the significant CPE prevalence rates recorded in different parts of India emphasize the need for controlling the further dissemination of CPE.

Conclusion: This study provides prevalence of carbapenemase production among enterobacteriaceae, which shows a rise in carbapenem resistance. There is a need for awareness among clinicians and nexus should be maintained. Infection caused by carbapenem resistant enterobacteriaceae have little treatment option and have been associated with high mortality rates.

Preventing both, carbapenem resistant enterobacteriaceae transmission and infection have become important public health objective.

There is a need of awareness among clinicians to manage multi drug resistant organisms like carbapenem resistant enterobacteriaceae. Patient should not succumb as a result of inadvertent use of antibiotics as they approach to medical fraternity with hope to get cure. So antibiotic stewardship program must be inacted and strictly followed.

Acknowledgment: We extend our gratitude to Medical Superintendent, Civil Hospital, Ahmedabad, Dean of B. J. Medical College and Director Post Graduate studies and Reserch for their support in this study.

References:

1. Zhanell GG, Wiebe R, Dilay L, et al. Comparative Review of the Carbapenems. *Drugs* 2007; 67:1027-1052.
2. Deshpande P, Rodrigues C, Shetty A, et al. New Delhi Metallo-β lactamase (NDM-1) in *Enterobacteriaceae*: Treatment options with Carbapenems Compromised. *J Assoc Physician India* 2010; 58:147-149.
3. Phenotypic method for differentiation of carbapenemase in Enterobacteriaceae- study from North India- Priyadutta, Varshagupta, IJPM.
4. PravinK.Nair, consultant Microbiologist and Head of infection control, Holy spirit hospital, Mumbai, India Received: 25.3.2013, Accepted: 25.9.2013 copyright journal of microbiology and infectious diseases 2013, All rights reserve.
5. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty fifth informational supplement. CLSI document M100-S25. Wayne PA; available from: <http://www.clsi.org>.
6. Center for disease control and prevention 1600 clifton road Atlanta, GA 30329-4027, USA. [Nd. www.cdc.gov/hai/organisms/cre/cre-toolkit/backgrouhtml](http://www.cdc.gov/hai/organisms/cre/cre-toolkit/backgrouhtml)
7. Carbapenem resistance among e.coli and Klebsiellapneumoniae in a tertiary care hospital in south India- s nagaraj, spchandran, indian journal of medical Microbiology.
8. Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of carbapenemase in

Enterobacteriaceae: study from north india. Indian j PatholMicrobiol

9. Gupta E, MohantyS, etal. Emerging resistance to carbapenems in a tertiary care hospital in north india. Indian m Med Res 2006;124:95-98.2012;55:357-360
10. Wattal C, GoelN, OberoiJK,et al. Surveillance of multidrug resistant organisms in tertiary care hospital in Delhi,India.JAssoc Physicians india 2010;58:32-36.

Conflict of interest: None
Funding: None
Cite this Article as: Vagadia K, Shah N I, Soni S, Vegad M M, Mistry A, Suprabha. Prevalence of Carbapenem Resistant Enterobacteriaceae in Various Clinical Specimen. Natl J Integr Res Med 2017; 8(2):80-83