International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Carbapenem Resistant Mechanism in Carbapenem Resistant Gram Negative Bacilli Other Than Carbapenemase

Sibin P S[1], Anuranjini C[2], Jeeva Rani[3]

[1]Microbiologist, Department of Medical Microbiology, VPS Lake Shore Hospital and Research Centre, Ernankulam, Cochin, Kerala.
[2]Assistant Professor, Department of Medical Microbiology, School of Health Sciences, Kannur University, Kerala.
[3]Microbiologist, Department of Medical Microbiology, Aster Malabar Institute of Medical Sciences, Calicut, Kerala.

Corresponding Author: Sibin P S

ABSTRACT

Introduction - Infections have been the major cause of disease throughout the history of human population. In the middle of the nineteenth century antibiotics are discovered as a powerful weapon in the battle against infectious diseases. Antibiotic resistance has been detected in many bacterial species. As each new class of antibiotic introduced into the clinical use, resistance starts to develop in the microorganism they are intended to kill. Both innate and acquired mechanisms contribute to the development of antibiotic resistance in bacteria.
Objectives - The present study was conducted to analyze the prevalence of Carbapenem resistance in gram negative bacilli and their carbapenem resistance mechanisms other than carbapenemase in tertiary care hospital.
Methods - The objectives of this study includes to analyze the carbapenemase producers and non carbapenemase producers by Modified Hodge Test (MHT) and to detect the presence of Efflux pump mechanism in MHT positive and negative strains by Ethidium Bromide (EtBR) agar cartwheel method.
Result - Klebsiella was found to be predominant form of carbapenem resistant gram negative bacilli. Among the 144 MHT negative strains all the strains showed either Efflux pump or Impermeability mechanisms. By Ethidium Bromide agar Cartwheel method only 10% strains were positive for efflux activity. Current study also indicates that the carbapenem resistant Pseudomonas was mainly due to Efflux activity. But in the case of carbapenem resistant Klebsiella was mainly due to impermeability mechanism. The overall prevalence of carbapenem resistant Gram Negative Bacilli was found to be 10%.

Key Words: Carbapenem resistance, Modified Hodge Test, Efflux pump, Gram Negative Bacilli, Klebsiella.

INTRODUCTION

Infections have been the major cause of disease throughout the history of human population. In the middle of the nineteenth century antibiotics are discovered as a powerful weapon in the battle against infectious diseases. Scientific antibiotic discovery started by Alexander Fleming, who observed inhibition of growth on his agar plate on which he was growing Staphylococcus species. It was later found that a microorganism that was later to be

called Penicillium notatum was the cause of inhibition of the Staphylococcus around it as a result of excreting some chemical into the media. That marked the beginning of the discovery of penicillin which together with several other different antimicrobial agents was later to save millions of humans and animals from infectious disease causing organisms. However bacteria and other organisms are remarkably resistant and can develop ways to survive drugs meant to kill or weaken them. Such antibiotic resistance or drug resistance is largely due to the increasing use of antibiotics. Therefore antibiotic resistance is the major challenge in the 21st century.

Antibiotic resistance has been detected in many bacterial species. As each new class of antibiotic introduced into the clinical use, resistance starts to develop in the microorganism they are intended to kill. Both innate and acquired mechanisms contribute to the development of antibiotic resistance in bacteria.

Carbapenem play a critically important role in our antibiotic armamentarium. Of the many hundreds of different beta lactams, carbapenems possesses the broadest spectrum of activity and greatest potency against Gram positive and Gram negative bacteria. As a result, they are often used as "last line agents" or "antibiotic of last resort" when patients with infections become gravely ill or are suspected of harbouring resistant bacteria. [1-3] Unfortunately, the recent emergence multidrug resistance (MDR) pathogens seriously threaten this class of life saving drugs. [4] Several recent studies were available from various countries including India. [5-7] Enterobacteriaceae are the members of the intestinal flora. They are most common cause of infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis, and catheter associated infections.

Mechanisms associated with carbapenem resistance are complex and involve various genes. Multidrug efflux system, Impermeability and production of Carbapenemases (Enzyme that inactivate carbapenems) are the most important molecular mechanisms that mediate carbapenem resistance in Enterobacteriaceae. [8-12] Antibiotic efflux in bacteria was first reported in the late 1970s for tetracyclines in Escherichia coli and attributed to the plasmid encoded Tet protein. Impermeability due to the loss of OprD porin is the most common mechanism described in Pseudomonas aeruginosa. Carbapenem resistant Enterobacteriaceae have been reported worldwide as a consequence of large acquisition of Carbapenemase genes. The first Carbapenemase producer in Enterobacteriaceae (NmcA) was identified in 1993. Since then, a large variety of carbapenemase has been identified in Enterobacteriaceae belonging to 3 classes of beta lactamases: the Ambler class A, B and D beta lactamases. CLSI published a recommendation that carbapenems susceptible Enterobacteriaceae with elevated MICs or reduced disc diffusion zone sizes be tested for the presence of carbapenemases using the Modified Hodge Test (MHT). Ethydium Bromide (EtBr) agar cartwheel method is easy to perform, there by facilitating the rapid identification of isolates to detect Multidrug resistance mediated by efflux and EDTA is the agent that increase the membrane permeability, therefore resistance due to impermeability can be detected by invitro susceptibility testing by adding this agent.

In the view of above, the present study was conducted to analyze the prevalence of Carbapenem resistance in gram negative bacilli and their carbapenem resistance mechanisms other than carbapenemase in tertiary care hospital. The carbapenems are β-lactum type of antibiotics with an exceptionally broad spectrum of activity coverage of Gram positive and Gram negative aerobes and are stable almost all bacteria. The first carbapenem to be identified in the mid 1970s was thienamycin, a compound

produced by the soil organism Streptomyces cattleya. [13-14]

Carbapenems inhibit cell wall synthesis by binding to most high molecular weight PBPs. They traverse the outer membrane of gram negative bacteria through specific outer membrane protein (OMPs) to each the periplasmic space. The most significant OMP is Opr D in pseudomonas aeruginosa. Although variations exist depending on the specific agent, they preferentially bind to PBPs 1a, 1b, 2 and 4 and to a lesser extent PBP3, which is the primary target of amino – penicillins and cephalosporins. This low affinity with PBP3 is through to be responsible for the formation of sphere forms without the production of long filaments of bacterial lysis. The affinity of carbapenems to multiple PBPs of various bacteria contributes to the broad spectrum of activity of these agents. [15] The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta lactam resistant bacteria. [13] Carbapenem antibiotics are the last treatment option for severe, life threatening infections caused by gram negative enteric bacilli with multiple drug resistance. Therefore, an understanding of carbapenem resistance is important. Carbapenem resistance among members of the family Enterobacteriaceae is mediated by the 1. Production of beta lactamases (Carbapenemases) that hydrolysis the carbapenems 2. Active pumping of the antibiotics out of the cell using complex "Efflux pumps" 3. Changes in outer membarane porins that block the entry of these antibiotics (Impermeability). [15] Evidence of increasing incidence of infections due to carbapenem resistant enterobacteriaceae is reported from various countries. Guh Ay et al., [16] reported that 7 US communities (Colorado, Georgia, Maryland, Minnesota, New Mexico, New York and Oregon) the high prevalence of CRE during the period of 2012- 2013 Multidrug resistant organisms such as CRE have been labelled as a "serious threat to public health" by the Centres for Disease Control and prevention and "one of the greatest threats to human health".

The present study was intended to analyze the mechanisms of carbapenem resistance and other than carbapenemase production in gram negative bacilli isolated from clinical samples in tertiary care hospital. The objectives of this study includes to analyze the Carbapenemase producers and non carbapenemase producers by Modified Hodge Test (MHT) and to detect the presence of Efflux pump mechanism in MHT positive and negative strains by Ethidium Bromide (EtBR) agar cartwheel method. Another objective was to detect the Impermeability in MHT positive and negative strains by augmented in vitro susceptibility test by adding EDTA.

MATERIALS AND METHODS

The study was carried out in Malabar Institute of Medical Sciences, Calicut over the period of January to March 2016.Clinical samples such as urine, pus, blood, body fluids catheterized specimens, sputum, respiratory specimens and tissue specimens were collected aseptically in a sterile container. Immediately after collection, the samples were transported to microbiology laboratory and processed. Whenever there was delay in processing the samples were transferred to appropriate transport media depending on the specimen and preserved under appropriate condition as per the standard protocols. All the samples were processed in appropriate media within 2-4 hours of collection. The bacterial isolates obtained were identified based on their morphological, cultural and biochemical characters. The unidentified organisms are identified by using automated culture system VITEK 2 compact. Modified Hodge Test were performed for the detection of Carbapenemase and non Carbapenemase, positive results were later confirmed by (for impermeability) Meropenem – EDTA disc synergy and

detection of Efflux pump mechanism by using Ethidium bromide.

MODIFIED HODGE TEST – FOR THE DETECTION OFCARBAPENAMSASE ACTIVITY:

The test was performed as suggested by Lee et al., [17] The Modified Hodge Test (MHT) detects Carbapenemase production in isolates of Enterobacteriaceae. Modified Hodge (clover leaf) Test involving disorted carbapenem inhibition zones. Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (E. Coli ATCC 25922) towards a carbapenem disc where the concentration of the antibiotic is low. The result is a characteristic clover leaf like indentation. MHT positive test has a clover leaf like indentation of E. coli 25922 growing along the test organism growth streak within the disc diffusion zone. MHT negative test has no growth of the E. coli 25922 along the test organism growth streak within the disc diffusion.

MEROPENEM – EDTA DISC SYNERGY TEST:

The test strains were lawn cultured and 10 microgram meropenem disc and meropenem with EDTA were placed 20 mm apart. Following incubation, carbapenem resistant strains doesn't give sensitive zone. Metallo-beta-lactamase enzymes require zinc for their action. Since EDTA is a strong chelator of zinc, it chelates the zinc present in the medium making the zinc unavailable to the enzyme and thus suppresses the MBL activity and strains shows increased zone of diameter. Increased zone diameters of 6mm than the meropenem alone were considered to be positive. The detection of impermeability indicated by the formation of inhibitory zone greater than normal discs by adding permeabilizing agent like EDTA.

DETECTION OF EFFLUX AND NON EFFLUX ACTIVITY – BY USING ETHEDIUM BROMIDE CARTWHEEL METHOD:

The principle of this test is flurometric assay, the paasage of the common substrates of Efflux pumps EtBr across the cytoplasmic membrane and its subsequent intracellular accumulation inside the bacterial cell. EtBR traverses the bacterial cell wall and once inside, it can be concentrated to a point where it fluorescence when excited by Ultra Violet light (UV). Efflux pump of MDR bacteria recognize this substance and are able to extrude out in to the medium. [18]

Prepared the MHA plates containing EtBR concentration 20µL. MHA plates was prepared fresh on the previous or same day of the experiment and kept protected from the light. MHA plates were then divided into as many as 12 sectors by radial lines, forming a cartwheel pattern. Prepared 0.5 % McFarlands dilutions of the overnight cultures of the bacterial isolates. In a straight line streak the test cultures or swabbed on the EtBr – MHA plates. The MHA plates were incubated at 37^0C for 16 hours. After this period MHA plates examined under a gel imaging system, the presence of fluorescent growth indicates absence of efflux activity where as absence of fluorescence growth indicates presence of efflux activity.

RESULT

During this period of study total of 5324 various clinical specimens were subjected to isolation of bacterial pathogens and 2510 gram negative organisms were isolated and identified in hospital. Based on morphological, biochemical characters and antibiotic susceptibility patterns of each gram negative isolates were analyzed and those isolates which showed meropenem and imipenem resistance were selected for screening carbapenem resistance other than carbapenemase. Among 2510 gram negative isolates 10% (250) are carbapenem resistant gram negative bacilli. These isolates include Klebsiella, E. coli, Enterobacter,

Chromobacter, Acinetobacter, and Pseudomonas species.

Klebsiella was found to be predominant form of carbapenem resistant gram negative bacilli. From the total of 250 sample 138 Klebsiella were isolated. Followed by E.coli (87), Enterobacter (20), Pseudomonas (3), Acinetobacter and Chromobacter (1) each. Among these samples 143 (57.6%) Carbapenem resistant Gram negative bacilli were isolated from the urine sample. Apart from the urine sample Klebsiella and Enterobacter species, the most of the carbapenem resistant Gram Negative Bacilli isolated from the sputum samples 36 (14%). 27 (11%) carbapenem resistant Gram Negative Bacilli were isolated from the blood samples, and remaining 44 Carbapenem resistant Gram negative bacilli were isolated from the pus, swabs and exudates respectively

Modified Hodge Test (MHT) was the easy and simple test to be performed to detect carbapenemase producing bacteria. From this test classify the organisms as carbapenemase producers and non producers (may be efflux pump or impermeability mechanism).

Table 1 shows carbapenemase positive and negative strains by MHT.

Total number of strains	No. of strains showing positive in MHT	No. of strains showing negative MHT
Klebsiella	63 (45.7%)	75 (54.3%)
E. coli	30 (34.4%)	57 (65.5%)
Enterobacter	13	7
Chromobacter	0	1 (100%)
Acinetobacter	0	1
Pseudomonas	0	3
Total	106(42.4%)	144(57.6%)

Present study it was observed that 54.3% (75) of the Klebsiella and 65.5% (57) of the E.coli were the MHT negative strains, therefore it indicates that these Klebsiella and E.coli is having other than carbapenemase mechanisms. But in the case of Enterobacter species carbapenemase mechanism was predominant. Only the isolate of Pseudomonas, Acinetobacter and Chromobacter species were obtained which are MHT negative. In our study it revealed that high prevalence of other than carbapenemase mechanism is predominant such as impermeability and efflux pump. Out of 250 strains collected, total 42.4% (106) shows carbapenemase producers and remain 57.6% (144) are non carbapenemase producers.

Table 2 reveals the Efflux pump, Impermeability mechanism and carbapenemase activity in MHT positive strains.

Number of MHT positive strains	Efflux pump by EtBr Method	Impermeability by adding EDTA
Klebsiella N=63	0	63
E. coli N=30	10	20
Enterobacter N= 13	13	0
Chromobacter N=0	0	0
Acinetobacter N= 0	0	0
Pseudomonas N= 0	0	0
Total N=106	23	83

In order to identify the efflux pump mediated carbapenem resistance in MHT positive strains Ethidium Bromide agar Cartwheel method was used. Since only 22%strains shows the Efflux pump mechanism. In our study only E. coli and Enterobacter species showed Efflux pumps mediated carbapenem resistance. Whereas Klebsiella strains does not shows efflux mechanism. To identify the Impermeability mechanisms we added EDTA, an agent that increases the permeability. Out of 106 strains 78% shows the impermeability. 100% of Klebsiella species and 19% E. coli were exhibited this mechanism. In this current study it indicates carbapenemase positive strains are also shown the impermeability and efflux mechanisms. In carbapenem resistant Gram Negative Bacilli a single mechanism may not be sufficient to cause a clinically relevant degree of resistance.

Table 3 Depicts the Efflux pump and Impermeability mechanism in MHT negative strains.

Number of MHT negative strains	Efflux pump by EtBr Method	Impermeability by adding EDTA
Klebsiella N=75	6	69
E. coli N=57	5	52
Enterobacter N= 7	0	7
Chromobacter N=1	0	1
Acinetobacter N= 1	0	1
Pseudomonas N= 3	3	0
Total N=144	14	130

Among the 144 MHT negative strains all the strains showed either the Efflux pump or Impermeability mechanisms. By the Ethidium Bromide agar

Cartwheel method only 10% strains were positive for efflux activity. Current study also indicates that the carbapenem resistant Pseudomonas was mainly due to Efflux activity. But in the case of carbapenem resistant Klebsiella was mainly due to impermeability mechanism and all the Acinetobacter, Enterobacter, Chromobacter and majority of E.coli strains were having impermeable mechanism. In Chromobacter, Acinetobacter and Enterobacter species, efflux pump mediated resistance mechanism were absent in our study, where as 90% strains were showed carbapenem resistance due to impermeability by augmented in vitro susceptibility assay.

DISCUSSION

A CRE prevalence rate of rate of 12.26% have been obtained in tertiary care hospital in Mumbai, India over a period of 12 months, from which majority of isolates were detected in urine sample. [19] In this present study the prevalence of Carbapenem resistant gram negative bacilli rate was 10%. Among the samples analyzed in the laboratory 57% carbapenem resistant Gram Negative Bacilli are isolated from urine sample followed by sputum (14.4%) and blood (11%) samples. Klebsiella was the most prevalent carbapenem resistant gram negative bacilli from the total samples followed by E.coli and Enterobacter species. CRE that can cause different types of health care associated infections, wound or surgical site infections and meningitis. They are normally found in human intestine. In health care settings CRE infections commonly occurs among sick patient who are received treatment for other conditions such as diabetes or patients undergo any transplantation procedure or an stem cell implantation and long term stay in hospital along with exposure to antimicrobial therapy. Patients whose care requires devices like ventilators, and patient who are taking long course of certain antibiotics are the most risk for Klebsiella infections. [20-22] Organisms isolated from the blood samples showed Klebsiella was the most prevalent carbapenem resistant Enterobacteriaceae followed by E.coli which may be indicate the blood stream infections. European authorities report local outbreak in numerous countries across the continent, which a high proportion of CRKP (Carbapenem resistant Klebsiella pneumoniae) in blood stream isolates in Greece, Italy and Cyprus. Multiple countries in the Middle East and southern Mediterranean basis, South America and Asia are affected. CRKP has become endemic in the Indian subcontinent. CRKP blood stream (BSI) is associated with an immense case fatality rate of 40% to 70%. [23-29]

From our study it is revealed that 144 carbapenem resistant Gram Negative Bacilli strains (57.6%) are non carbapenemase producers and remaining 106 (42.4%) shows the carbapenemase production. Which indicates the high prevalence of other than carbapenemase mechanisms such as efflux and impermeability are common. Active Efflux pumps transport drug through the bacterial envelope and limit the intracellular accumulation of toxic components. It was well established the multidrug resistance efflux pumps encoded by bacteria can confer clinically relevant resistance to antibiotics. [29]

The MHT negative strains are subjected to efflux activity, among this 14 out of 144 non carbapenemase producers shows efflux activity. Klebsiella (59 out of 75), E. Coli (52 out of 57) and Enterobacter, Chromobacter and Acinetobacter were the most prevalent organism shows carbapenem resistance through the impermeability mechanism. Enterobacter causing nosocomial infection involving the urinary tract, lower respiratory tract, skin and soft tissue, biliary tract, wounds, intravenous catheters and central nervous system. [30] Fuchen Yang et. al., [31] reported that the Ertapenem resistant Enterobacter cloacae in the Taiwanese university hospital mainly due to the active efflux pump mechanism. Athul Khajuria et al., [32] conducted a study

in tertiary care hospitals in Central India and the study states that MBL production contribute carbapenem resistance in Enterobacter species.

When analyzed MHT positive strains by EtBr method, it showed that 33% of E. coli strains were carbapenem resistance which was mediated by efflux pumps. The outer membrane of gram negative bacteria performs the crucial role of providing an extra layer of protection to the organism. By combining a highly hydrophobic lipid bilayer with pore forming proteins of specific size exclusion properties, the OM acts as selective barrier. The permeability properties of this barrier, therefore, have a major impact on the susceptibility of organisms to major antibiotics. Porin protein play an important role in the permeability of the outer membrane of gram negative bacteria to small hydrophilic molecules, such as beta lactam antibiotics.

In MHT negative strains 90% shows the impermeability mechanism. Among the 92% of Klebsiella, 91% E. coli and 100% Enterobacter species showed carbapenem resistance which was mediated by impermeability mechanism. EDTA was the excellent agents that increase the permeability of bacterial cellwall. In MHT positive strains the present data proves that not only carbapenemase mechanism but also other mechanisms like Efflux and Impermeability have provide major resistant mechanism. Current study strongly supported the only 22% Enterobacteriaceae were the Efflux pump mechanism but 78% shows the Impermeability. The study strongly supported the Yohei Doi et al., 2006 [15] that gram negative bacteria although a single mechanism may not be sufficient to cause a clinically relevant degree of resistance, it occurs through an interplay involving carbapenemase production, impaired Impermeability, and enhanced Efflux.

Among this most of the Carbapenem resistant bacteria were isolated from the urine samples (57%) followed by sputum (14%) and blood (11%) samples. Carbapenem resistant Gram Negative Bacilli were the major nosocomial pathogen causing significant morbidity and mortality. Carbapenem resistant Klebsiella species poses a major problem for clinical therapeutics. Modified Hodge Test (MHT) can be used to detect carbapenem resistance. The present study identified that other than carbapenemase were the major cause of resistance in Gram negative bacteria and 57.6% were the non carbapenemase mediated mechanism. The outer membrane barrier can produce significant levels of resistance in carbapenem resistant Gram Negative Bacilli.

CONCLUSION

In Conclusion the overall prevalence of carbapenem resistant Gram Negative Bacilli were found to be 10% (250/2510). Carbapenem resistance Klebsiella and E. coli were mostly due to the impermeability mechanisms. In carbapenem resistant Gram Negative Bacilli although a single mechanism may not be sufficient cause a clinically relevant degree of resistance, frank resistance occurs through an interplay involving carbapenemase production, impaired permeability and enhanced efflux.

In future, the design of new antimicrobial preparations might aim to ensure the accumulation of active agent within the cell, relaying on several principle strategies to do so :(A) Increasing uptake of the antimicrobial compounds by (1) Enhancing self promoted uptake mechanisms (2) Combining the active agents with a permeabilizer (3) A synergistic physical process, and or (B) increasing accumulation of the antimicrobials by reducing the activity of multidrug efflux system.

REFERENCES

1. Bradley JS, Garau J, Lode H et al. Carbapenems in clinical practice: a guide to their in serious infection. Int. J. Antimicrob. Agents 1999;11(2): 93-100.
2. Paterson DL. Recommendation for treatment of severe infections caused by

Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Clin. Microbiol. Infect. 2000; 6(9):460–3.
3. Paterson DL. Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin. Microbiol. 2005;18 (4): 657–686.
4. Queenan AM, Bush K. Carbapenemases: the versatile beta lacatamases. Clini Microbiol Rev 2007;20 (3):440-458.
5. Gopalakrishnan R., Sureshkumar D. Ch anging trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India.2010; 58Suppl:25–31.
6. Nicasio A. M., Kuti J. L., Nicolau D. P.. 2008. The current state of multidrug-resistant gram-negative bacilli in North America. Pharmacotherapy.2008;28(2): 235–49
7. Rossi F. The challenges of antimicrobial resistance in Brazil. Clin Infect Dis.2011; 52(9):1138–43.
8. Bush K. Jacoby KG, Medeiros AA. A functional classification scheme for βlactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995;39(6):1211–33
9. Poole K. *Pseudomonas Aeruginosa*: Resistance to the Max. *Frontiers in Microbiology*. 2011;2:65. doi:10.3389/fmicb.2011.00065.
10. Hammami S. Ghozzi R. Burghoffer B et al. Mechanisms of carbapenem resistance in non metallo beta lactamase producicng clinical isolates of Pseudomonas aeruginosa from a Tunisian hospital. Pathol Biol (Paris). 2009; 57(7-8):530-5
11. El Amin N. Giske CG. Jalal S. Keijser B et al. Carbapenem resistance mechanisms in Pseudomonas aeruginosa: alterations of porin oprD and efflux proteins do not fully explain resistance patterns observed in clinical isolates. APMIS .2005;113(3): 187-196
12. Martin SI. Qin X. Zerr DM. Weissman SJ. Molecular diversity in mechanisms of carbapenem resistance in paediatric Enterobacteriaceae. International journal of antimicrobial agents. 2012;39 (1): 52-57.
13. Kahan JS et al. Theinamycin a new betalactum antibiotic.Discovery, taxonomy, isolation and physical properties. J Antibiot (Tokyo) 1979;32: 1-2.
14. Nunez LE. Mendez C. Brana AF et al.The synthetic gene cluster for the beta lactum carapenem Theinamycin in Streptomyces catteleya. Chem boil. 2003; 301-311.
15. Yohei doi and Henry F Chambers. Mandell, Douglas & Benetts Principles and practice of infectious diseaseas By John E Benett, Raphael Dollin, Martin J. Blaser: Chapter 22. Other beta lactum antibiotics P-293.
16. Guh AY. Bulens SN. Mu Y et al. Epidemiology of carbapenem resistant enterobacteriaceae in 7 US communities. 2012-2013. J Antibiot. 2015; 314(14):1479-87
17. Lee K. Chong Y. Shin HB et al. Modified Hodge and EDTA-disk synergy tests to screen metallo β-lactamase producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 2001;7(2):88-91.
18. Marta Martins. Matthew P McCusker. Miguel Viveiros et al. A Simple Method for Assessment of MDR Bacteria for Over-Expressed Efflux Pumps. The open Micr. Jrnl. 2013; 7: 72-82.
19. Pravin K Nair. Michelle S Vaz. Prevalence of carbapenem resistance Enterobactriaceae from a tertiary care hospital in Mumbai, India. JMID. 2013;3(4):207-210.
20. Centers for Disease Control and Prevention 1600 Clifton Road Atlanta, GA 30329-4027, USA,800-CDC-INFO (800-232-4636) TTY: (888) 232-6348.
21. Chitnis AS. Caruthers PS. Rao AK et al. Outbreak of carbapenem resistant ennterobacteriaceae at a long term acute care hospital: Sustained reducing in transmission through active surveillance and targeted interventions. Infection control and Hospital Epidemiology. 2012; 33 (10):984-92.
22. Choi JP. Cho SJ. Lee SJ et al. Influx of multi drug resistant, Gram negative bacteria (MDR GNB) in a public

hospital among elderly patients from long term care facilities: a single centre pilot study. Archives of Gerontology and Geriatrics.2012; 54(March- April) :19-22.
23. Amit S. Mishali H. Kotlovsky T et al. Bloodstream infections among carriers of carbapenem-resistant Klebsiella pneumoniae: etiology, incidence and predictors. CMI. 2015 ;21(1): 30–34.
24. Albiger B, Glasner C, Struelens M, Grundmann H, Monnet D, the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill. 2015;20(45):pii=30062. DOI:http://dx.doi.org/10.2807/1560-7917.ES.2015.20.45.30062
25. Najaraj S. Chandran SP. Shamanna P et al. Carbapenem resistance among Escherichia coli and Klebsiella pneumonia in a tertiary care hospital in south India. Indian J Med Microbiol. 2012; 30(1):93–5.
26. Borer A. Saidel-Odes L. Riesenberg K et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumonia bacteremia. Infect Control Hosp Epidemiol. 2009;30(10):972–6.
27. Patel G. Huprikar S. Factor SH et al. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol. 2008;29(12):1099–106.
28. Ben-David D. Kordevani R. Keller N et al. Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. Clin Microbiol Infect. 2012;18)1):54–60
29. Pidock LJ. Multidrug resistance efflux pumps not just for resistance. Nat Rev microbial. 2006;4(8): 629-36.
30. Sanders WE Jr. Sanders CC. Enterobacter spp. Pathogens poised to flourish at the turn of the century. Clin.Microbiol.Rev. 1997;10(2):220-41.
31. Fu-Chen yang. Jing Jou Yan. Kuei-Hsiang Hung et al. Charectaraization of Ertapenem – Resistant Enterobacter cloacae in a Taiwaneese University Hospital. J Clin Microboiol. 2012; 50(2): 223-226.
32. Athul Khajuria. Ashok Kumar. Praharaj et al. Carbapenem Resistance among Enterobacter species in a Tertiary care Hospital in Central India. Chemotherapy Research and practice.2014; volume 2014 (2014), Article ID: 972646-52.

How to cite this article: Sibin PS, Anuranjini C, Rani J. Carbapenem resistant mechanism in carbapenem resistant gram negative bacilli other than carbapenemase. Int J Health Sci Res. 2017; 7(11):47-55.

****