International Journal of scientific research and management (IJSRM)
\||Volume||3||Issue||3||Pages|| 2307-2312||2015||
Website: www.ijsrm.in ISSN (e): 2321-3418

Study on Detection of Carbapenemases In Clinical Isolates Of Family Enterobacteriaceae

P.Vasundhara Devi[1]*,P.Sreenivasulu Reddy[2], Maria Sindhura John[2]

1.Professor 2.HOD 3.TUTOR

Department of Microbiology,Narayana Medical College,Nellore,A.P

Corresponding Author: P.Vasundhara Devi

Professor,Department of microbiology,Narayana Medical College,Nellore,A.P

Email: dr.pvasundhara@yahoo.com

ABSTRACT:

Background: Enterobacteriaceae group of organisms are inhabitants of the intestinal tract.They are the source of community and hospital acquired infection.the carbapenems are recommended as last choice in treating in serious infections caused by multi drug resistance Gram negative bacilli and the emergence of Carbapenem resistant Enterobacteria is therefore worrisome as the antimicrobial armamentarium consequently is restricted.**Objective:** The study is under taken with a view to generally screen the Gram negative rods for the production of Carbapenemase as these enzymes do not always produce resistant breakpoints for Carbapenems using standardized susceptibility testing methods.**Materials and Methods:**A total of 200 samples were processed. The samples inoculated onto Nutrient agar, Blood agar, Macconkey agar and the colonies tested for motility, catalase and oxidae test. sugar fermentation methods and biochemical tests were performed. Antibiotic sensitivity was done by Kirby bauer disc diffusion method.Carbapenemase detection was done by Modified Hodge method.**Results:**Among 200 samples processed, the highest no.of samples were taken from the age group 41-50yrs(45). Highest isolated Enterobacteriaceae was Escherichia coli(102) followed by klebsiella (88).130 samples showed resistance to Carbapenem antibiotics.Among them,50 Enterobacteriaceae showed Modified Hodge test positive.In which, Eschericia coli was the highest(42)of 84%followed by Klebsiella sps(6)of 12%.**Conclusion:** our study findings are contrary to the belief. Therefore it is recommended that large sample based studies may be taken up in which may throw better light.a

Key words: Enterobacteriaceae, Carbapenemase, Kirby bauer disc diffusion, Modified Hodge test

INTRODUCTION:

Enterobacteriaceae are the Gram negative enteric bacilli are common causes of wide variety of infections involving diverse anatomical sites in both healthy and compromised host. Enterobacteriaceae includes Escherichia coli, Klebsiella, Proteus, Enterobacter, Serratia, Citrobacter, Margonella, Providencia and Edwardsiella. They are component of normal animal and human colonic flora and or of the flora of a variety of environmental habitats, including long term care facilities and hospitals.

The GNB possess an extra cytoplasmic outer membrane which are critical determinants in pathogenesis causing Urinary tract infection, Pneumonia, Bacteremia, Intra abdominal abscesses, Biliary tract infections, Nosocomial sinusitis etc. Carbapenen resistance Enterobacteriaceae has been reported world wide as a consequences of largely of acquisition of Carbapenemase genes.(1)

The Carbapenemase are a class of beta lactamases antibiotics that differ from the penicillins by the substitutionnof a carbon atoms for a sulphur atom and by the addition of a double bond to the five membered ring of the penicillin nucleus(2) .Imipenem, Meropenem, Etrapenem and Doripenem are the four beta lactam antibiotics of the carbapenem class(3)

Mechanism of action of Carbapenems exhibits bactericidal activity by binding to the penicillin binding proteins(98).which are responsible for for elongation and cross linking the peptidoglycon of the bacterial cell wall. This binding results in impairment of construction of the cell wall, inhibition of cell growth frequently, cell lysis and death.(4)

Carbapenems are now frequently used as reversed drug in treating infections caused by multidrug resistant Gram negative bacilli. These antibiotics are stable to beta lactamases including the extended spectrum beta lactamases and AmpC produced by gram negative bacilli. Unfortunately resistance to these antibiotics started emerging from 1990 and has been reported world wide over the years with varying frequencies(5).Pseudomonas aeruginosa and Acinitobacter sps in particular are more associated with Carbapenem resistance(6,7)

Detection of carbapenemase is difficult. It can be detected by phenotypic as well as genotypic methods (8).Among phenotypic tests Modified Hodge test is relatively easy and simple method to be performed in a laboratory.

The study is under taken with a view to generally screen the Gram negative rods for the production of Carbapenemase as these enzymes do not always produce resistant breakpoints for Carbapenems using standardized susceptibility testing methods.

MATERIALS AND METHODS:

A total of 200 samples were collected from patients of all age groups with clinical evidence admitted to medical MICU, surgical (SICU), Neuro NICU and pediatric PICU wards of Narayana General hospital ,Nellore. This study was conducted from October 2012 to October 2013 in the department of microbiology with the help of various clinical departments in Narayana Medical College, Nellore. over a period of 1 year the samples were processed.

All samples for microbiologicl processing were collected in appropriate sterile universal containers.Samples collected from the patients were Endotracheal aspirates from suction tips of patients on Ventilators, Sputum, Urine, Pus and Blood.

Processing Of Samples:

First smears were prepared on clean microscopic glass slides with samples and Gram staining was performed and observed for Gram reactions, size, shape, arrangement of organisms, pus cells, squamous epithelial cells, etc. Then the samples inoculated onto Nutrient agar, Blood agar, Mac conkey agar. The plates were incubated at 37c for 18-24 hrs. After overnight incubation,the isolated colonies again Gram staining was performed and with the colonies of Gram negative bacilli, Motility, Catalase and Oxidase test were performed.

Further isolation was done with all required Sugars and Biochemical tests. Sugar fermentation tests with sugars like Glucose, Lactose,Ssucrose,Maltose, Mannitol, Xylose etc. for Genus level identification

Biochemical tests like Indole, Methyl red, VogesProskauer ,Simmons citrate, Urease, TSI,

Nitrate reduction test, OF test etc were performed for Species level identification

Antibiotic Sensitivity Testing:

Antibiotic sensitivity testing was done by Kirby bauer disk diffusion methd on Muller Hinton agar using antibiotics like Meropenem, Imipenem, Gentamicin, Cefotaxime, Cefixime, Ciprofloxicin, Amikacin. Zone size were measured according to CLSI guide lines.

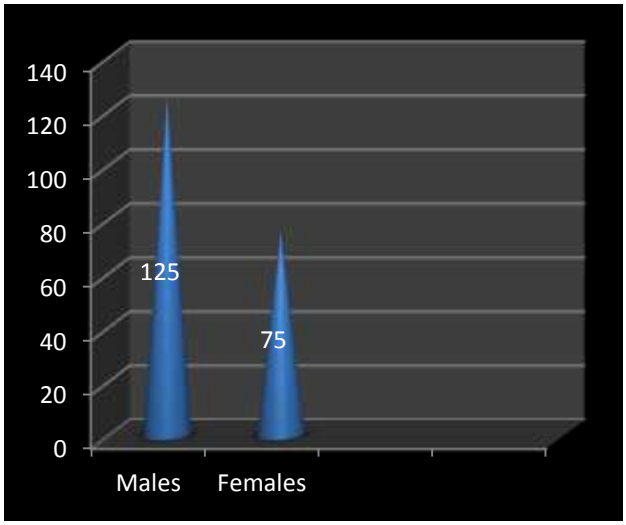
Modified Hodge Test:

The Meropenem and Imipenem resistant strains were subjected to MHT for detection of Carbapenemases. An overnight culture suspension of E.coli ATCC 25922 which was adjusted to 1:10 dilution of the 0.5 McFarland standard was inoculated on the surface of Muller Hinton agar plate evenly. After the brief drying at the room temperature,10µg Ertapenem disk was placed in the centre of the plate. Meropenem resistant test strain from an overnight culture was streaked heavily from the edge of the disk to the edge of the plate. After 24 hrs the presence of a distorted or clover leaf shaped inhibition zone due to Carbapenemase production by the test strain was considered positive(9)

RESULTS:

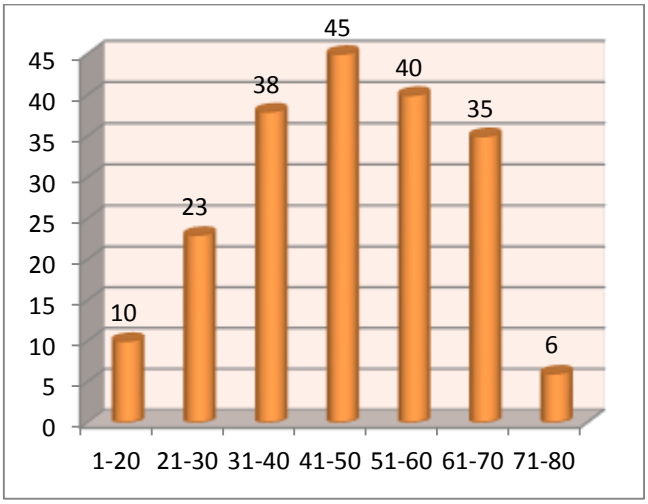
During the study period extending from over a period of 1 years from october 2012 to october 2013. During this period 200 clinical samples were received from Narayana General hospital to the Department of Microbiology Narayana Medical College.

1.Gender wise distribution of Enterobacteriaceae



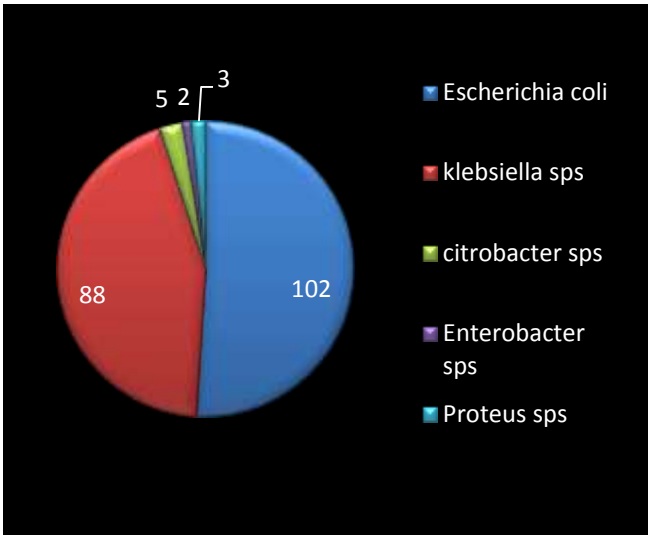
Among 200 samples processed, most of the samples were collected from Males 125 and 75 samples were collected from Females.

2.Age wise distribution of total samples



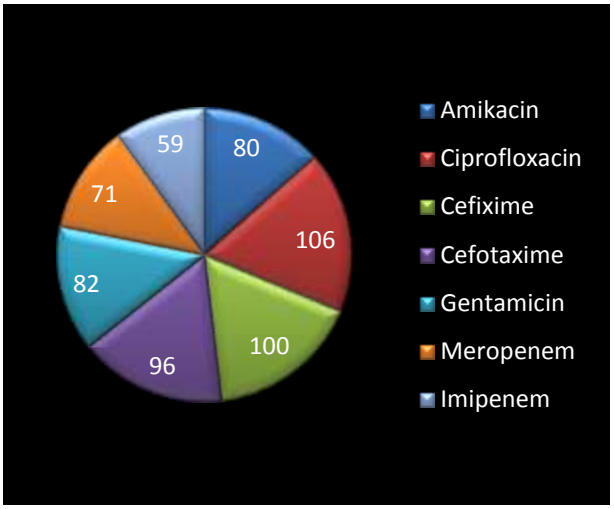
Among 200 samples, The Highest no.of samples were taken from the age group 41-50yrs(45) followed by 51-60yrs (38).Minimum no.of samples are collected from the age group 71-80 yrs(6).

3.prevalance of Enterobacteriaceae in total clinical samples



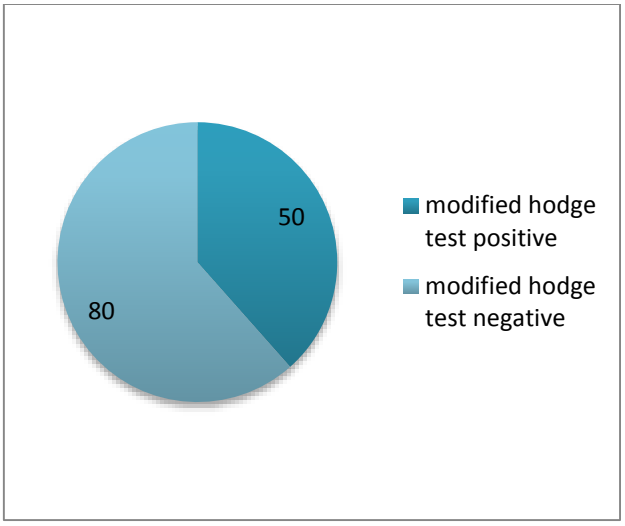
Among the clinical samples collected, highest isolated enterobacteriaceae is Escherichia coli(102) followed by Klebsiella sps(88). Citrobacter sps, Enterobacter sps,Pproteus sps isolation was lowest.5,2,3, respectively.

4.Prevalnce of Carbapenem resistance of Enterobacteriaceae isolates by Disk diffusion method



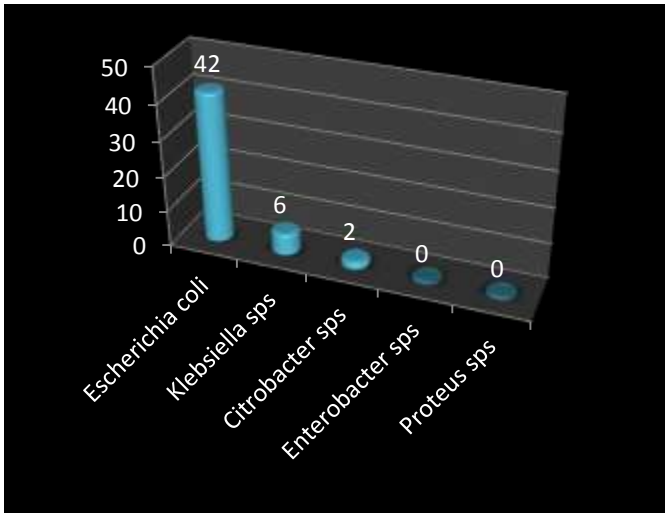
Out of 200 samples processed,130 samples showed resistance to carbapenem antibiotics.Among them Meropenem resistance was 42.3%(55 isolates),Imipenem 39%(50 isolates),Imipenem+Meropenem 19%(25 isolates)

5. Modified Hodge Test



Among 130 carbapenem resistance isolates,50 Enterobacteriaceae showed Modified Hodge test positive and 80 isolates were Modified Hodge test negative.

6.Prevalance of Carbapenemase resistance among Enterobacteriaceae isolated by Modified Hodge test



Among 50 Modified Hodge test positive , Eschericia coli was the highest (42)of 84%.followed by Klebsiella sps (6) of 12%.Ccitrorobacter sps(2) showed 4% resistance to Carbapenems. Enterobacter sps, Proteus sps showed 100% sensitive to Carbapenems.

DISCUSSION:

For more than two decades Carbapenems have been considered as the treatment of last resort for managing multidrug resistant infections caused by Enterobacteriaceae. The increased occurance of MDR and resistance to Carbapenems has been a

cause of concern to public health. Klebsiella pneumonia and Escherichia coli in particular are most often associated with Carbapenem resistance.

The present study documents the Carbapenem resistance among Enterobacteriaceae in a total of 200 samples collected from patients admitted in Narayana General Hospital, Nellore.

Among the 200 samples processed majority of samples from Males 125(62.5%)samples and from Females 75(37.5%)samples.(Table-1)

In the 200 samples collected , maximum no.of samples were collected from the age group 41-50yrs(22.5%) were 45 samples.(Table-2)

Among the Enterobacteriaceae isolates Carbapenem resistance by disk diffusion method were Meropenem 55 (42.3%), Imipenem 50 (39%), both Imipenem and Carbapenem 25(19.23%).Similar findings were seen in Gomty Mahajan et al in 2011,42(31.8%) isolates were Meropenem resistance.(Table-4)

Among Enterobacteriaceae 50(39%)were positive for Carbapenemase production by Modified Hodge test(Table-5). Similar data was seen in Gomaty Mahajan et al in 2011, Out of 42 carbapenem resistance isolates,47.6%(20/42) isolates were found to produce Carbapenemase enzyme by Modified Hodge test and remaining 22 were found to be negative(10). S.Rai et al, showed out of 102 isolates ,92 isolates were positive for Carbapenemase production by Modified Hodge test(11). Priya Datta et al showed 19 isolates positive by Modified Hodge test out of 330 isolates(12). K.V Ramana et al showed 385 positive isolates of Carbapenemase production out of 1072 isolates by Modified Hodge test(13).

Escherichia coli remains remains at the top with 42(84%) followed by Klebsiella 6(12%), Citrobacter sps 2 (4%),among Entero bacteriaceae producing Carbapenemase detected by Modified Hodge test(Table-6). Similar findings wrere repoted by Amjad et al,he reported E.coli 38%,Klebsiella sps 17%,Citrobacter sps 2%(14).

Since Carbapenemase resistance is mediated by several mechanisms, cross resistance is commonly seen among related antibiotics. Although there are various specific tests to detect the underlying mechanism of Carbapenemase resistance, Modified Hodge test is simple, easy to perform and cost effective test which can be used conveniently used to screen Carbapenemase production

REFERENCES:

1.Queenam AM,Bush K.Carbapenemases the versatile β – lactamases.Clin Microbial Rev.2007;20:440-58

2.Spencer BA,Richard III JW,McCoy LF,Carino E,Washington J,Edgar P et al.Antibiotic activity of Meropenem on Multi resistant gram negative organisms.J Burns Surg 2002;1:12-17

3.Jones RN,Sarder HS,Fritsche TR.Comparative activity of Doripenem and three other carbapenems tested against gram negative bacilli with various beta lactamase resistant mechanism.Diagn Microbiol Infect Dis 2005;52:71-74.

4.Hellinger W,Brewe N.Carbapenems and monobactams: Meropenem and Aztreonam.Mayo Clinic Proc 1999;74:420-34

5.Deshpande LM,Fritsche TR,Jones RN.Molecular epidemiology of selected multidrug resistant bacteria: A global report from the SENTRY Antimicrobial surveillance program.Diagn Microbiol Infect Dis.2004;49:231-6.

6.Taneja N,Aharwal SM,Sharma M.Imipenem resistance in nonfermentors causing nosocomial urinary tract infections.Indian J Med Sci 2003;57:294-9

7.Finkelstein R,Rabina G,Kasis I,Mahamid I.Device associated,device – day infection rates in an Israeli adult general intensive care unit.J Hosp Infect 2000;44:200-5

8.Tenover CF.Mechanisms of antimicrobial resistance in Bacteria.AM J Med 2006;119(6A)

9.Lee K,Chong y,Shin HB,Kim YA,Yong D,Yum JH.Modified Hodge and EDTA – disk synergy tests to screen metallo beta lactamase producing strains of pseudomonas and acinetobacter species.Clin Microbiol Infect 2001;7:88-91

10. Gomty Mahajan, Sheevani Sheemar, Shashi Chopra (2011) Carbapenem resistance and phenotypic detection of carbapenemases in clinical isolates of acinetobacter baumanii Indian Journal of Medical Sciences2011;1:18-25.

11. S Rai, V Manchanda, NP Singh, IR Kaur**.** Zinc-dependent carbapenemases in clinical isolates of family Enterobacteriaceae. Indian Journal of Medical Sciences, 2011;3: 275-279.

12. Priya Datta**,** Varsha Gupta**,** Shivani Garg**,** Jagdish Chander .Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from north India.Indian Journal Of Pathology And Microbiology 2012,3:357-360.

13. KV Ramana**,** Ratna Rao**,** CH. V Sharada**,** MA Kareem**,** L Rajashekar Reddy**,** MS Ratna Mani.Journal Of Natural Science And Biology 2013;2:346-348

14.Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol 2011 December;3(4):189-93.