Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4176
DOI URL: http://dx.doi.org/10.21474/IJAR01/4176

RESEARCH ARTICLE

CARBAPENEM RESISTANT GRAM NEGATIVE ISOLATES FROM BURN WOUND INFECTION IN A TERTIARY CARE CENTRE.

Dr. Lancy J, Seema A Nayar, Dr. Sam Mathew, Dr.Thara Ann Jose and Dr. Sahira.
Govt. Medical College, Thiruvananthapuram, Kerala.

Manuscript Info

Manuscript History

Received: 01 March 2017
Final Accepted: 01 April 2017
Published: May 2017

Key words:-
carbapenem, Carbapenemase, Modified Hodge Test, Multidrug resistant bacteria.

Abstract

Carbapenems are beta lactam antibiotics with the broadest spectrum of activity used for the treatment of infections caused by multi drug resistant (MDR) strains of gram negative bacteria especially in hospitalized people. Carbopenems are often considered as the last line of therapy. During the last few years, carbapenem resistance has been increasingly reported among the group of Enterobacteriance and nonfermenters. This may be attributed to the production of carbopinemases / metallo-beta-lactamases (MBLs). Most often carbopenem resistant bacteria have been reported from urinary infection, septicaemia wound infection and pneumonia. The present study shows the high prevalence rate of such strains (67.92%) among the isolates obtained from burn wound infection at Govt. Medical College, Thiruvananthapuram. A total no. of 22 patients were admitted following blast injury at Puttigal, a village at Kollam District on 10-04-2016 Kerala, from whom 56 samples of exudates and 10 samples of blood were collected from third day of admission. The age group of the patients being 16 yrs to 70 yrs and the extent of bruns ranging from 20% to 70%. Majority of the bacterial isolates obtained in culture from exudates and blood were multidrug resistant (75%). The predominant species was pseudomonas aeruginosa (39.58%) carbapenemase production wasdetected by phenotypic screening methods such as Imepenem resistance by disc diffusion technique by Kirby – Bauer Method, combined disc test, modified Hosge test and E-test. Of the total no. of 53 multidrug resistant gram negative isolates obtained in the study, 100% of E.coli, 71.43% of pseudomonas aeruginosa, 70.59% ofAcinetobacter baumanii and 53.85% of klebsiella pneumoniae, were carbapenemase producers. All the strains were susceptible to Colistin and polymyxins. The high prevalence of carbapenemase resistant strains highlights the need for active approach by both clinician and microbiologist to initiate infection control measures to prevent their dissemination.

Introduction:-

Burn wound infections rank among the most serious forms of trauma resulting in anatomic, physiologic and immunologic stresses especially when burn involves >50% of the total body surface area. Immunosuppression

Corresponding Author:- Dr. Lancy J.
Address:- Govt. Medical College, Thiruvananthapuram, Kerala.

resulting from severe burn injuries predispose to many infectious complications. The organisms causing burn wound infections may be endogenous or exogenous, which include bacteria, fungi and viruses. Gram negative bacteria, accounts for >50% of burn wound infections. Nowadays, there is increased incidence of multi-drug resistant gram negative bacteria reported from hospitalised patients. Carbapenems were used to treat such infections. Concern has arisen in recent years, over increasing resistance to carbapenems as there are few therapeutic options. So rapid detection of carbapenemase production in the multi-drug- resistant gram negative isolates is mandatory and the tests should be done as a routine in the clinical laboratories in future.

Aim of the Study:-

1. To isolate and identify the bacterial pathogens causing burn wound infection
2. To study the antibiotic susceptibility pattern of the isolates obtained from clinical specimens collected from burn wound infection.
3. To detect carbapenemase producing bacteria among the gram negative multidrug resistant strains obtained in the study by phenotypic methods

Materials and Methods:-

Study design	:	Descriptive study
Study group	:	Patients admitted on 10-04-2016 following blast injury and developed burn wound infection.
Study period	:	2 months (from the day of admission to discharge)
Study setting	:	Dept. of Microbiology, Surgery, orthopaedics, Plastic surgery, Neurosurgery and Dermatology, Govt. Medical College Hospital, Thiruvananthapuram

Methodology:-

Collection of samples:-

Exudates from the burn wound infection sites were collected using sterile double swabs after cleaning the site with sterile normal saline on the third day after admission in the hospital. 2 samples of blood each 5 ml was collected under a septic precautions from patients with suspected sepsis and directly inoculated into the blood culture bottle containing 50ml of Brain Heart Infusion broth. Samples were transported to the 24 hrs. clinical Microbiology laboratory at Govt. Medical College Hospital, Thiruvananthapuram immediately after collection.

Methodology:-

Processing of samples:-

Gram straining of the exudate was done in the laboratory with the material collected in one swab to study the morphology and gram reaction of the bacteria and to find out the presence of pus cells. The other swab was used for inoculating the culture plates- Blood agar, Chocolate agar, Mac Conkey agar and Mannitol salt agar and blood cultures are incubated at 37^0C. After overnight incubation the culture plates are examined for the appearance of colonies. Smear was prepared from a single colony and gram staining was done. Identification of the organism was done by the colony morphology, gram staining and relevant biochemical reactions.

Identification of gram negative Bacteria:-

Gram negative bacilli are identified by gram staining, colony morphology on blood agar and Mac Conkey agar, oxidase test, catalase test and other relevant biochemical reactions.

Pseudomonas aeruginosa was identified by positive High Leifson's oxidative Test and Arginine dihydrolasetest.Acinetobacterbaumanni was identified by oxiolative reaction on O/F medium and pink colonies on 10% lactose medium. E.coli and Klebsiella pneumoniae were identified by lactose fermenting colonies on Mac Conkey agar and relevant biochemical reactions.Antibiotic sensitivity testing of the isolates was performed by Kirby-Bauerdisc diffusion methods using Muller – Hinton agar according to CLSI guidelines.

Quality control:E.Coli – ATCC 25922, Pseudomonas aeruginoa – ATCC 27853.

Phenotypic screening methods for MBL production:-

Screening for metallo-beta-lactamase production was performed in Imepenem resistant isolates of pseudomonas aeruginosa, Acinetobacter baumanii, Klebsiela pneumonia and E.coli.

Screening for carbapenem Resistance:-
The isolates tested by Kirby – Bauer disc diffusion method using Imepenem (10ug) and meropenem (10ug) discs on Mueller – Hinton agar with zone diameter 19mm were further checked for the production of metallobetalactamases.

Combined Disc Test:-
Test organism was inoculated on to Muller – Hinton agar as recommended by CLSI guidelines. Two imepenem discs (10ug) were placed on the platewith a distance of 25mm apart. EDTA solution was added to one of them. The zone of inhibition around Imepenem disc and imepenem EDTA disc were compared afterovernight incubation at 37^0C. An increase in zone diameter of 7mm or more around the imepnenem – EDTA disc as compared to that of the Imepenem disc alone was considered positive for MBL production.

Modified Hodge test – method – I:-
Imepenem (10µg) disc was placed on Muller – Hinton agar plate inoculated with 0.5 McFarland turbidityE.Coli ATCC 25922 strain. The test strain was streaked radically from the edge of the disc to the periphery of the plate. After overnight incubation at 37^0C, the presence of a distorted inhibition zone indicated thecarbapenem – hydrolysing activity of the test strain.

Modified Hodge Test (MHT) – Method II:-
0.5 MacFarland turbidity suspension of the E.coli ATCC 25922 is prepared and inoculated onto Muller Hinton Agar plate as for routine disc diffusion procedure. Allow the plate to dry. Place a 10 ugErtapenem susceptibility disc in the centre of the test area. After that in a straight line, streak the test organism from the edge of the plate. Repeat the same with QC strain in another direction (positive control) and negative control incubate overnight at 35 degree C +/- 2 degree C for 24 hrs.

Positive: After 24 hours of incubation, examine the plate for clover – leaf type indentation at the intersection of the test organism and the E.coli 25922, within the zone of inhibition of the carbapenem susceptibility disc.

Negative : if there is not growth of E.coli 25922 along the test organism growth streak within the disc diffusion Zone the test is considered as negative for carbapenemase production.

Phenotypic confirmation of MBL production by E-Test:-
An E-test strip containing Imepenem and Imepenem EDTA was used to do the test. A reduction in MIC of Imepenem of three or more two – fold dilution in the presence of EDTA was interpreted as a positive test indicating MBL production.

Results:-

A total no. of 78 samples were collected under sterile precautions and sent to the 24 hours central clinical microbiology Laboratory at Govt. Medical College, Thiruvananthapuram.

Table I:- Samples Analysis.

Sl.No.	Nature of specimen	Total No.	Culture positives
1	Exudate	56	44(78.57%)
2	Blood	10	3(30%)
3	Tracheal aspirate	30	2 (66.66%)
4	Sputum	3	1 (33.33%)
5	Urine	3	1 (33.33%)
6	Central line tip	1	1 (100%)
7	Bone tissue	1	0 (0%)
8	BAL fluid	1	0 (0%)
	Total	78	52 (66.66%)

Table 2:- Distribution of cases according to gender.

Gender	**No . of percentage**
Male	19 (86.36%)
Female	3 (13.64%)
Total	22 (100%)

Table 3:- Distribution of cases according to age.

Age group	No . of percentage
12-20	2(9.09%)
21-30	4(18.18%)
31-40	5 (22.73%)
41-50	7 (31.82%)
51-60	3(13.64%)
61-70	1(4.55%)
Total	22 (100%)

Table 4:- Distribution of cases according to extent of burns.

Extent	No. and percentage
1-10%	1 (4.55%)
11-20%	3 (13.64%)
21-30%	2 (9.09%)
31-40%	43 (13.64%)
41-50%	6 (27.27%)
51-60%	5 (22.73%)
61-70%	0 (0%)
71-80%	2 (9.09%)
Total	22 (100%)

Table 5:- Monomicrobial isolates.

Sl. No.	Organism	No. of percentage
1	Pseudomonas aeruginosa	16 (40%)
2	Acinetobacter baumanii	11(27.5%)
3	Klebsiella pneumonia	10 (25%)
4	MRSA	1 (2.5%)
5	Staphylococcus aureus	1 (25%)
6	Enterococci	1 (25%)
	Total	40 (100%)

Table 6:- Polymicrobial isolates

Sl. No.	Organisms	No. of percentage	Total isolates
1	Pseudomonas aeruginosa and MRSA	2(50%)	Pseudomonas (3)
2	Acinetobacter baumanii and MRSA	1 (25%)	MRSA (3)
3	Acinetobacter baumanii and Pseudomonas aeruginosa	1 (25%)	Acinetabacterbanmanii (2)
	Total	4 (100%)	8

Table 7:- Blood culture isolates.

Sl. No.	Organism	No. of percentage
1	Pseudomonas aeruginasa	1 (33.33%)
2	Acinetobacter baumanii	1 (33.33%)
3	E.Coli	1 (33.33%)
Total		3 (100%)

Table 8:- Analysis of other clinical specimens and isolates.

Sl. No.	Sample	Total No.	Culture positive	Culture negative
1	Central line tip	1	1 (100%)	0%
2	Tracheal aspirate	3	2 (66.66%)	1 (33.33%)
3	Sputum	3	1 (33.33%)	2 (66.66%)
4	Urine	3	1 (33.33%)	2 (66.66%)
	Total	10		

Table 9:- Carbapenemace producers among multi-drug resistant gram negative isolates (n=53)

Sl. No.	MDR bacteria	Total no. tested	MBL positive
1	Pseudomonas aeruginosa	21(39.22%)	15(71.43%)
2	Acinetobacter baumanii	17(32.01%)	12 (70.59%)
3	Klebsiella pneumoniae	13(24.53%)	7(53.85%)
4	Escherichia coli	2(3.77%)	2(100%)
	Total	53(100%)	36(67.92%

Table 10:- Antibiotic susceptibility pattern of the multi-drug resistant gram negative isolates.

	MDR Strain	Pseudomonas aeruginosa n=21		Acinetobacter baumanii N=17		Klebsiella pneumonia n=13		Escherichia coli n = 2
Antibiotic tested	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
1. Ampicillin (10µg)	NT	NT	0	17 (100%)	NT	NT	0	2(100%)
2. Gentamicin (10µg)	0	21 (100%)	0	17 (100%)	0	13(100%)	0	2(100%)
3. First generation cephalosporius	NT	NT	0	17(100%)	0	13(100%)	0	2 (100%)
4. Amikacin (30ug)	0	21(100%)	0	17(100%)	0	13(100%)	0	2 (100%)
5. Ciprofloxazin (5ug)	0	21(100%)	0	17(100%)	0	13(100%)	0	2 (100%)
6. Third generation cephalosporius	7(33.3%)	14(66.66 %)	0	17(100%)	12(15.38 %)	11(84.62 %)	0	2(100%)
7. Cefaperazonesulbactum (75/30ug)	4(19.04%)	17(80.85 %)	0	17(100%)	15.38%)	11(84.62 %)	0	2(100%)
8. Piperacillin Tazobactum (100/10ug)	1(19.04%)	17 (80.95%)	NT	NT	NT	NT	NT	NT
9. Imeperem (10ug)	6(28.57%)	15(71.43 %)	5(29.41%)	12(70.69 %)	6(46.15 %)	7(53.85 %)	0	2(100%)
10. Meropenem (10ug)	6(28.57%)	15(71.43)	5(29.41%)	12(70.59 %)	6(46.15 %)	7(53.85 %)	0	2(100%)
11. Tigecycline (5ug)	6(28.57%)	15(71.43 %)	5(29.41%)	12(70.59 %)	6(46.15 %)	7(53.85 %)	0	2(100%)
12. Colistin (10ug)	21 9100%)	0	5(29.41%)	12(70.59 %)	6 (46.15%)	7(3.85%)	0	2(100%)
13. Polymyxin (300 units)	21(100%)	0	5(29.41%)	12(70.59 %)	6(46.15 %)	7(53.85 %)	0	2(100%)

Among the third generation cephalosporins, ceftazidime was used fro testing pseudomonas aeruginosa and ceftriaxone was used for testing Acinetobacter baumanii, klebsiella pneumoniae and E.coli.

Table 11:- Detection of carbopenemase producing isolates in MDR gram negative bacteria (n=36)

Sl. No.	Organism	Imepenem resistance by Disc diffusion method	Combined disc method	Modified Hodge test	E-test
1	Pseudomonas aeruginosa	15(41.67%)	15(41.67%)	15(41.67%)	15(41.67%)
2	Acinetobacter baumanii	12 (33.33%)	12 (33.33%)	12 (33.33%)	12 (33.33%)
3	Klebsiella pneumoniae	7(19.44%)	7(19.44%)	7(19.44%)	7(19.44%)
4	Escherichia coli	2(5.55%)	2(5.55%)	2(5.55%)	2(5.55%)
	Total	36(100%)	36(100%)	36(100%)	36(100%)

Discussion:-

The present study was aimed at identifying carbapenem resistance in gram negative bacterial isolates from clinical samples received at the 24 hrs clinical Microbiology Laboratory at Govt. Medical College Hospital, Trivandrum from patients admitted with burn wound infection following blast injury. A total no. of 78 samples were collected from 22 patients. Culture positivity was 78.57% in exudates and 30% in blood culture. Among the culture positive, 90.90% were monomicrobial and 9.09% were polymicrobial. Of the total no. of 59 clinical isolates obtained in the study. 53 (89.83%) were gram negative bacteria and only 6 isolates (10.17%) were gram positive bacteria. All the gram negative bacterial isolates were multi-drug resistant (89.83%). Carbapenemase production was detected by different phenotypic methods. Among the gram negative bacterial isolates. Screening methods like antimicrobial susceptibility testing by Kirby- Bauer disc diffusion method using Imepenem (10ug) and menopenem (10ug) discs as per CLSI guidelines, combined disc test and Modified Hodge test.Carbapenemase producers were confirmed by E-Test.Among the carbapenemase producers, the predominant species were pseudomonas aeruginosa (41.67%) Klebsiella pneumoniae (19.44%) and E.coli 5.55%). The high prevalence of multi drug resistant gram negative organisms is considered as a warning sign for the emerging spread of antibiotic resistance which requires urgent need for implementation of strict antibiotic policy and infection control measures.

The high percentage of MDR isolates is probably due to empirical use of broad specimen antibiotics and nonadherence to hospital antibiotic policy. Once MDR strains become established in the hospital environment they can persist for months. Therefore careful microbiological surveillance and in vitro testing before the start of antibiotic therapy an restrictive antibiotic policy may be of great help in prevention and treatment of MDR isolates in burns units and thus reduction of overall infections related morbidity and mortality.

In spite of may phenotypic tests, PCR is considered as the gold standard for testing carbapenemase resistant strains which is not available in the routine diagnostic laboratories.All the MDR strains of gram negative isolates in our study were sensitive to colistin. The growing prevalence and difficulty of treating such multidrug resistant Enterobacteriaceae has led to a renaissance of the use of antibiotics such as colistin which was discovered in the 1950s but rarely used until recently due to unattractive levels of toxicity.The prevalence rate of carbapenem resistance in our study was 67.92% among the patients admitted with burn wound infection. Theprevalence rate is quite high when compared to many other studies. More recently many countries have experienced a dramatic upswing in the prevalence of carbapenem resistant Enterobacteriaceae with the highest prevalence rate of 60%.

Conclusion:-

Carbapenemases are diverse enzymes that vary in their ability to hydrolycecarbaoenems and other betatactums. Detection of cabapenemase is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection associated mortality. Among the beta lactamases, the carbapenemases especially tranferablemetallobeta-lactamases are the most feared because of their ability to hydrolyse virtually all drugs in that class including the carbapenems. The transmissible enzymes can be acquired unpredictably by important nosocomial pathogens such a pseudomonas aeruginosa, acinetobacterbaumanii and members of the family Enterobacteriaceae. In addition to their resistance to all beta-lactams, the MBL producing strains are frequently resistant to aminoglycosides and fluroquinolones. However they are usually susceptible to polymyxins. The rapid detection of cabapenemase production is necessary to initiate effective infection control measures to prevent their dissemination.

References:-

1. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clinical Micro boil. Review 2007:20.
2. Clinical Laboratory standards Institute / NCCLS performance standards for Antimicrobial susceptibility Testing:18th Informational Supplement (2012) CLSI /NCCLS M100-S18.
3. Oneman EW. Colour Atlas and Text book of Diagnostic Microbiilogy. 6th edition. Philadelphia:Lippinecott. Williams and Wilkins; 2006.p.211-302.
4. Galani I, Rekatsina PD et al, Evaluation of different laboratory tests for the dectection of metallo-beta-lactamase production in Enterobacteriaceae. J. Antimicrob. Chemotherapy 2008:61:548-553.
5. Manoharan A., Chatterjee S, Mathai D; SARI study group. Detection and characterization of metallo-beta-lactamase producing pseudomosas aeruginosa. Indian J Medmicrobiol 2010:28:241-2.
6. Nagaraj S. et al. Carbapenem resistance among Escherichia coli and Klebsiella Pneumoniae in a tertiary care hospital in South India. Indian J Med. Microbiol 2012:30:93-95.
7. Sonal Asthana, PurvaMathur and VibhorTak. Detection of Carbapenemase production in gram negative bacteria. Journal of Laboratory physicians: 2014 "6(2) 69-75.
8. Patrice Nordmann, Laurent Poirel and LourentDortet. Rapid detection of Carbapenemase producing Enterobacteriaceae. Emerging Infections Disease (2012) vol:18:N0.9.
9. Deshpande L, et al 2006. Occurrence and characterization of Carbapenemase- producing Enterobacteriaceae: report from the Sentry Antimicrobial Surveillance Program 9200-2004. Microb-Drug Resist 12:223-230.
10. Landmasn D. et al. 2005. Evaluation of techniques for detection of Carbapenem- resistant Klebsiella pneumonia in stool Surveillance Klebsiella. J. Clin. Microbiol. 43:5639-5641.
11. Nordmann P et al. Global spread of carbapenemase producing Enterobacteriaceae. Emerg Infect Diseases 2011;17:1791-1798.