Original Article

Phenotypic Detection of Carbapenemase Producing Drug Resistant Gram Negative Bacteria in Rajshahi Medical College Hospital, Bangladesh.

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Abstract

Background: Carbapenem resistance is a major and on-going public health problem globally and locally. It occurs mainly among Gram negative pathogens. Most of the Carbapenemase producing bacteria are multidrug resistant bacteria including 3rd generation of cephalosporin and carbapenems. **Objective:** To isolate and identify Carbapenemase producing drug resistance gram negative bacteria with their antibiogram in Rajshahi Medical College Hospital (RMCH). **Methods:** A Cross sectional descriptive study was carried out in different surgical units of RMCH. A total of 250 wound swabs collected from the respective patients as sample referred to Microbiology department of Rajshahi Medical College for investigation. The specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 370 C for 24 hours. The isolated bacteria were identified by their colony morphology, pigment production, haemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests. Susceptibility tests of the bacterial isolates were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media. Carbapenemase producing bacteria were identified phenotypically by modified Hodge test. **Results:** Out of a total 250 samples, culture yielded growth were 213(85.2%) and 37(14.8%) had yielded no growth. Female were predominant 146(58.4%) in comparison to male 104(41.6%) with a male female ratio 1: 1.4. Maximum 47.2% cases were within the age group of 19-30 years. Among the culture positive isolates, Gram negative organisms were higher (58.8%) than gram positive (41.2%). S. aureus was the predominant organism 71(30.8%) followed by P.aeruginosa 67(29%), E.coli 43(18.7%) and Klebsiella spp. 20(8.7%). Among 67 isolated P. aeruginosa and 82 isolated Enterobacteriaceae, 18(26.8%) and 14(17%) were carbapenemase producers respectively. **Conclusion:** It may be concluded that, most of the isolated Carbapenemase producing gram negative bacteria are multidrug resistant and causes complicated infections. So, It is necessary to perform sensitivity test prior to start antimicrobial therapy for proper management and routine screening test for carbapenemase producing should be practiced to detect the carriers and treat them adequately and also reduce and control the spread of antimicrobial resistance.

Key words: Gram negative bacteria; Carbapenemase producing bacteria; Multidrug resistant bacteria, Modified Hodge test.

Introduction

Antimicrobial resistance is the ability of bacteria to resist the effects of an antimicrobial agent that reduces the effectiveness of drugs designed to cure or prevent infections. Antimicrobial resistance is mounting threat to the control of infectious diseases both globally as well as locally in Bangladesh. Infections with resistant microbes not only results in greater morbidity and mortality, but also increase the health care cost.[1]

Carbapenemases are specific β-lactamases with the ability to hydrolyze carbapenems. These periplasmic enzymes hydrolyze carbapenems preventing the drug from reaching the PBP target. In addition, overproduction of class C β-lactamases (Amp C betalactamases), can lead to carbapenem resistance, especially when combined with other resistance mechanisms (e.g., porin loss). Adequate detection of carbapenemase-producing GNB is crucial for infection control measures and appropriate choice of antimicrobial therapy.[2]

The prevalence of carbapenem resistant Enterobacteriaceae and Pseudomonas aeruginosa in Bangladesh 9.8% and 53.7%, in India 44.3% and 10%, in Italy 44%, and 19% , in US 18%.[3,4,5]

Regarding the antimicrobial resistance rates of carbapenemase producing Enterobacteriaceae in Bangladesh to third generation cephalosporins 80%-100%, to fluroquinolones, aminoglycosides, monobactum 60%-80%, to carbapenem 10%-30%.6 The antimicrobial resistance rates of carbapenemase producing Pseudomonas aeruginosa in Bangladesh to third generation cephalosporins 90%-100%, to fluroquinolones, aminoglycosides, monobactum 65%-80%, to carbapenem 15%-30%.[7,8]

A number of simple phenotypic tests, most of them in the disk diffusion format, have been described and evaluated as methodologies for the specific detection of carbapenem resistant organisms. Phenotypic confirmation may be performed using one or two methods, the Modified Hodge Test (MHT) and the carbapenemase inhibition tests. MHT is used for detection of diffusible carbapenemases, and the inhibition tests are used to distinguish between the different classes of carbapenemases.[9]

Knowledge of the Carbapenemase producing drug resistant gram negative bacteria in and extent of drug resistance of these isolates against different antimicrobial classes in a Rajshahi region will therefore be useful in order to provide locally applicable data and to guide selection of appropriate antibiotics for empirical therapy.[10]

Methods

A Cross sectional descriptive study was carried out in different surgical units of Rajshahi Medical College Hospital (RMCH). Patients with surgical wound in surgical indoor of RMCH constituted the study population. A total of 250 wound swabs collected from the respective patients during the period of July 2017 to June 2018 as samples referred to Microbiology department of Rajshahi Medical College for culture and further investigations. Antimicrobial susceptibility of 231 bacterial isolates from wound swab specimens were analysed in the present study. All the specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 370 C overnight. If culture plates showed the growth of bacteria then it was identified by their colony morphology, pigment production, haemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests.

The identified bacteria were sub cultured and processed for drug sensitivity test. Susceptibility tests of the bacterial isolates with different antimicrobials were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media by commercially available antimicrobial disks. Carbapenemase producing bacteria were identified phenotypically by modified Hodge test.[11]

Detection of carbapenemase:

Screening for carbapenemase: The 2013 recommendations of Clinical and Laboratory Standard Institute (CLSI), isolates with reduced susceptibility to meropenem and imipenem (diameters of zones of inhibition ≤ 13 mm) by disk diffusion method were screened for the production of carbapenemase.[12]

Modified Hodge Test:

Procedure (CLSI 2017):

1. An inoculum of 5ml E.coli (ATCC 25922) was prepared with sterile normal saline and standardized by 0.5 McFarland standard.
2. The inoculum was diluted by adding 4.5ml of sterile normal saline with 0.5ml of standard inoculum.
3. The diluted inoculum of E.coli (ATCC 25922) was spread to MH agar plate with a cotton swab.
4. The plate was allowed to dry 3-10 minutes.
5. Then an imipenem disk (10µg) was placed at the centre of the plate.
6. A straight line was drawn with the help of an inoculating wire loop containing identified test bacteria from margin of imipenem disk to the of the MH plate.
7. Four identified test bacteria were tested in a single MH Plate and incubated overnight at 37oC in a aerobic condition.

Reading and interpretation of results:

Reading was taken after 24 hours to see clover leaf type of indentation at the intersection of the bacteria and E.coli (ATCC 25922) within the zone of inhibition.c

Positive result was indicated by the presence of clover leaf like indentation of the E.coli (ATCC 25922) along the streak line of test bacteria within the zone of inhibition.

Negative result showed no growth of the E.coli (ATCC 25922) along the test bacterial streak within the zone of inhibition.

Ethical clearance for the study was taken from the Instutional Review Board and concerned authority, Rajshahi Medical College & Hospital.

Results

Out of 250 samples, 213(85.2%) samples were culture positive while 37(14.8%) samples were culture negative. A total of 231 organisms were isolated from the culture. Among them gram negative bacteria were predominated, 136(58.8%) and gram positive bacteria were 95(41.2%).

Table I: Age and sex distribution of different clinical samples (N=250).

Age (Years)	Number of samples cultured (%)	Male N(%)	Female N(%)	Culture-positive cases (%)	Male N(%)	Female N(%)
19-30	118(47.2)	34(13.6)	84(33.6)	98(39.2)	29(11.6)	69(27.6)
31-40	53(21.2)	29(11.6)	24(9.6)	46(18.4)	26(10.4)	20(08)
41-50	37(14.8)	22(8.8)	15(06)	32(12.8)	20(08)	12(4.8)
>50	42(8.8)	19(7.6)	23(9.2)	37(14.8)	17(6.8)	20(08)
Total	250(100)	104(41.6)	146(58.4)	213(85.2)	92(36.8)	121(48.4)

Accordingly, age and sex distribution of study population is shown in following Table-I. Maximum 118(47.2%) cases {male 34(13.6%) and female 84(33.6%)} were found within the age group of 19-30 years. As a whole, males were 41.6% and females were

58.4% with a male and female ratio 1:1.4. Highest number of culture positive cases were seen in the age group of 19 to 30 years, 98 (39.2%) (Table I).

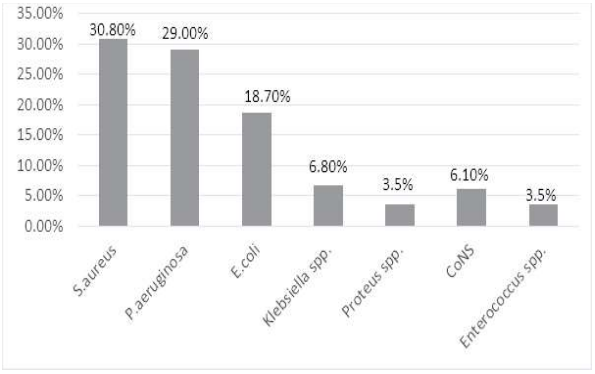

Figure I: Pattern of bacteria isolated from wound swabs (N=250).

Out of 250 samples, total 231 bacteria were identified. S.aureus was 71(30.8%) followed by P.aeruginosa was 67(29%), E. coli was 43(18.7%) and Klebsiella spp. was 20 (8.7%) (Figure I)..

Table II: Frequency of carbapenemase producing bacteria.

Isolates	Total No. of org. Tested	No. of positive org. confirmed by phenotypic method (%)
Carbapenem resistant *Pseudomonas aeruginosa*	67	18 (26.8%)
Carbapenem resistant *Enterobacteriaceae*	82	14 (17%)

Among 67 isolated Pseudomonas aeruginosa 18 (26.8%) were phenotypically confirmed by modified hodge test as carbapenem resistant Pseudomonas aeruginosa and among 82 isolated Enterobacteriaceae, 14(17%) were phenotypically confirmed by modified hodge test as carbapenem resistant Enterobacteriaceae (Table II).

Table III: Antimicrobial resistance pattern among the carbapenemase producing Pseudomonas aeruginosa and Enterobacteriaceae.

Antimicrobial agents	*Pseudomonas aeruginosa* N=(18)	*Enterobacteriaceae* (N=14)
Imipenem	08(44.4%)	06(42.8%)
Azithromycin	17(94.4%)	13(92.8%)
Ciprofloxacin	13(72.2%)	09(64%)
Ceftriaxone	18(100%)	14(100%)
Cefepime	11(61.1%)	10(71.4%)
Piperacillin/tazobactam	07(58.3%)	07(50%)
Meropenem	10(55.6%)	09(64%)
Aztreonam	14(77.8%)	10(71.4%)
Amikacin	08(44.4%)	08(57.1%)
Cefuroxime	18(100%)	14(100%)
Colistin	01(5.6%)	01(7.1%)
Cefixime	18(100%)	14(100%)
Doxycycline	09(66.7%)	11(78.8%)
Amoxiclav	15(83.3%)	12(85.7%)

All the carbapenemase producers were 100% resistant against ceftriaxone, cefuroxime and cefixime. Besides that ciprofloxacin 64% to 72%, amikacin 50 to 57%, meropenem 58 to 64%, aztreonam 71 to 78%, cefepime 61 to 71%, amoxiclav 83 to 86% and azithromycin 90 to 92% resistant. Colistin and imipenem were showed lower resistance 5 to 7% and 42 to 44% against carbapenemase producers respectively (Table III).

Discussion

Out of 250 wound swab samples obtained in the Microbiology lab from various departments of RMCH, Rajshahi for aerobic culture and sensitivity, 85.2% yielded positive culture whereas 14.8% yielded no growth. Study is nearly similar with the study of Nahar *et al.*[13] and Negi *et al.*[14] Study is nearly dissimilar with the study of Begum *et al.*[15] and Khan *et al.*[16] The reason for this high occurrence of culture positivity may be due to the fact that most of the study population were belonged to lower middle and lower socioeconomic

group with poor knowledge about personal hygiene, poor sanitation system in hospital, overcrowding of patients in hospital contribute to high rate of cross infection, inadequate measures for prevention of the spread of resistant pathogen in hospital environment.

Table I showed age and sex distribution of wound infection cases. Among them 104 (41.6%) were male and 146 (58.4%) were female. The female is predominant due to a good number of cases were taken from Obstetrics and Gynae department. The wound infection rate was higher in the female age groups than male. This higher infection cases in female patients may be due to the presence of poor nutrition, co-morbidity, malignancy, immunosuppression and hematological disorders. This study is nearly correspond with the study of Tasnim *et al.*[17] and Sharma *et al.*[18] Study is nearly dissimilar with the study of Khanam *et al.*[19] and Kumari *et al.*[20] Maximum 118 (47.2%) cases were found within the age group of 19-30 years. This study is nearly similar with the study of Tasnim *et al.*[17] and Erku *et al.*[21] This 19-30 age groups are predominant due to a good number of cases were taken from obstetrics and gynae unit and burn unit.

In this study, out of a total 250 samples, gram negative bacteria accounted for higher isolation rate (Gram-positive 41.2% and Gram-negative 58.8%) than gram positive bacteria. This study is nearly similar with the study of Nahar *et al.*[13] and Kaur *et al.*[22] Study is nearly dissimilar with the study of Roy *et al.*[23] and Rai *et al.*[24]

In this study, S.aureusis were the most frequent isolates 71(30.8%). Study is similar with the study of Nahar *et al.*[13] and Kumari *et al.*[20] Finding is dissimilar with Roy *et al.*[23] and Bhatnagar *et al.*[25] The high prevalence of S. aureus infection may be because it is an endogenous source of infection, improper personal hygiene, inappropriate hospital sanitation and contamination of surgical instruments. With the disruption of natural skin barrier S.aureus, which is a common bacterium on surfaces, easily find their way into wounds. P.aeruginosa is the second 67(29%) most common bacterial isolates. This study was similar with the study of Begum *et al.*[15] and Sharma *et al.*[18] This high isolation may be due to the fact that, Pseudomonas is a common nosocomial bacterial agent found in the hospital environment and also causes cross contamination among admitted patients and develops resistant to commonly used antimicrobial agents. Study is nearly dissimilar with the study of Chaudhary *et al.*[26] and Kumari *et al.*[20]

The isolated Pseudomonas aeruginosa and Enterobacteriaceae those were resistant to meropenem and ceftriaxone by producing carbapenemase were further detected by modified Hodge test and found 26.8% and 17% were carbapenemase producers respectively.

Regarding Pseudomonas aeruginosa, this is nearly similar with the study of Subramaniyan *et al.*[8] and Righi *et al.*[5] But study nearly dissimilar with the study of Barai *et al.*[3] and Kaur *et al.*[22]

Regarding Enterobacteriaceae, this is nearly similar with the study of Kaur *et al.*[22] and Dewakar *et al.*[27] But study is nearly dissimilar with the study of Barai *et al.*[3] and Pawar *et al.*[28]

In this study all the carbapenemase producing strains of Pseudomonas aeruginosa and Enterobacteriaceae are 100% resistant to ceftriaxone, cefuroxime and cefixime. Nearly

60%-70% resistance is observed against ciprofloxacin, and aztreonam and relatively lower resistance is observed against colistin, imipenem and meropenem. In other word,Colistin, imipenem and meropenem are effective against carbapenemase producing strains. This study is nearly similar with Farjana *et al.*[7] Subramaniyan *et al.*[8] and Rit *et al.*[29]

It is well established that carbapenemase producing, strains of bacterial infections are now challenging in management. It is necessary to perform sensitivity test prior to start antimicrobial therapy for proper management and routine screening test for carbapenemase producing should be practiced to detect the carriers and treat them adequately and also reduce and control the spread of antimicrobial resistance.

Identification of the risk factors associated with carbapenem resistant bacterial infections is necessary to guide appropriate empirical antibiotic therapy, thus, reducing unfavorable outcomes and morbidity. It is also important that the epidemiology of carbapenem resistance in gram negative bacteria be understood to implement adequate infection control measures.

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