



Original Article

Phenotypic Detection of Carbapenemase-producing WHO-declared Deadliest Drug-resistant Bacteria in the Rajshahi Region

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Abstract

Background: Carbapenem resistance is a major and ongoing public health problem globally and locally. It occurs mainly among Gram-negative pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Most of the carbapenemase-producing bacteria are multidrug resistant, including 3rd generation of cephalosporin and carbapenems. It may be intrinsic or mediated by transferable carbapenemase-encoding genes. This type of resistance gene is already widespread in certain parts of the world, mainly Europe, Asia, and South America.

Objective: To isolate and identify WHO-declared carbapenemase-producing deadliest drug resistance bacteria with their antibiogram in the Rajshahi region.

MATERIALS METHOD: Cross sectional descriptive study was done from July 2017 to June 2018. Wound swab was collected in different surgical and burn units of Rajshahi Medical College Hospital. The specimens were inoculated in blood agar, nutrient agar, and MacConkey's agar media and incubated aerobically at 37^o C for 24 hours. 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 by using the modified Hodge test.

Results: Out of the total 250 samples, culture yielded growth in 213(85.2%) cases, and 37(14.8%) yielded no increase. Females were predominant 146(58.4%) in comparison to males 104(41.6%), with a male-female ratio of 1: 1.4. A maximum of 47.2% of cases were between 19-30 years old. 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* 47(20.3%), *E.coli* 43(18.7%), and *Acinetobacter baumannii* 07 (3%). Among seven isolated *Acinetobacter baumannii*, 47 isolated *P. aeruginosa*, and 82 isolated *Enterobacteriaceae*: 6(85.7%), 33(70.2%), and 53(64.6%) were MDR; and 4(57.1%), 12(25.5%) and 14(17%) were carbapenemase-producers respectively.

Conclusion:

Most of the isolated carbapenemase-producing bacteria are multidrug resistant, and they tend to cause complicated infections. In addition, the expression of specific virulent factors, difficulty in diagnosis, and the non-availability of newer generation antibiotics make them one of the deadliest bacteria.

Keywords: Gram-negative bacteria; Carbapenem; Carbapenemase-producing bacteria; Multidrug resistant bacteria.

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Introduction

Antimicrobial resistance is a growing global public health threat that seriously affects the

management of infectious diseases. In recent years, infections due to multidrug-resistant bacteria, mortality, and morbidity rates are being increased significantly.¹ It is estimated that each

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year about 7,00,000 death occurs due to diseases caused by antibiotic-resistant bacteria, and that could be 10 million by the year 2050.² Each year, over 25,000 deaths in the European countries, 23,000 in the United States, 38000 in Thailand, about 80,000 in China and 58000 in India were reported from multidrug resistant bacterial infections.^{3,4}

On 27th February 2017, WHO published the list of antibiotic-resistant 12 families of bacteria that pose the greatest threat to human health. These are named the deadliest drug-resistant bacteria. Antibiotics towards those the listed bacteria showed resistance to highly effective multiple antibiotics, including carbapenems and third-generation cephalosporins.² The list is divided into three urgency categories- critical, high, and medium; representing how badly we need new antibiotics to treat their respective superbugs.

Priority –1: CRITICAL

1. *Acinetobacter baumannii*- carbapenem resistant
2. *Pseudomonas aeruginosa*- carbapenem resistant
3. *Enterobacteriaceae*-carbapenem resistant, ESBL producing.

Priority -2: HIGH

1. *Enterococcus faecium*- vancomycin resistant
2. *Staphylococcus aureus*- methicillin resistant, vancomycin- intermediate and resistant
3. *Helicobacter pylori*- clarithromycin resistant
4. *Campylobacter spp.* –fluoroquinolone resistant
5. *Salmonella* –fluoroquinolone resistant
6. *Neisseria gonorrhoeae*-3rd generation cephalosporin resistant, fluoroquinolone resistant.

Priority-3: MEDIUM

1. *Streptococcus pneumoniae*-penicillin-non-susceptible
2. *Haemophilus influenzae* - ampicillin resistant
3. *Shigella spp.*- fluoroquinolone resistant

The prevalence of carbapenem resistant *Enterobacteriaceae*, *Acinetobacter baumannii* and

Pseudomonas aeruginosa in Bangladesh were 9.8%, 55% and 53.7% respectively, in India were 44.3%, 56% and 10% respectively and in US were 18%, 22% and 60.3% respective.^{5, 6, 7, 8,9,10}

Regarding the antimicrobial resistance rates of Carbapenemase-producing *Enterobacteriaceae* in Bangladesh to third generation cephalosporins 80%-100%, fluoroquinolones, aminoglycosides, monobactam 60%-80% , to the carbapenem 10%-30%.^{11,12} The antimicrobial resistance rates of Carbapenemase-producing *Acinetobacter baumannii* in Bangladesh to third-generation cephalosporins are 90%-100%, fluoroquinolones, aminoglycosides, monobactam 70%-80%, to carbapenem 25%-60%.The antimicrobial resistance rates of Carbapenemase-producing *Pseudomonas aeruginosa* in Bangladesh to third-generation cephalosporins are 90%-100%, to fluoroquinolones, aminoglycosides, monobactam 65%-80%, to carbapenem 15%-30%.^{13,14,15}

Carbapenemase produces the deadliest drug-resistant bacteria in nosocomial infections. The extent of drug resistance of these isolates against different antimicrobial classes in a Rajshahi region will be useful to provide locally applicable data and guide the selection of appropriate antibiotics for empirical therapy.^{16,17}

Materials and Methods

The antimicrobial susceptibility of 231 bacterial isolates from wound swab specimens was analyzed in the present study. Aerobic culture and sensitivity tests were done in the Microbiology department of Rajshahi Medical College. All the samples 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, hemolysis on a blood agar plate, motility test, Gram staining, and relevant biochemical tests. The identified bacteria were subcultured, processed for drug sensitivity tests, and preserved for further use.¹⁸ 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.

Detection of Carbapenemase:

Screening for Carbapenemase: The 2013 recommendations of the 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. The carbapenemase producers were confirmed by a modified Hodge test.¹⁹

Results

Table-I: The frequency of bacterial isolates according to the specimens (N=250).

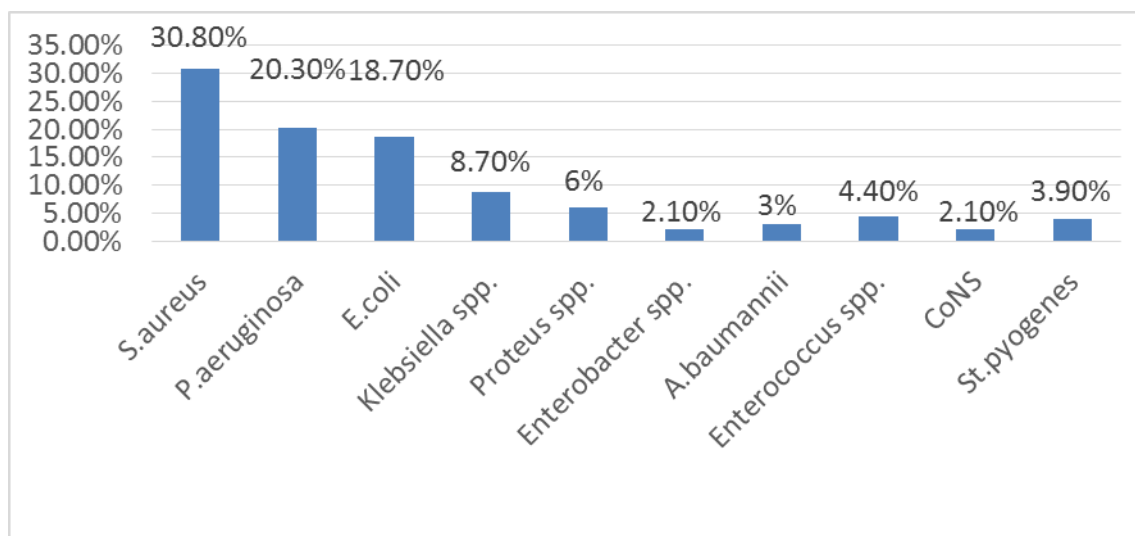
Specimen	Number of Samples	Culture positive	Culture negative
Wound swab	200(80%)	172(68.8%)	28(11.2%)
Burn wound swab	50(20%)	41(16.4%)	09(3.6%)
Total	250(100%)	213(85.2%)	37(14.8%)

A total of 250 specimens were collected from wound infection cases cultured in different bacteriological culture media. Among them, 200 (80%) were surgical site infections, and 50 (20%) were from burn wound infections. Among 250 samples, culture yielded growth in 213 (85.2%) cases, and culture-negative cases were 37 (14.8%).

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

Age (Years)	Number of samples cultured(%)	Male(%)	Female(%)	Culture-positive cases(%)	Male(%)	Female(%)
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(8)
41-50	37(14.8)	22(8.8)	15(6)	32(12.8)	20(8)	12(4.8)
>50	42(8.8)	19(7.6)	23(9.2)	37(14.8)	17(6.8)	20(8)
Total	250(100)	104(41.6)	146(58.4)	213(85.2)	92(36.8)	121(48.4)

Accordingly, the age and sex distribution of the study population are shown in following Table II. Four age groups were 19-30 years, 31-40 years, 41-50 years, and >50 years. 118(47.2%) cases were within the age group of 19-30 years, and 37(14.8%) patients were within the age group of 31-40 years.



Among the 231 isolates, gram-negative bacteria predominated were 136(58.8%), and gram-positive bacteria were 95(41.2%). *S.aureus* were the predominant bacteria with 71(30.8%) cases, followed by *P.aeruginosa* were 47(20.3%) cases, *E. coli* were 43(18.7%) cases, and *Acinetobacter baumannii* were 07(3%) cases.

Table-III: Frequency of Multidrug resistant bacteria (N=250).

Organisms	Total isolates	MDR isolates (%)
<i>Acinetobacter baumannii</i>	07	06(85.7%)
<i>Pseudomonas aeruginosa</i>	47	33(70.2%)
<i>Enterobacteriaceae</i>	82	53(64.6%)

Table-III shows multidrug resistance patterns among isolated *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*. Among 07 isolated *A. baumannii*, 47 isolated *P. aeruginosa*, and 82 isolated *Enterobacteriaceae*, 06(85.7%), 33(70.2%), and 53(64.6%) were MDR, respectively.

Table -IV: Frequency of Carbapenemase producing bacteria.

Isolates	Total No. of org. Tested	No. of positive org. confirmed by phenotypic method (%)
Carbapenem resistant <i>Acinetobacter baumannii</i>	07	04 (57.1%)
Carbapenem resistant <i>Pseudomonas aeruginosa</i>	47	12 (25.5%)
Carbapenem resistant <i>Enterobacteriaceae</i>	82	14 (17%)

Table -VI shows, Among 07 isolated *Acinetobacter baumannii*, 47 isolated *Pseudomonas aeruginosa*, and 82 isolated *Enterobacteriaceae*, 04 (57.1%), 12 (25.5%), and 14 (17%) were phenotypically confirmed by modified Hodge test as carbapenem resistant respectively.

Table -V: Antimicrobial resistance pattern among the carbapenemase-producing *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*.

Antimicrobial agents	<i>Acinetobacter baumannii</i> (N=04)	<i>Pseudomonas aeruginosa</i> N=(12)	<i>Enterobacteriaceae</i> (N=14)
Imipenem	01(25%)	02(16.7%)	02(14%)
Azithromycin	02(50%)	05(41.6%)	06(43%)
Ciprofloxacin	03(75%)	08(66.7%)	09(64%)
Ceftriaxone	04(100%)	12(100%)	14(100%)
Ceftazidime	04(100%)	12(100%)	14(100%)
Cefotaxime	04(100%)	12(100%)	14(100%)
Meropenem	02(50%)	03(25%)	02(14%)
Aztreonam	03(75%)	07(58.3%)	07(50%)
Gentamicin	03(75%)	06(50%)	08(57%)

Table V shows the antimicrobial resistance pattern among the carbapenemase-producing *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*. The carbapenemase producers were 100% resistant against ceftriaxone, cefotaxime, and ceftazidime. Besides that, ciprofloxacin is 64% to 75%, gentamicin 50 to 75%, aztreonam 50 to 75%, and azithromycin 33 to 50% resistant. Imipenem and meropenem showed lower resistance of 14 to 25% and 14 to 50% against carbapenemase producers.

Discussion

Antibiotic resistance caused by the deadliest drug-resistant bacteria is a global problem. World health organization (WHO) described antimicrobial-resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world.² 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. Our study is nearly similar to the study of Nahar *et al.* in Bangladesh and Negi *et al.* in India.^{20,21,22} Our study is about dissimilar to the study of Begum *et al.* in Bangladesh and Khan *et al.* in India.^{23,24,25} 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 groups with poor knowledge about personal hygiene, flawed sanitation system in a hospital, overcrowding of patients in hospitals contribute to high rate of cross infection, inadequate measures for prevention of the spread of the resistant pathogen in a hospital environment.

The present study noted that single isolated cases were more common in 195(91.5%) than multiple 18(8.5%). Our study is nearly similar to the study of Nahar *et al.* in Bangladesh and Saaiq, Ahmad and Zaib in Pakistan.^{21,4} The failure to isolate an organism was 14.8% in the present study may be because the patients had received antibiotics, either systemic or topical or both before sample collection, or it may be that samples were collected from sites where there were no organisms, or there was the presence of anaerobic bacterial infection.

Table II shows the age and sex distribution of various infection cases. Among them, 104 (41.6%) were male, and 146 (58.4%) were female. The female is predominant because a good number of cases were taken from Obstetrics and Gynaecology department. The wound infection rate was higher in the female age groups than in males. This higher infection cases in female patients may be due to poor nutrition, co-morbidity, malignancy, immunosuppression, and hematological disorders.²⁶ Our study is nearly similar to the study of Sharma *et al.* in India and Rajbahak *et al.* in Nepal.^{43,38} Our study is nearly dissimilar from the study of Khanamet *et al.* in Bangladesh and Kumari *et al.* in India.^{25,26} A maximum of 118 (47.2%) cases were found within the age group of 19-30 years. Our study is nearly similar to the study of Tasnim *et al.* in Bangladesh and Roku *et al.* in Ethiopia.^{2,27} These 19-30 age groups are predominant due to a good number of cases being taken from obstetrics and gynaecology unit and burn unit.

The isolation of bacteria and their sensitivity pattern changes from place to place and from time to time. In this study, Out of a total of 250 samples, gram-negative bacteria accounted for a higher isolation rate (gram-positive 41.2% and gram-negative 58.8%) than gram-positive bacteria. Our study is nearly similar to the study of Nahar *et al.* in Bangladesh and Kaur *et al.* in India.^{21,28} Our study is essentially dissimilar from the study of Roy *et al.* in Bangladesh; Rai *et al.* in Nepal.^{29,30}

Acinetobacter baumannii, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* are resistant to meropenem and ceftriaxone by producing carbapenemase was further detected by the modified Hodge test and found 57.1%, 25.5%, and 17% were carbapenemase producers respectively.

Regarding *Acinetobacter baumannii*, this study is similar to Ferdous *et al.* in Bangladesh and Kaur *et al.* in India.^{8,9} But different findings were reported by Nahar *et al.* in Bangladesh and Subramaniyan *et al.* in India.^{31,32}

Regarding *Pseudomonas aeruginosa*, this is nearly similar to the study of Subramaniyan *et al.* in India and Righi *et al.* in Italy.^{32,33} But our study is nearly

dissimilar to the study of Barai *et al.* in Bangladesh and Kaur *et al.* in India.^{34,9}

Regarding *Enterobacteriaceae*, this is nearly similar to the study of Kaur *et al.* in India and Diwakar *et al.* in India.^{28,35} But our study is nearly dissimilar to the study of Barai *et al.* in Bangladesh and Pawar *et al.* in India.^{34,36}

In this study, all the carbapenemase-producing strains of *Enterobacteriaceae* are 100% resistant to ceftriaxone, cefotaxime, and ceftazidime. Nearly 60%-70% resistance is observed against ciprofloxacin and aztreonam, and relatively lower resistance is observed against imipenem and meropenem. In other words, imipenem and meropenem are effective against carbapenemase-producing strains. Our study is similar to Islam *et al.* in Bangladesh and Anitha *et al.* in India.^{11,37}

Carbapenemase-producing strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are 100% resistant to ceftriaxone, cefotaxime, and ceftazidime, and relatively lower resistance is observed against imipenem and meropenem. Our study is nearly similar to Farjana *et al.* in Bangladesh and Subramaniyan *et al.* in India.^{13,32}

In this study, most of the patients were treated with 3rd generation cephalosporin and showed resistance to other antibiotics, indicating that carbapenemase-producing strains are multidrug resistant. The resistant pattern of carbapenemase-producing *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* to meropenem and ceftriaxone may be due to the random use of 3rd generation of cephalosporins and carbapenem without culture and sensitivity which lead to the emergence of resistance in carbapenemase producing bacteria and their dissemination throughout the hospital.

Conflict of interest: None declared

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