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°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.

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
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. 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 37°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.\(^1\)

**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(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, 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 (%) |
|----------------------------|----------------|------------------|
| *Acinetobacter baumannii*  | 07             | 06(85.7%)        |
| *Pseudomonas aeruginosa*   | 47             | 33(70.2%)        |
| *Enterobacteriaceae*       | 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. aeruginous*, 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 *Acinetobacter baumannii* | 07                        | 04 (57.1%)                                             |
| Carbapenem resistant *Pseudomonas aeruginosa*   | 47                        | 12 (25.5%)                                             |
| Carbapenem resistant *Enterobacteriaceae*       | 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 | *Acinetobacter baumannii* (N=04) | *Pseudomonas aeruginosa* (N=12) | *Enterobacteriaceae* (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. 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. Our study is about dissimilar to the study of Begum et al. in Bangladesh and Khan et al. in India. 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 Saaq, Ahmad and Zaib in Pakistan. 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 Gynae 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.28 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 Khanam 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 gynae 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 ceftaxone 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.8,35 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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