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Prevalence of Multidrug Resistant Bacteria Isolated from Infected Wound Patients in Tertiary Care Hospital of Rajshahi

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Abstract: ***Background:*** The threat of Multidrug resistant bacteria on the overall health sector has led to its recognition as the deadliest bacteria in the world. Most of the multidrug resistant bacteria are resistant to commonly used antibiotics including 3rd generation of cephalosporin, Fluroquinolones and carbapenems also. So, treatment of different infections caused by multidrug resistant bacteria are gradually becoming difficult and it also increased the mortality and morbidity. Objective: The aim of the study was to detect multidrug resistant bacteria isolated from infected wound patients in Rajshahi region. Materials and ***Methods:*** A cross sectional type of descriptive study was done during the period of July 2017 to June 2018. Wound swab was collected in different surgical units of Rajshahi Medical College Hospital. The specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 370 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. Multidrug resistant bacteria were identified by disk diffusion method against different classes of antimicrobials. ***Results:*** Out of total 250 samples, Culture yielded growth were 213(85.2%) and total 231 bacteria were identified. Among them 136 (58.8%) isolates were gram negative and 95 (41.2%) isolates were gram positive. S. *aureus* was the predominant organism 71(30.8%) followed by *E.coli* 48(20.8%), *Aeruginosa* 47(20.3%) and *Klebsiella spp*.20 (8.7%).Overall, 231 bacterial isolates were obtained and 135 (58.4%) were identified as MDR. The overall MDR among gram positive and gram-negative bacterial isolates were 55.8% and 60.3% respectively. Among gram positive bacteria's. aureus, Cons, and Enterococcus spp.39(55%),08(57.1%) and 06(60%) were identified as MDR respectively. Among gram negative bacteria, *E.coli*, *Aeruginosa, Klebsiella spp., Proteus spp*. and *Acinetobacter spp*. 28(58.3%), 30(63.8%), 12(60%), 08(57.1%) and 04(57.1%) were identified as MDR respectively. Vancomycin, linezolid and Imipenem were the most sensitive drugs against gram positive bacteria. Colistin and Imipenem were the most sensitive drugs against gram negative bacteria. ***Conclusion:*** The study highlights a high rate of multidrug-resistant bacterial pathogens causing wound infections, emphasizing the urgent need for effective antimicrobial stewardship and infection control measures.

Keywords: Multidrug resistant bacteria, Post operative wound infection, Antibiogram profiles.

Original Researcher Article

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Article at a glance:

Study Purpose: *To contribute to existing knowledge or propose new ideas.*
Key findings: *Among gram positive bacteria's. aureus, cons, and Enterococcus spp.39(55%),08(57.1%) and 06(60%) were identified as MDR respectively. Among gram negative bacteria, E.coli, Aeruginosa, Klebsiella spp., Proteus spp. and Acinetobacter spp. 28(58.3%), 30(63.8%), 12(60%), 08(57.1%) and 04(57.1%) were identified as MDR respectively.*
Newer findings: *Prevalence of isolated MDR bacteria were higher from previous study (40%) than this study (58.4%).*
Abbreviations: *MDR: Multidrug-resistant, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, Pseudomonas spp.: Pseudomonas species.*

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INTRODUCTION

Nosocomial infection can be defined as those occurring within 48 hours of hospital admission, 3 days of discharge or 30 days of an operation. They affect 1 in 10 patients admitted to hospital.[1] The prevalence rates of nosocomial infection in many countries ranged from 9.2% to 21.4%.[2] Reported that the nosocomial infections rate could be as high as 26% to 65% in developing countries and the rate of nosocomial infections varied from 4.8% to 11% in developed countries .[3]Surgical site infections are known to be one of the most common causes of nosocomial infections worldwide and account for nearly 20% to 25% of all nosocomial infections.[2] In Dhaka ,The predominant bacteria isolated from surgical site infections were *Staphylococcus aureus* 40.45% followed by *Escherichia coli* 28.18%.*Pseudomonas aeruginosa* 15.45%, *Enterococci* 8.18%, *Klebsiella* 4.09%, *Acinetobacter*2.27% and *Proteus* 3.36%.[4] Multidrug resistant (MDR) was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories. Bacteria can have developed resistant against antibiotics by different mechanisms.

One of the mechanisms of drug resistant is the production of beta-lactamase enzymes which hydrolyse the beta-lactam drugs like penicillin's, cephalosporins, monobactam and carbapenems.[5] The development of antimicrobial resistance is a natural phenomenon in microorganisms. In Bangladesh it is accelerated by the selective pressure exerted by overuse and misuse of antibiotics in therapeutic and non-therapeutic purposes in humans and animals. Irrational use of antibiotics is the greatest driver of resistance. Noncompliance of the patients with prescriptions and easy availability of antibiotics without prescription in Bangladesh also influence the emergence of resistance.[6] Tremendous use of antibiotic by the physicians and general public leads to the development of severe drug resistance phenomena in Bangladesh.[7] Although the circumstances of antimicrobial resistance may vary with geographical locations, the prevalence of different bacteria in nosocomial infections varies. So, every hospital should have antibiotic prophylaxis protocol and protocol must be reviewed and updated regularly.[8]

MATERIALS AND METHODS

Antimicrobial susceptibility of 231 bacterial isolates from wound swab specimens were analysed in the present study. Aerobic culture and sensitivity tests were done in the Microbiology department of Rajshahi Medical College. All the specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 37^0 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. 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.[9]

MULTIDRUG RESISTANCE TESTING

Multidrug-resistance test was performed by disk diffusion method according to the criteria set by the (CLSI, 2017) against different classes of antimicrobials: For gram positive bacteria-Cephalosporin class (cefuroxime, ceftriaxone); Aminoglycosides class (amikacin); Fluroquinolones class (ciprofloxacin), Tetracycline class (doxycycline); Penicillin class (amoxicillin and clavulanic acid); Glycopeptides class (vancomycin); Macrolides class (azithromycin) and Lacosamide's class (clindamycin) and Carbapenem class (imipenem) were used. For gram negative bacteria-Cephalosporin class (cefuroxime, ceftriaxone); Aminoglycosides class (amikacin); Fluroquinolones class (ciprofloxacin), Tetracycline class (doxycycline); Penicillin class (amoxicillin and clavulanic acid); Monobactam class (aztreonam); Macrolides class (azithromycin) and Polypeptides class (colistin) and Carbapenem class (imipenem) were used. Gram positive and gram-negative bacteria were tested for drugs selected from all ten classes of antimicrobials. The antimicrobial disks used for the test were all from (Oxoid Ltd. England). These drugs were selected based on the national list of medicines to treat infections, prescription frequencies and availability. In order to monitor quality (potency) of disks, a standard strain of *Aeruginosa* (ATCC-27853), *Aureus* (ATCC-25923) and *E.coli* (ATCC-25922) were tested at regular interval and whenever new batches of antimicrobial discs were used.[10]

RESULT

Table 1: Age and sex distribution of wound 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, age and sex distribution of study population is shown in following Table-1. Maximum 118(47.2%) cases were found within the age group of 19-30 years. As a whole, males were 41.6% and females were 58.4% giving 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%).

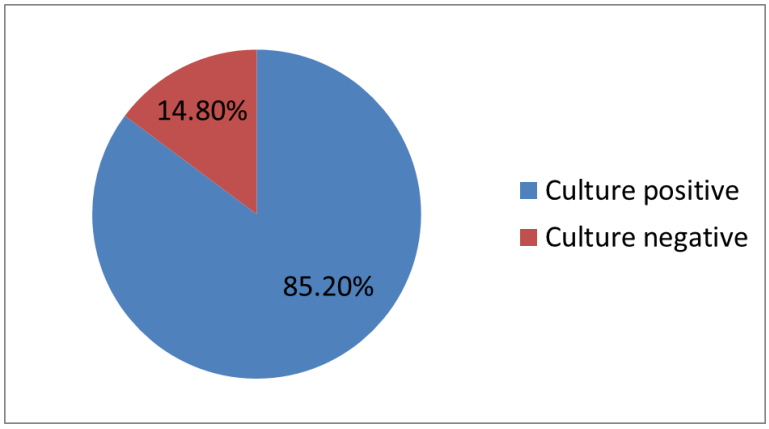

Figure 1: Distribution of culture positive and negative cases (N=250)

Out of 250 samples, 213(85.2%) samples were culture positive while 37(14.8%) samples were cultures negative.

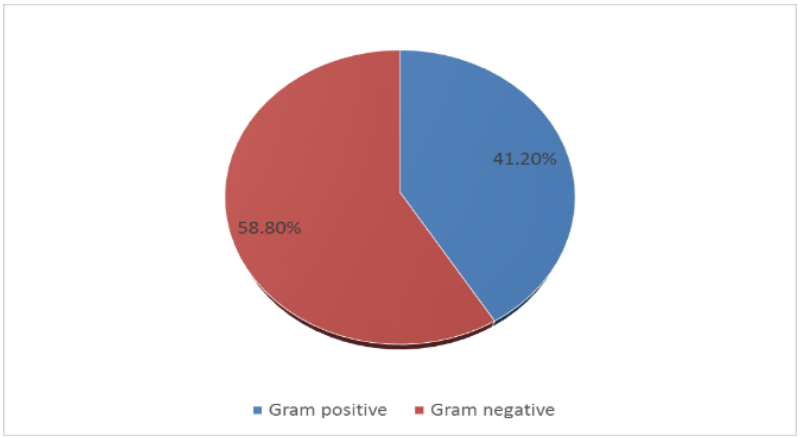

Figure 2: Distribution of Gram positive and Gram-negative bacteria (N=250)

Figure -2 shows distribution of gram-positive and gram-negative isolate among culture positive cases. Among the total 231 isolates, Gram negative bacteria were predominated 136(58.8%) and gram-positive bacteria were 95(41.2%).

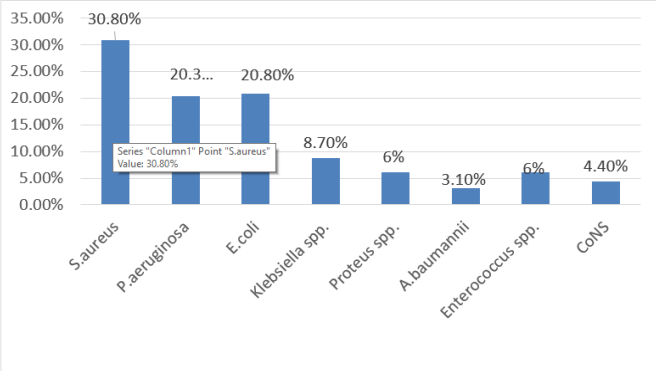

Figure 3: Pattern of bacteria isolated from clinical samples (N=250)

Out of 250 samples, total 231 bacteria were identified. *Aureus* was 71(30.8%) followed by *E. coli* was 48(20.8%), *Aeruginosa* was 47(20.3%) and *Klebsiella spp.* was 20 (8.7%).

Table 2: Antimicrobial susceptibility pattern of gram-positive bacteria

Antimicrobial agents	***Sauers* (N=71)**	***Cons* N= (14)**	***Enterococcus spp.* (N=10)**
Imipenem	16.9%	21.4%	20%
Azithromycin	56.5%	50%	50%
Ciprofloxacin	53.5%	42.9%	50%
Ceftriaxone	91.5%	85.7%	90%
Cefuroxime	94.5%	92.9%	90%
Vancomycin	5.6%	7.1%	10%
Linezolid	8.5%	14.3%	10%
Amikacin	32.3%	35.7%	40%
Amoxicillin/Clavulanic acid	63.4%	57.1%	60%
Cotrimoxazole	70.4%	78.6%	80%
Doxycycline	49.3%	64.3%	70%
Clindamycin	26.8%	28.6%	30%

Table-2 shows antimicrobial susceptibility pattern of gram-positive bacteria. All the gram-positive bacteria were highly resistant against ceftriaxone, cefuroxime, cotrimoxazole and amoxiclav. Vancomycin and linezolid showed lower resistance against gram positive bacteria.

Table 3: Antimicrobial susceptibility pattern of gram negativities bacteria

Antimicrobial agents	***E. coli* N= (48)**	***Pseudomonas aeruginosa* N= (47)**	***Klebsiella spp.* N=(20)**	***Proteus spp.*N=(14)**	***Acinetobacterbaumannii* (N=07)**
Imipenem	8.9%	12.7%	15%	14.3%	14.3%
Azithromycin	50%	63.8%	70%	64.3%	71.4%
Ciprofloxacin	54.2%	51.1%	50%	50%	57.1%
Ceftriaxone	83.3%	91.5%	90%	92.9%	85.7%
Cefuroxime	87.5%	93.6%	95%	92.9%	100%
Cotrimoxazole	75%	85.1%	80%	85.7%	85.7%
Doxycycline	58.3%	55.3%	65%	64.3%	71.4%
Aztreonam	45.8%	59.6%	60%	57.1%	57.1%
Amikacin	37.5%	42.6%	30%	35.7%	42.9%
Amoxicillin/Clavulanic acid	35.4%	46.8%	40%	42.9%	42.9%
Colistin	4.2%	6.4%	10%	7.2%	14.3%
Piperacillin/Tazobactam	25%	25.5%	20%	28.6%	28.6%

Table-3 shows the antimicrobial susceptibility pattern of gram-negative bacteria. All the gram-negative bacteria were highly resistant against ceftriaxone, cefuroxime, cotrimoxazole, doxycycline and aztreonam. Colistin, imipenem and piperacillin/tazobactam showed lower resistance against gram negative bacteria.

MULTIDRUG-RESISTANT PATTERN OF ISOLATES:

Table 4: MDR gram positive bacteria identified from infected wound

Bacteria	Antimicrobial classes resisted to No (%)								Average
	R3	R4	R5	R6	R7	R8	R9	R10	
S. aureus	03(4.2)	05(7.1)	12(16.9)	08(11.3)	05(7.1)	03(4.2)	02(2.8)	02(2.8)	**39(55)**
CoNS	01(7.1)	01(7.1)	02(14.2)	02(14.2)	01(7.1)	01(7.1)	00	00	**08(57.1)**
Enterococcus spp.	00	01(10)	2(20)	01(10)	01(10)	01(10)	00	00	**06(60)**

Key: R3 - R10 = resistance of bacteria to 3,4,5,6,7,8,9 or 10 classes of antimicrobials tested.

In this study, the overall MDR rate of gram-positive bacteria was 55.8%. Multidrug-resistant (MDR) status of gram-positive bacteria was tested against 10 classes of antimicrobials respectively. This means, 55% of *Aureus,* 57.1% of Coagulase negative *Staphylococci* (Cons) and 60% of *Enterococcus spp*. we're becoming MDR. Moreover, 16.9% of *Aureus* showed resistance to five antimicrobial classes. About 14.2% of Cons was resistant to six classes and 20% of *Enterococcus spy* was resistant to five classes as well (Table 4).

Table 5: MDR gram negative bacteria identified from infected wound

Bacteria	Classes of antimicrobial resisted to No (%)								Average No (%)
	R3	R4	R5	R6	R7	R8	R9	R10	
E. coli(48)	02(4.2)	04(8.3)	08(16.7)	04(8.3)	03(6.3)	03(6.3)	02(4.2)	02(4.2)	**28(58.3)**
P. aeruginosa(47)	02(4.3)	03(6.4)	09(19.1)	05(10.6)	04(8.5)	03(6.4)	02(4.3)	02(4.3)	**30(63.8)**
Klebsiella spp.(20)	01(5)	01(5)	02(10)	03(15)	02(10)	01(5)	01(5)	01(5)	**12(60)**
Proteus spp(14)	01(7.1)	01(7.1)	02(14.2)	02(14.2)	01(7.1)	01(7.1)			**08(57.1)**
Acinetobacter spp.(07)	00	00	1(14.3)	1(14.3)	1(14.3)	1(14.3)			**04(57.1)**

Key: R3 - R10 = resistance of bacteria to 3,4,5,6,7,8,9 or 10 classes of antimicrobials tested.

Then again, the overall MDR rate of gram-negative bacteria was 60.3%. Multidrug-resistant (MDR) status of gram-negative bacteria was tested against 10 classes of antimicrobials respectively. Relatively higher rate of MDR was seen among *P. aeruginosa, Klebsiella* and *E. coli* species accounting average resistance of 63.8%, 60% and 58.3% respectively. Additionally, 19.1% of *P. aeruginosa* and 16.7% of *E. coli* species were resistant to five classes. About 15% of *Klebsiella* spp. also showed resistant to six classes. The average MDR rate of *Acinetobacter* spy was found out to be 14.3% (Table 5).

DISCUSSION

Out of 250 clinical samples obtained from different surgery departments of RMCH, Rajshahi, 85.2% had positive culture whereas 14.8% had no growth. This study was nearly similar with the study of Hasan *et al.*[11]and Mama *et al.*[12] but dissimilar with the study of Begum *et al.*[13] and Bastola *et al.*[14] Table 1 shows age and sex distribution of various infection cases. Among them 104 (41.6%) were male and 146 (58.4%) were female. 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 haematological disorders. This study was nearly similar with the study of Tasnim *et a.l*[15] and Rajabhat *et al*[16].but study was nearly dissimilar with the study of Khanam *et al.*[17] and Bastola *et al.*[14] Out of a total 250 samples, gram negative bacteria were higher isolation rate

(Gram-positive 41.2% and Gram-negative 58.8%) than gram positive bacteria.

This study were nearly similar with the study of Hasan *et al.*[11]and Roopashree *et al.*[18] but nearly dissimilar with the study of Abedin *et al.*[19]and Rajabhat *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. *Aureus* were the most frequent isolates 71(30.8%). Study was similar with the study of Hasan *et al.*[11]and Asres *et al.*[20] but findings were dissimilar with Roy et al.[21]andUpreti *et al.*[22] The high prevalence of *S. aureus* infection may be because it is an endogenous source of infection and contamination of surgical instruments.

With the disruption of natural skin barrier *Aureus*, which is a common bacterium on surfaces, easily find their way into wounds. Among Gram negative bacteria, *E. coli* was the 48(20.8%) most common bacterial isolates. This study was similar with the study of Roy *et al.*[21]andRoopashree*et al.*[18] Study was nearly dissimilar with the study of Jubair *et al.*[23] Kaur *et al.*[24] The overall MDR among gram positive and gram-negative bacterial isolates were 55.8% and 60.3% respectively. In this study, among 136 isolated gram-negative bacteria 82(60.3%) were identified as MDR bacteria. This study was nearly similar with the study of Alam *et al.*[25]and Raza *et al.*[26] but nearly dissimilar with the study of Adhikari *et al.*[27] and Agyepong *et al.*[28] Among 95 isolated gram-positive bacteria 53(55.8%) were identified as MDR bacteria. This study was nearly similar with the study of Alam *et al.*[25] and Tilahun *et al.*[29] but nearly dissimilar with the study of Godber et al.[30] and Roopashree *et al.*[18]

The isolated MDR strains gram positive bacteria were highly resistant to ceftriaxone, cefuroxime, cotrimoxazole and amoxiclav. But relatively lower resistance was observed against vancomycin and linezolid. This study was nearly similar with Abedin *et al.*[24] and Alam *et al.*[25] In this study MDR strains of Gram-negative bacteria were highly resistant to ceftriaxone, cefuroxime, cotrimoxazole, doxycycline and aztreonam. Colistin, imipenem and piperacillin/tazobactam showed lower resistance against gram negative bacteria. This study was nearly similar with Abedin *et al.*[19] and Alam *et al.*[25]This variations may be due to differences in local conditions, prevention protocols, antibiotic policy as well as duration of study, variation in host and immune status of the host.

Declarations

I, hereby, declare that the submitted Research Paper is my original work and no part of it has been published anywhere else in the past.

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

Conflict of interest: None declared.

Consent: Informed written consent was taken from each patient or patient's attendant.

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