Prevalence of Multidrug Resistant Bacteria Isolated from Wound Infection in a Tertiary Care Hospital at Rajshahi.

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Abstract

Background: Multidrug resistant bacteria is a major and on-going public health problem both globally and locally. The threat of multidrug resistant bacteria is recognised as the most dead listed bacteria in the world. Most of the multidrug resistant bacteria are resistant to commonly used antibiotics including 3rd generation of cephalosporins, fluorquinolones and carbapenems. So treatment of different infections caused by multidrug resistant bacteria are gradually becoming more 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. **Methods:** Cross sectional type of descriptive study was done during the period of July 2017 to June 2018. Wound swab were collected in different surgical units of Rajshahi Medical College Hospital. The specimens were inoculated in blood agar, nutrient agar and MacConkey's agar plates and incubated aerobically at 37^0 C for 24 hours. Bacterial isolates were identified and susceptibility test was done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar plates. Multidrug resistant bacteria was identified by disk diffusion method against different classes of antimicrobials agents. **Results:** Out of total 250 wound samples, culture yielded growth in 213(85.2%) cases. Among 213 isolates, 136 (58.8%) were gram negative and 95 (41.2%) were gram positive bacteria. Maximum 118 (47.2%) cases were within the age group of 19-30 years and show growth in 98 (39.2%) cases of which males were 29 (11.6%) and females were 69 (27.6%). Among the isolated bacteria *S. aureus* was 71 (30.8%), *E.coli* 48 (20.8%), *P.aeruginosa* 47 (20.3%) and *Klebsiella spp.* 20 (8.7%). Out off, 231 bacterial isolates, 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 identified as MDR were 39 (55%), 08 (57.1%) and 06 (60%) respectively. Among gram negative bacteria *e.g.E.coli, P.aeruginosa, Klebsiellaspp., Proteus spp. and Acinenobacter spp.* were 28(58.3%), 30(63.8%), 12 (60%), 08(57.1%) and 04(57.1%) identified as MDR.Vancomycin, linezolid and imipenem were the most sensitive drugs against gram positive and colistin & imipenem against gram negative bacteria.

Conclusion: This study indicated that MDR of bacterial isolates of wound infection was very high and many of them were also identified as resistant to three or more classes of antimicrobials agents. Such widespread resistance to antimicrobial classes is something serious and few treatment options remain for these patients. Now the time has come to take urgent action other wise we are heading to a post- antibiotic era in which common infections and minor injuries can kill may live. Strict health policies should be applied to use antibiotics, continuously monitor and report antibiotic resistance.

Key words: resistant bacteria, post-operative wound infection, antibiogram profiles.

Introduction

Wound infection is one of the cause of nosocomial infection which can be defined as infection occurring within 48 hours of hospital admission or 3 days after discharge or 30 days of an operation.[1] It occurs 1 in 10 hospital patients. The prevalence rates of nosocomial infection in many countries ranged from 9.2% to 21.4%.[2] and could be as high as 26% to 65% in developing countries and 4.8% to 11% in developed countries.[3] Surgical site infections are the most common causes which account for nearly 20% to 25% of all nosocomial infections.[2] The isolated bacteria from surgical site infections in Dhaka were *Staphylococcus aureus* (40.45%), *Escherichia coli* (28.18%), *Pseudomonas aeruginosa* (15.45%), *Enterococci* (8.18%), *Klebsiella* (4.09%), *Acinetobacter* (2.27%) and *Proteus* (3.36%).[4]

Antimicrobial resistance reduces the effectiveness of drugs designed to cure or prevent infections. Multidrug resistant is define as the development of resistant to at least one agent in three or more antimicrobial classes. Bacteria can develop resistant against antibiotics by different mechanisms. One of the mechanisms of drug resistant is the production of beta-lactamase enzymes which hydrolysed the beta-lactam ring containing drugs like penicillins, cephalosporins, monobactum and carbapenems. Beta-lactamase enzymes may be penicillinase which destroys penicillin, cephalosporinase which destroys cephalosporins and carbapenemase which destroys carbapenems.[5]

In a recent study in Bangladesh has shown that more than 70% infecting bacteria were resistant against at least one of the antibiotics commonly used to treat infection.[6] An other study carried out in different parts of Bangladesh on 2014 reported that multidrug resistance bacterial infections in Dhaka was 79%, Chittagong was 60% and Rajshahi was lower percentage.[7] Different studies in India, Pakistan, Afghanistan, Nepal and Bangladesh stated that one child dies in every five minutes because of infections with multidrug resistant bacteria.[8]

The development of antimicrobial resistance is a natural phenomenon of bacteria. In Bangladesh it is accelerated by overuse or misuse of antibiotics in therapeutic and non-therapeutic purposes both in humans and animals. Irrational use of antibiotics are 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.[9] The knowledge of the multidrug resistant bacteria in nosocomial infections and extent of drug resistance of these isolates against different antimicrobial classes in Rajshahi region will be useful to provide local data and also help to selection of appropriate antibiotics for empirical therapy.[10,11]

Methods

A total of 250 wound swabs were aseptically collected from infected wound in different surgical unites of Rajshahi Medical College Hospital, Rajshahi and inoculated on blood agar, nutrient agar and Mac Conkey's agar plates in the Department of Microbiology, Rajshahi Medical College. All the plates were incubated aerobically at 37^0C over night for visible growth of bacteria. The growth was then identified by their colony morphology, pigment production, haemolysis on blood agar plate, motility test, Gram stain and relevant biochemical tests. Susceptibility tests of bacterial isolates with different antimicrobial agents were also done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar plate.[12]

Multidrug-resistance test was also performed by modified Kirby Bauer disk diffusion method according to the criteria set by the CLSI, 2017 against different classes of antimicrobials.Ten classes of antimicrobials were used for susceptibility test against both gram positive and gram negative bacterial isolates. Gram positive bacteria were tested with cefuroxime & ceftriaxone from cephalosporin class, amikacin from aminoglycosides class, ciprofloxac in from fluoroquinolones class, doxycycline from tetracycline class, amoxicillin and clavulanic acid from penicillin class, vancomycin from glycopeptides class, azithromycin from macrolides class and clindamycin from lincosamides class and imipenem from carbapenem class. Similarly Gram negative were also tested with cefuroxime & ceftriaxone from cephalosporin class, amikacin from amino glycosides class, ciprofloxacin from fluro quinolones class, doxycycline from tetracycline class, amoxicillin and clavulanic acid from penicillin class, aztreonam from monobactum class, azithromycin from macrolides class and colistin from polypeptides class andimipenem fromcarbapenem class. These antimicrobials were selected based on the national list of medicines to treat infections and availability. The used antimicrobial disks were from Oxoid Ltd., England. The standard strains of *P.aeruginosa* ATCC-27853, S.aureus ATCC-25923 and *E.coli* ATCC-25922 were tested to monitor quality of disks for every new batches of antimicrobial discs.[13]

Results

Table-I: Age and sex distribution of study population (N=250).

Age (years)	Number of samples cultured N (%)	Male N (%)	Female N (%)	Culture-positive cases N (%)	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)

Age and sex distribution of study population is shown in Table 1. Among 118 (47.2%) sample cultured, male and female were 34 (13.6%), 84 (33.6%) respectively and cultured yielded growth in 98 (39.2) cases of which male and female were 29 (11.6), 69 (27.6) respectively in the age group 19 to 30 yrs. It is the highest number of culture positive cases. Similarly in age group 31- 40 yrs. male and female were 29 (11.6) and 24 (9.6) out of 53 (21.2) cases and culture positive was 46 (18.4) cases of which males were 92 (36.8) and females were 121 (48.4).The male and female ratio was 1:1.4.).

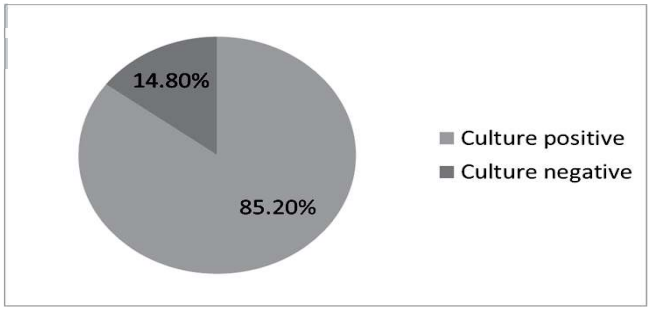

Figure I: Distribution of culture positive and negative cases (N=250).

Figure- I showed culture positive cases. Out of 250 samples, 213(85.2%) were culture positive and 37(14.8%) were cultures negative.

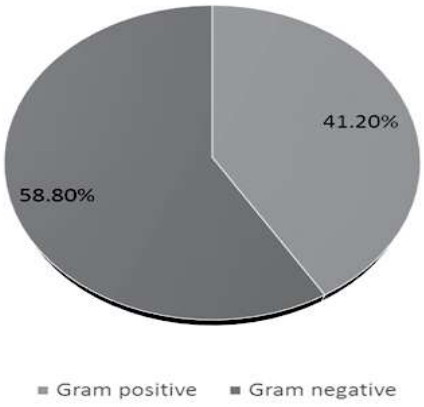

Figure-II: Distribution of Gram positive and Gram negative bacteria (N=250).

Figure-II showed the distribution of Gram-positive and Gram-negative isolate among culture positive cases. Among 231 isolates, Gram negative bacteria was 136 (58.8%) and Gram positive bacteria was 95 (41.2%).

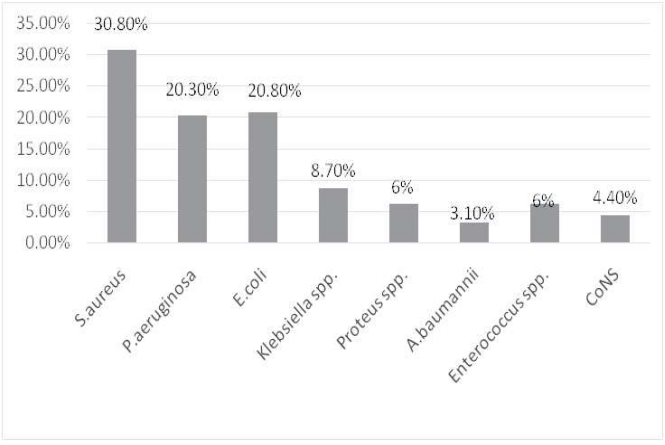

Figure-III: Identified bacteria from wound swab samples (N=250).

Figure-III showed the identified bacterial isolates from wound swab samples. Out of 250 samples 231(85.2%) were culture positive and S.aureus was 71(30.8%), E.coli was 48(20.8%), P.aeruginosa was 47(20.3%) and Klebsiella spp. was 20 (8.7%).

Table-II : Antimicrobial resistance pattern of Gram positive bacteria.

Antimicrobial agents	*S.aureus* (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 showed antimicrobial resistance pattern of Gram positive bacterial isolates. 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-III : Antimicrobial resistance pattern of Gram negative bacteria:

Antimicrobial agents	*E.coli* (N=48) %	*Pseudomonas aeruginosa* (N=47) %	*Klebsiella spp.* (N=20) %	*Proteus spp.* (N=14) %	*Acinetobacter baumannii* (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-III showed the antimicrobial resistance 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.

Table IV: MDR pattern of identified Gram positive bacteria

Bacteria	NO. of resistantto antimicrobial classes. 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)

NB: R3 to R10 = no. of resistant to 3,4,5,6,7,8,9 and 10 classes of antimicrobial.

Table-IV showed multidrug resistant of Gram positive bacteria against 10 classes of antimicrobials. 55% of S.aureus, 57.1% of Coagulase negative Staphylococci and 60% of Enterococcus spp. were becoming MDR. However, 16.9% of S.aureus were resistance to fiveantimicrobial,14.2% of CoNS was resistant to six and 20% of Enterococcus sppwas resistant to five classes of antimicrobials.

Table V: MDR pattern of identified Gram negative bacteria.

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)

NB: R3 to R10 = no. of resistant to 3,4,5,6,7,8,9 and 10 classes of antimicrobial.

Table- IV showed multidrug-resistant status of Gram negative bacteria against 10 classes of antimicrobials. MDR of Gram negative bacteria was 60.3%. Relatively higher rate of MDR was seen in *P.aeruginosa* (63.8%) *Klebsiella* (60%) and *E.coli* (58.3%) but low in *Acinetobacter* spp (14.3%). In addition, 19.1% *P. aeruginosa* & 16.7% *E.coli* were resistant to five classes and 15% *Klebsiella* spp. were resistant to six classes of antimicrobial.

Discussion:

A total of 250 clinical samples were collected from different unites of surgery departments of RMCH, Rajshahi for aerobic culture and sensitivity. Culture has yielded growth in 85.2% and no growth in 14.8% cases. This study was nearly similar with the study of Nahar *et al.*[14] and Negi *et al.*[15] but dissimilar with the study of Begum et *al.*[16] and Khan *et al.*[17] Regarding age and sex distribution, 104 (41.6%) were male and 146 (58.4%) were female. Female also predominant over male in all age groups. The female is more common than male due to a good number of cases were taken from Obstetrics and Gynae Department. This higher infection cases in female patients may be poor nutrition, co-morbidity, malignancy, immuno suppression and hematological disorders. This finding was nearly similar with the study of Tasnim *et al*[18] and Sharma *et al*[19] but nearly dissimilar with the study of Khanam *et al.*[20] and Kumari *et al.*[21] In the present study maximum [118 (47.2%)] cases were found within the age group of 19-30 years which is nearly corresponding with the study of Tasnim *et al.*[18] and Erku *et al.*[22] This maximum cases in this age group 19-30 yrs. are due to a good number of cases were taken from obstetrics & gynae and burn units.

In this study Gram negative bacteria was 58.8% and Gram-positive was 41.2% which was nearly similar with the study of Nahar *et al.*[14] and Roopashree *et al.*[23] but nearly dissimilar with the study of Abedin *et al.*[24] and Rai *et al.*[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 group with poor knowledge about personal hygiene, poor sanitation system and overcrowding of patients in hospital contribute to high rate of cross infection and inadequate measures for prevention of the spread of resistant pathogen in hospital environment.

In our study *S.aureus were* the most frequent isolates [71(30.8%)] which was similar with the study of Nahar *et al.*[14] and Asres *et al.*[26] but dissimilar with Roy *et al.*[27] and Upreti *et al.*[28] The high prevalence of *S. aureus* infection may be, it is an endogenous source of infection, commonly find in the hospital environment and normal flora of skin. *E.coli* was the 48 (20.8%) which is similar with the study of Roy *et al.*[27] and Roopashree *et al.*[23] but nearly dissimilar with the study of Jobayer *et al.*[29] & Kaur *et al.*[30]

The isolated MDR strains of Gram positive bacteria were highly resistant to ceftriaxone, cefuroxime, cotrimoxazole and amoxiclav but relatively lower resistance against vancomycin and linezolid. This finding is nearly similar with Abedin *et al.*[24] and Alam *et al.*[31]
On the other hand MDR strains of Gram negative bacteria were highly resistant to ceftriaxone, cefuroxime, cotrimoxazole, doxycycline and aztreonam but lower resistance against colistin, imipenem and piperacillin/tazobactam. This finding is nearly similar with Abedin *et al.*[24] Mostofa *et al.*[37] and Alam *et al.*[31] This variations may be due to differences in local conditions, prevention protocols, antibiotic policy as well as duration of therapy and variation of immune status of the host. It is well established that infection by MDR strains are now challenging in management. It is necessary to perform sensitivity test prior to start antimicrobial therapy, detect the MDR carriers by screening test, adequate treatment and reduce or control the spread.

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