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Phenotypic Detection of *MRSA* And *ESBL* Producing Bacteria with their Antimicrobial Resistance Pattern Isolated from Infected Wound Patients in Rajshahi Region

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Abstract: ***Background:*** Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) producing bacteria are significant public health threats, both globally and locally, due to their multidrug resistance, including resistance to third-generation cephalosporins and carbapenems. ***Objective:*** This study aimed to detect MRSA and ESBL-producing bacteria and analyze their antimicrobial resistance patterns in infected wound patients from the Rajshahi region. ***Materials and Methods:*** A cross-sectional descriptive study was conducted from July 2017 to June 2018, collecting wound swabs from surgical units at Rajshahi Medical College Hospital. Specimens were cultured on blood agar, nutrient agar, and MacConkey's agar, and incubated at 37°C for 24 hours. Bacterial susceptibility was tested using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar. MRSA was identified by Cefoxitin disk diffusion, and ESBL-producing bacteria were detected via the disk diffusion test. ***Results:*** Out of 250 samples, 213 (85.2%) yielded bacterial growth, identifying a total of 231 bacterial isolates. Among these, 136 (58.8%) were gram-negative, and 95 (41.2%) were gram-positive. Females were more predominant (146, 58.4%) compared to males (104, 41.6%), with a male-to-female ratio of 1:1.4. The most common isolate was *S. aureus* (71, 30.8%), followed by *Pseudomonas aeruginosa* (47, 20.3%). Of the *S. aureus* isolates, 53.5% were MRSA. Additionally, 41.3% of gram-negative isolates were ESBL producers, with high resistance to third-generation cephalosporins (65%) and carbapenems (40%). ***Conclusions:*** MRSA and ESBL-producing bacteria pose significant resistance challenges in wound infections in Rajshahi.

Keywords: Wound infection, antimicrobial susceptibility, *MRSA*, *ESBL*, Multidrug resistant bacteria.

Original Research Article

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Article at a glance:

Study Purpose: *To contribute the extend of existing knowledge or propose new findings.*
Key findings: *Among the 71 isolated S. aureus, 33(46.5%) were identified as MRSA by cefoxitin disk diffusion test. Among 136 isolated gram-negative bacteria, 60(44.1%) were phenotypically confirmed as ESBL producer by disk diffusion method.*
Newer findings: *Prevalence of MRSA and ESBL producing bacteria were higher from previous study (40.1% and 35.4%) than this study (46.5% and 44.1%).*
Abbreviations: MRSA *– Methicillin-Resistant Staphylococcus aureus,* ***ESBL*** *– Extended Spectrum Beta-Lactamase,* ***P. aeruginosa*** *– Pseudomonas aeruginosa,* ***CMT*** *– Cefoxitin Disk Diffusion Test.*

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (*MRSA*) is one of the main concerns among the drug-resistant isolates. *MRSA* is a major nosocomial pathogen worldwide and is potentially a great threat in medical therapy. Methicillin Resistant *Staphylococcus aureus* (*MRSA*) usually isolated from a variety of clinical specimens, but maximum isolation is from the wound infections and other pyogenic infections.[1] A significant

increase in the incident of *ESBL* related infections has been observed throughout the globe. *ESBL* genes are located on plasmids which allow efficient and rapid dissemination, and it also confers resistance to other classes of drugs including aminoglycosides, fluroquinolones and trimethoprim-sulfamethoxazole. This allows the organism to present as multidrug resistant phenotype .[2]

In Dhaka, the predominant bacteria isolated from wound infections were *Staphylococcus aureus* 40.45% followed by *Escherichia coli* 28.18%. *Pseudomonas aeruginosa* 15.45%, *Enterococci* 8.18%, *Klebsiella spp*.4.09%, *Acinetobacter* 2.27% and *Proteus* 3.36%.[3] The prevalence of methicillin resistant *Staphylococcus aureus* in Bangladesh 24.8%, India 45%, Nepal 58.3%and ESBL producing gram negative bacteria in Bangladesh 41.7%, India 40.1%, Pakistan 58.7%.[4,5,6,7] Regarding the antimicrobial resistance rates of *ESBL* producing gram negative bacteria in Bangladesh to third generation cephalosporins 80%-100%, to fluroquinolones, aminoglycosides, monobactum 60%-80%, to carbapenem 10%-30%.[8] The antimicrobial resistance rates of methicillin resistance *Staphylococcus aureus* in Bangladesh to penicillin 100%, third generation cephalosporins 80%-90%, to fluroquinolones, aminoglycosides, macrolids 60%-80%, to vancomycin and carbapenem 0%- 30%.[9]

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. The identified bacteria were sub cultured and processed for drug sensitivity test and preserved for further use.[10] 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.[11]

Detection of Methicillin resistant *Staphylococcus aureus* (*MRSA*)

Cefoxitin disk diffusion method:

All *Staphylococcus aureus* isolates were screened for methicillin resistance by using cefoxitin disk (30 µg). The inoculum size was adjusted with 0.5 McFarland's standard and incubating a lawn on Mueller Hinton agar at 35^0C for 24 hours with a cefoxitin disk (30 µg). According to the Clinical and Laboratory Standards Institute (CLSI) - 2015, a zone of growth inhibition around the cefoxitin disk of ≥22 mm ruled out *MRSA*; a zone size ≤21 mm indicated that the *mecA* gene is present and the isolate was reported as *MRSA*. Cefoxitin was used in place of oxacillin to detect *MRSA* as it is better inducer of the *mecA* gene, and test using cefoxitin give more reproducible and accurate results than tests with oxacillin.[11]

Detection of *ESBL* producing *Enterobacteriaceae*

Screening

Phenotypic confirmation

Screening for *ESBL*:

Screening for *ESBL* producing gram negative bacteria was carried out during AST by modified Kirby Bauer disk diffusion method. Cefotaxime, ceftazidime and ceftriaxone either alone or in combination when showed the desired zone of inhibition, indicated the presence of *ESBL* producing gram negative bacteria.[11]

According to CLSI guideline 2017 (Disk diffusion method):

Screening Antibiotics	Zone of inhibition
Ceftazidime (30 µg)	≤22 mm
or	
Cefotaxime (30 µg)	≤27 mm
or	
Ceftriaxone (30 µg)	≤25 mm

The use of more than one antimicrobial agent improves the sensitivity of *ESBL* detection.

Phenotypic confirmation by disc diffusion test (PCDDT):

After inoculation of a Mueller Hinton plate with the test organism, Ceftazidime 30 µg + Ceftazidime/Clavulanate 30/10 µg and Cefotaxime 30 µg + Cefotaxime/Clavulanate 30/10 µg these four disc were placed 20 mm apart. The plate incubated at 35^{0}C for 18-20 hours. A ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone phenotypically confirmed ESBL.[11]

RESULTS

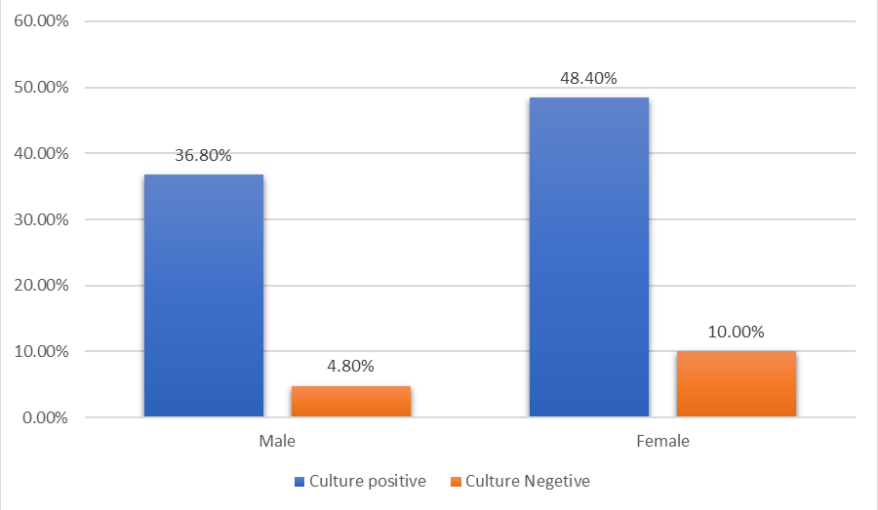

Figure 1: Sex distribution of culture positive cases

Figure-1 shows the sex distribution of culture positive cases. The female cases were 146 and culture yielded growth of 121 (48.4%) cases and culture negative were 25 (10%). The male cases were 104 and culture showed growth of 92 (36.8%) cases and culture negative were 12 (4.8%).

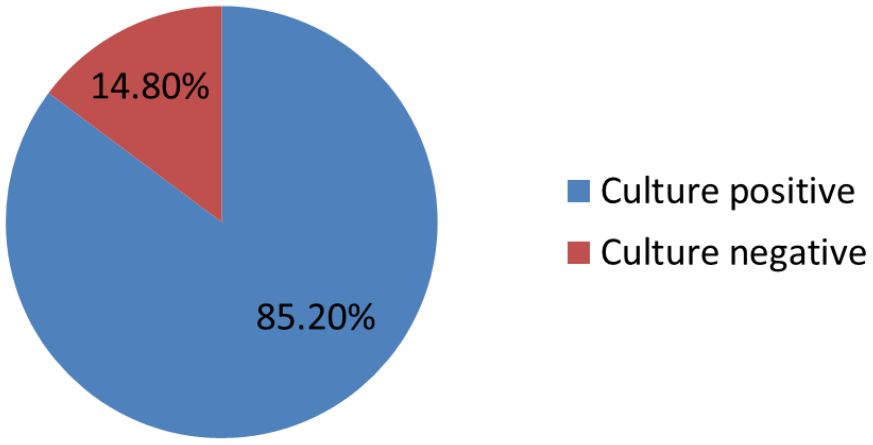

Figure 2: Frequency of culture positive and negative cases (N=250)

Figure 2 shows culture positivity of isolated organisms. Out of 250 samples, 213(85.2%) samples were culture positive while 37(14.8%) samples were culture negative.

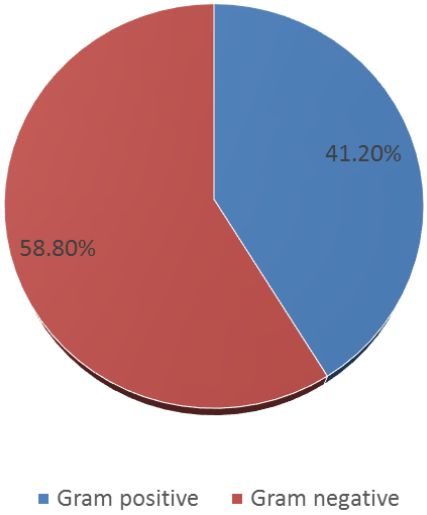

Figure 3: Frequency of gram positive and gram-negative bacteria (N=250)

Figure 3 shows the 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%).

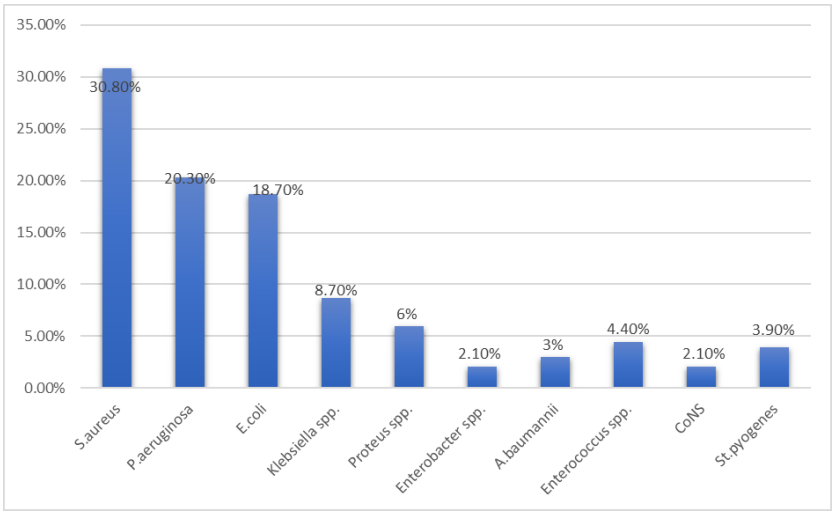

Figure 4: Pattern of bacteria isolated from wound samples (N=250)

Figure 4 shows the identified species of bacteria from wound samples. Out of 250 samples, total 231 bacteria were identified. *S.aureus* was 71(30.8%) followed by *P.aeruginosa* was 47(20.3%), *E. coli* was 43(18.7%) and *Klebsiella spp.* was 20 (8.7%).

Table 1: Frequency of *ESBL* and MRSA producing bacteria

Isolates	Total No. of org. Tested	No. of positive org. confirmed by phenotypic method (%)
ESBL producing gram negative bacteria	136	60 (44.1%)
Methicillin resistant *S.aureus*	71	33(46.5%)

Among the 71 isolated *S. aureus*, 33(46.5%) were identified as *MRSA* by cefoxitin disk diffusion test. Among 136 isolated gram-negative bacteria, 60(44.1%) were phenotypically confirmed as *ESBL* producer by disk diffusion method.

Table 2: Antimicrobial resistance pattern of *MRSA* producing *S. aureus* (N=33)

Antimicrobial agents	*MRSA* strain (Percentage %)
Imipenem	09(27%)
Azithromycin	15(45%)
Flucloxacillin	33(100%)
Amikacin	22(67%)
Ciprofloxacin	25(75%)
Ceftriaxone	31(94%)
Vancomycin	03(9%)
Amoxiclav	24(72%)
Cefuroxime	29(88%)
Linezolid	07(21%)
Cotrimoxazole	30(91%)
Cefixime	31(94%)
Doxycycline	21(63%)

Table 2 shows antimicrobial resistance pattern of *MRSA*. All the *MRSA* was 100% resistant against flucloxacillin, ceftriaxone and cefixime was 94%, cotrimoxazole 30(91%), cefuroxime was 88%, ciprofloxacin was 75% and amoxiclav was 72% resistant. Vancomycin, Linezolid and imipenem showed lower resistance 9%, 21% and 27% against MRSA respectively.

Table 3: Antimicrobial resistance pattern among ESBL producing gram negative bacteria (N=60)

Antimicrobial agents	*ESBL* strain (Percentage %)
Imipenem	05(8%)
Ciprofloxacin	28(47%)
Ceftriaxone	54(90%)
Ceftazidime	52(87%)
Cefuroxime	50(83%)
Azithromycin	30(50%)
Aztreonam	20(42%)
Amikacin	32(53%)
Piperacillin/tazobactam	17(28%)
Colistin	03(5%)
Cefepime	15(25%)
Cofotaxime	53(88%)
Cefixime	54(90%)

Table 3 shows the antimicrobial resistance pattern among ESBL producing gram negative bacteria. All *ESBL* producers were 80%-90% resistant against ceftriaxone, cefixime, cefotaxime and ceftazidime. Colistin, imipenem and cefepime showed lower resistance 5%, 8% and 25% against ESBL respectively.

DISCUSSION

Out of 250 wound swabs obtained in the Microbiology laboratory from various departments of RMCH, Rajshahi for aerobic culture and sensitivity, 85.2% yielded positive culture whereas 14.8% yielded no growth. This study was nearly similar with the study of Khan *et al.*[12] and Narula *et al.*[13] but dissimilar with the study of Jobayer *et al.*[14] and Negi *et al.*[15] Figure I shows sex distribution of wound infection cases. Among them culture positive cases, 92 (36.8%) were male and 121 (48.4%) 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 was nearly similar with the study of

Khan *et al.*[12] and Sharama *et al.*[16] but dissimilar with the study of Sadia *et al.*[17] and Kumari *et al.*[18]

Out of a total 250 samples, Gram negative bacteria were accounted for 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 Sadia *et al.*[17] and Narula *et al.*[13] but nearly dissimilar with the study of Jobayer *et al.*[14] and Gangania *et al*.[19] The reason for this high occurrence of culture positivity may be due to the fact that most of the study population had 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. *S.aureus* were the most frequent isolates 71(30.8%) .Study were similar with the study of Sadia *et al.*[17] and Kumari *et al.*[18] but findings were dissimilar with Jobayer *et al.*[14] and Bhatnagar *et al*.[20] 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 *S.aureus*, which is a common bacterium on surfaces, easily find their way into wounds. Among gram negative bacteria, *P.aeruginosa* was the most common bacterial isolates 47(20.3%).This study was similar with the study of Chaudhary *et al.*[21] and Kumari *et al.*[18] Study was nearly dissimilar with the study of Begum *et al.*[22] Sharma *et al*.[16] The isolated gram negative bacteria were further tested to detect extended-spectrum beta lactamase (*ESBL*) producing strains. In this study *ESBL* producers were 44.1%.

This were nearly similar with the study of Haque *et al.*[6] and Kaur *et al.*[23] But different findings were reported by Yasmin *et al.*[24] and Negi *et al.*[15] This difference may be due to the fact that it is difficult to detect *ESBL* producers and its distribution varies between various geographical locations and hospitals. Methicillin resistant *S.aureus* (*MRSA*) is another therapeutic challenge like *ESBL* producing bacteria. Out of 71 *S.aureus* 33(46.5%) isolates were *MRSA.* This study was nearly similar with the study of Dutta *et al.*[25] and Rao *et al.*[5] But nearly dissimilar with the study of Hasan *et al.*[9] and Negi *et al.*[15] This difference may be due to *MRSA* infection is variable from different hospitals, geographical locations and countries depending on antibiotic policy.

The isolated *MRSA* strains were highly resistant to ceftriaxone, cefixime, ceftazidime, cefuroxime and flucloxacillin. But relatively lower resistance was observed against vancomycin, linezolid and imipenem. This study was nearly similar with Alam *et al.*[26] and Goswami *et al.*[27] In this study all the *ESBL* strains of gram-negative bacteria were highly resistant to ceftriaxone, cefuroxime, cefixime and `ceftazidime. Colistin, imipenem and cefepime are effective against *ESBL* strains. This study was nearly similar with Mostofa *et al.*[28] and Alam *et al.*[26] These 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 Instutional 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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