https://www.banglajol.info/index.php/BJMM/index

Bangladesh Journal of Medical Microbiology

January 2024, Volume 18, Number 1, Page 3-10

ISSN (Print) 2070-1810

ISSN (Online) 2072-3105

Original Article ***Open Access***

Comparison of Epidemiology of Patients Presented with Health Care Associated-Methicillin Resistant *Staphylococcus aureus* and Community Associated-Methicillin Resistant *Staphylococcus aureus* Infection attending at a Tertiary Care Hospital

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Abstract

Background: MRSA is an emerging pathogen worldwide and is usually detected in hospitals and other health care facilities. **Objective:** The purpose of the present study was to compare the epidemiology of patients presented with health care associated-methicillin resistant Staphylococcus aureus and community associated-methicillin resistant Staphylococcus aureus infection. **Methodology:** This study was designed as descriptive type of cross sectional type study. This study was carried out in the Department of Microbiology at Shaheed Suhrawardy Medical College, Dhaka. The specimens were collected from indoor as well as OPD of Shaheed Suhrawardy Medical College & Hospital, Dhaka. This study was conducted from January 2013 to June 2013 for a period of six (06) months. All patients at any age with both sexes presented with skin infection, soft tissue infection, urinary tract infection (UTI), burn wound, surgical site infection, orthopedic wound, puerperal sepsis as well as infection at anybody surface area who were attended at OPD as well as indoor department of hospital were selected as study population. The laboratory diagnosis was confirmed by microbiological test. **Results:** A total number of 290 patients presented with infections were enrolled for this study of which 145 patients were included in group A designated as CA-MRSA infection and the rest 145 patients were included in group B designated as HA-MRSA infection. Majority of the patients are in the 15 to 30 years' age group in both group A and group B which were 80(55.2%) cases in each. However less than 15 years' age group was the next most common in group A which is 22(15.2%) cases. The mean (±SD) age of group A and B was 30.43 (±16.131) and 29.43 (±15.648) years respectively. The difference of mean age between group A and B was not statistically significant (p=0.593). **Conclusion:** In conclusion history of smoking is found more common in group A than group B more urban dwellers in group A than group B.

Keywords: Epidemiology; health care associated-methicillin resistant; *Staphylococcus aureus*; community associated-methicillin resistant; infection

Bangladesh Journal of Medical Microbiology, January 2024;18 (1):3-10

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DOI: https://doi.org/10.3329/bjmm.v18i1.76947

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) first emerged as a clinically relevant human pathogen more than 3 decades ago[1]. The aggressive bacterium was first detected in hospitals and other health care facilities where vulnerable hosts, frequent exposure to

the selective pressure of intensive antimicrobial therapy, and the necessity for invasive procedures which further compromise host defenses created a favourable environment for dissemination. MRSA emerged as an important cause of health care-associated infections, particularly central line-associated bloodstream infection, ventilator-associated pneumonia, and surgical site infection. Despite the adoption of a number of measures to prevent spread, the incidence of MRSA infection at most USA hospitals has steadily increased over the past 20 years[2]. Complicating matters, the management of infection caused by MRSA remains a challenge for clinicians. A number of analyses suggest that MRSA infections are associated with increased mortality and cost of care when compared with those due to strains that are susceptible to methicillin. Cosgrove et al[3] identified a 2-fold increased risk of death associated with methicillin resistance.

The management and control of MRSA has been further complicated by dramatic changes in the epidemiology of transmission and infection observed over the past 2 decades. Specifically, *Staphylococcus aureus* strains resistant to methicillin, once exclusively linked to hospital care, have increasingly been detected among patients in the community who lack conventional risk factors for MRSA infection such as prior antimicrobial therapy or invasive procedures[4-5]. These community-associated MRSA (CA-MRSA) strains have demonstrated a predilection to affect specific populations. Clusters among school children and competitive athletes have been extensively described in both the scientific literature and the mass media[3,6]. CA-MRSA infection often manifests in characteristic clinical patterns including aggressive skin and soft tissue infections typically arising from an initial lesion often mistaken by patients and clinicians for a spider bite and necrotizing pneumonia[7].

CA-MRSA strains typically share a distinctive methicillin-resistance cassette that helps to explain the characteristic susceptibility of these strains to non–beta-lactam antimicrobial agents such as clindamycin and trimethoprim/sulfamethoxazole[8]. In addition, CA-MRSA isolates commonly over express a particular set of virulence factors, including the Panton-Valentine leukocidin[9]. While the specific relationship between these features and the unique clinical and epidemiological characteristics of CA-MRSA remain to be elucidated, the importance of these strains continues to grow. CA-MRSA has increasingly been linked to outbreaks of infection in hospitals and health care facilities, and there is some evidence that these strains are now the dominant cause of *staphylococcal* disease in some settings[10]. This present study was undertaken to compare the epidemiology of patients presented with health care associated-methicillin resistant *Staphylococcus aureus* and community associated-methicillin resistant *Staphylococcus aureus* infection.

Methodology

Study Design and Population: This study was designed as descriptive type of cross sectional type study. This study was carried out in the Department of Microbiology at Shaheed Suhrawardy Medical College, Dhaka. The specimens were collected from indoor as well as OPD of Shaheed Suhrawardy Medical College & Hospital, Dhaka. This study was conducted from January 2013 to June 2013 for a period of six (06) months. All patients at any age with both sexes presented with skin infection, soft tissue infection, urinary tract infection (UTI), burn wound, surgical site infection, orthopedic wound, puerperal sepsis as well as infection at anybody surface area who were attended at OPD as well as indoor department of hospital were selected as study population. The patients were voluntarily included in the study with their consent and they were neither supported nor additionally burdened financially. The sampling technique was purposive non-random sampling method. This purposive sampling was used as per inclusions and exclusion criteria.

Study Procedure: Specimens were collected according to the site of infection. Urine was collected if patients presented with UTI. Wound swab was collected by sterile swab stick from different infection site like skin infection, soft tissue infection, burn wound, surgical site infection and orthopedic wound infection. Blood was taken from septicemia patients.

Laboratory Methods: The laboratory diagnosis was confirmed by microbiological test described in literature9. All aseptic precaution was taken to prevent any contamination. Biosafety cabinet II was used for laboratory procedure. Specimens were inoculated onto routine culture media like blood agar media (HiMedia Laboratories Pvt. Ltd., India), MacConkey's agar media (HiMedia Laboratories Pvt. Ltd., India) and Chocolate agar media (HiMedia Laboratories Pvt. Ltd., India). Mannitol salt agar media (HiMedia Laboratories Pvt. Ltd., India), selective media for *Staphylococcus aureus* was used. Culture plate was incubated at 370 C for 24 hours and was examined the

colony growth. Incubation was performed in the aerobic incubator (Memmert Company Ltd., USA). Identification of S aureus was confirmed with a tube coagulase test[9]. Antimicrobial susceptibility testing was performed by using disk diffusion methodology according to Clinical Laboratory Standard Institute methodology[92]. The cut off value for the detection of resistant or sensitive was maintaining the values of CLSI. In the laboratory different bacteria were susceptible to different antibiotics. Thus a panel of antibiotics was used for the different isolated bacteria. The antibiotics were used to observe the sensitivity against MRSA like Azithromycin, amoxiclave, cloxacillin, flucloxacillin, linezolid, ceftriaxone, ciprofloxacin, cephradine, oxacillin, imipenem, cefixime and vancomycin.

Data Collection Technique: Data were collected using a preformed data collection sheet (questionnaire). Base line information was collected from the patient after exploration of different complaints and sign and symptoms. All information regarding clinical features and microbiological results were recorded in a data collection sheet. Data were collected by researcher herself. All data were compiled and edited meticulously. The data were screened and were checked for any missing values and discrepancy. All omissions and inconsistencies were corrected and were removed methodically.

Statistical Analysis: Computer based statistical analysis were carried out with appropriate techniques and systems. All data were recorded systematically in preformed data collection form (questionnaire) and quantitative data were expressed as mean and standard deviation and qualitative data were expressed as frequency distribution and percentage. Statistical analysis was performed by using window based computer software devised with Statistical Packages for Social Sciences (SPSS-17) (SPSS Inc, Chicago, IL, USA). 95% confidence limit was taken. Probability value <0.05 was considered as level of significance. Odd ratio (Odd ratio) was measured to see the risk estimation of the socioeconomic variables to CA-MRSA and HA-MRSA isolates. The association between qualitative variables was measured by Chi-Square test. Student's t test has been performed to see the association between quantitative variables. The sensitivity pattern of antibiotic between HA-MRSA and CA-MRSA were compared to see the statistical relation between them. The summarized data was interpreted accordingly and was then presented in the form of tables.

Ethical Consideration: Prior to the commencement of this study, the research protocol was approved by the ethical committee (Local Ethical committee) of Shaheed Suhrawardy Medical College, Dhaka, Bangladesh. The aims and objectives of the study along with its procedure, methods, risks and benefits of this study were explained to the respondent in easily understandable local language and then informed consent was taken from each patient. It was assured that all informed and records would be kept confidential and the procedure was helpful for both the physicians and the patients in making rational approach of the case management.

Results

A total 115 clinically and laboratory confirmed meningitis patients were enrolled in this study. In this study most of the cases of study population were in the age group 1 month to 5 years 97(84.3%) cases. The age distribution among the bacterial meningitis (35) showed the maximum 17(48.5%) in the age group 1 month to 1 year followed by 11(31.4%) in the age of more than 1 year to 5 years (Table 1).

Male was predominant in both group A and B which was 110(75.9%) vs 35(24.1%) cases and 112(77.2%) vs 33(22.8%) cases respectively. The male and female

Table 1: Distribution of Study Population According to Age (n=290)

Age Group	**Group A**	**Group B**	**Total**	**P value**
Less than 15 Years	17(11.7%)	22(15.2%)	39(13.4%)	
15 to 30 Years	80(55.2%)	80(55.2%)	160(55.2%)	
30 to 45 Years	19(13.1%)	18(12.4%)	37(12.8%)	
45 to 60 Years	19(13.1%)	17(11.7%)	36(12.4%)	0.234
More than 60 Years	10(6.9%)	8(5.5%)	18(6.2%)	
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	
Mean±SD	30.43±16.131	29.43±15.648	31.43±11.131	0.593

*Student's t test has been done to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant

ratio was 3.14:1 and 3.39:1 in group A and B respectively. The difference between male and female was not statistically significant (p=0.782) (Table 2).

Table 2: Distribution of Study Population According to Gender (n=290)

Gender	Group A	Group B	Total	P value*
Male	110(75.9%)	112(77.2%)	222(76.6%)	
Female	35(24.1%)	33(22.8%)	68(23.4%)	0.782
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

In group A, married patient was more common than unmarried which was 69(47.6%) cases and 72(49.7%) cases respectively. In group B, married patients were more common than unmarried which was 75(51.7%) cases and 68(46.9%) cases respectively. The difference was not statistically significant (p=0.597) (Table 3).

Table 3: Distribution of Study Population According to Marital Status (n=290)

Marital Status	Group A	Group B	Total	P value*
Married	69(47.6%)	75(51.7%)	144(49.7%)	
Unmarried	72(49.7%)	68(46.9%)	140(48.3%)	
Widow	4(2.8%)	2(1.4%)	6(2.1%)	0.597
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

Majority patients in both group A and B were Muslim which was 138(95.2%) cases and 136 (93.8%) cases respectively. Hindu was present in only 7(4.8%) cases and 9(6.2%) cases respectively. The difference was not statistically significant (p= 0.607) (Table 4).

Table 4: Distribution of Study Population According to Religion (n=290)

Religion	Group A	Group B	Total	P value*
Islam	138(95.2%)	136 (93.8%)	274(94.5%)	
Hindu	7(4.8%)	9(6.2%)	16(5.5%)	0.607
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups *Figure within parenthesis indicates percentage; *p value is not statistically significant

In group A mostly were student which was 43(29.7%) cases followed by labour, housewife, service holder and Business which were 39(26.9%) cases, 28(19.3%) cases, 22(15.2%) cases and 7(4.8%) cases respectively. In group B majority were student which was 45(31.0%) cases followed by labour, housewife, service holder and Business which were 41(28.3%) cases, 20(13.8%) cases, 20(13.8%) cases and 13(9.0%) cases respectively. The difference was not statistically significant (p= 0.702) (Table 5).

Table 5: Distribution of Study Population According to Occupation (n=290)

Occupation	Group A	Group B	Total	P value*
Service holder	22(15.2%)	20(13.8%)	42(14.5%)	
Business	7(4.8%)	13(9.0%)	20(6.9%)	
Student	43(29.7%)	45(31.0%)	88(30.3%)	
Labour	39(26.9%)	41(28.3%)	80(27.6%)	
Farmer	4(2.8%)	2(1.4%)	6(2.1%)	0.702
Housewife	28(19.3%)	20(13.8%)	48(16.6%)	
Unemployed	1(0.7%)	2(1.4%)	3(1.0%)	
Others	1(0.7%)	2(1.4%)	3(1.0%)	
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

Patients with primary education were the most common in both group A and B which was 46(31.7%) cases and 45(31.0%) cases respectively. Next to this was the SSC level which was 35(24.1%) cases and 36(24.8%) cases in group A and B respectively. Illiterate was found in 24(16.6%) cases and 22(15.2%) cases in group A and B respectively. HSC and graduate level patients were found in 27(18.6%) vs 13(9.0%) cases in group A respectively and 21(14.5%) cases in both groups. The difference in the educational status between group A and B was not statistically significant (p=0.601) (Table 6).

Table 6: Distribution of Study Population According to Education (n=290)

Education	Group A	Group B	Total	P value*
Illiterate	24(16.6%)	22(15.2%)	46(15.9%)	
Primary	46(31.7%)	45(31.0%)	91(31.4%)	
SSC	35(24.1%)	36(24.8%)	71(24.5%)	0.601
HSC	27(18.6%)	21(14.5%)	48(16.6%)	
Graduate	13(9.0%)	21(14.5%)	34(11.7%)	
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

Low socio-economic condition patient was found in 63(43.4%) cases and 48(33.1%) cases in group A and B respectively. Middle socio-economic condition patient was found in 82(56.6%) cases and 97(66.9%)

cases in group A and B respectively. The difference of the socio-economic condition between group A and B patients was not statistically significant (p=0.070) (Table 7).

Table 7: Distribution of Study Population According to Socio-economic Condition (n=290)

Socio-economic Condition	Group A	Group B	Total	P value*
Low Status	63(43.4%)	48(33.1%)	111(38.3%)	
Middle Status	82(56.6%)	97(66.9%)	179(61.7%)	0.070
High Status	0(0.0%)	0(0.0%)	0(0.0%)	
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant

History of smoking was present in 29(20.0%) cases and 24(16.6%) cases in group A and B respectively. Non-smoker was present in 116(80.0%) cases and 121(83.4%) cases in group A and B respectively. The difference in the history of smoking between group A and B patients was not statistically significant (p=0.447) (Table 8).

Table 8: Distribution of Study Population according to Smoking History (n=290)

Smoking History	Group A	Group B	Total	P value*
Smokers	29(20.0%)	24(16.6%)	53(18.3%)	
Non-smokers	116(80.0%)	121(83.4%)	237(81.7%)	0.447
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

Urban dwellers were found in 50(34.5%) cases and 46(31.7%) cases in group A and B respectively. Rural dwellers were found in 95(65.5%) cases and 99(68.3%) cases in group A and B respectively. The difference in the area of residence between group A and B patients was not statistically significant (p=0.618) (Table 9).

Table 9: Distribution of Study Population according to Area of Residence (n=290)

Residence	Group A	Group B	Total	P value*
Urban	50(34.5%)	46(31.7%)	96(33.1%)	
Rural	95(65.5%)	99(68.3%)	194(66.9%)	0.618
Total	**145(100.0%)**	**145(100.0%)**	**290(100.0%)**	

*Pearson Chi-Square test has been performed to see the association between two groups; *Figure within parenthesis indicates percentage; *p value is not statistically significant.

Discussion

MRSA is an emerging pathogen worldwide3. This bacterium is usually detected in hospitals and other health care facilities due to vulnerable hosts, repeatedly expose to the selective intensive antimicrobial therapy and invasive procedures[10]. This is enhanced by further weakness of host defenses; thus it creates a favourable environment for dissemination[6].

It has been found that MRSA is as an important cause of health care–associated infections, particularly central line-associated bloodstream infection, ventilator-associated pneumonia and surgical site infection[11]. The incidence of MRSA infection at most US hospitals has steadily increased over the past 20 years despite the adoption of a number of measures to prevent spread[2]. The management of infection caused by MRSA remains a challenge for clinicians.

MRSA was exclusively linked to hospital care which has increasingly been detected among patients in the community who lack conventional risk factors for MRSA infection such as prior antimicrobial therapy or invasive procedures[5]. CA-MRSA strains have demonstrated a predilection to affect specific populations. The infection caused by CA-MRSA often manifests in characteristic clinical patterns like aggressive skin and soft tissue infections typically arising from an initial lesion[7]. The specific relationship between these features and the unique clinical and epidemiological characteristics of CA-MRSA remain to be elucidated.

In this study a total number of 290 patients presented with infections were enrolled for this study of which 145 patients were included in group A and the rest 145 patients were included in group B. Patients who have fulfilled the criteria of CA-MRSA infection were designated as group A; on the other hand, patients who were satisfied the criteria of HA-MRSA infection were designated as group B. When the patients fulfilled the criteria the specimens were collected according to the site of the lesions. The distribution of study population according to age is recorded. It has been found that majority of the patients are in the 15 to 30 years' age group which are 80(55.2%) cases in each in both group A and group B. However less than 15 years' age group is the next most common in group A which is 22(15.2%) cases. The mean (±SD) age of group A and B is 30.43 (±16.131) and 29.43 (±15.648) years respectively. The difference of mean age between group A and B is not statistically significant (p=0.593). The mean is more or less same in both group A and B. However, Naimi et al[12] has reported that CA-MRSA

infection is more common among younger age group than HA-MRSA infection group. The fact is that the researcher has taken patients from paediatric hospital. This study has been also added that the mean age of the CA-MRSA is 30 years when the paediatric groups are excluded from the study. Thus this is similar to the present study. The patients were recruited from a tertiary care hospital where all age groups patients are available giving a similar age group in both groups. Invasive MRSA infections can be observed in all age spectrums[13]. Neonates, middle-aged individuals as well as the elderly can be affected[14]. However, the rate of rate of infection is highest among patients 65 years and older[13].

The distribution of study population according to sex is recorded. Male patient is predominant in both group A and B. The male and female ratio is 3.14:1 and 3.39:1 in group A and B respectively. The difference between male and female is not statistically significant (p=0.782). The result indicates that CA-MRSA infection is more commonly occurs in male patients than female. Waness[13] has reported that MRSA infection is more common among male patients. The reason may be due to more exposure to the environment by the male patients. Toeda et al[15] has reported that MRSA isolation was significantly higher in men than in women in clean operations and without complications. In age and disease, MRSA isolation was significantly higher in men than in women in all subgroups. Thus, this is can be said from this finding that gender may be a risk factor in the MRSA infection.

The distribution of study population according to marital status is recorded in this study. In group A, married patients (47.6%) are more common than unmarried (49.7%). In group B, married patients (51.7%) are more common than unmarried (46.9%). The difference is not statistically significant (p=0.597). From this study it has been found that married patients are more commonly recruited than unmarried. The distribution of study population according to religion is recorded. Majority patients in both group A (95.2%) and B (93.8%) are Muslim. Hindu is present in only 4.8% cases and 6.2% cases in group A and B respectively. The difference is not statistically significant (p=0.607). This indicates that the Muslims are more commonly recruited in this study; the reason is that Bangladesh is a country of 90.0% Muslim. Therefore, during data collection this is automatically shown that religion Islam is more commonly found in group A as well as B. In another report Naimi et al[12] has reported that race or ethnicity is significantly related to CA-MRSA and HA-MRSA infection though that study didn't show any relation with religion.

The distribution of study population according to occupation is recorded. In group A, mostly are student (29.7%) followed by labour (26.9%), housewife (19.3%), service holder (15.2%) and business (4.8%). In group B majority are student (31.0%) followed by labour (28.3%), housewife (13.8%), service holder (13.8%) and business (9.0%). The difference is not statistically significant (p=0.702). Different occupations are found in this study; however, predominantly students and labour. Usually the MRSA infection is commonly spread by direct contact. Among the labourer the personal hygiene doesn't maintain. Thus there is a more chance of infection occurs in this profession. There is no such evidence found where the reason of high infection among the students explained.

The distribution of study population according to education is recorded. Patients with primary education are the most common in both group A (31.7%) and B (31.0%). Patients with SSC level is 24.1% cases and 24.8% cases in group A and B respectively. Illiterate is found more in group A (16.6%) than in group B (15.2%). HSC and graduate level patients are found in 27(18.6%) vs 13(9.0%) cases in group A respectively and 21(14.5%) cases in both groups. The difference in the educational status between group A and B is not statistically significant (p=0.601). The distribution of study population according to socio-economic condition is recorded. Low socio-economic condition patient is more commonly found in group A (43.4%) than group B (33.1%). Middle socio-economic condition patient is found in 56.6% cases and 66.9% cases in group A and B respectively. The difference of the socio-economic condition between group A and B patients is not statistically significant (p=0.070). It has been found that illiterate or below primary level people are most commonly recruited in this study. In the government hospital majority of the patients are poor as well as illiterate. Thus there may be a selection bias occurred in this study. Illiterate people don't know the personal hygiene and are commonly infected with these types of contagious disease. Only hand wash can decrease the infection in 50.0% cases[16]. It has been found that low poor people are commonly infected with CA-MRSA and HA-MRSA[17].

The distribution of study population according to smoking history is recorded. History of smoking is present in 20.0% cases and 16.6% cases in group A

and B respectively. The difference in the history of smoking between group A and B patients is not statistically significant (p=0.447). It has been found from the result that patients with tobacco use are more vulnerable to CA-MRSA infection. Similar result has been reported by Naimi et al[12] and also has added that some other clinical conditions like diabetes mellitus, alcohol use as well as the others diseases are more commonly associated with CA-MRSA.

The distribution of study population according to area of residence is recorded. Urban dwellers are found more in group A (34.5%) than group B (31.7%). Rural dwellers are found more in group A (65.5%) than group B (68.3%). The difference in the area of residence between group A and B patients is not statistically significant (p=0.618). The distribution of study population according to types of specimens is recorded. Wound Swab is collected more from group A (77.2%) than B (66.9%). Blood is collected 17.2% cases from group A and 14.5% cases from B. Urine is collected from 5.5% cases and 9.7% cases in group A and B respectively. CSF is collected only from group B (1.4%). The difference among the types of specimens between group A and B is statistically significant (p=0.0001). It has been found that wound swab is the most common collected specimen. In majority of cases *Staphylococcus aureus* infects skin and soft tissue infection[2] though it can infect anywhere in the body. CSF collection is a cumbersome process and skilled hand as well as proper aseptic precaution is mandatory. That's why CSF is only collected in group B patients. On the other hand, specimen was collected from different body sites like nasal orifice, throat swab, tip of the catheter, conjunctival swab, umbilical swab and pleural fluid and these specimens are grouped as others. Kawsar et al[18] in Bangladesh has reported similar specimens for the isolation of *Staphylococcus aureus*.

Conclusion

In conclusion, the findings of this study permit to conclude that history of smoking is found more common in group A than group B. Urban dwellers are found more in group A than group B. For further study, the following recommendations are proposed nationwide further large scale study should be carried out. More frequent culture and sensitivity testing should be carried out before giving antibiotic therapy. Study of antimicrobial resistant should be carried out to evaluate the reason of development of antibiotic resistant.

Acknowledgements
None

Conflict of Interest
The authors have no conflicts of interest to disclose.

Financial Disclosure
The author(s) received no specific funding for this work.

Authors' contributions
Akhter H, Rahman F, Yusuf MA conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Akhter H, Hossain F, contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Hossain F, Moureen A, Islam R involved in the manuscript review and editing. Akhter H, Yusuf MA, Zabeen AP as collector of Data and Data Analyst. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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How to cite this article: Akhter H, Moureen A, Zabeen AP, Hossain F, Islam R, Yusuf MA. Comparison of Epidemiology of Patients Presented with Health Care Associated-Methicillin Resistant Staphylococcus aureus and Community Associated-Methicillin Resistant Staphylococcus aureus Infection attending at a Tertiary Care Hospital. Bangladesh J Med Microbiol, 2024;18(1):3-10

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Article Info
Received: 7 October 2023
Accepted: 2 December 2023
Published: 1 January 2024

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