Original Article

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Prevalence of Nasal Carriage of Staphylococcus aureus among Medical and Veterinary Students

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ABSTRACT

Background: *Nasal carriage among hospital personnel is one of the important sources of staphylococci for causing nosocomial infection. The aim of the study was to determine the nasal carriage rate of S. aureus among medical and veterinary students.*

Materials and methods: *A cross-sectional study was conducted on 81 were medical students and 76 veterinary students. Presumptive S. aureus was identified from nasal swab following conventional bacteriological methods and was confirmed by detecting the presence of species-specific nuc gene by PCR. All staphylococci isolates were tested for antimicrobial susceptibility. Isolates displaying resistance to oxacillin and cefoxitin were further tested for the presence of mecA gene by PCR.*

Results: *About 80% of the total S. aureus isolates from medical students showed Multi-Drug Resistance (MDR) whereas about 50% of the total S. aureus from veterinary students were MDR. Among the 39 isolates obtained from medical students, 20 (51.3 %) were methicillin resistant and the rate of methicillin resistance among veterinary students was 22.2%. Only one factor presence of "Rhinorrhea" was found significantly associated with carriage of Staphylococcus sp. among medical and veterinary students.*

Conclusion: *Nasal carriage of is common and there is high level of resistance against Ampicillin, Penicillin, Ciprofloxacin and Erythromycin.*

Key words: Antimicrobial resistance; Coagulase negative staphylococci; MRSA; Nasal Carriage; Staphylococcus aureus.

Introduction

Staphylococcus aureus is a human commensal and also a frequent cause of wide range of infectious conditions, ranging from mild to severe skin infections to life threatening infections such as endocarditis, osteomyelitis and pneumonia.[1] The anterior nares of the nose is the main ecological niche where the organism resides in human beings.[2] In medical students and patients who are nasal carriers may be the source for the transmission and spread of S. aureus in these settings.

The ability to acquire resistance to multiple antimicrobial classes makes S. aureus a challenging pathogen to treat. S. aureus which are resistant to methicillin, referred to as Methicillin-Resistant S. Aureus (MRSA) causes high morbidity and mortality, and increased treatment costs[3].The emergence and global dissemination of MRSA has become a leading cause of bacterial infections in both health care and community settings, resulting in serious consequences. The aim of the study was to determine the nasal carriage rate of methicillin-susceptible and methicillin-resistant S. aureus among medical and veterinary students in Chattogram, Bangladesh.

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Materials and methods

Study design and study population

A cross-sectional study was conducted to determine the prevalence of nasal carriage of S. aureus from 76 students of Chattogram Veterinary and Animal Sciences University (CVASU) and 81 students of Institute of Applied Health Science (IAHS) between May 2022 and October 2022. All procedures were carried out under an approval of the Ethics Committee of CVASU [Approval no. CVASU/Dir (R&E)EC/2022/349/12].

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Collection and processing of nasal swab

Participants were recruited on a voluntary basis during their regular activities. One nasal sample from each participant was collected using a sterile swab and placed in 5 ml Mueller Hinton broth (HiMedia, India) supplemented with 6.5% NaCl and transported to the Microbiology Laboratory of Department of Microbiology and Veterinary Public Health, CVASU.

Isolation and identification of Staphylococcus aureus

The nasal swabs were incubated overnight at 37°C. Thereafter, 10 µL of overnight enrichment culture were streaked onto 5% bovine blood agar and incubated overnight at 37°C. Colonies displaying the characteristic appearance of staphylococci on blood agar (Pigmented, raised, medium-sized and haemolytic) were sub-cultured on to mannitol salt agar (Oxoid Ltd. UK) and incubated at 37°C for 24 hours. Colonies compatible with staphylococci (Bright yellow-coloured colonies) were selected and stained by Gram's stain and tested for catalase production by standard microbiological methods. Catalase-positive and Gram-positive cocci were considered as staphylococci. The presumptive positive colonies on mannitol salt agar were then sub-cultured onto blood agar and incubated at 37°C for 24 hours. After that, isolated bacterial colonies were picked up and transferred to a 10 mL test tube containing 5 mL of Brain Heartinfusion Broth (BHIB) (Oxoid Ltd. UK) and incubated at 37°C for 24 hours. Following incubation, the staphylococci isolates were stored at -80°C using 50% glycerol until further examination.

Coagulase test

Collection of horse plasma Whole blood from a horse was collected for performing coagulase test using anti-coagulant. The collected blood was centrifuged at 3000 rpm for 10 minutes using a centrifuge machine.

Tube coagulase test

The tube coagulase test was performed by adding 0.2 mL of the overnight culture grown in brain heart infusion broth to 0.5 mL of horse plasma in a glass tube. A control tube without horse plasma also was placed to validate the result. The Gram-positive isolates that were coagulase negative but positive for catalase production were considered as Coagulase-Negative Staphylococci (CoNS).

Identification of S. aureus by Polymerase Chain Reaction (PCR)

All suspected staphylococci isolates were confirmed by PCR using the primers described by Shome et al. and the coagulase-positive S. aureus isolates were confirmed by targeting species specific gene nuc as described previously.[4,5]

Extraction of bacterial genomic DNA

Boiling method was used to recover bacterial DNA[6]. Blood agar was used to pick a loop full of fresh colonies (Approximately 3-4), which were then transferred to a 1.5 mL Eppendorf tube containing 200 µL of ultrapure water. After that, the tubes were vortexed to create a uniform cell suspension. On the top of each tube, a ventilation hole was drilled to allow extra vapours to escape while the tubes were boiling. The tubes were then submerged for 15 minutes in ahot water bath at 99°C. The tubes were immediately submerged in -20°C for five minutes after boiling. After freezing, the tubes were submerged again in 99°C hot water for 10 minutes, and the tubes that had been boiled were submerged in -20°C for five minutes. Repeated high-temperature boiling followed by quick freezing caused the bacterial cell wall to disintegrate, releasing the DNA inside. The suspension-filled tubes were then centrifuged at 13000 rpm for 5 minutes. Each tube's 100 µL of supernatant, which included bacterial DNA, was collected and stored at -20°C until use.

Polymerase chain reaction

PCR assays were performed using primers described by Shome et al. and Sasaki et al.[4,6] Nuclease-free water was used as negative control, and one previously identified strain of S. aureus were used as positive control.Visualization of amplified PCR products by agar gel electrophoresis.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the obtained isolates was performed following CLSI guidelines with a panel of 11 antimicrobials including Ampicillin, Cefoxitin, Ceftriaxone, Ciprofloxacin, Erythromycin, Gentamicin, Meropenem, Oxacillin, Penicillin, Sulfamethoxazole-trimethoprim and Tetracycline.[7] Bauer-Kirby disk diffusion procedure was used to perform the antimicrobial susceptibility test.[8] Staphylococci isolates showing resistance against at least three groups of antimicrobial agents (≥3) were defined as Multi-Drug Resistant (MDR) isolates.[9]

Detection of antimicrobial resistance genes by PCR

All oxacillin and cefoxitin resistant isolates were considered for prediction of mecA-mediated resistance in staphylococci.[7] The phenotypic resistant isolates were further investigated for the presence of the mecA gene by PCR.[10]

Statistical analysis

All descriptive and analytical analyses were performed using STATA®13.0 software. The representative heat map was constructed using Graphpad Prism (Version 7.05).

Results

The screening of nasal carriage of staphylococci revealed the presence of this bacteria in 48.1% (n=81)

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of the medical students and 35.5% (n=76) of the veterinary students based on the results of growth characteristics, morphological appearance and biochemical properties of the bacteria. Overall, 10 (25.6%) and 6 (22.2%) coagulase-positive S. aureus isolates were obtained from medical and veterinary students, respectively.All S. aureus isolates which were positive for coagulase were also positive for the presence of nuc gene.All isolates which were phenotypically positive for staphylococci were confirmed by PCR. A single 894-bp PCR product was detected from the Staphylococcus positive isolates (Figure 1).

The results of antimicrobial susceptibility testing of coagulase-positive S. aureus and CoNS isolates revealed that all Staphylococcus isolates irrespective of coagulase reaction exhibited resistance to Ampicillin and Penicillin. Among medical students, all coagulase positive S. aureus isolates displayed resistance to Ciprofloxacin whereas 89.7% isolates were found resistant against this antimicrobial agent. In addition, resistance to Erythromycin and Oxacillin were detected in 70% S. aureus isolates. On the other hand, about 80% CoNS isolates showed resistance against Erythromycin. Among veterinary students, Resistance against Erythromycin was detected in 66.7% S. aureus isolates and 81% CoNS isolates. In addition, more than 75% CoNS isolates displayed resistance against ciprofloxacin. Both coagulase-positive S. aureus and CoNS isolates were found sensitive to gentamicin and meropenem.

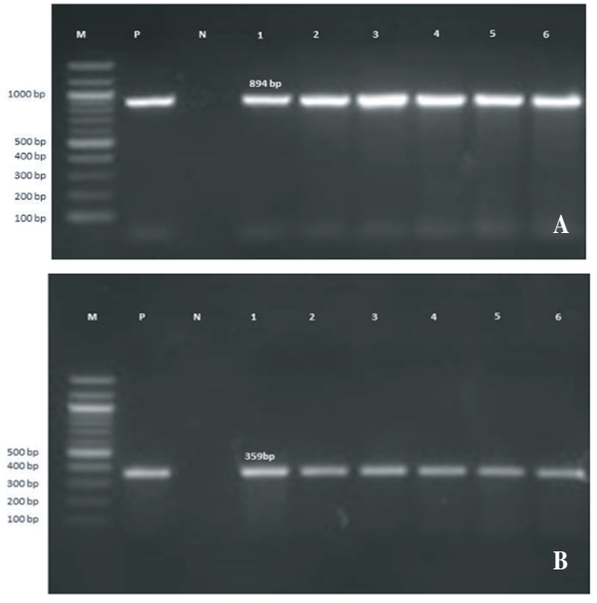

Figure 1 Electrophoresis on agarose gel showing the 894-bp PCR products (A) and the 359-bp PCR products (B) after amplification with specific primers. Amplifications were performed with chromosomal DNA from Staphylococcus isolates. Lanes: M = 100 bp DNA Marker, P = Positive control, N = Negative control, L1 - L6 = reaction specific for Staphylococcus.

Individual antibiogram profiles of all the isolates from medical and veterinary students are illustrated in Figure 2.

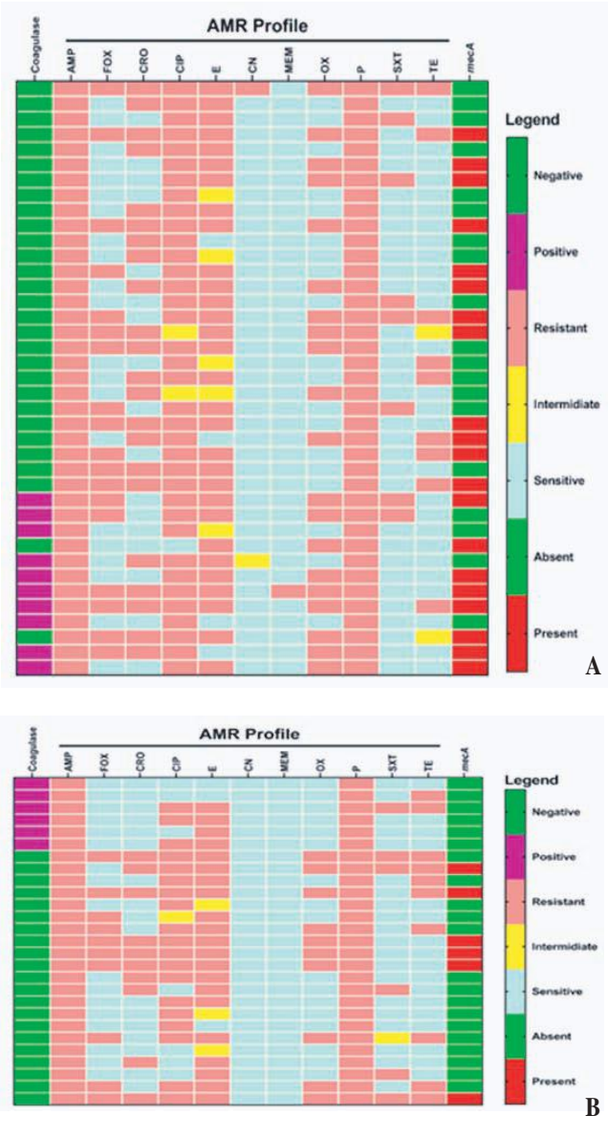

Figure 2 Heat map showing the distribution of antimicrobial resistance phenotype of methicillin resistant Staphylococcus aureus and methicillin resistant CoNS isolates obtained from medical (A) and veterinary (B) students. Each row represents one isolate.

AMP = Ampicillin, FOX = Cefoxitin, CRO = Ceftriaxone, CIP = Ciprofloxacin, E = Erythromycin, CN = Gentamicin, MEM = Meropenem, OX = Oxacillin, P = Penicillin, SXT = Trimethoprim-sulfamethoxazole, TE = Tetracycline.

Number and percentages of S. aureus and CoNS isolated exhibiting resistance to various number of antimicrobial classes are shown in Table I.

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Table I Number and percentages of S. aureus and CoNS isolated from medical and veterinary students exhibiting resistance to various number of antimicrobial classes

Coagulase test	From medical students: Number of antimicrobial classes to which isolates were resistant	From medical students: Number (%) of resistant isolates	From veterinary students: Number of antimicrobial classes to which isolates were resistant	From veterinary students: Number (%) of resistant isolates
Coagulase-positive S. aureus	2	2 (20%)	1	1 (16.7)
	3	3 (30%)	2	2 (33.3)
	4	1 (10%)	3	2 (33.3)
	5	4 (40%)	5	1 (16.7)
Coagulase-negative staphylococci (CoNS)	2	3 (10.3%)	1	1 (4.8)
	3	5 (17.2%)	2	3 (14.3)
	4	14 (48.3%)	3	4 (19.0)
	5	5 (17.2%)	4	6 (28.6)
	6	1 (3.4%)	5	4 (19.0)
	7	1 (3.4%)	6	3 (14.3)

A total of 5 and 14 resistance patterns with different combination of antimicrobial agents were observed in coagulase-positive S. aureus and CoNS isolates, respectively. About 50% of the total coagulase-positive isolates showed multi-drug resistance (i.e. Resistance to ≥3 antimicrobial classes) with a range from 3 to 5 different antimicrobials while about 81% of total CoNS isolates displayed multi-drug resistance. Approximately 14.3% of the CoNS isolates were resistant to seven antimicrobial classes.

Among the 39 isolates obtained from medical students, 20 (51.3 %) were positive for mecA gene and 6 (22.2%) out of the 27 isolates from veterinary students carried mecA gene. (Table II) Notably, all mecA genes were carried by both CoPS and CoNS isolates and finally classified as methicillin resistant isolates (Figure 3).

Table II Prevalence of mecA gene in methicillin resistant isolates obtained from medical and veterinary students

Source	Total no. of staphylococci isolates	Oxacillin-resistant isolates	Cefoxitin-resistant isolates	mecA positive isolates	Prevalence
Medical students	39	20	18	20	51.3
Veterinary students	27	10	10	6	22.2

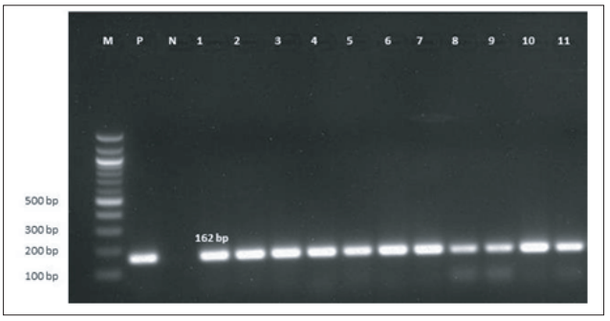

Figure 3 Gel Eelectrophoresis image of PCR products of Methicilline -resistant Staphylococcus isolates showing specific amplified bands 162 bp on 1.0 % agarose gel.
Lanes: M = 100 bp DNA Marker. L1-L1 = Methicilline -resistant Staphylococcus positive band, P = Positive control, N = Negative control.

Discussion

The nasal carriage of S. aureus varied based on the examined populations. In the present study, medical students have a higher rate of carriage compared to veterinary students. It may occur due to medical students practicing in the intensive care unit of hospital and may acquire S. aureus from the hospital.

In the current study medical and veterinary students were targeted where both preclinical and clinical students were included. The preclinical students have less chance of infection than clinical students, because they are not exposed to hospital patients. Medical students are at higher risk (48.15% S. aureus) than veterinary students (35.53%). Among 26 MRSA positive isolates 20 were medical students (80%) and 6 were veterinary students (20%). Any significant difference in these two groups might indicate a different risk potential in the two environments, community and hospital settings. Awareness could have been increased in the medical students to follow preventive measures such as washing the hand after touching the nose, wearing a gown and gloves to help prevention of transmission of infection.

Staphylococci obtained in the present study showed significant resistance to Penicillin (Both in medical and veterinary students which was 100%. This resistance pattern is closely similar same in both coagulase positive and coagulase negative isolates. For medical students, isolates were sensitive to Tetracycline, Trimethoprim-sulfamethoxazole, Meropenem, Gentamicin, whereas isolates obtained from veterinary students were sensitive to Oxacillin, Gentamicin and Meropenem. The indiscriminate use of antibiotics must end right away for the benefit of all people. For the use of antibiotics in various species of animals, proper legal

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protocol should be put in place. Similar to these results, high resistance rates to beta- lactams antimicrobials, such asampicillin and penicillin have been reported to S. aureus isolated from others previous study described by Legese et al.[11]

MRSA is a superbug for its resistance to beta lactam antibiotics.[12] MRSA encode mecA gene that allows the bacteria to produce penicillin binding proteins that are difficult to bacteria to bind medicine. Beta lactamase enzymes degrade the beta lactam antibiotics. Unfortunately, misuse or overuse of antibiotics like cephalosporins, fluoroquinolones, long term intensive care facilities, colonization, contact, very poor hand washing, living in crowed or unsanitary condition or using immune suppressive medications like corticosteroids are the risk factors.[12]

Limitations

This result cannot be generalized because the sample population was from selected community, comprising mainly students of two separate institutions as well as separate professionals. Due to resource constraints, the detailed genotypic characterization of S. aureus and CoNS that colonized in veterinary and medical students were not possible. Further research should be required to overcome these limitations.

Recommendation

Greater research into the sources and routes of transmission of microorganisms resistant to antibiotics is warranted, ideally using a One-Health perspective. It is critical to increase our understanding of how animal interactions and commerce (Direct transmission), farm management, and the larger farm environment (Indirect transmission) contribute to the spread of AMR and to pinpoint viable countermeasures to this phenomenon.

Conclusion

The screening of nasal carriage of Staphylococcus aureus among medical and veterinary students revealed that about 48.15% of medical and 35.53% of veterinary students were positive for this bacterium. S. aureus have acquired high level of resistance against Ampicillin, Penicillin, Ciprofloxacin and Erythromycin. A significant section of them showed multidrug resistance with a range of 3 to 5 antimicrobial agents. About 51% of isolates obtained from medical students and 22% of isolates from veterinary students carried mecA gene.

Disclosure

All the authors declared no competing in interests.

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