CHAPTER – I

Introduction

Staphylococcus aureus is both a human commensal and 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 (Lowy, 1998). Although this bacterium colonizes multiple body sites, the anterior nares of the nose is the main ecological niche where the organism resides in human beings (Wertheim et al., 2005). About 20 – 30% of the human population can harbor this bacterium in this niche (Krismer et al., 2017). By using a variety of proteins and several cell surface components, these bacteria can form a stable bond with nasal epithelial cells, leading to sustained carriage (Wertheim et al., 2005; Mulcahy and McLoughlin, 2016).

Nasal carriage of *S. aureus* can serve as a reservoir for the bacteria and can lead to the spread of infections to others. Within the first few days of life, *S. aureus* nasal colonization may start, and the horizontal transfer from a contaminated mother appears to be the main cause of *S. aureus* carriage in newborns. Hands can serve as a main vector for transmitting the bacteria from the surface to the nose. People who carry *S. aureus* in their nose are at increased risk of developing infections, especially if they have compromised immune systems. 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 (Gnanamani et al., 2017). 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. Cases of colonization or infection caused by MRSA are frequently reported in people who work with animals, including veterinary personnel.

MRSA is a major human pathogen with public health importance. In humans MRSA cause severe infectious disease, including food poisoning, pyogenic endocarditis, suppurative pneumonia, otitis

media, osteomyelitis and pyogenic infections of the skin and soft tissues. The number of illnesses brought on by MRSA is rising globally. MRSA consistently displays a multidrug resistance pattern not only to penicillin, but also to various antimicrobial classes, including macrolides, fluoroquinolones, aminoglycosides, tetracyclines and lincosamides (Algammal et al., 2020). One of the remaining effective treatments for MRSA infections is Vancomycin (Moise-Broder et al., 2004). It is quite concerning that vancomycin-resistant MRSA having recently been isolated in the USA (Lodise et. al., 2008). In Europe, bloodstream MRSA infection occurred in more than 170,000 patients in 2007 with 5400 deaths reported (Kock et al.,2010) . The economic burden associated with this infection was estimated as €380 million (ECDC/EMEA, 2009). The Centre for Disease Control and Prevention reported more than 80,000 bloodstream MRSA infections with 11,285 deaths in the United States in 2011 (CDC, 2013). A recent study in Bangladesh shows the prevalence rate of MRSA in clinical sample was 43.48% (Haq et al., 2011). Despite an increasing prevalence of MRSA in Bangladesh local data on its prevalence among students of medical and veterinary science are lacking.

Aims and objectives of the study

The overall aim of the study is to determine the nasal carriage rate of methicillin-susceptible and methicillin-resistant *S. aureus* among medical and veterinary students in Chattogram, Bangladesh. The specific objectives included -

1. To estimate the prevalence of nasal carriage of *S. aureus* and coagulase negative staphylococci (CoNS) among medical and veterinary students
2. To assess the antimicrobial resistance pattern of *S. aureus* and CoNS isolated from medical and veterinary students
3. To detect the methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant CoNS from medical and veterinary students
4. *To identify the risk factors associated with staphylococci.*

CHAPTER – II
Review of Literature

Members of the genus *Staphylococcus* are important human pathogens characterized as being catalase- positive, Gram- positive cocci that occur in pairs and clusters. Traditionally, members of this genus have been classified into two groups: coagulase-positive and coagulase- negative. In the coagulase-positive group, *Staphylococcus aureus* is the most important staphylococci. *Staphylococcus aureus* is a member of the Firmicutes, frequently found in the upper respiratory tract and commonly associated with nosocomial infections. It frequently found in the nasal cavity and skin or mucous membrane of both human and animals. *S. aureus* colonizes in the anterior nares of up to 50% of adults and about 15% of people consistently carry it (Rasigade et al., 2014). However, *S. aureus* is associated with various life-threatening diseases including pneumonia, osteomyelitis, endocarditis, septicemia, meningitis etc. (Loir et al., 2003).

Structure and morphology of *S. aureus*

S. aureus is 0.5-1.5 µm in diameter and spherical in shape without any flagella. The cell wall of *Staphylococcus* spp. has a strong, protective layer with a thickness of roughly 20–40 nm and a somewhat amorphous appearance. Below the cell wall there is located cytoplasm that is enclosed by the cytoplasmic membrane. Peptidoglycan is the basic component of the cell wall that makes up 50% of the cell wall mass. Another component of the cell wall is teichoic acids, a class of phosphate-containing polymers that make up around 40% of the mass of the cell wall. Teichoic acids come in two varieties: cell wall teichoic acids and cell membrane associated lipoteichoic acids, which either inserted into the bacterial lipid membrane or attached covalently to the peptidoglycan. Teichoic acids give the staphylococcal cell surface a negative charge and are important for the uptake and localization of metal ions, notably divalent cations, as well as the function of autolytic enzymes. About 90% of the weight of the cell wall mainly composed of Peptidoglycan and teichoic acid together and the rest is composed of surface proteins, exoproteins and peptidoglycan hydrolases (autolysins). Some of these components are involved in attaching the bacteria to surfaces and are virulence determinants (Harris et al., 2002).

Virulence and pathogenicity of *S. aureus*: Staphylococcal virulence factors can be classified based on their mechanism of action and pathogenicity as presented in the following table:

Table 1. Virulence factors of *S. aureus* and their function (Gnanamani et al., 2017)

Factors	Functions
Microbial Surface Components Recognizing adhesive matrix molecules (MSCRAMM)	Helping attachment to host tissues
Polysaccharide microcapsule Protein A Panton-Valentine Leukocidin (PVL) Alpha-toxin (Alpha hemolusin) Chemotaxis-inhibitory protein of S. aureus (CHIPS)	Breaking/evading the host immunity
Extracellular adherence protein (Eap) Proteases, lipases, nucleases, hyaluronatelyase, phospholipase C, metalloproteases (elastase), and Staphylokinase	Tissue invasion
Enterotoxins Toxic shock syndrome toxin-1 (TSST-1) Exfoliative toxins A and B	Induces toxinosis

The pathogenicity of *S. aureus* is primarily influenced by a trifecta of toxin-mediated virulence, invasiveness, and antibiotic resistance. The organism can cause sepsis by entering the blood and spreading in different organs. Diseases such as endocarditis, osteomyelitis, renal carbuncle, septic arthritis, and epidural abscess may occurred due to this hematogenous spread. Specific syndromes such as toxic shock syndrome, scalded skin syndrome and food borne gastroenteritis can also occur due to extra cellular toxins without a blood stream infection.

The main *S. aureus* toxin (α toxin) acts by two mechanisms. Each mechanism requires ADAM10 receptor that contains metalloprotease and disintegrin domains. First mechanism includes pore formation in a series of target cells by α toxin via formation of a heptameric pore. Secondly, epithelial, and endothelial breach caused by it via breaking adherens junctions and compromising the cytoskeleton (Figure 1).
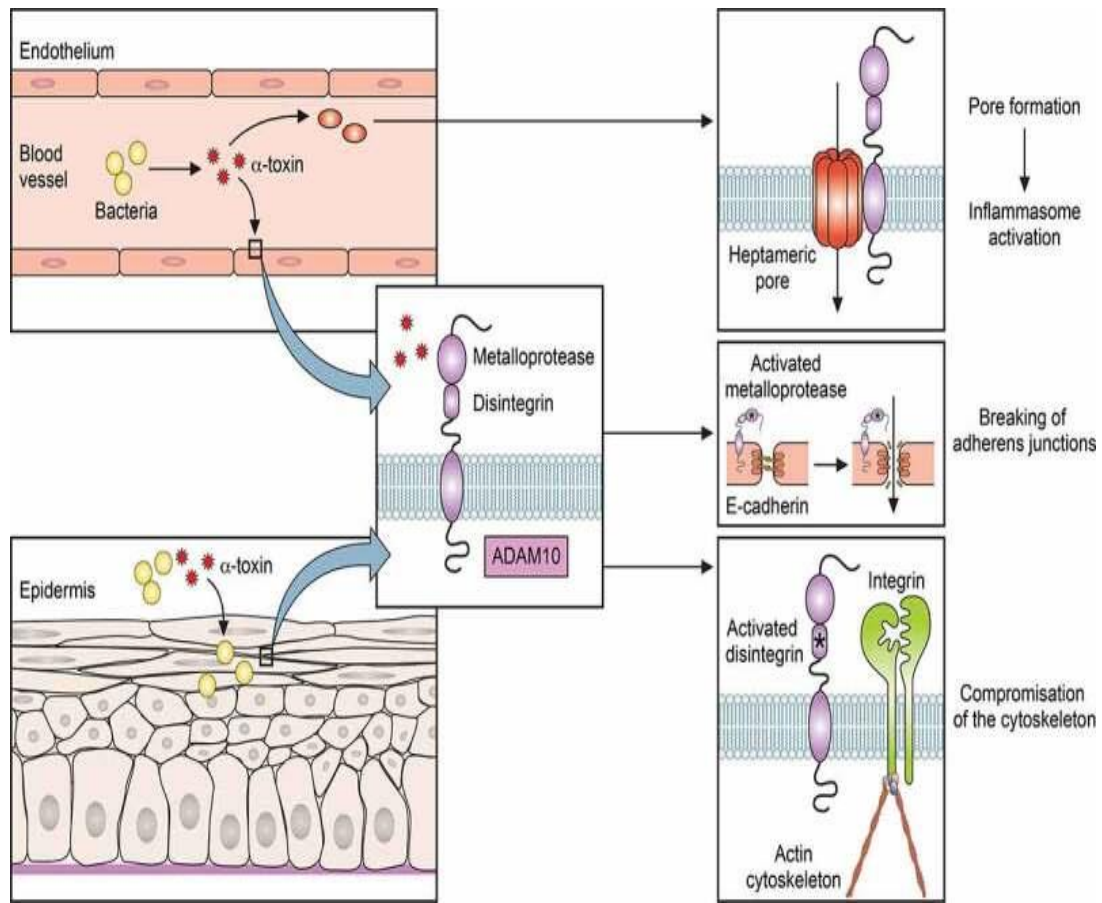
Figure 1: Importance of α-toxin in S. aureus infection (Cheung et al., 2021)

Moreover, *S. aureus* also act as an opportunistic pathogen where primary harm done by other pathogens or predisposing factors. For example, secondary infection by *S. aureus* commonly the ultimate reason for death in lung infection that have begun by a viral infection such as the flu (McCullers, 2014 and Morens et al., 2008). Furthermore, the organism may be inoculated into the skin from a site of carriage which results in different clinical manifestations of localized infections including carbuncle, cellulitis, and impetigo bullosa or wound infection.

Diseases caused by *S. aureus*

Staphylococcal skin infections: Staphylococcal diseases generally manifests as skin infections. Cellulitis or impetigo are two examples of superficial infections that can be focal with nodular abscesses (furuncles and carbuncles) or diffuse with vesicular pustules and crusting (Kwiatkowski et al., 2017).

Staphylococcal bacteremia: it is related to intravascular catheters or other foreign bodies. It may also occur without any obvious primary site (Holland et al., 2018).

Staphylococcal neonatal infections: Neonatal infections, such as skin lesions with or without exfoliation, bacteremia, meningitis, and pneumonia, typically manifest within six weeks of birth. On the other hand, patients with immune suppression and other viral infections can develop secondary pneumonia (Cailes et al., 2018).

Staphylococcal endocarditis: *S. aureus* endocarditis is an acute highly febrile illness often accompanied by visceral abscesses, embolic phenomena, pericarditis, subungual petechial, subconjunctival hemorrhage, purpuric lesions, heart murmurs, perivalvular abscess, conduction defects, and heart failure secondary to cardiac valve damage (Liesenborghs et al., 2020).

Staphylococcal toxic shock syndrome: any type of complicated *S. aureus* infection (eg, postoperative wound infection, infection of a burn, skin infection) or use of vaginal tampons may result in staphylococcal toxic shock syndrome. Methicillin-susceptible *S. aureus* (MSSA) has historically caused the majority of cases, but MRSA cases are on the rise (Krogman et al., 2017).

Staphylococcal osteomyelitis: occurs more frequently occur in children, causing chills, fever, and pain over the involved bone. Subsequently, the overlying soft tissue becomes red and swollen. The possibility of articular infection and its frequent effusion suggest septic arthritis rather than osteomyelitis (Kavanagh et al., 2018).

Spread and transmission of *S. aureus*

The skin, rectum, vagina, gastrointestinal system, and axilla are among the bodily areas where *Staphylococcus aureus* can be detected, with the anterior nares acting as the primary reservoir. *S. aureus* can enter the nasal mucosa through a cutaneous commensal site and can spread into the anterior nares if the host's defenses are defeated, making the host a *S. aureus* nasal carrier (Wertheim et al., 2005a). Nasal colonization in humans may start during the first few days of life (Maayan-Metzger et al., 2017). This has been shown in a cohort study that examined *S. aureus* nasal carriage in 100 infant-mother pairs for six months after delivery (Peacock et al., 2003). The carriage rate was nearly 40-50% throughout the first eight weeks of life before falling to 21 percent at six months. Additionally, 68% of infant-mother couples in this study had nasal carriage concordances, indicating the importance of environmental factors in *S. aureus* carriage (Peacock et al., 2003). Hands act as primary vector for *S. aureus* transmission after birth from surface to nose (Wertheim et al., 2005a). In a cohort study involving healthy hospital staff members and outpatients, nasal carriage was assessed using one or more swabs. Participants were asked to fill out a questionnaire on their nose-picking behavior, and it was discovered that there is a direct link

between this behavior and *S. aureus* nasal carriage. However, it is unknown whether patients who picked their noses more frequently had extra nasal sites colonized (Wertheim et al., 2006). Studies conducted on individuals who share a home have shown that these people frequently have genetically similar strains in their nares, which suggests horizontal transmission (Nouwen and Optima Grafische Communicatie, 2004; Muthukrishnan et al., 2013). Despite being rare, airborne transmission is another way that *S. aureus* could spread (Wertheim et al., 2005a). The danger of endogenous *S. aureus* spreading in the air increases and outbreak of the infection may occur with viral upper respiratory infections. In 1996, 8 out of 43 patients in a surgical ICU of a university hospital in the United States showed an outbreak of MRSA. According to the investigation a single physician was suffered an upper respiratory infection and detected as the source of outbreak and also was a nasal carrier of MRSA. The authors concluded their research by conducting an experimental clinical test on this physician to determine the airborne dispersal of *S. aureus*, and the results revealed that transmission of the bacteria was 40 times more likely to occur when he had a rhinovirus infection than when he did not. Dispersal was dramatically decreased when a mask was worn (Sherertz et al., 1996). On the other hand, healthcare workers are rarely sources of *S. aureus* transmission when there is not an outbreak and there are control measures (Price et al., 2017). Healthcare professionals' mobile devices might act as reservoir of *S. aureus* (Chang et al., 2017). In a recent study the likelihood of bacterial contamination of mobile phones of medical staff members' working in operating room was assessed. 72 healthcare professionals collected bacterial samples from their hands, anterior nares, and cellphones. The findings showed that *S. aureus* had been identified from the nares of 31 employees, from 8 mobile phones, and from 4 hands. 7/8 of the mobile phone strains were found to be genetically identical to nares-isolated strains, according to genotyping (Chang et al., 2017).
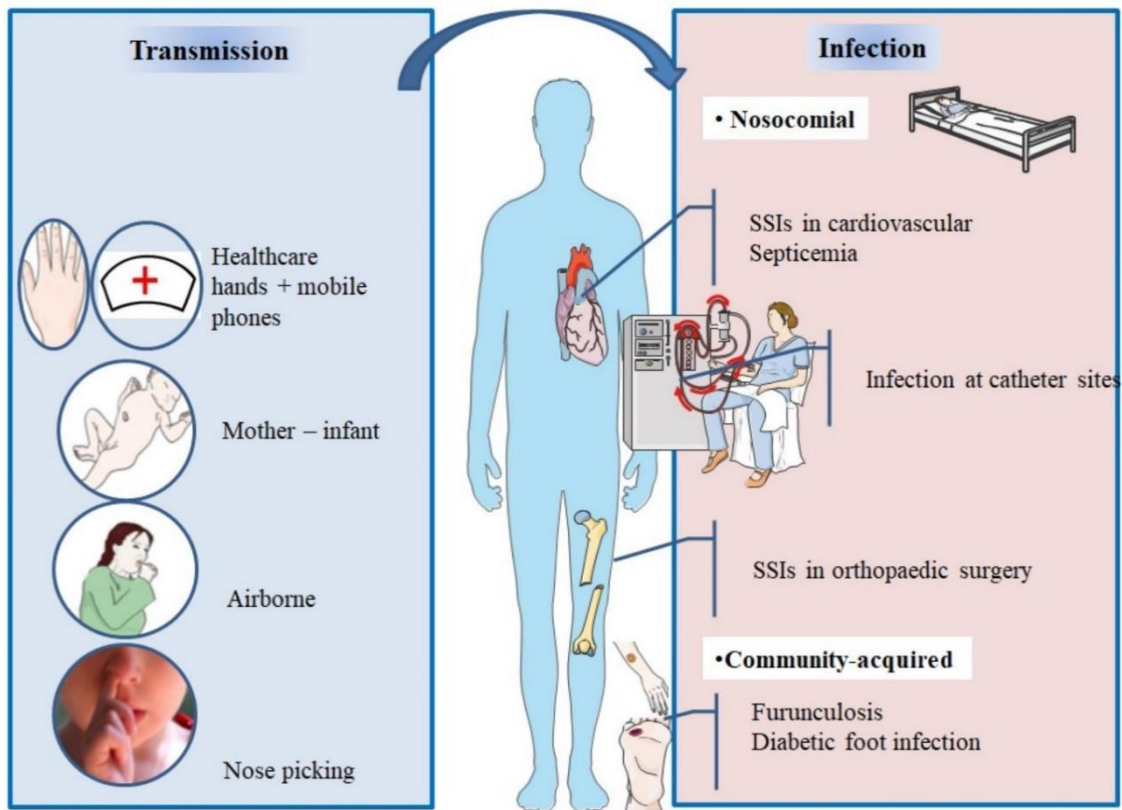
Figure 2: Main spread and transmission mechanism of *S. aureus* and impact of nasal carriage on subsequent infections (Sakr et al., 2018)

Mechanism of Colonization

The anterior part of the nares that is the vestibulum nasi is lined by a keratinized, stratified non-ciliated squamous epithelium, while the remainder of the nasal cavity, or its inner part, is coated with a ciliated columnar epithelium (Peacock et al., 2001; Weidenmaier et al., 2012). *S. aureus* has been described as habitat of both epithelia (Mulcahy et al., 2012). Additionally, nasal tissue of healthy volunteers was also described intracellular localization (Hanssen et al., 2017). As proven in vitro and in vivo (Mulcahy et al., 2012; Baur et al., 2014), *S. aureus* expresses adhesive molecules that are essential for the development of contacts with human cell surface components and are necessary for a successful colonization (Sakr et al., 2018; Figure 3).
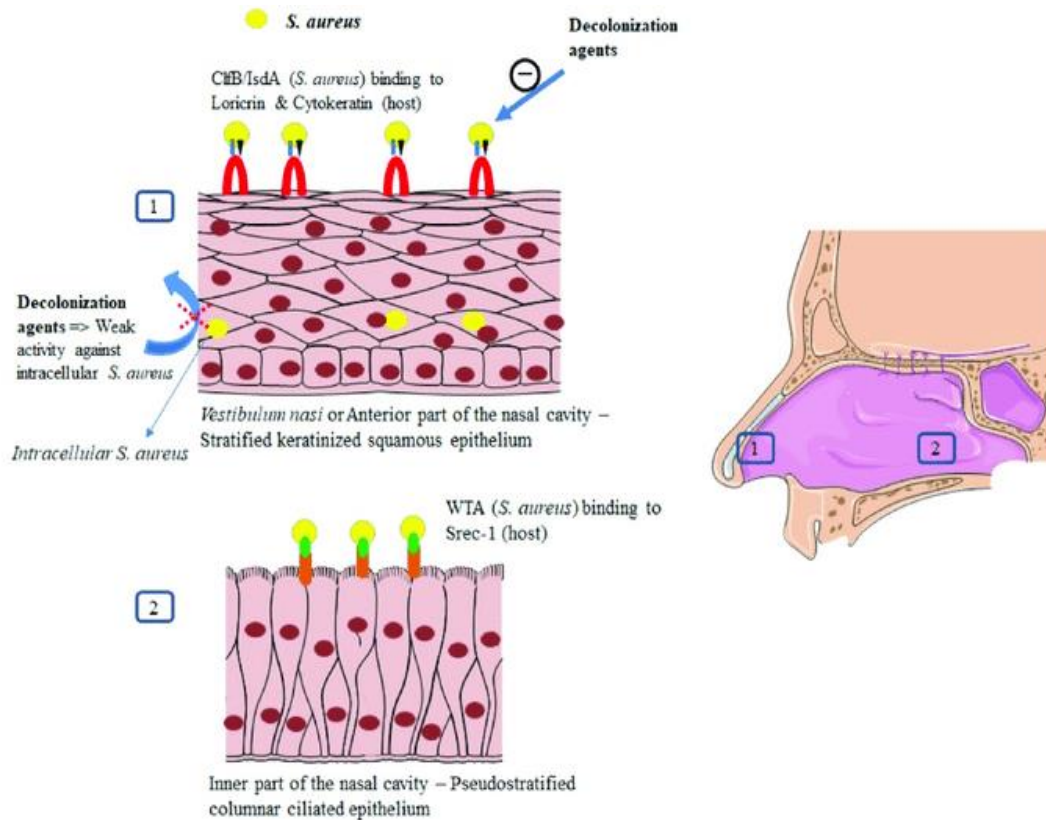
Figure 3: Mechanism of *S. aureus* nasal colonization (Sakr et al., 2018)

Individual risk factors for *S. aureus* nasal colonization

Nasal colonization is influenced by host factors including the underlying illness or conditions. According to certain studies, patients who were obese (Olsen et al., 2012) or infected with the human immunodeficiency virus (HIV) (Kotpal et al., 2016) experienced nasal carriage more frequently than those who were healthy. When compared to non-diabetic patients in the same population, this higher prevalence was also discovered among diabetes patients receiving dialysis (Luzar et al., 1990). Increased carriage rate also recorded with patients infected with other diseases including atopic dermatitis (Breuer et al., 2002), granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis), rheumatoid arthritis (Laudien et al., 2010), skin and soft tissue infections (Immergluck et al., 2017), granulomatosis with polyangiitis, and recurrent furunculosis (Demos et al., 2012). According to Liu et al. (2015) men and women had similar rates of carriage in healthy subjects, however men had larger bacterial densities. Numerous additional host characteristics, including as hormonal contraception (Zanger et al., 2012) and the presence of haemoglobin in nasal secretions (Pynnonen et al., 2011), have been thoroughly studied and

identified as additional predisposing factors. There was no correlation found between genetic factors and *S. aureus* carriage at the genomic level. It is interesting that some polymorphisms in host inflammatory response genes have been linked to *S. aureus* nasal carriage.

At the level of the immune system, polymorphisms in some protein-encoding genes and distinct AMPs expression profiles may be the factors that determine the varied carriage states. Vitamin D receptor polymorphisms were identified in Deoxyribonucleic Acid (DNA) isolated from peripheral blood leukocytes in a study involving 93 patients with type 1 diabetes. Analysis revealed a correlation between an elevated rate of *S. aureus* colonization and the existence of particular alleles encoding for vitamin D receptors (Panierakis et al., 2009).

S. aureus infection in healthcare workers

S. aureus nosocomial infections cause morbidity in hospitalized patients, extending the duration of hospitalization and driving up healthcare costs (Cosgrove et al., 2003). Infection by *S. aureus* has been linked with surgical wound, hospital-associated pneumonia, catheter-associated infections and bacteremia (Boucher et al., 2010). Due to the development of antibiotic resistance, particularly in the methicillin-resistant *S. aureus* (MRSA), the treatment has also become more complicated. *S. aureus* can colonize in healthcare personnel, and if infection control procedures are not followed it may be transmitted to patients under care of them. Therefore, the best target group to initially raise this awareness would be medical students. In order to serve this goal the prevalence of *S. aureus* among this group must be evaluated for the carriage status. (Stubbs et al., 1994) conducted an interesting study in which they examined the nasal carriage of *S. aureus* in Australian medical students. According to the degree of exposure in the hospital, medical students were divided into five groups in the study. The prevalence of *S. aureus* carriers did not differ across groups (35.2–42.6%), although it is important to note that among medical students in their clinical years as opposed to their pre–clinical years, there was an increase in resistant strains.

Hospital-associated methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequent cause of nosocomial infections and multidrug-resistant healthcare-associated illnesses (Zaha et al., 2019). Immunosuppression, hemodialysis, prolonged hospital stays, and old age are the main risk factors for MRSA (Garoy et al., 2019). MRSA is quite common in hospitals all around the world, with rates (>50%) being highest in North and South America, Asia, and Malta (Stefani et al., 2012).

In healthcare settings, meningitis, pneumonia, and infective endocarditis are a few of the life-threatening conditions that could result from MRSA infection (Lee et al., 2018). Compromised immune systems in inpatients, which can worsen the condition and can contracted by contact with hospital equipment, causal contact with the visitors, or healthcare workers themselves, are referred as contributing factors. Some of the variables that contribute to the spread of MRSA are poor hygiene of healthcare worker, insufficient barrier nursing, antibiotic resistance, a rise in possible carriers, and their usage of fomites. MRSA can be spread via surfaces that are contaminated as well as through direct hand contact with contaminated bodily fluid or stethoscopes (Jones et al., 1995), identity badges (Hogue et al., 2017), neckties (Pace-Asciak et al., 2018), and white coats (Sande and Basak, 2015), all of which are worn by healthcare professionals, mostly doctors and medical students.

S. aureus infection in veterinary professionals

In 30% of healthy individuals*, S. aureus* permanently colonizes the nasal mucosa and is momentarily present in up to 70% of them (von Eiff et al., 2001). MRSA carriers are uncommon (0.2%) in individuals who have never had any interaction with healthcare (Salgado et al., 2003). Colonization quadruples the likelihood of a subsequent infection. Colonizing MRSA strains may result in significant pneumonia or a purulent skin and soft tissue infection under certain circumstances. Livestock were mainly described as MRSA reservoirs among all animals (Witte et al., 2007). Animal hosts are adapted to the livestock-associated strains (LA-MRSA) (Fitzgerald, 2012). Humans may acquire colonized with these microorganisms after having regular and close contact with an MRSA-positive animal, but infection is uncommon (Cuny et al., 2015). Veterinary professionals are also at a higher risk of contracting MRSA apart from farmers and livestock breeders. A study was conducted to identify MRSA in veterinary professionals in Czech Republic in 2017. There were 134 attendees among which 88.8% were veterinarians, 4.4% were pharmacists/researchers and 3.7% were veterinary school students. Regarding the type of practice, 57% linked with small-animal practice, 42.3% in mixed practice and only 0.7% in livestock practice. In total, 29.9% samples confirmed *S. aureus* positive of which 6.72% were MRSA strains, all carrying *mecA* gene (Neradova et al., 2017).

MRSA is a significant pathogen that affects not just human but also livestock and small animals, and colonization itself increases the risk of subsequent infection. Several research conducted

worldwide have demonstrated the greater prevalence of MRSA transmission in veterinary professionals. In Europe, the rates vary from 0.7-19.2% (Žemličková et al., 2009). High prevalence data typically come from nations with highly established animal industry, like the Netherlands, Denmark, or Germany (Wulf et al., 2008; Moodley et al., 2008 and Cuny et al., 2009). International variations in prevalence rates are caused by factors such as the type of veterinary practice, the frequency of animal contact, the length of time since exposure, and the study design itself.

Antimicrobial resistance in *S. aureus*

Staphylococci are resistant to many antimicrobials and according to the history, AMR in staphylococci started at the beginning of the antibiotic era. Resistance to different antibiotics described below:

Beta-lactam resistance

Penicillin resistance

Penicillin G, the first beta-lactam antibiotic developed by Alexander Fleming in 1928, was first used as a chemotherapeutic treatment on humans in 1941 (Fletcher C, 1984). The antibiotic proved effective against Gram-positive infections as well as act as a strong weapon against Staphylococcal infection. The first reports of *S. aureus* strains resistant to penicillin surfaced a year after it was first used clinically. Penicillinase, an enzyme that is present in penicillin-resistant isolates cleaves the beta-lactam ring of penicillin and so renders the antibiotic inactive. The development and spread of penicillinase-mediated resistance in *S. aureus* is referred to as the first wave of resistance. The situation became pandemic after alarming spread in the 1960's. By the late 1960s, almost 80% of *S. aureus* isolates obtained from hospitals and community had developed penicillin resistance (Chambers and Deleo, 2009). Regardless of whether they originated from a hospital or the community, over 90% of Staphylococcal isolates expressed penicillinase enzyme by the early 2000s (Lowy, 2003).

Methicillin resistance

The discovery of methicillin, a penicillinase-stable semisynthetic penicillin used to counter the penicillinase resistance in *S. aureus*. Methicillin resistance (MRSA) was first documented in 1961, the same year that individuals began taking the antibiotic in clinics. After the first discovery, MRSA clones rapidly spread throughout the world, although only in nosocomial settings. This is known as the second wave of beta-lactam resistance in *S. aureus* infections (Enright et al., 2002). Methicillin resistance was caused by the presence of the *mecA* gene. Increased MRSA infection rate in hospitals resulted in high morbidity and mortality, as well as raised the expense of health treatment (Klein et al., 2007 and Köck et al., 2010).

The third wave of beta-lactam resistance in *S. aureus* emerged in the beginning of the 1990s as a result of reports of MRSA infections in the community. In the last ten years, community MRSA strains have spread throughout hospital settings, blurring the distinction between HA and CA MRSA (Mediavilla et al., 2012).

Quinolone resistance

Quinolones function as antibacterial agents by inhibiting DNA Gyrase and Topoisomerase IV, which are essential for de-supercoiling and separating concatenated DNA strands in bacteria. Due to point mutations in the GrlA subunit of topoisolmerase IV and the GyrA subunit of Gyrase, *S. aureus* gradually acquires resistance to quinolones. Another mechanism by which *S. aureus* develops quinolone resistance is the development of NorA efflux pumps (Hooper, 2000). Despite the fact that the mechanism of resistance and the genes responsible for its encoding are completely different, quinolone resistance and methicillin resistance are frequently linked in *S. aureus*. MRSA isolates implicated in acute bacterial skin and skin structure infections (ABSSSIs) in hospitals in 2008 which had a fluoroquinolone resistance rate of 70.3%. Due to the high incidence of quinolone resistance, even the use of third- and fourth-generation quinolones has been disallowed for the treatment of MRSA in hospital settings. Despite the fact that non-beta-lactam antibiotics like quinolones were once effective against CA-MRSA infections, the situation has changed recently due to an increase in the prevalence of multi-drug resistance CA-MRSA infections (Dalhoff, 2012).

Vancomycin resistance

Vancomycin, a glycopeptide antibiotic, was discovered in 1952 from a microbiological source (*Streptomyces orientalis*). Despite being given clinical approval in 1958, methicillin and other anti-staphylococcal penicillins that were less toxic but equally effective against penicillin-resistant staphylococci quickly exceeded vancomycin (Levien, 2006). Since vancomycin has been clinically effective in treating MRSA infections since the 1980s, it has become known as the "workhouse anti-MRSA" medicine (Rodvold and McKoneghy, 2014). In 2002, first report of *S. aureus* strain with a vancomycin MIC of greater than 128 mg/L was released. The bacterium exhibited the high-level vancomycin resistance gene *VanA* and was methicillin-resistant (Sievert et al., 2002). Rare reports of *S. aureus* strains resistant to vancomycin being identified came after this. These strains are all known as vancomycin-resistant *S. aureus* since they have all been demonstrated to have a high vancomycin MIC (> 8 mg/L) (VRSA).

Resistance to other antibiotics

Because HA_MRSA strains are frequently MDR phenotypic, drugs including sulphonamides, tetracyclines, aminoglycosides, chloramphenicol and clindamycin ruled out due to inactivity, leaving vancomycin as the backbone of treatment. *S. aureus*, especially MRSA, has frequently been found to be resistant to sulphonamides and trimethoprim (Then et al., 1992), tretracycline (Schmitz et al., 2001), aminoglycosides (Schmitz et al., 1999), chloramphenicol (Fayyaz et al., 2013), and clindamycin (Frank et al., 2002).

Methicillin resistant *S. aureus* (MRSA)

MRSA is a Gram-positive Staphylococcus strain that is resistant to widely used antibiotics known as betalactams such as methicillin, oxacillin, and penicillin. When a large mobile genetic element called staphylococcal cassette chromosome, mec (SCCmec) is present, it is called Methicillin resistant *Staphylococcus aureus* (MRSA). It possesses the *mecA* gene, which codes for PBP2a, an alternative penicillin binding protein with a poor binding affinity for all P-lactams (Ito *et al*., 1999). Since p-lactamase-insensitive penicillins were first used in medical practice, MRSA strain were first identified in hospital settings. Because of their capacity to develop multidrug resistance determinants, MRSA strains continue to pose a severe threat to health care. Methicillin-sensitive *S. aureus* (MSSA) can also cause disease outbreaks in hospitals (Kurlenda et al., 2009), MRSA is

particularly easy to spread throughout a hospital, and without the implementation of a special surveillance program with control procedures, there is a high risk of an epidemic in such hospital.

Prevalence of MRSA

MRSA has grown to be a global issue although its prevalence varies greatly between nations. While incidence rates are low in Scandinavia, The Netherlands, and Switzerland, they are consistently high in the USA, South America, Japan, and southern Europe (Styers et al., 2005; Talan et al., 2011; Moran et al., 2006). There are several investigations which suggested that rate of MRSA is increasing among healthy community-dwelling individuals. Even community acquired MRSA has been break through from its origin site to the community of hospital settings (Van Cleef et al., 2011). In certain hospitals, the CA-MRSA strains have even replaced the standard hospital-acquired MRSA strains (Garcia-Alvarez et al., 2012). According to research, there are large variations in the reported prevalence rates of CA-MRSA. This is partly due to the diverse criteria used to distinguish between CA-MRSA and HA-MRSA, but it is also due to the various contexts in which the investigations were conducted. It should be mentioned that only few studies have been carried out on community members who were chosen at random and were in good health. Since the majority of studies have been conducted on hospitalized patients or patients who have just been admitted, the 'real' prevalence of CA-MRSA has likely been overstated. Recently, prevalence rates of CA-MRSA has been reported through a meta-analysis of studies (Salgado et al., 2003). In 27 retrospective investigations and 5 prospective studies, the combined prevalence of CA-MRSA among MRSA isolates from hospitalized patients was 30.2% and 37.3%, respectively. The combined MRSA colonization rate among community members without healthcare contacts was 0.2%. The incidence of MRSA nasal carriage among young, healthy community members was found to be 0.7% in a Portuguese surveillance study (Sa´-Lea˜o et al., 2001). The prevalence of CA-MRSA following hospital admission has been reported to be 0.1% in Switzerland (Harbarth et al., 2005) and 0.03% in The Netherlands (Wertheim et al., 2004).

Detection method of *S. aureus*

Culture: The cultural properties of *Staphylococcus* is given below.

Bovine blood agar: Colonies are found surrounded by hemotoxic zone. This reaction occurs mainly due to hemotoxic reaction (Baired and Parkar,1980).

Mannitol Salt Agar: Colonies with bright yellow zone due to mannitol fermentation (Baired and Parkar.,1980).

Biochemical properties: The biochemical properties of this organism is given below-

Catalase test: This test is done for evaluation of gas bubbles of Hydrogen peroxide (Rusenova et al., 2017). The organism is Catalase positive.

Oxidase test: This test is done for oxidase positive bacteria that turn the broth dark blue within 5 to 6 minutes (Rusenova et al., 2017).

Coagulase test: This test is done for formation of clot (Rusenova et al., 2017). *Staphylococcus aureus* is coagulase positive.

Carbohydrate Dissimilation Test: The production of acid from maltose and mannitol under aerobic conditions is the indicator of Carbohydrate Dissimilation test (Baired- Parkar, 1980).

Characteristics on growth medium

Isolation of the organism can be done by streaking from the clinical specimen or from a blood culture onto solid media such as blood agar, tryptic soy agar, or brain heart infusion agar.

Specimens may be contaminated with other microorganisms can be inoculated onto mannitol salt agar plate containing 7.5% sodium chloride that allows the growth of halo-tolerant staphylococci. Being mannitol fermenting bacteria *S. aureus* gives yellow or golden colored colonies. On blood agar, round, raised, opaque, yellow to golden yellow colonies of 1-2 mm in diameter growth seen after inoculating 18-24 hours. Growth of the organism may be formed with or without hemolysis. Isolates should be sub cultured at least once on a non-selective medium after initial isolation before using in a diagnostic test which requires pure culture or heavy inoculum (El-Jakee et al., 2008).

Identification of toxins

In extreme situations like food poisoning and toxic shock syndrome, toxin identification is important. Different toxins produced by *S. aureus*, including enterotoxins A to D and TSST-1 that may be identified by using agglutination tests. The toxins present in the samples clumps the latex particles and determines the test result (Berube et al., 2013). For this purpose, commercial latex agglutination tests are available.

Disc diffusion test for MRSA

S. aureus is incubated on Mueller Hinton agar (MHA) impregnated with Oxacillin (1 or 5µg) and Cefoxitin (30µg) discs to carry the disc diffusion method. Identification of MRSA is done by assessing zone of inhibitions with oxacillin < 14 mm and/or cefoxitin < 21 mm (CLSI, 2007). Due to its simplicity of use and higher sensitivity, the cefoxitin disc diffusion test is thought to be superior to the oxacillin disc diffusion test. Cefoxitin activates the MRSA *mecA* gene, and the results have been resemblance to PCR (Broekema et al., 2009). As a result, due to environmental resource constrains, the Cefoxitin disc diffusion test can serve as an alternative to PCR for the detection of MRSA.

Oxacillin MIC test

Gradient plates of MHA containing 2% NaCI with doubling dilutions from 0.25 ng/ml to 256 ng/ml of oxacillin are prepared. *S. aureus* inoculum is prepared by diluting 0.5 9 McFarland equivalent suspension of a strain with sterile normal saline to the concentration of 10^4 CFU/ml. The plate's arc spot inoculated and incubated at 35°C for 24 hours. An oxacillin MIC of less than or equal to 2 µg/ml is indicative of susceptibility and that of > 2 mg/ml resistance (CLSI, 2007).

Chromogenic Media

For the direct detection of MRSA different selective and differential chromogenic media are used. This kind of medium includes antibiotics like cefoxitin as well as a particular chromogenic substrate. MRSA will produce colored colonies due to hydrolysis of chromogenic substances in the presence of antibiotics.

PCR: Polymerase chain reaction (PCR) is used for detection of *mecA* gene of *S. aureus*. This can be done by using *mecA* gene specific primers (Bhanderi and Jhala, 2011).

Nucleic acid amplification tests

Commercial nucleic acid amplification tests are available for the direct detection and identification of *S. aureus* in clinical specimens. Whereas the earlier versions of these tests required manual extraction of bacterial DNA and testing multiple specimens in large batches, integrated processing of specimens (extraction, gene amplification, and target detection) is now performed on highly

automated platforms with disposable reagent strips or cartridges. They are useful for screening patients for carriage of methicillin-sensitive S. aureus (MSSA) and MRSA (Kateete et al., 2010)

From the above mentioned reviews we can see that healthcare associated infections are increasing daily. Medical and veterinary students are more prone to infections due to their exposure to patients and animals. So the study was done to find out the prevalence of nasal carriage of *S. aureus* among the students of two institutions in Chattogram, Bangladesh.

CHAPTER – III

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 students of two institutions – Chattogram Veterinary and Animal Sciences University (CVASU) and Institute of Applied Health Science (IAHS) under the University of Science and Technology, Chittagong (USTC). The study was carried out during the period of May 2022 to October 2022.

Collection and processing of nasal swab

Participants were recruited on a voluntary basis during their regular activities. Before collecting samples, an informed consent form was made available to each participant who also completed a questionnaire regarding demographic and clinical information (Annexure 1). One nasal sample from each participant was collected using a sterile swab. The swab was introduced into nostrils, gently rotated, 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. All procedures were carried out under an approval of the Ethics Committee of CVASU [Approval no. CVASU/Dir (R&E)EC/2022/349/12].

Isolation and identification of *Staphylococcus aureus*

The nasal swabs kept at Mueller Hinton broth 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 hemolytic) 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 colored 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 heart

infusion 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. The resulting supernatant, the plasma, was then transferred to a sterile test tube using a sterile micropipette. The plasma was then stored at - 20°C for future use.

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. After gentle mixing, the tests were incubated at 37°C and examined after 2, 4, 6 and 24 hours. The presence of coagulates were considered when large organized coagulation of all the contents of the tube occurred which do not come off when inverted (Brasil, 2003). A control tube without horse plasma also was placed to validate the result.

Isolates that were catalase-positive and coagulase-positive were taken presumptively as *S. aureus*. 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. (2011) and the coagulase-positive *S. aureus* isolates were confirmed by targeting species specific gene *nuc* as described previously (Sasaki et al., 2010).

Extraction of bacterial genomic DNA

Boiling method was used to recover bacterial DNA (Ahmed et al., 2015). 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 vapors to escape while the tubes were boiling. The tubes were then submerged for 15 minutes in a

hot 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. (2011) and Sasaki et al. (2010). The primer sequences used for the PCR are shown in Table 2. PCR reactions were conducted with a 25 μL reaction volume. Proportions of different reagents used for PCR are given in Table 3. Negative and positive controls were used in each reaction. Nuclease-free water was used as negative control, and one previously identified strain of S. aureus were used as positive control.

Table 2. Primer sequences used in polymerase chain reaction (PCR) to detect staphylococci and *Staphylococcus aureus*

Gene	Primer name	Primer sequence (5´-3´)	Amplicon size (bp)	PCR condition	Reference
23S rRNA	SAS2F	AGCGAGTCTGAATAGGGCGTTT	894	Reaction mixtures were thermally cycled once at 94°C for 5 min, followed by 30 times at 94°C for 30 s, 60°C for 30 s, 72°C for 45 s and then once at 72°C for 10 min.	Shome et al., 2011
	SAS2R	CCCATCACAGCTCAGCCTTAAC			
nuc	au-F3	TCGCTTGCTATGATTGTGG	359	Reaction mixtures were thermally cycled once at 95°C for 2 min; 30 times at 95°C for 30 s, 56°C for 35 s, and 72°C for 1 min; and then once at 72°C for 2 min.	Sasaki et al., 2010
	au-nucR	GCCAATGTTCTACCATAGC			

Table 3. Contents of PCR reaction mixture for the detection of staphylococci and *Staphylococcus aureus*

SL.No	Contents	Volume
1	Thermo Scientific Dream Taq PCR Master mix (2x) ready to use	12.5µl
2	Forward primer	0.5 µl
3	Reverse primer	0.5 µl
4	Nuclease-free water	9.5 µl
5	DNA template	2 µl
	Total	25µl

Visualization of amplified PCR products by agar gel electrophoresis

A gel tray was assembled with setting proper teeth sized gel comb in the tray. Then, 1.5% agarose solution (Seakem® LE agarose, Lonza) was made and kept in a water bath at 50°C for cooling, and 5 µL ethidium bromide was added. Finally, the melted agarose was added to the gel tray and let to stand for roughly 20 minutes to allow the gel to solidify. The gel was placed in an electrophoresis tank that had 50 mL of 1X TAE buffer previously added to it. Then 5 µL of each PCR products were added. Items were loaded into the gel holes. In order to compare the amplicon size of the gene product, one hole was loaded with a DNA marker (Thermo scientific O'Gene ruler 100 kb). In each electrophoresis run, both negative and positive controls were used. Electrophoresis was conducted at 100 volts and 80 mA for 35 minutes. Finally, a UV Transilluminator was used to visualize the gel (BDA Digital, Biometra GmbH, Germany).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the obtained isolates was performed following CLSI guidelines (CLSI, 2020) with a panel of 11 antimicrobials including Ampicillin, Cefoxitin, Ceftriaxone, Ciprofloxacin, Erythromycin, Gentamicin, Meropenem, Oxacillin, Penicillin, Sulfamethoxazole-trimethoprim and Tetracycline. Bauer-Kirby disk diffusion procedure (Bauer et al., 1966) was used to perform the antimicrobial susceptibility test. Mueller-Hinton (MH) agar (Oxoid Ltd., UK) containing 2% NaCl was prepared according to the manufacturer's instructions. A bacterial turbidity equivalent to 0.5 McFarland standards was used as inoculum for each isolate.

For each isolate, the zone of inhibition around each disk was measured and interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI documents (CLSI, 2020) (Table 4). Methicillin resistance was determined by measuring zone diameter around oxacillin and cefoxitin discs. Staphylococci isolates showing resistance against at least three groups of antimicrobial agents (≥3) were defined as multi-drug resistant (MDR) isolates (Li et al., 2014).

Table 4. Interpretive categories and zone diameter breakpoints (CLSI, 2020)

Antimicrobial agent	Disc code	Disc concentration	Diffusion zone breakpoint (mm)		
			Sensitive	Intermediate	Resistant
Ampicillin	AMP	10 µg	≥29	-	≤28
Cefoxitin	FOX	30 µg	≥22	-	≤21
Ceftriaxone	CRO	30 µg	≥22	-	≤21
Ciprofloxacin	CIP	5 µg	≥21	16-20	≤15
Erythromycin	E	15 µg	≥23	14-22	≤13
Gentamicin	CN	10 µg	≥15	13-14	≤12
Meropenem	MEM	10 µg	≥22	-	≤21
Oxacillin	OX	1 µg	≥18	-	≤17
Penicillin	P	10 units	≥29	-	≤28
Trimethoprim-sulfamethoxazole	SXT	25 µg	≥16	11-15	≤10
Tetracycline	TE	30 µg	≥19	15-18	≤14

Detection of antimicrobial resistance genes by PCR

All oxacillin and cefoxitin resistant isolates were considered for prediction of *mecA*-mediated resistance in staphylococci (CLSI, 2020). The phenotypic resistant isolates were further investigated for the presence of the *mecA* gene by PCR (Larsen et al., 2008). The sequences of primers used for this gene are listed in Table 5. DNA extraction of the isolates was performed by boiling method as described in previous section. To run PCR assays 20 pmol/µL concentrations of the each primer was used. PCR was done in a 25 µL total reaction volume. PCR reaction mixture contained 9.5 µL of nuclease free milliQ water, 12.5 µL dreamtaq master mix (Thermo Scientific), 0.5 µL of each primer and 2 µL of DNA template. The cycle condition for PCR was 1 cycle at

94°C for 15 min (initial denaturation); 30 cycles at 94°C for 30 sec (denaturation), 59°C for 1 minute (annealing), 72°C for 1 minute (extension); and one cycle at 72°C for 10 min (final extension). For a negative control master mix without any DNA template and for a positive control a previously isolated positive strain were used. PCR products (amplicons) were stored at 4°C until analyzed by electrophoresis in 2% agarose gel.

Table 5. Oligonucleotide primers used in PCR to detect *mec*A gene

Name of Primer	Sequence (5'-3')	Size of amplified product (bp)	Reference
*mec*A (Forward)	TCCAGATTACAACTTCACCAGG		Larsen et al., 2008
*mec*A (Reverse)	CCACTTCATATCTTGTAACG	162	

Statistical analysis

All data were recorded into a Microsoft Excel 2010 spread sheet. The prevalence of nasal carriage of *S. aureus* and CoNS was calculated by considering the number of positive isolates as the numerator, divided by the number of students sampled as the denominator. Firstly, univariable logistic regression analysis was performed to identify possible risk factors, and subsequently, any factor having a *p*-value of ≤0.20 was selected to build the further multivariable logistic regression model. Any variables with a *p*-value of 0.05 was considered significant and kept in the final model. All descriptive and analytical analyses were performed using STATA®13.0 software. The representative heat map was constructed using Graphpad Prism (version 7.05).

CHAPTER – IV

Results

Prevalence of nasal carriage of *S. aureus* and CoNS

A total of 157 students were enrolled in this study, among them 81 were medical students and 76 were veterinary students. The screening of nasal carriage of staphylococci revealed the presence of this bacteria in 48.1% (n=81) 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 (Figure 4 and Figure 5). 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 6). Overall, 10 (25.6%) and 6 (22.2%) coagulase-positive *S. aureus* isolates (Figure 7) were obtained from medical and veterinary students, respectively. Coagulase-positive isolates irrespective of veterinary and medical students were confirmed by detection of *nuc* gene by PCR (Figure 8). All *S. aureus* isolates which were positive for coagulase were also positive for the presence of *nuc* gene.

Antimicrobial susceptibility testing of *S. aureus* and CoNS isolates obtained from medical students

The results of antimicrobial susceptibility testing of coagulase-positive *S. aureus* and CoNS isolates are shown in Table 6 and Table 7, respectively. The results revealed that all *Staphylococcus* isolates irrespective of coagulase reaction exhibited resistance to Ampicillin and Penicillin. 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.
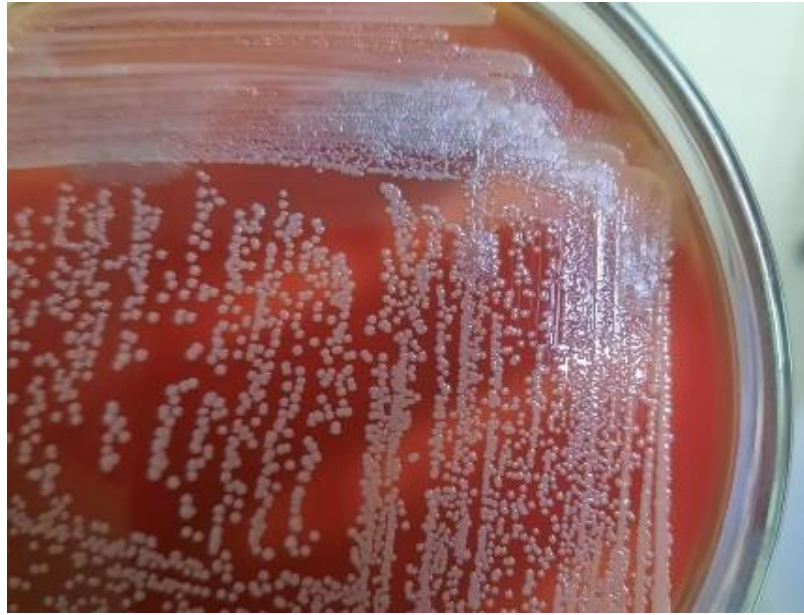
Figure 4. Characteristic growth of staphylococci on blood agar
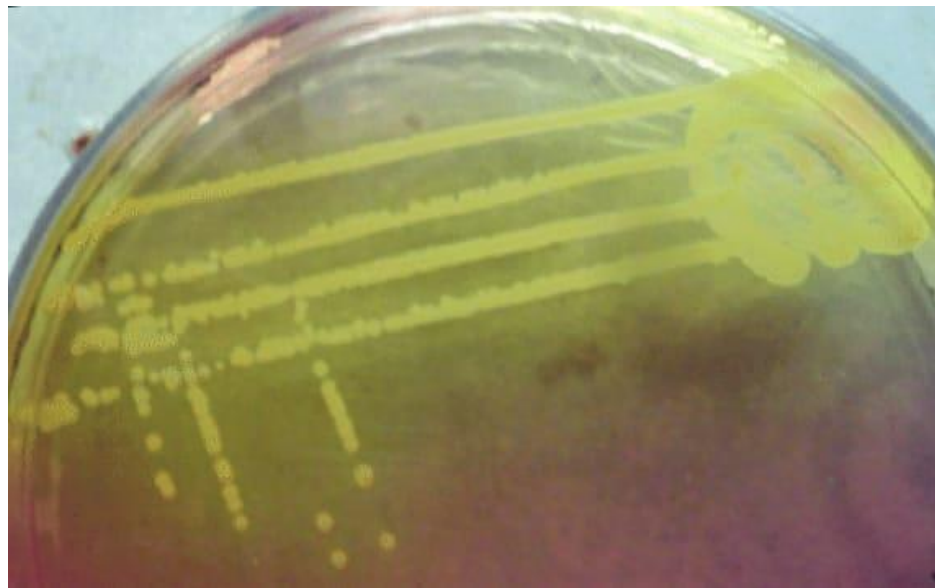
Figure 5. Colony morphology of staphylococci on mannitol salt agar

Figure 6. Electrophoresis on agarose gel showing the 894-bp PCR products 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*.

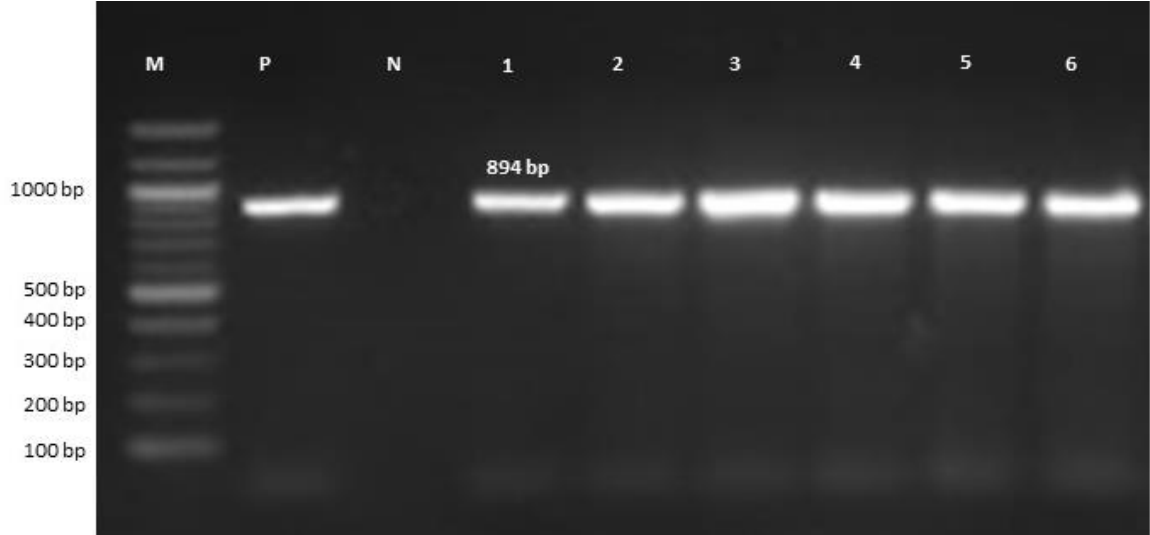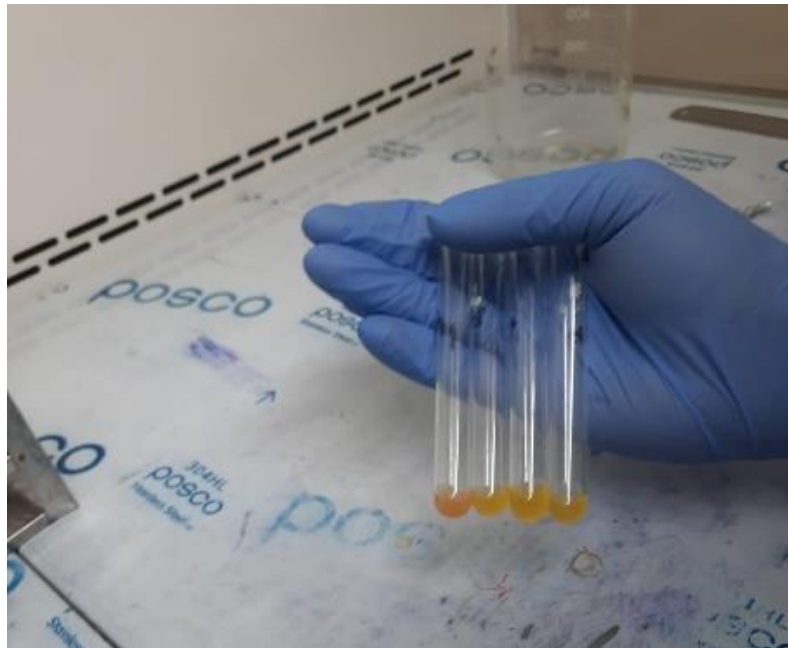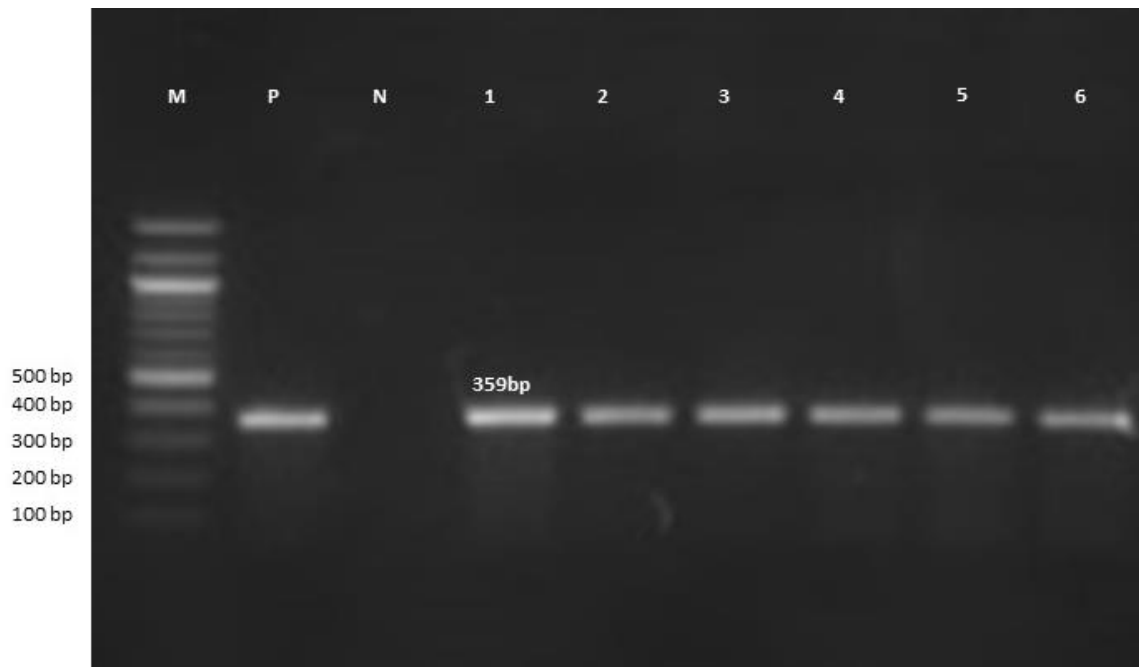
Figure 7. Result of coagulase test for *S. aureus*

Figure 8. Electrophoresis on agarose gel showing the 359-bp PCR products after amplification with specific primers. Amplifications were performed with chromosomal DNA from *Staphylococcus aureus* isolates. Lanes: M = 100 bp DNA Marker, P = Positive control, N = Negative control, L1 - L6 = reaction specific for *Staphylococcus aureus*.

Table 6. Antimicrobial susceptibility pattern of coagulase-positive *S. aureus* isolated from medical students (n=10)

Antimicrobial agents	Number of isolates		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin (AMP)	0	0	10 (100)
Cefoxitin (FOX)	5 (50)	0	5 (50)
Ceftriaxone (CRO)	6 (60)	0	4 (40)
Ciprofloxacin (CIP)	0	0	10 (100)
Erythromycin (E)	2 (20)	1 (10)	7 (70)
Gentamicin (CN)	9 (90)	1 (10)	0
Meropenem (MEM)	9 (90)	0	1 (10)
Oxacillin (OX)	3 (30)	0	7 (70)
Penicillin (P)	0	0	10 (100)
Trimethoprim-sulfamethoxazole (SXT)	8 (80)	0	2 (20)
Tetracycline	9 (90)	0	1 (10)

Table 7. Antimicrobial susceptibility pattern of coagulase-negative staphylococci (CoNS) isolated from medical students (n = 29)

Antimicrobial agents	Number of isolates		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin (AMP)	0	0	29 (100)
Cefoxitin (FOX)	16 (55.2)	0	13 (44.8)
Ceftriaxone (CRO)	11 (37.9)	0	18 (62.1)
Ciprofloxacin (CIP)	1 (3.4)	2 (6.9)	26 (89.7)
Erythromycin (E)	2 (6.9)	4 (13.8)	23 (79.3)
Gentamicin (CN)	28 (96.6)	0	1 (3.4)
Meropenem (MEM)	29 (100)	0	0
Oxacillin (OX)	16 (55.2)	0	13 (44.8)
Penicillin (P)	0	0	29 (100)
Trimethoprim-sulfamethoxazole (SXT)	23 (79.3)	0	6 (20.7)
Tetracycline	19 (65.5)	2 (6.9)	8 (27.6)

Antimicrobial susceptibility testing of *S. aureus* and CoNS isolates obtained from veterinary students

The overall results of antimicrobial susceptibility testing of coagulase-positive *S. aureus* and CoNS isolates are shown in Table 8 and Table 9, respectively. Like medical students, all staphylococci isolates from veterinary students were resistant to Ampicillin and Penicillin. 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.

Individual antibiogram profiles of all the isolates from medical and veterinary students are illustrated in Figure 9 and Figure 10, respectively.

Table 8. Antimicrobial susceptibility pattern of coagulase-positive *S. aureus* isolated from veterinary students (n=6)

Antimicrobial agents	Number of isolates		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin (AMP)	0	0	6 (100)
Cefoxitin (FOX)	6 (100)	0	0
Ceftriaxone (CRO)	6 (100)	0	0
Ciprofloxacin (CIP)	3 (50)	0	3 (50)
Erythromycin (E)	2 (33.3)	0	4 (66.7)
Gentamicin (CN)	6 (100)	0	0
Meropenem (MEM)	6 (100)	0	0
Oxacillin (OX)	6 (100)	0	0
Penicillin (P)	0	0	6 (100)
Trimethoprim-sulfamethoxazole (SXT)	5 (83.3)	0	1 (16.7)
Tetracycline	4 (66.7)	0	2 (33.3)

Table 9. Antimicrobial susceptibility pattern of coagulase-negative staphylococci isolated from veterinary students (n=21)

Antimicrobial agents	Number of isolates		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin (AMP)	0	0	21 (100)
Cefoxitin (FOX)	11 (52.4)	0	10 (47.6)
Ceftriaxone (CRO)	11 (52.4)	0	10 (47.6)
Ciprofloxacin (CIP)	4 (19)	1 (4.8)	16 (76.2)
Erythromycin (E)	1 (4.8)	3 (14.3)	17 (81)
Gentamicin (CN)	21 (100)	0	0
Meropenem (MEM)	21 (100)	0	0
Oxacillin (OX)	11 (52.4)	0	10 (47.6)
Penicillin (P)	0	0	21 (100)
Trimethoprim-sulfamethoxazole (SXT)	15 (71.4)	1 (4.8)	5 (23.8)
Tetracycline	13 (61.9)	0	8 (38.1)

Multi-drug resistance pattern of staphylococci isolated from medical students

Diversity of resistant phenotypes among the coagulase-positive *S. aureus* and CoNS isolates obtained from medical students are presented in Table 10 and Table 11, respectively. A total of 7 and 22 resistance patterns with different combination of antimicrobial agents were observed in coagulase-positive S. aureus and CoNS isolates, respectively. About 80% of the total coagulase positive *S. aureus* isolates showed multi-drug resistance (i.e. resistance to ≥3 antimicrobial classes) with a range from 3 to 5 different antimicrobials (Table 12) while about 98% of total CoNS isolates displayed multi-drug resistance. Approximately 4% of the CoNS isolates were resistant to seven antimicrobial classes (Table 12).

Figure 9: Heat map showing the distribution of antimicrobial resistance phenotype of methicillin resistant *Staphylococcus aureus* and methicillin resistant CoNS isolates obtained from medical 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.
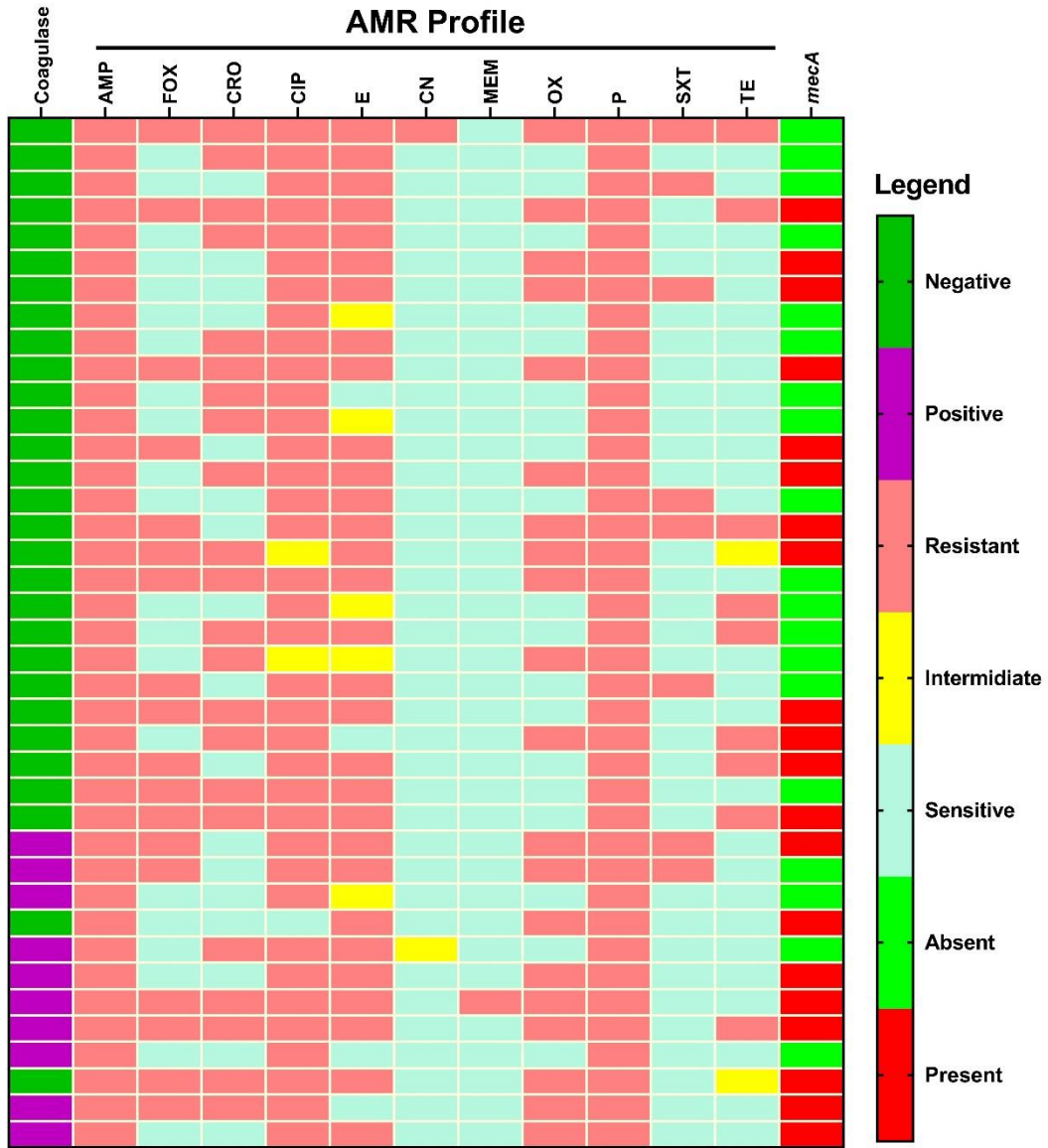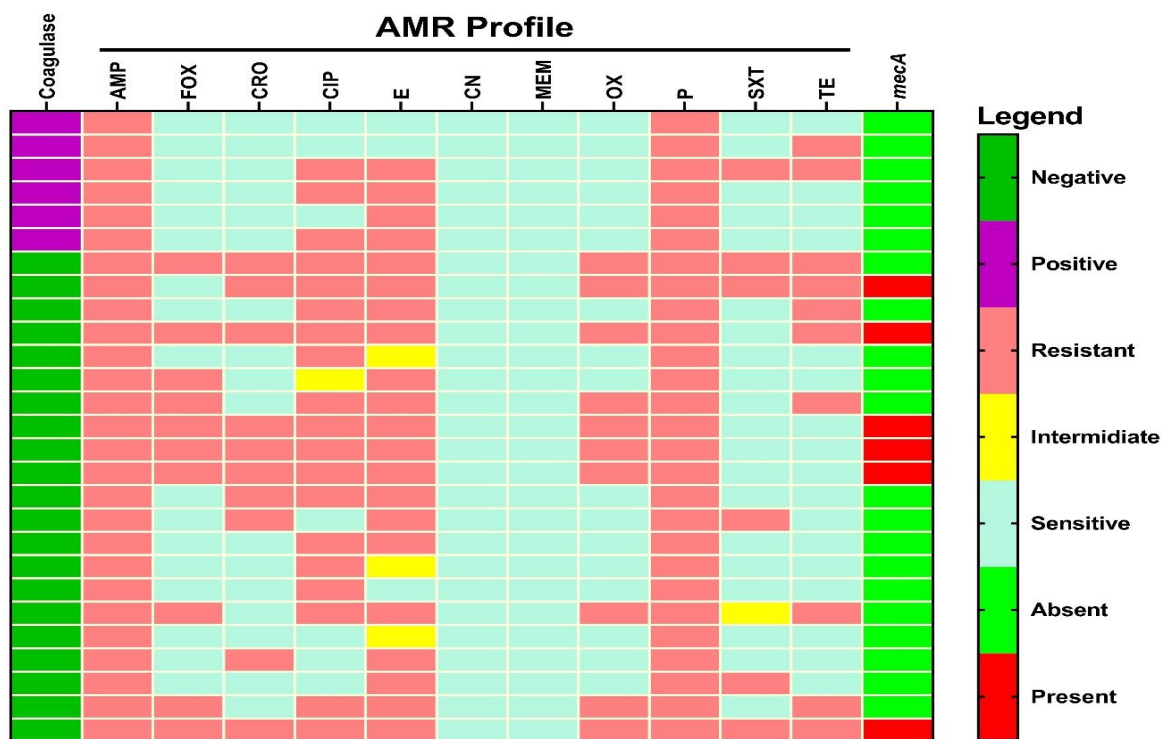
Figure 10: Heat map showing the distribution of antimicrobial resistance phenotype of methicillin resistant *Staphylococcus aureus* and methicillin resistant CoNS isolates obtained from veterinary students. Each row represents one isolate. AMP = Ampicillin, FOX = Cefoxitin, CRO = Ceftriaxone, CIP = Ciprofloxacin, E = Erythromycin, CN = Gentamicin, MEM = Meropenem, OX = Oxacillin, P = Penicillin, SXT = Trimethoprim-sulfamethoxazole, TE = Tetracycline.

Table 10. Antimicrobial resistance profile of coagulase-positive *Staphylococcus aureus* isolated from medical students

Sl. no.	Resistance phenotype	No. of isolates displaying resistance
1.	AMP-CIP-P	2
2.	AMP-CIP-E-OX-P	2
3.	AMP-CRO-CIP-E-P	1
4.	AMP-FOX-CRO-CIP-OX-P	1
5.	AMP-FOX-CIP-E-OX-P-SXT	2
6.	AMP-FOX-CRO-CIP-E-OX-P-TE	1
7.	AMP-FOX-CRO-CIP-E-MEM-OX-P	1

Table 11. Antimicrobial resistance profile of coagulase-negative staphylococci (CoNS) isolated from medical students

Sl. no.	Resistance phenotype	No. of isolates displaying resistance
1.	AMP-CIP-E-OX-P	1
2.	AMP-CIP-E-OX-P-SXT	1
3.	AMP-CIP-E-P-SXT	2
4.	AMP-CIP-P	1
5.	AMP-CIP-P-TE	1
6.	AMP-CRO-CIP-E-OX-P	1
7.	AMP-CRO-CIP-E-P	3
8.	AMP-CRO-CIP-E-P-TE	1
9.	AMP-CRO-CIP-OX-P-TE	1
10.	AMP-CRO-CIP-P	2
11.	AMP-CRO-OX-P	1
12.	AMP-E-OX-P	1
13.	AMP-FOX-CIP-E-OX-P-SXT-TE	1
14.	AMP-FOX-CIP-E-P	1
15.	AMP-FOX-CIP-E-P-SXT	1
16.	AMP-FOX-CIP-E-P-TE	1
17.	AMP-FOX-CRO-CIP-E-CN-OX-P-SXT-TE	1
18.	AMP-FOX-CRO-CIP-E-OX-P	3
19.	AMP-FOX-CRO-CIP-E-OX-P-TE	1
20.	AMP-FOX-CRO-CIP-E-P	2
21.	AMP-FOX-CRO-CIP-E-P-TE	1
22.	AMP-FOX-CRO-E-OX-P	1

Table 12. Number and percentages of *S. aureus* and CoNS isolated from medical students exhibiting resistance to various number of antimicrobial classes

Coagulase test	Number of antimicrobial classes to which isolates were resistant	Number (%) of resistant isolates
Coagulase-positive *S. aureus*	2	2 (20%)
	3	3 (30%)
	4	1 (10%)
	5	4 (40%)
Coagulase-negative staphylococci (CoNS)	2	3 (10.3%)
	3	5 (17.2%)
	4	14 (48.3%)
	5	5 (17.2%)
	6	1 (3.4%)
	7	1 (3.4%)

Multi-drug resistance pattern of staphylococci isolated from veterinary students

Resistant phenotypes among the coagulase-positive S. aureus and CoNS isolates obtained from veterinary students are shown in Table 13 and Table 14, respectively. 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 (Table 15) while about 81% of total CoNS isolates displayed multi-drug resistance. Approximately 14.3% of the CoNS isolates were resistant to seven antimicrobial classes.

Table 13. Antimicrobial resistance profile of *S. aureus* isolated from veterinary students

Sl. no.	Resistance phenotype	No. of isolates displaying resistance
1.	AMP-CIP-E-P	2
2.	AMP-CIP-E-P-SXT-TE	1
3.	AMP-E-P	1
4.	AMP-P	1
5.	AMP-P-TE	1

Table 14. Antimicrobial resistance profile of coagulase-negative staphylococci (CoNS) isolated from veterinary students

Sl. no.	Resistance phenotype	No. of isolates displaying resistance
1.	AMP-CIP-E-P	1
2.	AMP-CIP-E-P-TE	1
3.	AMP-CIP-P	3
4.	AMP-CRO-CIP-E-OX-P-SXT-TE	1
5.	AMP-CRO-CIP-E-P	1
6.	AMP-CRO-E-P	1
7.	AMP-CRO-E-P-SXT	1
8.	AMP-E-P-SXT	1
9.	AMP-FOX-CIP-E-OX-P-TE	3
10.	AMP-FOX-CRO-CIP-E-OX-P	3
11.	AMP-FOX-CRO-CIP-E-OX-P-SXT-TE	2
12.	AMP-FOX-CRO-CIP-E-OX-P-TE	1
13.	AMP-FOX-E-P	1
14.	AMP-P	1

Table 15. Number and percentages of *S. aureus* and CoNS isolated from veterinary students exhibiting resistance to various number of antimicrobial classes

Coagulase test	Number of antimicrobial classes to which isolates were resistant	Number (%) of resistant isolates
Coagulase-positive *S. aureus*	1	1 (16.7)
	2	2 (33.3)
	3	2 (33.3)
	5	1 (16.7)
Coagulase-negative staphylococci (CoNS)	1	1 (4.8)
	2	3 (14.3)
	3	4 (19.0)
	4	6 (28.6)
	5	4 (19.0)
	6	3 (14.3)

Prevalence of methicillin resistant *Staphylococcus* sp. obtained from medical and veterinary students

The distribution of *mecA* gene in staphylococci isolates is shown in Table 16. 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. Notably, all *mec*A genes were carried by both CoPS and CoNS isolates and finally classified as methicillin resistant isolates (Figure 11).

Table 16. Prevalence of *mec*A gene in methicillin resistant isolates obtained from medical and veterinary students

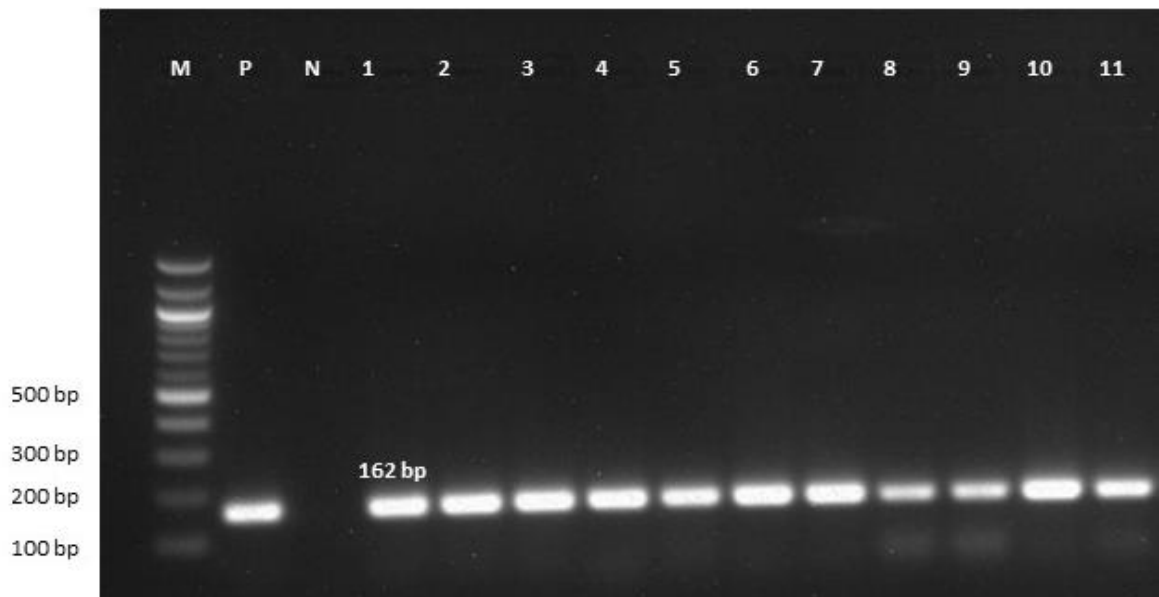| Source	Total no. of staphylococci isolates	Oxacillin-resistant isolates	Cefoxitin-resistant isolates	*mec*A positive isolates	Prevalence
Medical students	39	20	18	20	51.3
Veterinary students	27	10	10	6	22.2

Figure 11: 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.

Risk factors associated with the carriage of *Staphylococcus* sp. in different veterinary and medical students

In univariable logistic regression analysis only one factor presence of "Rhinorrhea" is significantly associated with carriage of *Staphylococcus* sp. in different veterinary and medical students (Table-12). However, none of the variables or factors was fit for multi-variable logistic regression analysis.

Table 17: Univariable logistic regression analysis of risk factors for the carriage of *Staphylococcus sp.* in different veterinary and medical students.

Variables	Co-variable	No. of students	No. students positive for *S. aureus* (%)	95% CI	*p*- value (Chi-square)
Age	Pre-clinical (<22 years)	95	39 (41.05)	18.29-32.36	0.75
	Clinical (>22 years)	62	27 (43.55)	11.65 -24.02	
Gender	Female	82	36 (43.90)	16.61-30.30	0.62
	Male	75	30 (40.0)	13.27-26.14	
Discipline (Institute)	Veterinary (CVASU)	76	27 (35.53)	11.64-24.02	0.10
	MBBS (IAHS)	81	39 (48.15)	18.29-32.35	
Body weight	Heavy	45	15 (33.33)	5.44-15.26	0.27
	Medium	98	46(46.94)	22.31-37.08	
	Thin	14	5 (35.71)	1.04-7.27	
Skin infection	yes	0	0	-	-
	no	157	66 (42.03)	34.21-50.16	
Rhinorrhea	yes	22	17 (77.27)	6.43-16.77	0.000*
	no	135	49 (36.30)	24.06-39.08	

Variables	Co-variable	No. of students	No. students positive for *S. aureus* (%)	95% CI	*p*- value (Chi-square)
Septicemia	yes	0	0	-	-
	no	157	66 (42.03)	34.21-50.16	
Previous hospitalized	yes	0	0	-	-
	no	157	66 (42.03)	34.21-50.16	
Previous surgical history	yes	0	0	-	-
	no	157	66 (42.03)	34.21-50.16	
Practice in hospital environment	yes	89	38 (42.70)	17.73-31.67	0.84
	no	68	28 (41.18)	12.19-24.73	
Use of antimicrobials	yes	2	1 (50.00)	0.01-3.49	0.81
	no	155	65 (41.94)	33.60-49.52	
Use of nasal drop	yes	0	0	-	-
	no	157	66 (42.03)	34.21-50.16	
Dwelling place	Student hall	74	35 (47.30)	16.04-29.61	0.20
	Own house	83	31 (37.35)	13.82-26.84	

CHAPTER – V

Discussion

S. aureus is an opportunistic pathogen which has multifactorial effect on respiratory tract, gastrointestinal tract, skin, perineum, vagina, axillae and pharynx. The present study was conducted to determine the prevalence of nasal carriage of *S. aureus* and CoNS from medical and veterinary students. The overall prevalence of *S. aureus* nasal carriage among medical and veterinary students were 25.6 and 22.2%, respectively. 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. But 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.

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 protocol should be put in place. Similar to these results, high resistance rates to beta- lactams antimicrobials, such as

ampicillin and penicillin have been reported to *S. aureus* isolated from others previous study described by Legese et al. (2018)

MRSA is a superbug for its resistance to beta lactam antibiotics (Ralston et al.,2018). MRSA encode *mec*A 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 (Ralston et al.,2018)

To prevent the MRSA colonization among medical and veterinary students, preventive measures like maintaining high standards of hygiene, thoroughly washing and drying hands before and after caring for a patient, touching potentially contaminated equipment or dressings should be practiced. Students should use hand wipes or hand gel before touching the patients. They should maintain hygiene before and after entering the ward. Infected patients should be isolated from others.

Students with rhinorrhea was observed in 77.27% isolates which was statistically significant (p value=0.000). 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.

When attempting to combat AMR, it is crucial to reduce the spread and transmission of resistant germs both inside and across animal and human populations. It is challenging to pinpoint the exact origin of resistant bacterial strains due to the capacity of bacteria to spread from one environment to another, sometimes over great distances and among various populations. Therefore, 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.

CHAPTER-VI

Conclusions:

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.

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Annex 01: Questionnaire

Title: Prevalence of nasal carriage of Staphylococcus aureus among medical and veterinary students

Name of the interviewer: Dr. Salina Akter

Serial number:

1) Particulars of the patient:

Name:

Age:

Gender:

Student of:

Height:

Weight:

2) **Symptoms**

		Yes	No	Don't know
1	Skin infections			
2	Previous respiratory infections			
3	Septicemia			
4	Previous Nasal infection			

3) **Health Care**

		Yes	No	Don't know
4	In the past 6 months have you been a patient in the hospital?			
5	In the past 6 months have you had surgery?			
6	In the past 6 months have you worked in a health care facility?			

7	In the past 3 months have you taken any antibiotics?			
8	In the past 6 months have you used intravenous drugs?			
9	In the past 6 months have you used nasal drops?			
10	Is there any history of use of topical antibiotic?			

4) **Living conditions**

		Yes	No	Don't know
10	Are you currently living in a dorm?			
11	In the last 6 months have you lived in a dorm?			
12	Have you been in contact with any pet animal in the past 6 months?			
13	Do you live in a crowded environment?			

5) Treatment

	Types	Name	Dose
a	Oral drugs		
b	Injection		
c	Nasal drop		
d	Exercise, walking, and other physical activity		