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Colonization, Virulence Factors, and Antimicrobial Susceptibility Patterns of Staphylococcus Aureus Isolated from the Anterior Nares of Medical and Paramedical Students

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Abstract

Background: Hospital environment and healthcare personnel form an integral part of healthcare system. Patients visit the hospital for various reasons. Some patients require hospitalization and others require medical and surgical interventions. Microbes present in the hospital environment and those colonized in the anterior nares, and on the skin of healthcare personnel could pose an increased threat of hospital acquired infections. Staphylococcus aureus (S. aureus) is one among many microbial species with potential to cause mild to severe infections that could be present colonized in the healthcare personnel. This study is aimed to evaluate the colonization of S. aureus in the anterior nares of medical and paramedical students.

Methods: Nasal swabs were collected from a total of 100 medical, and paramedical students. The swabs were cultured on blood agar, and the staphylococcal isolates were identified to the species level by using standard and conventional microbiological techniques. The colonies were counted on isolation (<10 colonies-scanty growth; <25 colonies-moderate growth; <50 colonies-moderate to heavy growth; and > 50 colonies; heavy growth). The virulence determinants including hemolysis, pigment production was assessed. The antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method.

Results: Microbes had grown in 97% of the nasal swabs. Most frequent bacterial isolates were Coagulase negative Staphylococcus (CONS), and diphtheroid bacteria (bacterial resembling Corynebacterium diphtheriae. S. aureus was isolated in 13% of the study participants.

Conclusion: The isolation rates of S. aureus were noted to be 13%. Increased resistance was noted against vancomycin, and commonly used antimicrobial agents. Most isolates demonstrated hemolysis on blood agar.

Keywords: *Staphylococcus aureus, diphtheroid, Healthcare personnel, medical and paramedical students, anterior nares, nasal swab, Coagulase negative Staphylococcus (CONS).*

Introduction

Staphylococci are a group of gram-positive cocci. These bacteria are noted to be normal colonizers both human and animals [1, 2]. In 25-40% of healthy humans, they can be colonized on skin, and mucus membranes [3]. Staphylococci are either coagulase positive or coagulase negative. The *Staphylococcus aureus* (*S. aureus*) is the coagulase positive variant and among the several coagulase negative Staphylococcal species, the *Staphylococcus saprophyticus* and the *Staphylococcus epidermidis* are frequently associated with human infections [4]. The *Staphylococcus hyicus* was reported to be having both the coagulase positive and the coagulase negative variants. *S. hyicus* was reported to cause exudative dermatitis in pigs [2].

S. aureus has been found to colonize the nasal mucosa as noted from the previous research studies. Because of the site of colonization of the staphylococci, both on the skin, mucosa, and the nasal orifices, there is an increased potential for these organisms to easily transmit among people and may therefore cause infections in the susceptible population.

Also, most staphylococcal species, although are present as normal human colonizers, have several virulence factors making them

potential/opportunistic pathogens in the susceptible humans [5, 6].

Staphylococci are also stringent and may survive in the environment for months in dried pus, thus increasing their transmissibility.

Among the staphylococcal species, the *S. aureus* is considered as a significant pathogen owing to its ability to cause mild to severe and superficial to deep seated infections. *S. aureus* has been identified to colonize in the anterior nares and become source of infection for susceptible population [3].

In a previous research study performed by me and my colleagues, we found that 16% school going children were colonized with *S. aureus*. Of the *S. aureus* isolates 19% were identified as methicillin resistant *S. aureus* (MRSA) [3].

Significance of methicillin resistant s. Aureus (MRSA)

Penicillin was among the first discovered antibiotics, which was found most effective against microbial infections. The discovery of penicillin in the 1920's was followed by its increased use during the II world war. Although, penicillin use was instrumental in minimizing the morbidity and the mortality during the II world war, its indiscriminate use later had caused the emergence of penicillin resistant staphylococcal strains. Resistance to penicillin during the 1970's had led to the discovery of modified penicillin's/synthetic penicillin's like the methicillin, and nafcillin. Later, it was observed that there were some *S. aureus* strains which were found to be resistant to the other antibiotics belonging to the cephalosporins, an alternate antibiotic group to penicillins.

Further research had revealed that the *S. aureus* strains which were resistant to the methicillin (MRSA) could develop resistance to other group of antibiotics [7]. Infections caused by these strains was posing treatment failures and resulting in mortality [8]. It was later identified that the MRSA strains were indeed showing a gene called as *Mec A* gene, probably coding for resistance [9]. Since most laboratories do not have access to molecular techniques, the phenotypic detection of the presence of *Mec A* gene was confirmed by checking with methicillin (5µg), oxacillin (1µg), or the more specific cephoxitin (2µg) by using the routine Kirby-Bauer disk diffusion method [10].

Vancomycin Resistance Among Staphylococcus Species

In 1999, for the first-time strains of *S. aureus* moderately resistant to the vancomycin (vancomycin intermediate sensitive *S. aureus*-VISA) were reported, followed by some of the *S. aureus* resistant to vancomycin (VRSA) [11]. Since vancomycin is the stock antibiotic which was used to treat deep-seated/invasive *S. aureus* infections, there were several cases of treatment failures resulting in severe morbidity and mortality [12].

Considering the potential of the *S. aureus* from being present as a normal colonizer in healthy individuals, to produce several potential virulence factors, to be able to develop resistance to most commonly use antimicrobial agents, and to be able to cause both endogenous and exogenous infections it is important to know the colonization frequency and antimicrobial susceptibility patterns of *S. aureus* among different groups of population [13].

In view of the close association of the health-care workers with various patient groups, it is important to analyse the health-care workers for the colonization of *S. aureus* [14, 15, 16]. The *S. aureus* colonized in the health-care workers may potentially be transmitted to the patients and lead to serious infections which can be difficult to treat.

Aim of The Study

Therefore, this study is undertaken to assess the colonization of various staphylococcal species with special reference to *S. aureus* colonization, virulence, and their antimicrobial susceptibility patterns.

Materials and Methods

This is a prospective, and cross-sectional study. A group of 100 students were recruited in the study. All the subjects included in the study were either medical (pursuing MBBS), para medical students pursuing B. Sc medical laboratory technology or B. Sc nursing students. All the students were briefed about the study protocol, and only those who volunteered were included in the study. An informed and written consent was obtained from each participant in the study. The study was approved by the institutional ethical committee (PIMR/IEC/2019-10000119).

Screening for colonization

A sterile cotton swab was used to obtain the nasal swab from each subject. The swab was carefully rolled inside both the nasal orifices, by gradually rolling and touching most areas of the nasal mucosa, and by taking care not to smear the surrounding skin surface. The swab is then immediately transported to the laboratory for further processing.

The nasal swabs were inoculated into blood agar and were incubated overnight at 35-37^{0}C. All the culture plates were checked for the presence of a visible growth, the next day. The specimens showing a visible growth were recorded. The colony colour, the presence of single or multiple bacterial growth was recorded along with the presence or absence of haemolysis production (alpha, beta, or gamma haemolysis) as shown in **Figure 1**.

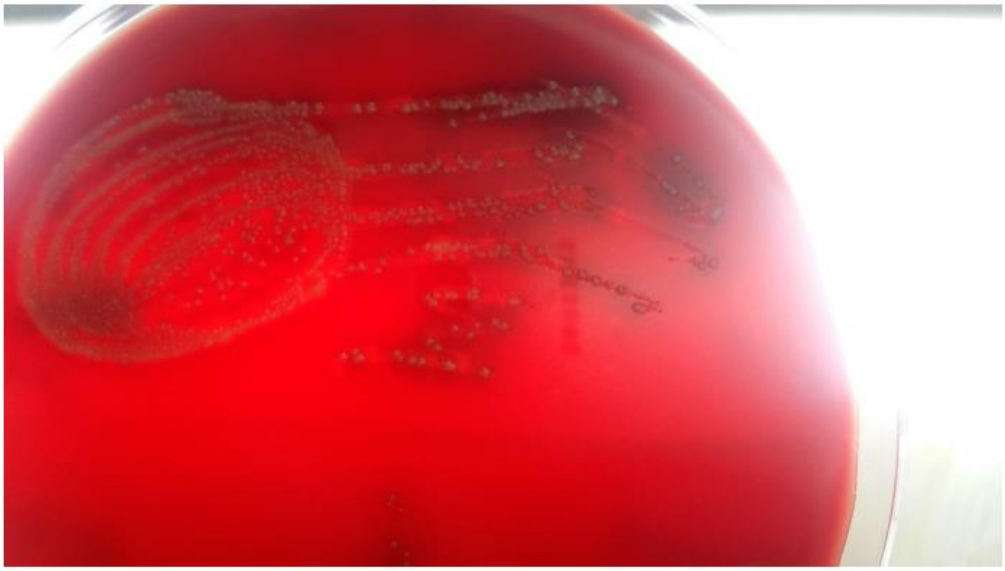


Figure 1. Staphylococcal species showing beta haemolytic colonies on blood agar

Gram's Stain

The colonies were gram's stained to confirm their gram's reaction, and were observed for the arrangement, if gram-positive cocci (GPC) were seen. When GPC's were seen, they were confirmed as either in clusters, tetrads, or in pairs. All those GPC's which were seen in clusters were suspected as belonging to the staphylococcal group as shown in **Figure 2**.

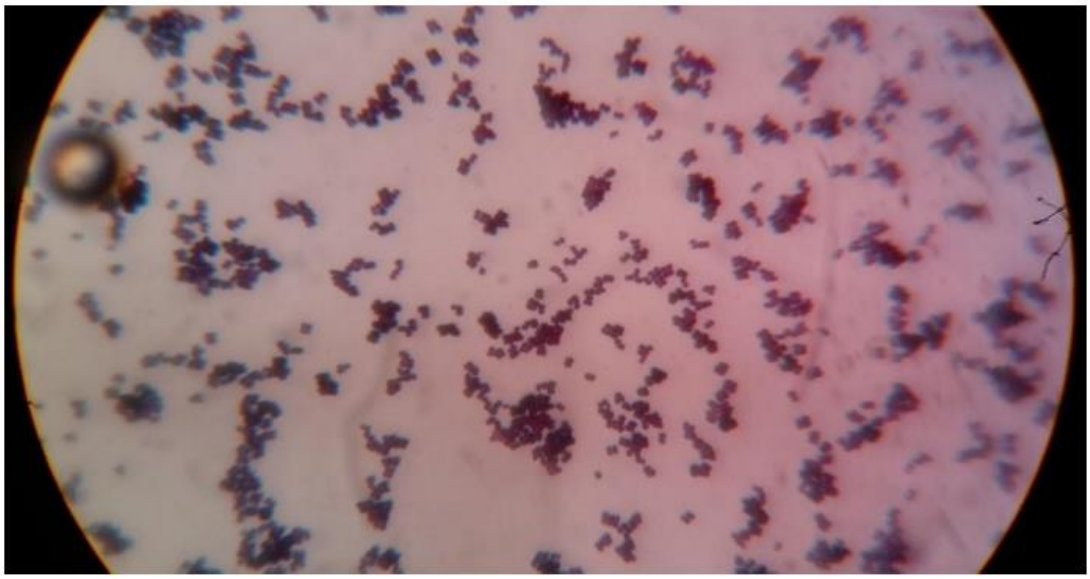


Figure 2. Staphylococcus seen as gram-positive cocci in clusters after grams staining

Colony Count

The growth obtained from the swabs were interpreted using the colony counting, where in the growth was categorized as <10 colonies (scanty growth), <25 colonies (moderate growth), <50 colonies (moderate to heavy growth), and > 50 colonies (heavy growth).

Catalase Test

A catalase test using the 3% hydrogen peroxide (H_2O_2) was used to confirm that they belong to *Staphylococcus* group (catalase positive), and *Streptococcus* group (catalase negative).

Coagulase Test

All the strains which were GPC's present in clusters, and were catalase positive, were identified as belonging to *Staphylococcus* group. These strains were checked for the production of coagulase enzyme. The strains were inoculated into a defined quantity of plasma, and after an overnight incubation at 37^0C, were checked for the presence of clot. Those strains showing the clot were identified as *S. aureus* or coagulase positive staphylococci (COPS), and those not forming were considered as coagulase negative staphylococci (CONS) as shown in **Figure 3**.

Figure 3. Staphylococcus species showing coagulase positive and coagulase negative reactions respectively

Antimicrobial Susceptibility Testing

All staphylococcal isolates were tested for their susceptibility against various antibiotics using the traditional Kirby-Bauer disk diffusion method. Here the strain to be tested was first inoculated in to the peptone water, and after an incubation od 1.5-2 hours at 37^0C, when a certain degree of turbidity (MacFarland's 0.5 standard) was obtained, a sterile cotton swab is immersed in the growth and lawn cultured on a Muller-Hinton agar (MHA).

Then various antibiotic discs were applied, and the plates were incubated overnight at 37^0C. After overnight incubation, the plates were observed for zones of clearance around the antibiotic discs, where there is no growth, also called zones of inhibition, indicating that the antibiotic is effective and did not allow microbial growth. If the bacterial growth was observed near the disc, and little or no zone of inhibition was seen, the antibiotic was considered as ineffective i.e. the bacteria is resistant to the antibiotic as shown in **Figure 4**.

Figure 4. Antimicrobial susceptibility testing result of the Staphylococcal species using Kirby-Bauer disk diffusion method

Results

Of the 100 nasal swabs cultured 97 (97%) grew bacteria. Among the bacteria isolated include the coagulase negative *Stahpylococcus* spp, and the *S. aureus* (13%). Few subjects were colonized with dual bacteria including the Gram-positive bacilli, which were identified as bacteria morphologically resembling *Corynebacterium diphtheriae* also called as diphtheroids or non-diphtheritic *Corynebacterium* species. The bacteria isolated were categorized based on the colony colour, coagulase production, haemolysis on blood agar, the types of growth on primary cultures, and the colony counts as shown in **Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, and Figure 10** respectively.

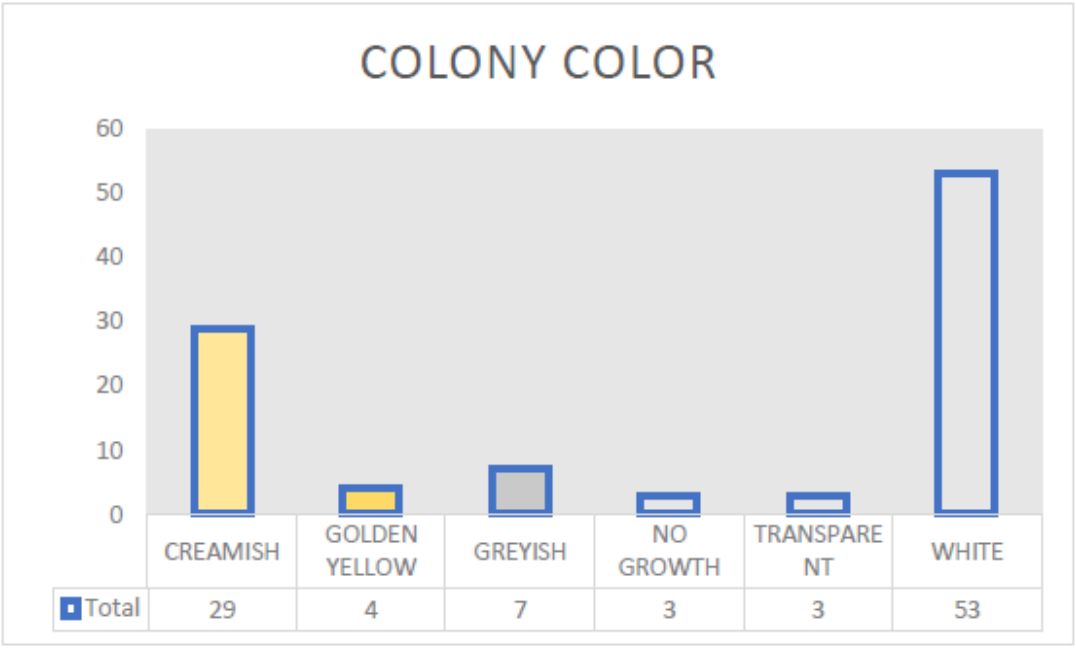


Figure 5. Categorization of the isolated *Staphylococcus* species based on colony colour

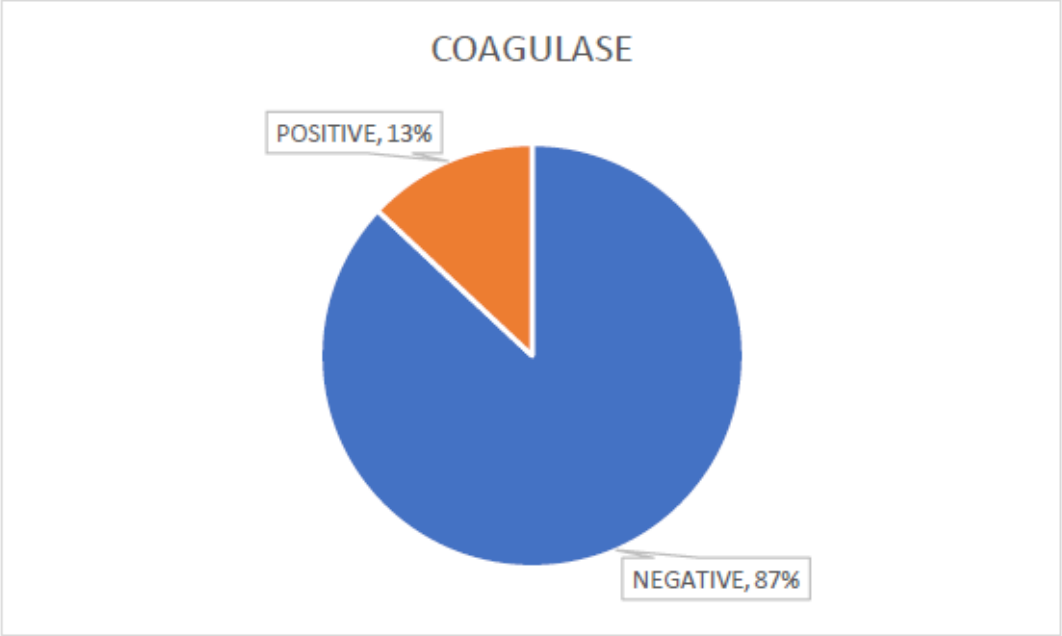


Figure 6. Characterization of the isolated Staphylococcus species based on coagulase test

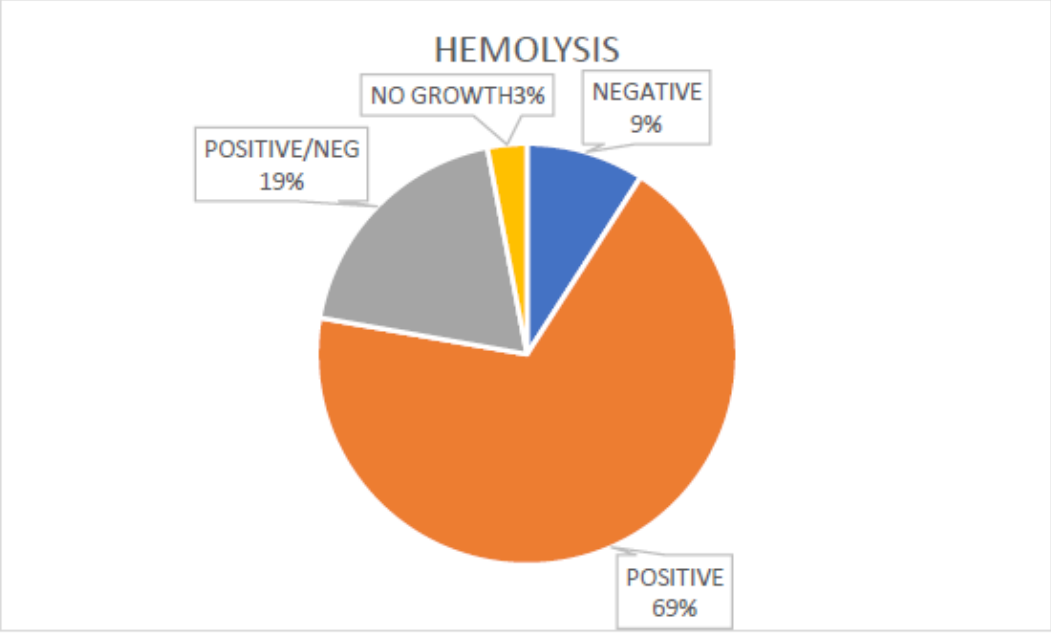
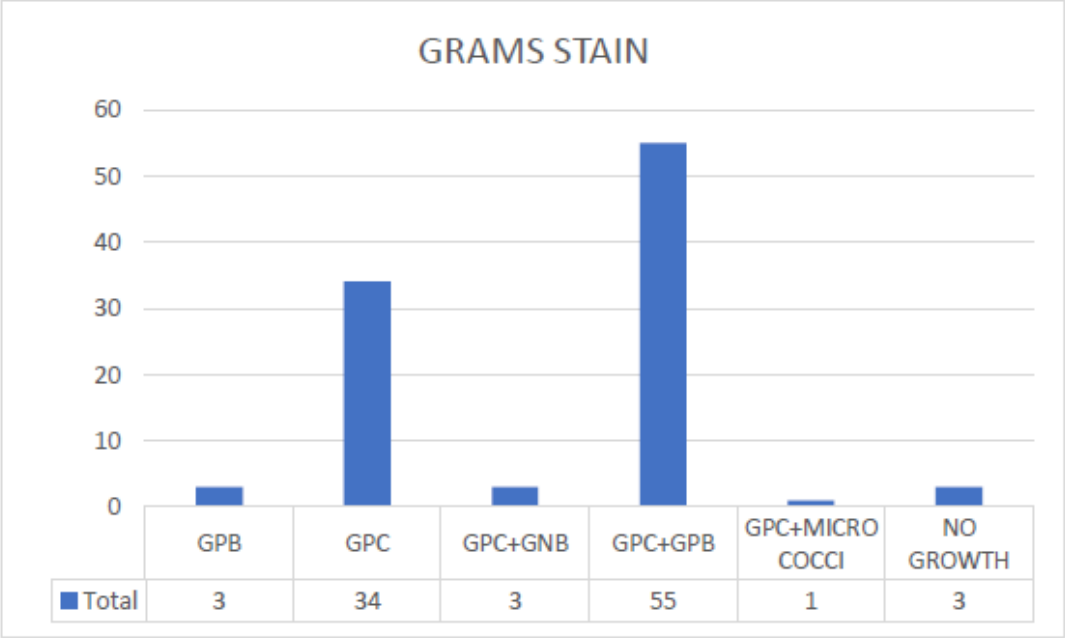


Figure 7. Categorization of the isolated *Staphylococcus* species based on haemolysis on blood agar



GPB: gram positive bacilli; GPC: gram positive cocci; GNB: gram negative bacilli

Figure 8. Type of growth on primary isolation from the nasal swabs

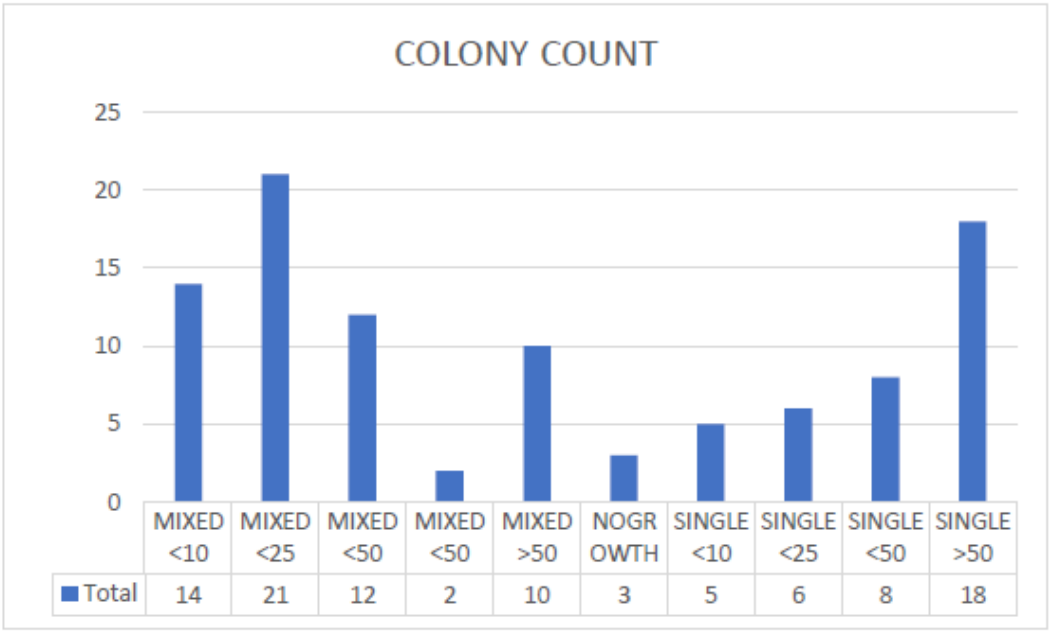
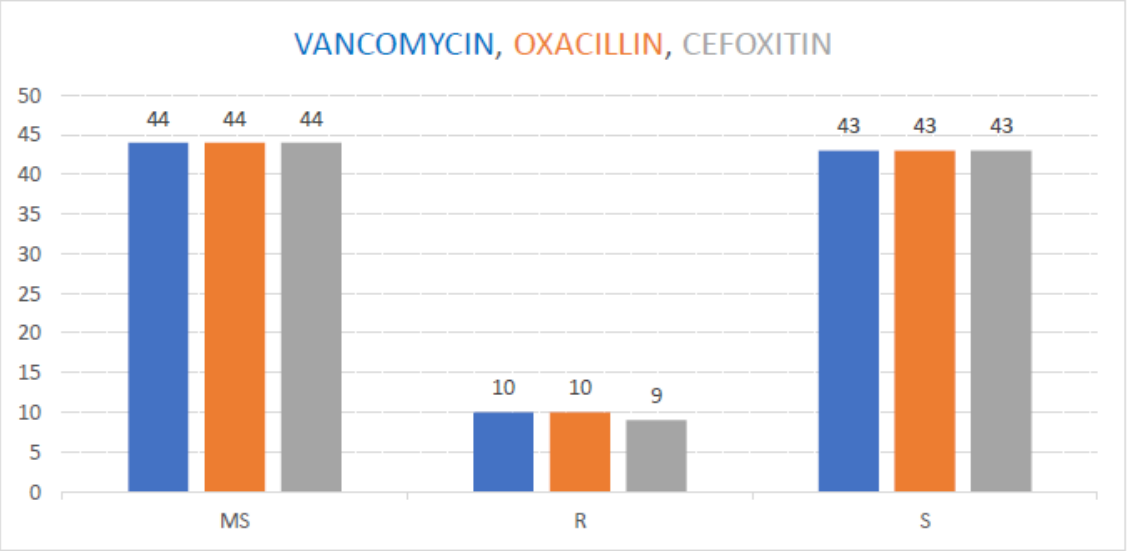


Figure 9. Categorization of the isolated Staphylococcus species based on colony counts

The antibiotic sensitivity testing of the isolated *Staphylococcus* spp was performed and interpreted with special reference to the methicillin resistance and vancomycin susceptibility as shown in **Figure 11**.



MS: moderately sensitive; R: resistant; S: sensitive

Figure 10. Vancomycin, Oxacillin, and Cefoxitin susceptibility patterns of the staphylococcal isolates

Discussion

Colony colour assumes significance because the pathogenic strains i.e. *Staphylococcus aureus* show golden yellow non-diffusible pigment [17, 18, 19, 20, 21]. Also, the cream coloured colonies usually have been noted to produce the coagulase enzyme. Most coagulase negative staphylococcal strains produce white coloured colonies and are assumed to be either non-pathogenic or opportunistic pathogens. In the present study 87% of the isolated Staphylococcal spp. were coagulase negative and only 13% were coagulase positive. When the colony colours were analysed, 53% showed white coloured colonies followed by cream coloured colonies (29%), and only 4% produced golden yellow coloured colonies. All the white coloured colonies were noted to be coagulase negative staphylococci, and all the golden yellow pigmented colonies were coagulase positive. Of the 29 cream coloured colonies only 9 (31%) were coagulase positive. These results suggest that pigmentation may not completely correlate with the colonization and pathogenicity.

Haemolysis is considered as an important virulence determinant among bacteria [22]. Most pathogenic bacteria were noted to possess the haemolytic properties. In the present study, 69% of the staphylococcal isolates showed haemolysis on blood agar. Few other nasal isolates revealed clones of bacteria, of which some were haemolytic and the others non-haemolytic (19%). All the coagulase positive staphylococcal isolates were positive for haemolysis and only 9% of all the staphylococcal isolates were noted to be non-haemolytic. These results suggest that the haemolytic properties can be positively correlated with the colonization of bacteria.

Assessment of the type of growth from the nasal cultures assumes significance due to the easy transmissibility [23, 24, 25, 26]. A pure growth of gram-positive cocci was observed in 34% of the study population and 55% of the cultures revealed mixed growth that included both the gram-positive cocci (*Staphylococcus* spp.), and gram-positive bacilli (non-diphtheritic *Corynebacterium* species). Mixed gram-negative bacilli, along with gram positive cocci were isolated in 3% of the population and only 1% revealed the growth of *Micrococcus* spp. Only 3% of the cultures showed single gram-positive bacilli and 3% were negative (no growth) in culture. These results suggest the fact that the *Staphylococcus* spp., and the non-diphtheritic *Corynebacterium* spp. can be present colonized in healthy subjects and such colonized individuals may act as reservoirs of bacteria and may transmit infections to susceptible population.

Colony counts have been successfully used to assess the urinary tract infections and colonization rates of several other bacterial like the *Campylobacter jejuni* in the intestine [27, 28]. The nasal cultures were assessed for growth by counting the number of colonies. Nasal cultures in this study revealed 59% mixed cultures and

among the mixed cultures 83% were showing <50 colonies and 20% showed >50 colonies. Pure growth of single colony was observed among 39% of cultures and 3% were negative for culture. Of the culture which showed pure growth, 46% were showing >50 colonies. The study results with respect to the type of growth suggests that in most mixed cultures, the colony count was <50 and many single cultures were showing >50 colonies. The presence of a pure colony may correlate well with the colonization of the bacterial species.

Not only the colonization, and the virulence factors, the assessment of antimicrobial susceptibility patterns of the microorganisms is significant in the health care perspective [29, 30, 31, 32]. The antimicrobial susceptibility patterns of the isolated Staphylococcus spp. revealed increased resistance the antibiotics that include the clindamycin (85%), erythromycin (90%), amoxycillin-clavulanic acid (92%), and piperacillin-tazobactam (92%). Moderate sensitivity was noted against the ceftriaxone55%, ciprofloxacin (62%), and co-trimoxazole (65%).

All the strains were sensitive to linezolid (100%), and 98% sensitivity was noted against imipenem. Variable sensitivity was observed against the aminoglycoside antibiotics amikacin (75%), and gentamicin (68%).

Conclusion

The study results noted an abnormally high rates of colonization among the medical, and para-medical undergraduate students. There was an overall colonization rate of 97% and 13% were colonized with the potentially pathogenic *Staphylococcus aureus*. Most isolates were showing the haemolytic properties, and many were producing pigmentation (cream, golden yellow), which may potentially predict their pathogenicity. The antimicrobial sensitivity patterns of all the isolates revealed increased resistance to the oxacillin/cefoxitin (methicillin resistant *Staphylococcus aureus*) and vancomycin. Variable susceptibility patterns were noted against other regularly used antimicrobial agents.

Future Perspectives

The health care persons, and the people constantly exposed to the patients and hospital environment are at increased risk of getting colonized with microorganisms. The anterior nares/nasal cavities have been known to colonize staphylococcal species, and especially S. aureus. The colonization of bacteria among healthy individuals causes no harm but if such microorganisms are transmitted to the susceptible populations, like the hospitalized patients, they may cause serious consequences. Also, the microorganisms present in the hospital environments may potentially be multi-drug resistant and pose an increased threat to the patients from the colonized healthcare workers. Therefore, regular screening of the people involved in the healthcare must be done, and necessary steps may be initiated to stop the transmission of such microorganisms to the already debilitated patients.

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