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Nasal Colonization of *Staphylococcus aureus* among Preclinical Medical Students: Implications as Future Sources of Infection in Hospitals

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ABSTRACT

Staphylococcus aureus, a leading pathogen in various human infections, can colonize different anatomical sites asymptomatically, posing risks to immunocompromised individuals. This study aims to determine the prevalence of asymptomatic nasal carriage of *S. aureus* among pre-clinical medical students. The study population comprised pre-clinical medical students at Universitas Ciputra. All fifth-semester students who voluntarily participated and provided informed consent were included, resulting in a total sample of 57 medical students. Nasal swab samples were collected on two occasions, ten months apart, and *S. aureus* was identified by culture method. Data were processed using descriptive statistics to determine the prevalence of *S. aureus* carriage. The prevalence rates of intermittent and persistent carriers were calculated and compared between the two sampling occasions to identify patterns of carriage. *S. aureus* nasal carriage was found in 10 (17%) participants. Nearly all participants (98%) had more than one bacterial type on nasal swab, with only 1 (2%) showing *S. aureus* as the sole bacterium. Among the isolated S. aureus, 1 (10%) was Methicillin-Resistant *S. aureus* (MRSA). Among the 37 individuals who participated in both samplings, *S. aureus* was detected at least once in the nasal swabs of 18 individuals (49%): 13 participants (35%) were intermittent carriers, and 5 participants (14%) were persistent carriers. Our study reveals that a significant number of pre-clinical medical students are asymptomatic carriers of *S. aureus* in their nasal passages, either intermittently or persistently. As they transition into healthcare roles, these carriers may act as reservoirs for transmission of this bacteria within healthcare settings.

Keywords: *Staphylococcus aureus*, nasal carrier, medical students, transmission, healthcare.

INTRODUCTION

Staphylococcus aureus is a significant pathogen causing a range of infection, from mild to severe and life threatening infection. It has been reported to cause significant number of bacteremia and infective endocarditis (1), osteoarticular (2-4), skin and soft tissue (5), pleuropulmonary (5), and device related infections(6). Generally, the mortality rate linked with *S. aureus* infections exceeds that of other bacterial infections due to the potent virulence and antibiotic resistance of *S. aureus*, as well as the severity of the infections it induces (7).

In addition to its pathogenic potential, *S. aureus* exhibits a notable capacity for asymptomatic colonization, wherein it establishes commensal relationships with its human host without eliciting overt clinical manifestations. This colonization phenomenon encompasses a spectrum of niches within the human microbiome, including but not limited to the nares, oropharynx, rectum, and skin (8).

While seemingly harmless, the colonization of *S. aureus* can lead to significant clinical implications as described above, especially among individuals with predisposing factors for severe staphylococcal infections. These risk factors include a multitude of immunological, physiological, and environmental variables that enhance the progression from asymptomatic colonization to manifest disease. It includes but not limited to age, chronic alcoholism, immunosuppressive treatment, ultimately or rapidly fatal underlying diseases (6). Colonization can play a crucial role in the pathogenesis of *S. aureus* infections, particularly in patients undergoing surgery, those on hemodialysis or continuous ambulatory peritoneal dialysis (CAPD), and those with intravascular

devices or colonized with methicillin-resistant *S. aureus* (MRSA). Importantly, this susceptible subset of the population may suffer from severe staphylococcal infections either due to internal colonization or external transmission facilitated by interpersonal contact or environmental reservoirs (9).

Transmission dynamics play a pivotal role in the dissemination of *S. aureus* within healthcare settings and communities. Common modes of transmission encompass direct contact, respiratory droplets, fomites, and environmental contamination, each serving as conduits for the dissemination of this ubiquitous pathogen (9). Furthermore, the carriage dynamics of *S. aureus* exhibit considerable heterogeneity, characterized by persistent carriers who sustain prolonged colonization and intermittent carriers demonstrating sporadic colonization patterns. Each carriage type confers distinct epidemiological implications and may variably contribute to the overall burden of *S. aureus* within a given population.

The aim of this study is to determine the prevalence of *S. aureus* nasal colonization among medical student. In the context of medical education and clinical practice, elucidating the prevalence of *S. aureus* colonization among nascent healthcare professionals assumes paramount significance. A cohort study focusing on nasopharyngeal colonization rates among medical students, particularly those yet to embark on clinical rotations, serves as a surrogate for community-based carriage prevalence. Notably, medical students, upon transitioning to clinical rotations, assume roles as frontline healthcare providers, thereby underscoring the imperative of characterizing their colonization status to mitigate the risk of nosocomial transmission to vulnerable patient populations within hospital settings.

METHODS

The study population comprised pre-clinical medical students at Universitas Ciputra who have not undergone clinical rotation during period 2022-2023. All fifth-semester students who voluntarily participated and provided informed consent were enrolled in this study, resulting in a total sample of 57 medical students. The study was conducted in Microbiology Laboratory, School of Medicine, Universitas Ciputra Surabaya and has been approved by the ethical committee of Universitas Ciputra Surabaya (Approval number 097/EC/KEPK-FKUC/I/2024).

Sample was obtained from anterior nasal swab to both nostril using the same disposable sterile cotton swab, which then directly plated into blood agar plate (5% sheep blood). The blood agar plate was incubated at 37°C for 24 hours until visible colonies were seen. The predictive colonies were then stained by Gram staining method, and thus showing Gram positive coccus were then subsequently confirmed to be catalase positive and coagulase positive to be determined as *S. aureus*. Catalase test was performed using 3% hydrogen peroxide on a glass slide, and coagulase test was performed using Staphaurex™ Latex Agglutination Kit. Sampling was conducted twice with a ten-months interval between each.

The antibiotic susceptibility analysis of isolated *S. aureus* was carried out using the Kirby Bauer disk diffusion method on Mueller Hinton agar plates. The interpretation was conducted following the guidelines outlined in the Clinical and Laboratory Standards Institute (CLSI) standard M100. Data of *S.aureus* positivity and antibiotic sensitivity profile were processed using descriptive statistics to determine the prevalence of *S. aureus* carriage and its resistance pattern. The prevalence rates of intermittent and persistent carriers were calculated and compared between the two sampling occasions to identify patterns of carriage.

RESULT AND DISCUSSION

Characteristic of The Participants

S. aureus remains a formidable pathogen, causing a spectrum of infections ranging from mild to life-threatening. Our study focused on nasal colonization among medical students prior to clinical rotation, a population with critical implications for nosocomial transmission. Understanding

colonization rates and antibiotic sensitivity profiles is vital for implementing effective infection control measures and treatment strategies. A total of 57 subjects participated in this study.

They were medical students at their second year of study who have not experienced any clinical rotation. This consists of 12 male (21%) and 45 female (79%), with age range of 19-41 years old (median 19.9, IQR 19.5- 20.2). Almost all participants (96.5%) have finished senior high school as their last educational level at the time of sampling.

Personal hygiene might affect the colonization of *Staphylococcus aureus* to the skin. As many as 50 (87.7%) participants take a bath at least 2 times a day, while the remaining 7 (12.3%) only take a bath once daily. Regarding the atopic history, 6 (10.5%) participants has history of asthma, 2 (3.5%) participants has history of allergic rhinitis, 1 (1.8%) with history of atopic dermatitis, and the remaining 48 (84.2%) did not have any atopic history. For those participants with known history of allergy, 8 (14.0%) participants reported allergies to dust, 7 (12.3%) participants reported allergies to seafood, 1 (1.8%) participant reported allergy to egg, while the remaining 41 (71.9%) participants did not report any history of allergy.

Tabel 1. Characteristics of the Study Participants

Characteristic	Number (%)
Sex	
Male	12 (21)
Female	45 (79)
Age	
<20 years	34 (60)
20 – 30 years	22 (38)
>30 years	1 (2)
Last Educational Level	
High school	55 (96.5)
Bachelor's degree	2 (3.5)
Bathing Habits	
1x/day	7 (12.3)
≥ 2x/day	50 (87.7)
History of atopic disease	
Asthma	6 (10.5)
Allergic Rhinitis	2 (3.5)
Atopic Dermatitis	1 (1.8)
No atopic history	48 (84.2)
History of Allergic	
Dust	8 (14.0)
Seafood	7 (12.3)
Egg	1 (1.8)
None	41 (71.9)

Characterization of *Staphylococcus aureus* Nasal Colonization

We systematically swabbed both nostrils of the 57 participants in our study and successfully identified *S. aureus* from nasal swabs obtained from 10 individuals, indicating a prevalence rate of 17%. Among the 10 participants with identified *S. aureus* carriage, 9 were female, while 1 was male. Specifically, we observed S. aureus nasal carriage in 9 out of 45 female participants, resulting in a prevalence of 20%, which was higher than the prevalence of 8.3% among males, where only one carrier was detected among the 12 male participants. However, the limited sample size in this study hinders the ability to draw meaningful conclusions regarding the

difference in prevalence. A similar study conducted by Kuehnert and Liu showed no difference in S. aureus nasal colonization between men and women(10).

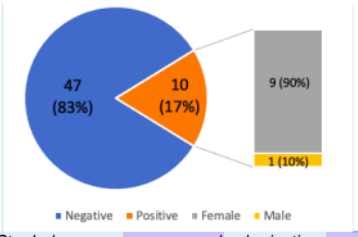

Figure 1. Prevalence of *Staphylococcus aureus* nasal colonization among medical students

Bacterial Growth Patterns of The Nasal Swabs

Furthermore, we conducted an analysis of bacterial growth patterns from the nasal swabs. Nearly all samples (98%) exhibited the presence of more than one type of bacterial colony. Interestingly, only one sample showed the growth of a single type of colony. It is noteworthy that this particular sample exhibiting monoclonal growth was also the sample where *S. aureus* was detected.

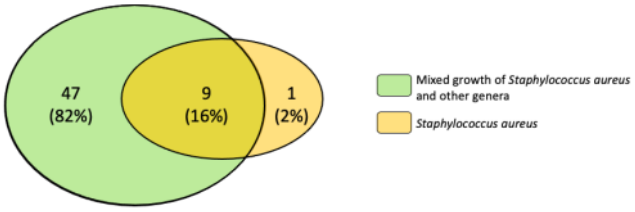

Figure 2. Bacterial Growth Pattern from Nasal Swabs of medical students

Our analysis of bacterial growth patterns revealed a predominance of polymicrobial colonization, with only one participant exhibiting monoclonal growth. Notably, this participant was also the sole carrier of *S. aureus*. The complex composition of the highly variable nasal microbiota influences the promotion or inhibition of *S. aureus* nasal colonization. This is governed by factors such as competition for nutrients, trace elements, and epithelial attachment sites, alongside variations in susceptibility to host defense molecules and the production of antimicrobial substances by other bacteria. These mechanisms, as discussed by Laux and Krismer, determine the competitive dynamics among nasal bacteria (11, 12).

Antibiotic Sensitivity Pattern on The Isolated *Staphylococcus aureus*

In addition to characterizing the nasal colonization rates of *S. aureus* among medical students, we conducted an assessment of the antibiotic sensitivity profile of the isolated *S. aureus*. Understanding the susceptibility of these strains to commonly used antibiotics is crucial for informing appropriate treatment strategies and addressing the challenges posed by antibiotic resistance.

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As shown in figure 3, the sensitivity analysis of the 10 isolated *S. aureus* strains revealed significant findings. All isolates exhibited full sensitivity (100%) to Ciprofloxacin and Gentamicin. However, susceptibility to Penicillin was notably lower, with only 30% of isolates showing sensitivity. Similarly, only 40% of isolates were susceptible to Azithromycin. In contrast, most isolates showed relatively high sensitivity to Trimethoprim/Sulfamethoxazole (80%) and Clindamycin (70%). Remarkably, all isolates were fully sensitive (100%) to Linezolid. Notably, 10% of the isolated *S. aureus* strains were resistant to Cefoxitin, thus was identified as Methicillin-resistant *S. aureus* (MRSA).

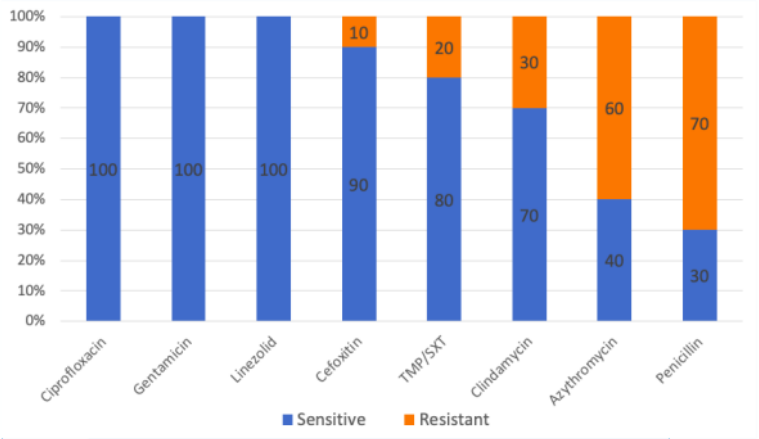

Figure 3. Antibiotic sensitivity profile of the isolated *Staphylococcus aureus*

Assessment of those antibiotic sensitivity profiles revealed concerning trends in resistance, particularly to Penicillin and Azithromycin. While Ciprofloxacin, Gentamicin, and Linezolid demonstrated robust efficacy, resistance to traditional antimicrobial agents like Trimethoprim/Sulfamethoxazole and Clindamycin underscores the necessity of prudent antibiotic and ongoing surveillance to monitor the emerging resistance, particularly concerning Methicillin-resistant *S. aureus* (MRSA). Notably, 10% of the isolated *S. aureus* in the initial sampling of this study was MRSA. While the prevalence may vary with a larger sample size, the detection of MRSA nasal carriers among medical students prior to clinical rotation is a particular concern due to the potential for transmission to others, including patients in hospital settings. Medical students, which were often overlooked among healthcare workers, may serve as a potential conduit for *S. aureus* transmission to patients, particularly due to their limited awareness of standard infection control measures during the initial stages of clinical practice, thereby increasing the risk of transmission during this phase (13).

Nasal Carriage Pattern of *Staphylococcus aureus*

After a ten-months interval from the initial sampling, a follow-up assessment was conducted to investigate the nasal carriage patterns of *S. aureus* among participants, discerning between persistent and intermittent carriers. The total number of participants in the second sampling was 43 individuals. From these 43 participants included in the second sampling, 14 yielded positive results. Within this group of 14 individuals, 9 individuals were newly positive,

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having previously tested negative, indicating the emergence of new cases of nasal carriage within the studied population. As a result, the prevalence of *S. aureus* nasal carrier in this second sampling was 33%. This result revealed fluctuating nasal carriage patterns, with a prevalence of approximately 17% observed during the initial occasion and around 33% during the subsequent occasion, aligning with previous reports of colonization rates ranging from 10% to 35% in healthy populations, including medical students prior to clinical rotation (13-16). The nearly twofold difference in prevalence between the two occasions can partly be attributed to variations in sample sizes and the potential for negative periods during first sampling among intermittent nasal carriers.

Furthermore, from the initial cohort of 57 participants, only 37 samples were obtained for the second sampling. Among these 37 individuals who participated in both samplings, *S. aureus* was always absent in the nasal swabs of 19 individuals (51%). Conversely, it was detected at least once in the nasal swabs of 18 individuals (49%): 13 participants (35%) exhibited its presence on a single occasion, either during the first or second sampling, thus indicating intermittent carriage. Meanwhile, 5 participants (14%) showed *S. aureus* nasal colonization on both occasions, indicating persistent carriage (Fig 4). Notably, among the 10 participants who were previously identified as carriers, 9 were available for the second sampling. Among these 9 participants, 5 (50%) tested positive and 4 (40%) tested negative on the second sampling.

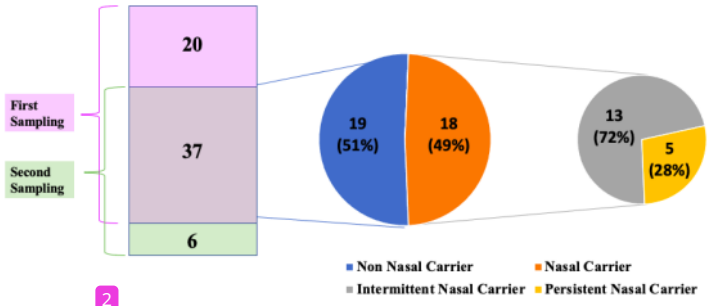

Figure 4. Nasal Carriage Pattern of *Staphylococcus aureus* among medical students

In addressing the classification of carriers, various methodologies were employed across studies, resulting in differing criteria. However, a universally accepted definition for carrier status remains elusive, particularly concerning the optimal number of swabs and the threshold for positivity. Some research identified persistent carriers as individuals consistently testing positive for *S. aureus* across all nasal swabs (17). Conversely, other studies established cut-off values for the carrier-index, calculated as the ratio of positive swabs to total swabs per person (18, 19). In addition, Nouwen et al proposed a culture rule using two nasal swabs a week apart to differentiate *S. aureus* carriers, with persistent carriers identified by both swabs showing bacterial counts exceeding 10^3 colony forming unit (CFU), while intermittent carriers are determined if only one culture is positive or if both cultures have a low CFU count (20). In this study, participants were designated as persistent carriers if both nasal swab samples, collected ten months apart, yielded positive results for *S. aureus*, while intermittent carriers were characterized by *S. aureus* detection on a single occasion.

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This differentiation holds significant clinical implications. Persistent carriers may pose a higher risk for transmission within healthcare settings, as they maintain a continuous source of *S. aureus*. Targeted interventions, such as decolonization protocols or enhanced infection control measures, may be warranted to mitigate the risk of infection and transmission from persistent carriers, especially for MRSA carriers (21, 22). Conversely, intermittent carriers, while exhibiting variable colonization patterns, may still contribute to transmission during periods of positivity. Therefore, strategies aimed at reducing transmission, such as hand hygiene promotion and environmental disinfection, should be implemented consistently to minimize the risk posed by intermittent carriers.

Comparative analysis with existing literature reveals variability in colonization rates and antibiotic sensitivity profiles across populations and settings. While our study focused on medical students, similar investigations among healthcare workers and community populations (23-26) provide valuable insights into transmission dynamics and reservoirs of *S. aureus*.

Limitations of our study include its cross-sectional design and single-center setting, which may limit generalizability. Future research incorporating longitudinal surveillance and multi-center collaboration would enhance our understanding of colonization dynamics and facilitate the development of evidence-based infection control strategies.

CONCLUSION

Our study provides valuable insights into the prevalence, antibiotic sensitivity profile, and dynamics of nasal carriage of *S. aureus* among medical students. A substantial proportion of pre-clinical medical students are asymptomatic carriers of S. aureus in their nasal passages, either intermittently or persistently. As these students transition into healthcare roles, they may serve as reservoirs for the transmission of this bacterium within healthcare settings. These results underscore the importance of continuous monitoring and focused interventions to mitigate the risk of nosocomial transmission and combat emerging antibiotic-resistant organisms, particularly MRSA, from medical students serving as primary healthcare providers to the most vulnerable patients in healthcare settings.

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CONFLICT OF INTEREST

This study was funded by the internal research grant (DIP) from the Research and Community Service Institute (LPPM) of Universitas Ciputra, for the fiscal year 2023/2024. Authors declare no conflict of interest.

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PAGE 1

PAGE 2

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PAGE 6

PAGE 7

PAGE 8

PAGE 9