



Compelling Evidence of Bacterial Pharyngeal Colonization among Clinical Sciences Students

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Upper respiratory tract is one of the commonest sites for microbial colonization, and the colonized individuals are at risk of infection, and medical students are frequently exposed to a variety of infections agents and are more likely to get colonized by them. The current study aimed To determine the frequency of pharyngeal bacterial colonization among clinical students. Methods: A total of 140 throat swab was collected from study participants, among them, 70 clinical students were enrolled, and 70 nonclinical subjects were recruited as a control group. Isolated bacteria were identified using ordinary laboratory techniques (Gram staining and biochemical tests). Result: Among clinical participants, there were 16 (80%) of them in the age group 20–25 years of age, Frequency of pharyngeal carriage in studied subjects were 29(20.7%), 23(32.9%) of clinical sciences students were significantly carry the virulent bacterial pathogen, while only 6 (8.6%) of the control group have clinical pharyngeal bacteria (P-value ≤ 0.001). *S. aureus* was carried among 11 (15.7%) clinical sciences students, followed by *S. pneumoniae* 3 (4.3%), *P. aeruginosa*, and *Haemophilusspp* 2 (2.9%). Penicillin and Ciprofloxacin resistance was high frequent, statistically, significant differences were also revealed in the clinical sciences students group who have had a

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past history of respiratory infection, were 13/16 (80%), and 6/7 (85.7%) of them frequently carried gram-positive bacteria and negative bacteria respectively (p. value =0.023)

Conclusion: Clinical students complain of a high frequency of bacterial throat colonization, and then multidrug-resistant of bacterial strain. Such evidence may increase the possibility that clinical students play role in the transmission and dissemination of the infection for others.

Keywords: Clinical students; sore throat; coagulase-positive Staphylococci; antimicrobial sensitivity.

1. INTRODUCTION

Throat infections are among the most widespread and serious infections that compel an individual to seek medical attention. It represents some of the most common bacterial diseases encountered affecting people of all ages [1,2]. These infections are one of the leading causes of morbidity and mortality in critically ill patients. The nose and throat are the frequent sites of infection because they come in direct contact with the physical environment and are exposed to air-borne microorganisms [3]. The prevention of hospital-acquired infection depends on the continuous and concerted efforts of all those who design, administer, and work in hospitals. Microbiologists - whose training and experience should have made them familiar with the causes of communicable disease and the sources and routes of transmission of pathogenic microorganisms - should play a leading part in these activities [4].

The upper respiratory tract is one of the commonest sites for microbial colonization. The colonized individuals are at risk of infection and can be a source of transmission of pathogens. Medical students are frequently exposed to a variety of infections agents and are more likely to get colonized by them. The respiratory tract can be infected by a variety of bacteria, both gram-positive and gram-negative such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Staphylococcus aureus* usually colonize the upper respiratory tract of humans, which may lead to devastating complications. Although the diseases that they cause may range from mild to severe, in most cases, the microbes remain localized within the respiratory system. Fortunately, most of these infections also respond well to antibiotic therapy [5,6].

Clinical science student Healthcare-associated infections (HAIs) are a major cause of morbidity and mortality in patient populations seeking medical treatment, they are most frequently caused by viral, bacterial, and fungal pathogens,

and pharyngitis is primarily caused by bacteria and viruses. [7,8]. Microorganisms may survive in the healthcare setting for various periods of time if adequate ventilation is not provided which is it raises the danger for hospital inhabitants, patients, and clinical students who spend an extra hour in the hospital [9]. Pharyngeal colonization by *S. aureus* remains the most frequent bacteria colonize upper respiratory tract is thought to be a risk factor for nosocomial infections; removing it is important in stopping such infections. [10,11]. MRSA is the leading cause of nosocomial infections as well as other multidrug-resistant healthcare-associated infections [12,13].

MRSA is most frequent in hospitals worldwide with the highest rates (>50%) documented in North and South America, Asia, and Malta, and MRSA-associated infection in healthcare settings may be correlated with life-threatening status. The high frequency of immuno-compromised patients is one of the risk factors which can be acquired via healthcare instruments or casual contact from visitors or healthcare workers themselves [14]. In the present study, we aimed to identify bacteria that colonize the pharynx of clinical students and pre-clinical students comparing to nonclinical participants in Al Kharj, Saudi Arabia.

2. MATERIALS AND METHODS

2.1 Study Design

A prospective, analytical, cross-sectional study providing a comparison of bacteria from the throat of clinical and nonclinical students was conducted over a period between September-December 2020. Approval was taken from a collage of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University, Alkharj-KSA. All participants were recruited after signed of informed consent.

2.2 Study Area and Study Participants

A total of 140 subjects were recruited by nonprobability sampling methods. subjects who

participated in the study were interned students (clinical sciences students) with at least 6 months of hospital experience (interns) who work at different departments at Al Kharj hospitals. And pre-clinical students were (Students at level 3, 4 regarded as nonmedical) as a control group. Clinical science students, all participants using antibiotics two weeks before collection, medical sciences students on long leave (more than three months), and preclinical sciences students who had been hospitalized within the previous six months were excluded from the study. A formalized questionnaire was designed to gather demographic data, such as gender, hospital exposure, recent antibiotic exposure, respiratory illness, etc.

2.3 Methods

2.3.1 Samples collection

The throat swab was collected aseptically using sterile Evepon swab sticks, by swabbing the posterior oropharynx, and immediately transported in sterile cotton plugged test tube to the microbiology laboratory at a collage of applied medical sciences, Prince Sattam Bin Abdul Aziz University for further analysis.

2.3.2 Culture and bacterial identification

Specimens were directly inoculated on 5% sheep blood agar and chocolate agar, MacConkey (MAC) agar, and Mannitol Salt Agar (MSA), then they were incubated at 37°C in a candle jar with increased CO₂. A combination of criteria including growth and chemical reactions on primary or selective media, type of hemolysis present, colony characteristics, and Gram staining reactions are used in the identification of isolated bacteria. Common gram-positive cocci, such as staph and strep can often be identified by morphology on Gram staining and results of catalase and coagulase tests. Gram-negative rods (bacilli) are identified using tests that demonstrate biochemical reactions such as oxidation or fermentation of the sugars set.

2.3.3 Antibiotic susceptibility

Testing of the bacterial isolates for antibiotic sensitivity was performed on Mueller–Hinton agar by the Kirby–Bauer disc diffusion method, diameters of the inhibition zone were measured with a slide gauge to the nearest millimetre. Test results were only approved once diameters of the

inhibition zone were within the range of standards. Bacterial isolates were tested against the following antimicrobial agents: Penicillin (10 µg), Erythromycin (15µg), Ciprofloxacin (10µg), Gentamicin (10µg), Clindamycin (2µg), Ceftriaxone (30 µg), Tetracycline (30 µg), Vancomycin (30 µg), Azithromycin (30 µg), Cotrimoxazole (25µg). Each disc was placed on the Mueller–Hinton agar medium with forceps under aseptic conditions forceps to ensure that it was affixed to the agar incubated overnight at 37°C.

2.4 Statistical Analysis

Data were analyzed using the SPSS software version 26.0, and descriptive statistics (e.g., frequency and percentage), chi-square test, were used. The threshold for statistical significance was $p < 0.05$.

3. RESULT

A total of 140 throat swabs were collected from study participants, among them, 70 were clinical intern students at the different hospitals at Al-kharj province were enrolled in the study, and 70 were non-clinical subjects (include pre-medical students and employees) recruited as a control group. Among clinical sciences participants, there were 65(92.4%) of them in the age group 20–25 years of age, where 37(52.9%) of nonclinical students aged between 26–30 years old. Regarding gender distribution; there is an equal distribution between males and females 36(51.4%), and 37(52.9%) respectively. 80(57.2%) of participants have no history of past infections, while (21.4%), (28.5%) and (7.1%) of clinical students were have had a past history of sinusitis, pharyngitis, and otitis media respectively, all demographic data were displayed in Table 1.

The bacteriological profile of pharyngeal swab cultures of clinical sciences students and the control group are summarized in Table 2. The frequency of pharyngeal carriage in studied subjects was 29(20.7%), 23(32.9%) of clinical sciences students significantly carried virulent bacterial pathogen, while only 6 (8.6%) of the control group have clinical pharyngeal bacteria (P -value ≤ 0.001). *S. aureus* was carried among 11 (15.7%) clinical sciences students, followed by *Streptococcus pneumoniae* 3 (4.3%), *Pseudomonas aeruginosa*, and *Haemophilusspp* 2 (2.9%). The equal distribution rate of isolated

bacteria among the control group was revealed 1 (1.4%) for all gram-positive and negative strains (*S. aureus*, *S. pneumonia*, *S. viridans*, and *K. pneumoniae*, etc). Frequency of antibiotic resistance pattern of isolated gram-positive and negative bacteria among clinical and nonclinical students summarized in Tables 3, 4. Statistically, significant differences were revealed in Penicillin and Ciprofloxacin (P. value ≤ 0.023 , 0.001) respectively. Statistically significant differences were also revealed in the clinical sciences students group who have had a past history of respiratory infection, were 13/16 (80%), and 6/7 (85.7%) of them frequently carried gram positive bacteria and negative bacteria respectively (p. value =0.023) Table 5. There was no correlation reported in the past history of respiratory infection in the control group.

4. DISCUSSION

Hospital-associated infection transmitted by health workers looks like the tip of an iceberg, as healthcare workers are assumed to be significant inhabitants for nosocomial pathogens transmission, and medical students, notable members of the healthcare team, might be a reservoir too. On the other hand, the human respiratory tract is an ideal area for microbial colonization, therefore, as a result, it is critical to evaluate healthcare workers and clinical students as well for virulent pathogen colonization. Since current study aimed to ascertain clinical sciences intern students at different hospitals at Al Kharj for pharyngeal bacterial carriage.

Table 1. Characteristics and Demographic data of Medical and Nonmedical students

Characteristics	Total N=140	Clinical Students n=70(%)	Non-Clinical Students n= 70 (%)	P. value
Gender				
Male	75 (53.6%)	36 (51.4%)	39 (55.7%)	0.768
Female	65 (46.4%)	34 (48.6%)	31 (44.3%)	
Age				
20-25 years old	98 (70%)	65 (92.4%)	33 (47.1%)	0.131
26-30 years old	42 (30%)	5 (7.1%)	37 (52.9%)	
History of past infection				
No	80 (57.2%)	30 (42.9%)	50 (71.4%)	0.034
Sinusitis	30 (21.4%)	15 (21.4%)	15 (21.4%)	
Pharyngitis	22 (15.7%)	20 (28.5%)	2 (2.9%)	
Otitis media	8 (5.7%)	5 (7.1%)	3 (4.3%)	
Recurrent Infection				
No	45 (32.1%)	6 (8.5%)	39 (55.7%)	0.021
$\geq 1-2$ Months	40 (28.6%)	31 (44.3%)	9 (12.9%)	
$\geq 2-4$ Months	55 (39.3%)	33 (47.1%)	22 (31.4%)	
Total	140 (100%)	70 (100%)	70 (100%)	

Table 2. Frequency of total bacteria isolated from Clinical and Non Clinical students

Isolated bacteria	Total n=140 (%)	Clinical Sciences Students n=70 (%)	Non-Clinical Students n=70 (%)	P. value
<i>Staphylococcus aureus</i>	12 (8.6%)	11 (15.7%)	1 (1.4%)	0.001
<i>Streptococcus pneumonia</i>	4 (2.9%)	3 (4.3%)	1 (1.4%)	0.692
<i>Pseudomonas aeruginosa</i>	2 (1.4%)	2 (2.9%)	-	0.964
<i>Streptococcus viridans</i>	2 (1.4%)	1 (1.4%)	1 (1.4%)	0.841
<i>Corynebacterium spp</i>	1 (0.7%)	1 (1.4%)	-	0.632
<i>Klebsiella pneumonia</i>	3 (2.1%)	2 (1.4%)	1 (1.4%)	0.621
<i>Haemophilus spp</i>	3 (0.7%)	2 (2.9%)	1 (1.4%)	0.991
<i>Acinetobacter spp</i>	2 (1.4%)	1 (1.4%)	1 (1.4%)	0.127
Total	29 (20.7%)	23(32.9%)	6 (8.6%)	

Table 3. Frequency of antibiotic resistance pattern of isolated gram positive bacteria among Clinical and Non Clinical students

Pattern	Total n=19 (%)	MIC	Clinical Students n=16 (%)	Non Clinical n=3 (%)	P. value
Penicillin	10(52.6%)	≤ 0.11	9 (56.1%)	1 (33.3%)	0.033
Erythromycin	15(78.9%)	≤ 28	13 (68.8%)	2 (66.7%)	0.021
Ciprofloxacin	11(57.9%)	≤ 1	11(68.8%)	0	0.112
Gentamicin	4(21.1%)	≤ 32	3 (18.8%)	1 (33.3%)	0.657
Clindamycin	3(15.8%)	≤ 0.23	3 (18.8%)	0	0.134
Ceftriaxone	1(4.3%)	≤ 3	1 (6.3%)	0	0.361
Tetracycline	1(5.1%)	≤ 4	1 (6.3%)	0	0.138
Vancomycin	0	0	0	0	-

Table 4. Frequency of antibiotic resistance of isolated gram negative bacteria among clinical and nonclinical student

	Total n=10 (%)	MIC	Clinical Students n=7 (%)	Non Clinical n=3 (%)	P value
Penicillin	5 (50%)	≤ 0.13	4 (57.1%)	1 (33.3%)	0.053
Azithromycin	4 (40%)	≤ 2	4 (57.1%)	1 (33.3%)	0.136
Ciprofloxacin	3 (30%)	≤ 1	3 (42.9%)	0	0.103
Ceftriaxone	4 (40%)	≤ 3	3 (42.9%)	1 (33.3%)	0.145
Co-trimoxazole	0	-	0	0	-

Table 5. Frequency of isolated bacteria among study group and History of past and recurrent infection

Characteristics	Clinical Students n=23 (%)		P. value	Non-Clinical Students n= 6 (%)		P. value
	Gram positive n=16 (%)	Gram negative n=7 (%)		Gram positive n=3 (%)	Gram negative n=3 (%)	
History of past Respiratory infection						
No	3 (18.8%)	1 (14.3%)	0.023	1 (33.3%)	-	0.134
Sinusitis	2 (12.5%)	1 (14.3%)		1 (33.3%)	2 (66.7%)	
Pharyngitis	10 (62.5%)	4 (57.1%)		1 (33.3%)	-	
Otitis media	1 (6.1%)	1 (14.3%)		-	1 (33.3%)	
Recurrent Respiratory Infection						
No	2(12.5%)	1(14.3%)	0.051	2 (66.7%)	1 (33.3%)	0.121
1-2 Months	10 (62.5%)	5 (71.4%)		1 (33.3%)	1 (33.3%)	
2-4 Months	4 (25%)	1 (14.3%)		-	1 (33.3%)	

The overall rate of pharyngeal pathogens carriage was reported among study participants 30 (21.4%), while about third 22 (31.5%) is acquisition rate among clinical sciences group; half of the 11 (18.6%) carry *S. aureus*. Our finding much increased than other reported studies, such as Zakai SA et al in Saudi Arabia [15] who reported 18.7% of medical students to carry MSSA strain, also (25,7%) asymptomatic colonization rate of *S. aureus* were revealed by

Szymanek-Majchrzak K et al. [16], as well as Zhou K et al. [17]. Increases rates of bacterial colonization among clinical science medical students are expected results and can be associated with hospitalization exposure and frequent patients contact. Among study, subjects enrolled regardless of gender and their age range between 20-30 years old, majority of clinical science students 65/70 (92.4%) were in the age group between 20-25 years old. with

regard to the history of past infection, 30(42.9%) of clinical science group had no history of past infection where reminder (57%) suffer from past respiratory infections two months later, in contrast, to control group almost of them 50 (71.4%) had no history of past infection; findings were statistically significant (P-value 0.034). Interestingly (91.4%) of clinical science group have a recurrent respiratory infection for 1-2/ and 2-4 months. Our finding was supported by those who revealed a crucial correlation between the acquiring of *S. pneumoniae* and respiratory symptoms was noticed in cohorts of Hajj pilgrims [18]. This could clarify why clinical students in our study have increased in colonization rate due to frequent exposure to microorganisms from patients, and health care workers during their training in the hospital, thus they play a significant role in being a source and means of transmitting nosocomial infections. Additionally, younger age, low experience about clinical practice and preventive measures as well as collection time of samples (winter seasons), plays crucial risk factors.

The overall prevalence rate of bacterial throat colonization was higher in the clinical sciences group rather than control group 22(31.4%), and 8 (11.4%) respectively, this attributed to the completely sophisticated environment in a hospital environment. Our finding is in agreement with other studies, Dharm Raj Bhatta et al [19] who found a colonization rate of around 40%, and [17,20,21].

On the other hand among diverse numbers and sorts of isolated bacteria that have been detected in the throats of both medical students and control groups, *S. aureus* was most prevalent among clinical students followed by *S. pneumoniae*, and *K. pneumoniae*, and relatively low frequency of member of Enterobacteriaceae. It is important to note that *S. pneumoniae*, *K. pneumoniae*, and *H. influenzae* are the most widespread causative bacterial pathogens correlated with acquired respiratory tract infections [22], and then they are possible aetiological agents of pneumonia, pharyngitis, otitis media, and para-nasal sinusitis (which are common symptoms among our clinical sciences students).

All isolated bacteria were normal flora which is harmless in healthy persons; nevertheless, flora can be transformed into an inflammatory agent once triggered by another microorganism, or any

stimulus (such as acquiring of antibiotic resistance gene) because the colonization process is complicated [23,24]. Hence health care personnel including clinical students are susceptible to bacterial infection, this conclusion was supported by many studies [25,26], who revealed the ultimate knowledge scores for SPs were low, primarily in the domains of hygiene practices, strict management, and personal protective equipment, implying inadequate and inefficient guidance obtained by medical students via the standard curriculum, leaving them vulnerable to health facility-related infections. Antibiotic resistance pattern of isolated gram-positive bacteria among clinical and nonclinical students disclosed that (56.1%) of isolated gram-positive among clinical students were statistically resistant to Penicillin, and (68.8%) resistant to Erythromycin (P-value 0.03, and 0.02) respectively. However, all isolates of gram-negative bacteria among clinical sciences students were resistant to Penicillin and Azithromycin. Our finding is inconsistent with many studies who stated an antimicrobial resistance pattern of nasal and pharyngeal specimens, especially Staphylococci (MRSA) Okamo B et al, [27] conclude that elevated antimicrobial resistance rate of isolates in tertiary care units, due to a combination of excessive pressure caused by the widespread use of antimicrobial drugs, greater patient and health professional density, and unsatisfactory infection control practices. Antimicrobial-resistant organisms are more widespread in the hospitalized inhabitants. This is attributable to a combination of factors, including elevated amounts of antimicrobial use, vast reservoirs of bacteria, and the facilitation of transmission through close personnel and resident contact within health facilities, Adlan et al [28]. So this concerning actuality implies the need for increased knowledge and awareness between medical sciences students regarding preventive and control measures of infection to reduce nosocomial infection and healthcare-associated infection as much as possible.

5. SIGNIFICANCE STATEMENT

The strength of this study lies in comparing clinical students with nonclinical participants belonging to two different environments, as well as a collection of our data during the winter season (December). However, a study had a complaint from some limitations; First: the transmission of isolated pathogens from the

sample of medical students to the control group, patients, or the other healthcare personnel, and vice versa was not included in the current study. Second: the results of this study cannot be generalized to other medical students, because the sample size was too small, as well as the sample was collected from clinical students at Al Kharj, KSA. Third: the current study depends on classical microbiological techniques rather than advanced molecular methods. Finally, Methicillin-Resistant Staphylococcus aureus (MRSA) hasn't been identified

6. CONCLUSION

Clinical students complain of a high frequency of bacterial throat colonization, and then multidrug-resistant of bacterial strain. Such evidence may increase the possibility that clinical students play role in the transmission and dissemination of the infection for others. Efforts should be adequate to raise the knowledge and awareness among medical students, in addition to strong adherence to infection control protocols to minimize the transmission of nosocomial diseases in healthcare institutions.

DATA AVAILABILITY

The data generated and analyzed in the current study are included in the article

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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