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Gram Positive Bacteria Carriage among Health Care Workers: An Under-Reported Source of Infections?

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Abstract

***Staphylococcus aureus* and *Streptococcus pyogenes* are two highly infectious pathogens implicated in a significant percentage of healthcare associated infections. They produce wide range of infections, from mere folliculitis & furuncles, cellulitis, myositis, & glomerulonephritis to conditions with very significant morbidity such as necrotizing fasciitis & Toxic Shock syndrome, and thus represent an important subset of infections that need to be tackled urgently. To assess prevalence of nasal as well as oropharyngeal carriage of *Staphylococcus aureus* & *Streptococcus pyogenes* among health-care workers and its antimicrobial resistance pattern. One nasal swab & two oropharyngeal swabs were collected from each participant, with one nasal & oropharyngeal swab cultured on blood agar & mannitol salt agar for *Staphylococcus aureus*, and the second oropharyngeal swabs were cultured on Crystal violet blood agar for *Streptococcus pyogenes*, further subjected to susceptibility test by disc diffusion method on Muller-Hinton agar as per CLSI guidelines 2019. Prevalence of *Staphylococcus aureus* carriage was 9% which includes 4% It is nasal, 4.5% oropharyngeal & 0.5% both. Prevalence of MRSA, MLS_B & mupirocin resistant *Staphylococcus aureus* was 1.5%,4% & 0%respectively. Prevalence of oropharyngeal carriage of *Streptococcus pyogenes* was 1.5%. This study feature the need of screening of Health-care workers for nasal as well as oropharyngeal carriage of *Staphylococcus aureus & Streptococcus pyogenes* & further its antimicrobial resistance pattern.**

Keywords: *Staphylococcus aureus*, *Streptococcus pyogenes,* healthcare workers, nasal carriage, antibiotic susceptibility

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INTRODUCTION

Among *staphylococci* species *Staphylococcus aureus* is one of the most virulent, & can produce a broad spectrum of infections, from mere folliculitis & furuncles, to conditions with very significant morbidity such as Toxic Shock syndrome & necrotizing fasciitis[1]. *Streptococcus pyogenes*, is also responsible for a broad range of infections, ranging from sore throat to cellulitis, myositis, & glomerulonephritis[2]. Both *Staphylococcus aureus* & *Streptococcus pyogenes*, although a part of normal human flora[3,4], are implicated in a significant percentage of post-op surgical site infections (SSIs), & have been frequently isolated from nose & throat swab samples of healthcare workers (HCWs)[5].

Resistance to antimicrobial agents has become an important concern worldwide today. HCWs are implicated to be sources of nosocomial Methicillin Resistant *Staphylococcus Aureus* (MRSA) infections not just in surgical settings, but also in ICUs[7,8]. Thus, proper screening is necessary to prevent spread of such infections in the future.

Earlier considered insignificant, studies in recent years have observed to the contrary that this carriage may actually be responsible for a considerable proportion of post-op SSIs, & HDU infections, data which needs to be further validated. Thus, this study was carried out to assess the prevalence of carriage of *Staphylococcus aureus* & *Streptococcus pyogenes,* & its antimicrobial resistance pattern.

MATERIALS AND METHODS

A cross sectional study was conducted in AVBRH & JNMC, Sawangi (Meghe) Wardha. from june 2019 to September 2019. 200 health care workers (Staff nurses and ward attendents) participated in the study. Exclusion criteria was HCWs who received antibiotics for the past 30 days or those suffering from any upper respiratory tract infections .

Study was conducted after approval from Institutional ethical committee. Details of the study were explained to each participant. 1 nasal swab & 2 oropharyngeal swabs were collected from each participant.

One nasal swabs & one oropharyngeal swab were cultured on blood agar as well as on Mannitol Salt agar for isolation of *Staphylococcus aureus* & identified as per standard microbiological procedures[8].

Second oropharyngeal swabs were immediately cultured on Crystal violet blood agar by "streak & stab" technique for isolation of *Streptococcus pyogenes*. and identified as per standard microbiological procedures[8].

Antibiotic susceptibility testing of *Staphylococcus aureus* isolates

All the *Staphylococcus aureus* isolates were subjected to in vitro antibiotic susceptibility testing by disc diffusion test on Muller-Hinton agar as per CLSI guidelines 2019[9].

Phenotypic detection of MRSA, Macrolide-Lincosamide-Streptogramin (MLS_B) resistance and high level mupirocin resistance was done following CLSI guidelines2019.

MRSA - All the *Staphylococcus aureus* isolates were subjected to in vitro antibiotic susceptibility testing for cefoxitin (30µg) by disc diffusion test on Muller-Hinton agar. Isolates giving inhibition zone diameter of ≤ 21 mm for cefoxitin (30µg) disc were labeled as oxacillin or methicillin resistant and a zone diameter of ≥ 22 mm as oxacillin or methicillin sensitive.

Macrolide-Lincosamide-Streptogramin (MLS_B) resistance was identified using a double disc test with erythromycin (15 µg) disc and clindamycin (2 µg) disc. Briefly, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg).

Clindamycin susceptible phenotype(MS)- *Staphylococcus aureus* isolates showing resistance to erythromycin (zone size ≤13mm) and sensitive to clindamycin ((zone size ≥21mm)

Inducible clindamycin resistance phenotype (MLS_Bi) - *Staphylococcus aureus* isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having Inducible clindamycin resistance phenotype. Hazy growth within the zone of inhibition around clindamycin even if no D-zone is apparent was taken as clindamycin resistant.

Constitutive clindamycin resistance phenotype (MLS_Bc) - *Staphylococcus aureus*

isolates showing resistance to erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm)

High level mupirocin resistance - All the *Staphylococcus aureus* isolates were subjected to in vitro antibiotic susceptibility testing of Mupirocin (200 µg) by disc diffusion test on Muller-Hinton agar. No zone for Mupirocin(200 µg) was labeled high level mupirocin resistance.

Control strains used were *Staphylococcus aureus* ATCC 25293 and confirmed strains of MRSA and Inducible clindamycin resistance from microbiology laboratory JNMC.

Antibiotic susceptibility testing of *Streptococcus pyogenes* isolates

All the *Streptococcus pyogenes* isolates were subjected to in vitro antibiotic susceptibility testing on Muller-Hinton agar with 5% sheep blood as per CLSI guidelines 2019[9].

Macrolide-Lincosamide-Streptogramin (MLS_B) resistance phenotypes were identified using a double disc test with erythromycin (15 µg) disc and clindamycin (2 µg) disc. Briefly, erythromycin (15 µg) disc was placed at a distance of 13 mm (edge to edge) from clindamycin (2 µg).

Clindamycin susceptible phenotype(MS)-*Streptococcus pyogenes* isolates showing resistance to erythromycin (zone size ≤15mm) and sensitive to clindamycin (zone size ≥19mm).

Inducible clindamycin resistance phenotype (MLS_Bi) - *Streptococcus pyogenes* isolates showing resistance to erythromycin (zone size ≤15mm) while being sensitive to clindamycin (zone size ≥19mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having Inducible clindamycin resistance phenotype. Hazy growth within the zone of inhibition around clindamycin even if no D-zone is apparent was taken as clindamycin resistant.

Constitutive clindamycin resistance phenotype(MLS_Bc)-*Streptococcus pyogenes* isolates showing resistance to erythromycin (zone size ≤15mm) and clindamycin (zone size ≤15mm). Control strains used was *Streptococcus pyogenes* ATCC 19615.

OBSERVATION AND RESULTS

Among 200 participants 39(19.5%) were male & 161(80.5%) female. The age of participants ranged from 25 yrs to 50 yrs. Carriage of *Staphylococcus aureus* was seen in 18 participants (prevalence 9%) which included 8 (4%) having only nasal carriage, 9 (4.5%) with only oro-pharyngeal carriage and 1(0.5%)with both nasal & oro-pharyngeal carriage. Carriage of *Streptococcus pyogenes* was seen in 3 participants (prevalence1.5%) which included 2 with both *Staphylococcus aureus* & *Streptococcus pyogenes* carriage. Age and sex were not found significantly associated with carriage ($p > 0.05$).

All the *Staphylococcus aureus* isolates were sensitive to Rifampicin, Mupirocin, Vancomycin & Amikacin. 73.68% isolates were found sensitive to Ciprofloxacin followed by Clindamycin (68.42%), erythromycin (57.90%) & penicillin(10.53%). Resistance pattern of *Staphylococcus aureus* is represented in table 2. Among 3 Streptococcus pyogenes isolates, 2 isolates (66.6%) were susceptible to Erythromycin. One isolates showed Macrolid resistance,MS phenotype. 3/3 isolates (100%) were sensitive to Penicillin, Clindamycin, Ciprofloxacin, Tetracycline, Bacitracin, Vancomycin & Amikacin.

DISCUSSION

Colonisation with pathogenic organisms among HCW is one of the risk factor for nosocomial & community acquired infections. Nasal &

Table 1. Segregation of organisms isolated by swabbing method

Organism	Participant		Total
	Nursing Staff (n=152)	Ward attendents (n=48)	
Staphylococcus aureus	15	3	18
Streptococcus pyogenes	3	0	3

Table 2. Resistance pattern of *Staphylococcus aureus* isolates

Total	MRSA	MLS_{Bi} Phenotype	MLS_{Bc} Phenotype	MS Phenotype	High level mupirocin resistance
Total samples n=200	3 (1.5%)	5 (2.5%)	1 (0.5%)	2 (1%)	0 (0%)
S aureus isolates N=19	3 (15.79%)	5 (26.32%)	1 (5.26%)	2 (10.53%)	0 (0%)

oro-pharyngeal colonisation by *Staphylococcus aureus* and oro-pharyngeal colonization with *Streptococcus pyogenes* may be one of the risk factor in such conditions.

In our study, *Staphylococcus aureus* carriage was seen in 18 participants (prevalence 9%). The findings correlate with findings of Yamasaki F et al. who reported a prevalence of 11.1%[10]. The prevalence was found to be lower than other studies reviewed in literature, including Indian, such as Kumar P et al. who found the prevalence to be 47.62[11], and International literature as well, such as Esposito S et al. who obtained 25.7%, El AilaNA et al. who observed 25.5% and Boisset S et al., who obtained a value of 38.8%.[4, 6, 12].

Prevalence of nasal carriage in current study(4.5%) is less than the study carried out in the same setting and study population in 2013(14.58%)[13]. This can be due to the strict implementation and monitoring of infection control practices including screening and treatment of nasal carriers.

On assessment of antibiotic susceptibility, only 2/19 (10.53%) isolates were found to be sensitive to Penicillin. Overuse and indiscriminate prescribing of penicillin congeners is being implicated as the cause for this increased resistance. 11/19 isolates (57.90%) were found to be sensitive to erythromycin. Multiple studies have reported sensitivity in the range from 33% to 91%[14-16].

13/19 isolates (68.42%) in this study were found to be susceptible to clindamycin. Sensitivity reported in other studies includes 94% by authors Pathak, et al., 91.3% in study of Fomda, et al., etc[16,17]. The lower sensitivity in this study may be because of overuse of this antibiotic.

100% isolates were senisitive to Amikacin in this study which correlates with finding of other studies.[14, 17].

Sensitivity to Ciprofloxacin was observes in 73.68% of the isolates. Susceptibility to ciprofloxacin varies in different population from 27.06% to 87.8%[16-18].

In our study, Vancomycin was found sensitive in 100% isolates. It correlates with study on nasal isolates of *Staphylococcus aureus* by other authors[14-17].

All 19 *Staphylococcus aureus* isolates, were tested for methicillin resistance (MRSA), MLS_B resistance and High level mupirocin resistance. In this current study, 15.79% of the isolates were MRSA. MRSA findings correlates with studies of other authors like 10% in study of M C Cormak, et al. and 11.1% in study of Yamasaki F, et al.[10, 19]. MLSBi phenotype was seen in 26.32% of isolates. among *Staphylococcus aureus* nasal isolates Inducible clindamycin resistance was found to be higher in our study, compared with other studies. It was reported as 16.7% and 16.40% in nursing students and Health care workers respectively.[20, 21]. No isolate showed high level Mupirocin resistance. Similar findings were found in study of Lonekke, et al. and Jimei Du, et al.[22, 23].

Carriage of *Streptococcus pyogenes* was noted in 3 participants (prevalence1.5%) which includes 2 with both *Staphylococcus aureus* and *Streptococcus pyogenes* carriage.

Assessment of antibiotic susceptibility pattern for *Streprococcus pyogenes* was carried out. In the present study all the isolates were sensitive to penicillin by disc diffusion method. This finding correlates with finding obtained in studies from authors S. Sharma, et al.[24] Penicillin

sensitivity was 91.3% in study from D Ray, et al.[25] Erythromycin resistance was found in 1/3 isolates (33.3%) which was comparable to other studies[24, 26].

CONCLUSION

Staphylococcus aureus nasal carriage Screening and treatment is routine infection control practice followed in hospitals.

In the present study 18/200 nursing staff were *Staphylococcus aureus* carriers which include 8 nasal, 9 oropharyngeal and 1 both. This draw attention towards the need of screening of Health care workers for nasal as well as oropharyngeal carriage. Studying antimicrobial resistance pattern of the isolates is important. There is also a need of molecular studies to find out causal association of carriage with HAIs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Approved by Institutional ethical committee of DMIMS (Deemed to be university), Wardha.

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