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RESEARCH ARTICLE

A STUDY ON NASAL CARRIAGE OF STAPHYLOCOCCUS SPECIES AMONG MEDICAL AND PARAMEDICAL STUDENTS IN A TERTIARY CARE HOSPITAL, COIMBATORE

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ABSTRACT

Introduction: Staphylococci are a part of the normal flora of the human body. They are ubiquitous in nature and they cause a variety of infections ranging from suppurative lesions like folliculitis, abscess, wound infections to urinary tract infections, toxic shock syndrome etc. The carriage of this species can be of nasal, vaginal or perineal origin. The emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) has evoked the need to screen for carriers since the treatment options available are very limited.

Aim: A cross- sectional study was conducted among the medical and paramedical students to detect the nasal carriage of Staphylococcal species with emphasis on Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Materials and Methods: Nasal swabs were collected from the subjects and the identification of different Staphylococcal species was done by correlating the results of Gram stain by light microscopy, Colonial morphology by culture, Biochemical reactions and Antibiotic Susceptibility Test results. The results were tabulated and the data was statistically analysed with IBM SPSS version 20 software.

Results: Out of the 310 samples that were collected, 191 (61.61 %) samples showed Staphylococcal growth. A total of 75 (39.26%) isolates out of the 191 isolates were found to be *Staphylococcus aureus*. Out of which 13(6.8%) isolates were Methicillin-Resistant *Staphylococcus aureus*.

Conclusion: The nasal carriage of Staphylococcal species, especially MRSA in healthy individuals carries a potential risk. In this study, a 6.8% incidence of MRSA was noticed among 191 Staphylococcal samples. Topical methods like nasal sprays or ointments of mupirocin or chlorhexidine can be employed to prevent the spread of the species.

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INTRODUCTION

Staphylococcus is an ubiquitous facultative anaerobe appearing as gram positive cocci in clusters on light microscopy. Among many other species of Staphylococcus genus, *Staphylococcus aureus* still remains as a significant and potential nosocomial pathogen because of its diversity, severity and increased frequency causing a wide array of infections affecting namely the skin and soft tissue, musculoskeletal system, respiratory system, central nervous system, endovascular system & genitourinary system. (Lowy, 1998) Undoubtedly large proportion of healthy population harbour and carry *Staphylococcus aureus* and also Coagulase negative Staphylococcus (CONS) as normal commensal flora or as colonizer of various body sites especially the nose, skin surfaces and etc., During favourable conditions, Staphylococcal infection spreads by direct contact or indirectly by toxin production especially in health care settings. (Cosgrove *et al*., 2003) This could be a reason for increased incidence and rapid spread of staphylococcal infections and (or) diseases where the original reservoir(s) of infection still remains unclear. (Christian Cespedes *et al*., 2002) The situation appears worse in the hospitals. (Sah *et al*., 2013) Heterogeneity of the disease and the unique ability of *Staphylococcus aureus* to develop resistance to most of the new antibacterial antibiotics, indicates the ability of adaptation and survival in various conditions. (Como-Sabetti *et al*., 2009) Emergence of the "Superbug" namely Methicillin Resistant *Staphylococcus aureus* (MRSA) in the 1980s is still posing a major challenge for the clinicians to treat the staphylococcal infections which are more common

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in hospitals. Perhaps MRSA remains as a danger not only in hospitals (HA-MRSA) but also in the community (CA-MRSA) (Boerlin and Reid-Smith, 2008). mec A gene is a partial genetic sequence of Staphylococcal Cassette Chromosome mec (SCC mec) coding for Penicillin Binding Protein 2A (PBP2A). Mutation of mecA gene by horizontal gene transfer results in methicillin resistant mystery bug "the MRSA" rendering resistance to various beta-lactam antibiotics like penicillins and cephalosporins. (Maple *et al*., 1989) Nasal carriage of drug resistant Staphylococci viz., MRSA is a potential risk factor for the establishment of infection and or disease (Van Rijen *et al*., 2008) not only in immunocompromised states or immunosuppressant states like infancy, childhood, pregnancy, elderly age group, patients suffering from HIV, patients on prolonged corticosteroid therapy, patients on cancer chemotherapy, health care providers like doctors, nurses, laboratory technicians and etc., but also in immunocompetent individuals. Anterior nares are considered as a main reservoir of *Staphylococcus aureus* both in adults and in children. About 35-50 % of apparently healthy adult population harbour *Staphylococcus aureus* at any instant. (Bannerman, 2003) This study aims to screen for nasal carriage of Staphylococcus species among the high risk group vide the medical and paramedical students as they are potentially endangered target groups in a health care setting.

Aims and objectives

(i) To identify the presence of *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) in the anterior nares of medical and para-medical students and also detect the antimicrobial susceptibility patterns.
(ii) To also identify the presence of other species of Staphylococci especially coagulase negative Staphylococcus (CONS), *viz.,* Methicillin Resistant Coagulase negative Staphylococci (MRCONS) and Methicillin Sensitive Coagulase negative Staphylococci (MSCONS) and also their Antimicrobial Susceptibility Test patterns.
(iii) To screen for beta lactamase enzyme producing *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

A cross-sectional study was conducted among the medical (Group I) and paramedical students (Group II) in the Diagnostic Microbiology section of Central Service Laboratory after obtaining Institutional Human Ethics Committee (IHEC) clearance and informed written consent from study participants. A total of 617 students comprising of 437 medical and 180 paramedical students were included in the study.

Study Design & Methodology: Students who had given informed written consent for research study were involved in the research by giving a self administered questionnaire to fill. Also nasal swabs were collected under sterile precautions with sterile, dry cotton swabs from anterior nares of each nostril about 1 cm depth from the subject by inserting the swab and then gently rotating the swab three times. The swabs were then transported in Amie's transport media with charcoal (Hi Media, Mumbai, India) at a temperature of 4ºC - 8ºC to the Diagnostic Microbiology Laboratory at Karpagam Faculty of Medical Sciences and Research, Coimbatore, Tamil Nadu, preferably within 2 hours of collection and processed immediately. The collected swabs were subjected to the following.

DAY 0:

a) The nasal swabs were inoculated in 5% Sheep Blood Agar plate (SBAP), Mac Conkey`s agar plate (MAC), Nutrient agar (NA) and Mannitol Salt Agar plate (MSAP). The plates were incubated at 37ºC overnight.
b) Gram Stain was performed for all nasal swabs and results noted.

DAY 1: (After 24 hours)

a) The plates were looked for the colony morphology and type of hemolysis in SBAP, lactose fermentation in MacConkey's agar plate, yellow colonies in Mannitol salt agar plate & golden yellow pigments in Nutrient agar plate. The plates were incubated up to 48 hours to be called as culture negative (no growth).
b) Slide catalase and tube coagulase tests were performed for suspected Staphylococcal colonies.
c) Biochemical reactions like Mannitol fermentation and Modified Hugh Leifson test (OF Test) were performed.
d) The Antibiotic Susceptibility Testing (AST) was performed on Mueller Hinton Agar (Hi Media Laboratories, Mumbai/India) according to the CLSI 2014 with bacterial suspension of 1.0 Mac Farland turbidity standard. The following antibiotics were tested by Kirby-Bauer disk diffusion procedure namely-Penicillin (10µg), Vancomycin (30µg), Erythromycin (5µg), Ciprofloxacin (1µg), Co-trimoxazole (25µg), Ceftriaxone (30µg), Clindamycin (2µg), Cefoxitin (30µg), Linezolid (30µg), Teicoplanin (30µg), Amoxicillin (10µg), Amoxyclav (30µg), Furazolidone (100µg) & Bacitracin (30 units).

Control strain: *Staphylococcus aureus* ATCC 25923 was used as reference standard control strain for every batch of culture and susceptibility testing.

Screening for Methicillin Resistance: As Methicillin is an unstable compound, Methicillin resistance was tested by using Cefoxitin disc (30µg) by Kirby-Bauer disk diffusion method on Mueller Hinton Agar where cefoxitin is the surrogate marker for Oxacillin/methicillin resistance.

DAY 2: (After48 hours)

a) Biochemical reactions were read and the results were noted.
b) Antibiotic Susceptibility Test patterns were read and subsequent identification of MRSA, MSSA, MRCONS, MSCONS& D-test positive Staphylococci done.

The results were tabulated and the data was statistically analyzed with IBM SPSS version 20 software. The

identification of different Staphylococcal species was done by correlating the results of Gram stain by light microscopy, Colonial morphology by culture, Biochemical reactions and Antibiotic Susceptibility Test results.

OBSERVATION AND RESULTS

A total of 617 students from both medical and paramedical fields were included in the study. Characteristic of the targeted study population is depicted in Table 1.

Table 1. Characteristics of study population

Field of Study		Males (No.)	Females (No.)	Total
Medical students Group 1	I MBBS (Pre-clinical)	68	82	150
	II MBBS (Para-clinical)	69	76	145
	III MBBS (Clinical)	62	80	142
Para-medical students Group 2	I year nursing	03	57	60
	II year nursing	02	58	60
	III year nursing	03	57	60

Table 2. Biochemical tests done to identify Staphylococcus species

Biochemical tests	Positive	Negative	Remarks
Slide Catalase test (N=233)	191	42	(i) Slide Catalase is positive, probably Staphylococci (ii) Slide Catalase is negative, probably not Staphylococci
Tube Coagulase test (N=191)	75	116	(i) Tube coagulase detecting free coagulase is positive, probably *Staphylococcus aureus sub spp anaerobius, S. hyicus, S. intermedius*, *S. lutrae or S. schleiferi sub spp. coagulans.* (ii)Tube coagulase is negative, probably Coagulase negative Staphylococci (CONS) namely *Staphylococcus saprophyticus, S. cohnii subsp. cohnii,S. warneri, S. epidermidis,S. cohnii subspp. urealyticum, S. captitus subspp. captitus, S. hominis, S. caprae or S. lugdunensis.*
Mannitol Fermentation test (N=191)	75	116	*Staphylococcus aureus* ferments mannitol sugar producing yellow color.
Modified Hugh and Leifson test (OF test) where (N=191)	(i) Fermentative reaction in 75 isolates, probably *Staphylococcus aureus*, (ii) Oxidative reaction in 116 isolates probably Micrococci or CONS		

Table 3. Characterisation of Staphylococcal species

Staphylococcal Isolates	No.	%
Methicillin-resistant *Staphylococcus aureus* (MRSA)	13	6.8 %
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	62	32.46 %
Methicillin-resistant Coagulase-Negative Staphylococcus (MRCONS)	27	14.13 %
Methicillin-sensitive Coagulase-Negative Staphylococcus (MSCONS)	89	46.59 %
Total	191	

Table 4. Distribution of Staphylococcal isolates among Medical (Group 1) and Para-medical (Group 2) students

Staphylococcal species	Medical Students		Paramedical students	
	No.	%	No.	%
MRSA (13)	10	76.92 %	03	23.07 %
MSSA (62)	56	90.32 %	06	9.67 %
MRCONS (27)	26	96.29 %	01	3.70 %
MSCONS (89)	84	94.38 %	05	5.61 %
Total (N=191)	176		15	

Table 5. Antibiotic susceptibility pattern of Staphylococcus isolates

S.No	Antibiotics	MRSA (N=13, 6.8 %)		MSSA (N= 62, 32.46 %)		MRCONS (N= 27, 14.13 %)		MSCONS (N=89, 46.59%)	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
1.	Cefoxitin	00	13	62	00	00	27	00	89
2.	Erythromycin	00	13	48	14	12	15	67	22
3.	Clindamycin	00	13	57	05	24	03	75	14
4.	Penicillin	00	13	19	43	00	27	26	63
5.	Vancomycin	13	00	62	00	27	00	89	00
6.	Linezolid	13	00	62	00	27	00	89	00
7.	Teicoplanin	13	00	62	00	27	00	89	00
8.	Amoxicillin	00	13	48	14	00	27	80	09
9.	Amoxy-Clav	00	13	50	12	00	27	53	36
10.	Ciprofloxacin	04	07	53	09	03	24	21	68
11.	Co-Trimoxazole	13	00	38	24	21	06	81	08
12.	Ceftriaxone	00	13	57	05	00	27	68	21
13.	Furazolidone	13	00	62	00	27	00	89	00
14.	Bacitracin	00	13	00	62	00	27	00	89

A total of 310 out of 617 (50.24%) students voluntarily consented for the research study. Sex distribution had a mild to moderate female preponderance where female constituted 186 out of 310 (60%) compared to males with 124 out of 310 (40%). The nasal swabs were obtained from these 310 subjects and were inoculated into different agar plates. Out of the 310 samples, 233(75.16%) samples were culture positive and 77 (24.83%) samples were culture negative or sterile. Among the 233 clinical isolates, 75 (32.18%) were of *Staphylococcus aureus*, 116 (49.78%) were of Coagulase negative *Staphylococcus aureus* (CONS) and 42 (18.02%) samples were of bacteria other than Staphylococci which is represented in the Figure 1 below.

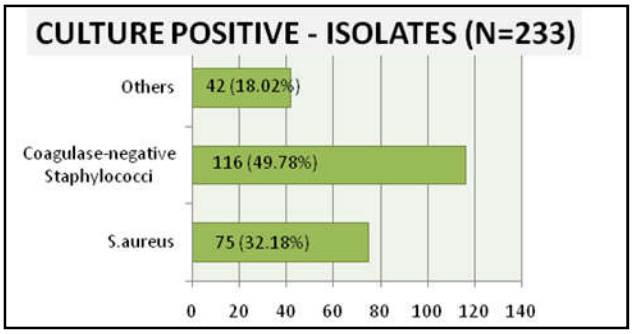

Figure 1. Bacterial Culture positive isolates

Out of the 116 samples, 27 (23.27%) isolates were Methicillin Resistant CONS and 89(76.72%) isolates were Methicillin Sensitive CONS. And out of 191 samples, 75 (39.26%) were identified as *Staphylococcus aureus*. Among 75 *Staphylococcus aureus* isolates, 'D Test' was positive in 8 isolates (10.66%) and 56 (74.66%) isolates produced golden yellow pigment on Nutrient Agar Plate. All the 75 (100%) *Staphylococcus aureus* bacterial isolates produced yellow colonies on Mannitol Salt Agar.

DISCUSSION

Out of 617 students who were included in the study, only 310 students consented for the study (50.2%) among which 124 students were males (40%) and 186 students were females (60%). Among the 310 students, 273 (88.06%) subjects belonged to the medical students (Group 1) and 37 (11.93%) subjects belonged to the paramedical students (Group 2) Among 310 nasal swabs cultures, 233 samples led to clinical isolation of culture positives (75.16%) and 77 samples ended up as culture negative (24.83%). Among the 233 culture positive bacterial isolates, 191 were Staphylococcal species which accounted for 61.61% of total study participants (N=310).A total of 75 samples out of 191 Staphylococcal isolates showed growth of *Staphylococcus aureus* (39.26%) which was similar to a study done (Goyal *et al*., 2002) which reported 37.33% prevalence of *Staphylococcus aureus*. Many research studies done elsewhere (Bannerman, 2003; Sharon Rainy Rongpharpi *et al*., 2013) reported the prevalence of *Staphylococcus aureus* ranging from 20-50%. Prevalence of Coagulase negative Staphylococcus (CONS) was 116 (60.73%). The prevalence of MRSA, MSSA, MRCONS & MSCONS were 6.8%, 32.46%, 14.13% & 46.59% respectively among healthy medical and paramedical students. Studies done in India reported 11-16% prevalence of MRSA. (Sharon Rainy Rongpharpi *et al*., 2013; Vinodhkumaradithyaa *et al*., 2009) One study also done in India had reported least prevalence (1%) of MRSA. In the current study, prevalence of MRCONS was 14.13% which was similar to a study done in India (Shobha *et al*., 2005) where the incidence was about 14%. Also, D-test was positive in 8 isolates out of 75 (10.66%) *Staphylococcus aureus* isolates. In Group2, about 3 grew MRSA (8.1%) out of 37 paramedical students, whereas about 10 out of 273 medical students grew MRSA (3.66%). Also among 191 clinical isolates of Staphylococcal species, MSCONS was predominant (N=89, 46.59%). Penicillin resistance was observed in all 40 (100%) clinical isolates of MRSA (13) & MRCONS (27), whereas MSSA and MSCONS showed 69.35% and 70.78% respectively. Similarly, resistance to Ceftriaxone was also observed in all 40 (100%) clinical isolates of MRSA & MRCONS, whereas MSSA and MSCONS showed 8.06% and 23.59% respectively. All the MRSA and MRCONS isolates were sensitive to Vancomycin, Linezolid and Teicoplanin. Inducible resistance identified by 'D' test was positive in 8 isolates out of 75 isolates (10.66%) conferring resistance to both erythromycin and clindamycin. Measures to prevent the spread of hospital bound infections are:i) Periodic screening for nasal carriage of Staphylococcal species especially MRSA among health care providers every 6 months, ii) Following stringent Sterilization & Disinfection and or Hospital Infection Control practices while providing patient care with the support of functional and effective CSSD and HICC, iii) Isolating subjects harboring MRSA from work and providing them with early specific therapy like nasal sprays or ointments of mupirocin or chlorhexidine will help in the prevention of spread of infection, hand washing should be strictly followed before touching a patient, iv)Before clean/aseptic procedures, after body fluid exposure risk, after touching the patient & after touching the patient surrounding as it is a simple and an effective tool and v) Creating awareness and imparting knowledge about drug resistant bugs like MRSA for primordial prevention of the Staphylococcal infections.

Limitations of the study

The limitations of the study were as follows:

a) Getting consent from the students for taking a nasal swab even after explaining the procedure;
b) Since the sampling was interventional, the anxiety and the apprehension of the subject hunched to be a limitation;
c) Contamination of the culture and antibiotic sensitivity plates hindered the interpretation of the results;
d) Doctors, nursing staff, dietary workers, sanitary workers and attendees were not included in the study. Hence, screening them for carriage of Staphylococcal species can be carried out as Phase II project;
e) As this is a cross-sectional study, the subjects who harboured MRSA were neither subjected to treatment nor referred to a physician for further management.

Conclusion

Staphylococcal species remains as an undeniable part of normal commensal flora of the nose of healthy individuals.

MSCONS (46.59%) were the predominant isolate followed by MSSA (32.46%). In the current study, the nasal carriage of MRSA was 13(6.8%) out of 310 subjects which attributes to the disease causing potential especially in high risk groups. Prevalence of MRSA in healthy individuals and evolving drug-resistance could be potential target for translational research in the future. Molecular methods to detect mecA gene of MRSA is a promising research tool to confirm the bacterial identification & resistance pattern.

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