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Study of Nasal Carriage of *Staphylococcus aureus* with Special Reference to Methicillin Resistance among Nursing Staff

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of nosocomial infections and more prevalent in the community settings.

Objectives: To determine the frequency of nasal carriage of *S. aureus* and MRSA among nursing staff and to assess the Antibiogram profile of isolated strains of *S. aureus* with special reference to methicillin sensitivity. Material and methods: Hospital based cross sectional study was conducted in Department of Microbiology, Krishna Institute of medical sciences, Karad in the year 2015. By purposive sampling technique, a total of 50 nursing staff was enrolled and the specimens were collected from their anterior nairs and sent for isolation and Antibiotic susceptibility testing in microbiology laboratory as per Standards. The data were complied and analyzed for prevalence of *Staphylococcus aureus* (carrier state) and significant association.

Results: The prevalence of *Staphylococcus aureus* (carrier state) was 52% observed in hospital nursing staff. The frequency of isolates of *S. aureus* in female and male staff observed was 16 and 10 respectively. The proportion of MRSA reported in male and female staff was 20% and 43.75% respectively with overall of 34.61%. A higher percentage of resistance by MRSA was shown to Penicillin, Oxacillin and Cefoxitin i.e. 100% followed by Levofloxacin (55.5%), Tetracycline (50%), Trimethoprim/Sulphamethoxazole (44%), Clindamycin (33%), Ciprofloxacin and Erythromycin (22%), Gentamicin (11%).

Keywords: *Staphylococcus aureus*; MRSA; prevalence; nursing staff

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Introduction

Staphylococcus aureus (S. aureus) has been recognized as an important pathogen in human diseases. Methicillin-Resistant *Staphylococcus Aureus* (MRSA) is a major cause of nosocomial infections. In recent year MRSA have also become more prevalent in the community settings [1]. Susceptibility to the infection depends on factors such as immunity and general state of health. The ecological niches of *S. aureus* strains are mainly anterior nares of human beings [2]. The three patterns of carriage of *S. aureus* are described viz. persistent carriers (20%), intermittent carriers (60%) and non-carriers (20%).

Health care workers (HCWs) are considered as an important reservoir of *S. aureus*. Several studies have reported that, the rate of nasal carriage of *S. aureus* among HCWs ranges from 16.8% - 56.1% [3]. There are many reports of outbreaks of skin infections as well as more serious conditions such as pneumonia or blood stream infections from various healthcare settings. Over the period of 20 – 30 years, MRSA strains have been present in hospitals as Hospital Acquired MRSA (HA-MRSA); they have become a major cause of hospital- acquired infection. Community Acquired MRSA (CA-MRSA) emerged worldwide in late 90s [4]. Nasal carriage of MRSA is recognised as a risk factor for subsequent infection of endogenous origin. Control of transmission from patient to patient or healthcare worker to patient is critical. The hospital reservoir for MRSA includes recognized and unrecognized colonized or infected patients, as well as previously colonized or infected patients readmitted to

the hospital. Early and appropriate Infection Control Measures (ICM) is key elements to reduce MRSA transmission and to control the hospital reservoir [5]. An active surveillance program needs to be undertaking to identify and eliminate the carriage in healthcare workers and reduce the hospital acquired infection rates in health care establishments. As the nursing staff is in the close approximates for the patient care, and if they are carriers, there is risk of epidemic in hospitals.

In context to Indian health care delivery system, inadequate and unequal health care structure, lack of trained health work force, population and hospital ratio, nurse-patient ratio are adversely affected. In and out patient department, nursing staff remains in close vicinity for a long period of times and has to work under poor facilities. We have hypothesized that MRSA nasal carriage among nursing staff adversely affects the patients' health care. To combat future incidence of nosocomial acquired MRSA, and to provide preventive benefits to the nursing staff, the study was designed to estimate the prevalence of nasal carriages of *S. aureus* and MRSA among nursing staff attached to rural tertiary care hospital.

Material and Methods

A cross sectional study was conducted in the Department of Microbiology, Krishna Institute of Medical Sciences (KIMS) Karad located in rural western Maharashtra, India. The study was planned in the year 2015 and the study subjects considered were nursing staff working at Krishna Hospital and Medical Research Centre, a tertiary health care centre under KIMS Karad. By using Purposive sampling technique, a total of 50 nursing staff were included as study subjects in the study viz. 21 males and 29 females. Self designed, validated structured proforma was used to collect the information pertained to demographic data. The study subjects were interviewed by investigator during working hours.

Microbiology

Specimens were collected from anterior nares with sterile cotton swabs, the same swabs were used for both the nostrils of nursing staff with informed consent during May –June 2015. Swabs were cultured on 5% blood agar, incubated at +35 ± 1°C and examined for growth after 24-48hours. Each specimen has been subjected to microscopic study under Grams stain and has been inoculated in Blood agar, MacConkey's agar and Mannitol-salt agar. The organism was identified by microscopy using Gram staining technique, colony characteristics: Colonies are large (2-4mm in diameter), circular, convex smooth, and show hemolysis on Blood agar. After colony smear and Gram staining of bacterial colonies, the biochemical identification was carried out by following tests [6]; Catalase test, Slide coagulase test and tube coagulase test, Mannitol salt agar for fermentation, Urease test, Sugar fermentation test for detection of acid production by using Glucose, Maltose, Sucrose and Mannitol.

Antibiotic susceptibility test

All the isolated strains of *S. aureus* were subjected to antibiotic susceptibility testing by Kirby-Bauer Disc diffusion technique according to the CLSI guidelines using following antibiotics; Penicillin(P 10 units/disc), oxacillin30mcg/disc), Gentamicin(GEN 10 mcg/disc), Ciprofloxacin(CIP 5mcg/disc), Levofloxacin(LE 5 mcg/disc), Erythromycin(E 15mcg/disc), Clindamycin(CD 2 mcg), Linezolid(LZ30mcg/disc),Vancomycin(VA10mcg),Tigecycline(TGC 15mcg), Tetracycline (TE 30mcg/disc), Nitrofurantoin(NIT 300mcg/disc), Trimethoprim/ sulfamethoxazole(COT 1.25mcg/ disc and 23.75mcg/disc) ,cefoxitin discs(CXX 30 mcg/disc) of HI MEDIA were used. Interpretation of test was done as per guidelines 2013 (M100 S23).Quality control of the test was done by Standard Strain (*Staphylococcus aureus* ATCC 25923) [7].

Screening for methicillin resistance was done using cefoxitin disc(30µg) screen test and 6µgm/ml of oxacillin in Mueller-Hinton agar supplemented with NaCl (4% w/v, 0.68 mol/L). For dilution 0.5 MacFarlands Standards is used. Control strains of *S. aureus* ATCC 25923 are used. The entire surfaces of the plate medium was inoculated using sterile cotton swab soaked in inoculums and antibiotic discs (Hi Media) are applied where the plates were inverted and incubated at 35-37°C for 24 hours. The Zone diameter around the disk is measured using measuring scale and the results were interpreted as susceptible, moderately susceptible or resistant as per recommendations.

Analysis: Data were compiled and entered into MS Excel and presented in tabular form. Data analysed into frequency percentage and association was worked out by using chi-square test. Statistical significant difference was considered if p value is < 0.05 at 95% confidence interval.

Ethical consideration: Institutional ethical clearance and institutional data collection permission was obtained before study planned.

Results

A total of 50 nursing staff include, 21 males and 29 females were in age ranges from 18 to 58 years respectively **(Table 1).**

Table 1 show, a total of 10(47%) *S. aureus* strains were isolated from 21 male nursing staff were in age group ranges from 18-58 years. Methicillin susceptibility and resistance in context to *S. aureus* was 80% and 20% respectively.

Among the female participants, upper and lower age reported was 58 and 18 years respectively with high percentage of subjects, 37.9% were in age group was 28-38 yrs. A total of 16, *S. aureus* strains were isolated from the participants of which maximum, 76% were Methicillin susceptibility and resistance strains were of 24 % **(Table 2)**.

Table 3 depicts, the frequency of isolates of *S. aureus* are of 1.3 times higher in females as compared to males as indicated by

Table 1: Isolation of *Staphylococcus aureus* in male staff

Age group (Yrs)	Male	S.aureus	Methicillin susceptibility (%)	Methicillin Resistance (%)
18-28	6	2	2(100%)	0(0%)
28-38	6	3	1(84%)	2(20%)
38-48	4	2	2(100%)	0(0%)
48-58	5	3	3(100%)	0(0%)
Total	21	10	8(80%)	2(20%)

Table 2. Isolation of *Staphylococcus aureus* in female staff

Age groups (yrs.)	Female	S.aureus	Methicillin susceptibility (%)	Methicillin Resistance (%)
18-28	9	4	3(89%)	1 (11%)
28-38	11	6	4(82%)	2(18%)
38-48	6	4	1(50%)	3(50%)
48-58	3	2	1(70%)	1(30%)
Total	29	16	9(76%)	7(24%)

Table 3. Gender distribution of Isolates and MRSA profile.

Isolates@	Females	Males	MRSA profile	Females	Males
S.aureus	16	10	Total Isolates	16	10
No isolations	13	11	Resistance(MRSA)	7	2
Total	29	21	%	43.75%	20%
χ2@: p-0.8, OR-1.3(CI-0.4-4.1), Fisher's Exact #: p-0.63, OR-2.1(CI-0.3-12)					

Table 4: Antibiogram Profile of MSSA.

S.No	Name of antibiotic	Susceptible	Resistance	% of Susceptibility
1.	Penicillin	0	17	0%
2.	Oxacillin	17	0	100%
3.	Gentamicin	15	2	88%
4.	Ciprofloxacin	10	7	58%
5.	Levofloxacin	9	8	52%
6.	Erythromycin	4	13	23%
7.	Clindamycin	5	12	29%
8.	Linezolid	17	0	100%
9.	Vancomycin	17	0	100%
10.	Tetracycline	8	9	47%
11.	Nitrofurantoin	17	0	100%
12.	Tigecycline	17	0	100%
13.	Trimethoprim/ sulfamethoxazole	10	7	58%
14.	Cefoxitin	17	0	100%

Odd's ratio, however difference was not statistically significant. Similarly MRSA profile also shows, an overall proportion of resistance observed among female and male identified *S. aureus* isolates was 43.75% and 20% respectively with Odd's of 2.1 times higher in females as compared to males, however the difference was not statistically significant (p>0.05).

Table 4 shows, A total of 14 antibiotics were tested against the 17 MSSA strains and all the *S. aureus* strains isolates were sensitive to Oxacillin, Cefoxitin, Linezolid, Vancomycin, Nitrofurantoin, Tigecycline i.e. 100% followed by Gentamicin (88%), Ciprofloxacin, Trimethoprim/Sulphamethoxazole (58%), levofloxacin(52%), Tetracycline (48%), Clindamycin (29%), and Erythromycin (23%) (Table 4).

Table 5 shows, most of the Methicillin resistant *S. aureus* were resistant to multiple drugs. A total of 14 antibiotics were tested against the 9 MRSA strains and a higher percentage of resistance was seen to Penicillin and Oxacillin, i.e. 100% followed by Levofloxacin (55.5%), Tetracycline (50%), Trimethoprim/ Sulphamethoxazole (44%), Clindamycin (33%), Ciprofloxacin and Erythromycin (22%) and Gentamicin (11%).

Discussion

Healthcare Workers (HCWs) constitute an important reservoir of *S. aureus*. The screening of the nasal carriage in HCWs is an important component in the control of MRSA in any health care facility. The identification of the colonized staff members allow the appropriate management of these persons, to prevent the spread to others. MRSA may cause problems in the hospital infection control programs. Among the Gram positive pathogens, *S. aureus* continues to cause skin and soft tissue infections in the community as well as invasive infections in the hospitalised patients. In a recent Europe-wide survey, the most common organisms were *S. aureus* (71%) with 22.5% being MRSA [8].

Nasal colonization with *S. aureus* especially among healthcare personnel plays as a significant role in the increasing prevalence of resistant community acquired *S. aureus* infections [9]. The principal mode of MRSA transmission within an institution is from patient to patient via the transiently colonized hands of hospital personnel who acquire the organism after direct patient contact or after handling the contaminated materials [10]. This study revealed that 26(52%) of nursing staff are carriers of *S. aureus* in their anterior nares among which 9(34.61%) were methicillin resistant. Nasal carriage rate of MRSA have been reported in a range of 6% to 17.8% among adults in health care workers in hospital settings elsewhere in the world [11] . Radhakrishna et al. stated prevalence of *S. aureus* and MRSA as 17.5% and 14.3% respectively [11]. Askarian et al. from Sharin, Iran stated prevalence of nasal carriage of methicillin sensitive *S. aureus* (MSSA) was 25.7% and was 5.3% with highest nasal carriage of MRSA in surgical wards and emergency department [12]. In our study prevalence of *S. aureus* is higher as compared to other studies, could be due to disproportionate patient to nursing staff ratio, patient bed ratio and poor implementation of hospital infection control policy. Most of the health care staff is working more than 10 years of duration this could also be another reason for high prevalence and MRSA.

Rongpharpi et al., stated prevalence of *S. aureus* as 52.8% and that of MRSA is 11.43% [3]. Kakhandki et al., stated prevalence of 43.6% for *S. aureus* and that of 12% for MRSA [10]. Our study shows prevalence of *S. aureus* 52% which simulates with most of the above studies, and that of MRSA 22 % is higher.

Elimination of nasal carriage

Strategy to interrupt transmission of *Staphylococcus aureus* by elimination of nasal carriage and thereby preventing subsequent infection should be implemented. Continuous surveillance is needed to more accurately access the prevalence, geographical distribution and epidemiology of hospital acquired infections. Elimination can be done by three approaches as follows, first is local application of antibiotics and disinfectants or both e.g. Mupirocin, second is administration of systemic antibiotics like Rifampicin as it is proven as the most effective agent, and the third is bacterial interference i.e, active colonization with a strain of *S. aureus* which has minimal pathogenic properties but is able

Table 5: Antibiogram profile of MRSA,

S.no.	Name of antibiotic	Susceptible	Resistance	% of Resistance
1.	Penicillin	0	9	100%
2.	Oxacillin	0	9	100%
3.	Gentamicin	8	1	11.1%
4.	Ciprofloxacin	7	2	22.2%
5.	Levofloxacin	4	5	55.5%
6.	Erythromycin	7	2	22.2%
7.	Clindamycin	6	3	33.3%
8.	Linezolid	9	0	0%
9.	Vancomycin	9	0	0%
10.	Tetracycline	4	5	55%
11.	Nitrofurantoin	9	0	0%
12.	Tigecycline	9	0	0%
13.	Trimethoprim/ sulfamethoxazole	5	4	44.4%
14.	Cefoxitin	0	9	100%

to prevent colonization by more virulent strains by competition for binding sites in the nose. Mupirocin is the cornerstone of decolonization regimens, a successfull strategy to prevent healthcare associated Staphylococcal infections. However, new MRSA colonization has been reported even after the use of Mupirocin. Prolonged widespread or uncontrolled use and multiple course of Mupirocin are all associated with development of Mupirocin resistance [13].

Recommendations

Surveillance of MRSA should be undertaken in a systematic way and the feedback should be routinely given to the respective healthcare authorities. The inappropriate or unnecessary use of antibiotics should be avoided. This will reduce the likelihood of the emergence and spread of strains with reduced susceptibility to glycopeptides, i.e. Vancomycin-intermediate *S. aureus*/glycopeptide-intermediate *S. aureus* (VISA/GISA) and Vancomycin-resistant *S. aureus* (VRSA).

Screening for MRSA carriage in selected patients and clinical areas should be performed according to locally agreed criteria based upon assessment of the risks and consequences of transmission and infection. Nasal and skin decolonization should be considered in certain categories of patients. The general principles of infection control should be adopted for patients with MRSA, including patient isolation and the appropriate cleaning and decontamination of clinical areas. Inadequate staffing, especially amongst nurses, contributes to the increased prevalence of MRSA.

Conclusion

The study concluded that, 52% of hospital nursing staff is *Staphylococcus aureus* carriers and proportion of MRSA and MSSA was 34% and 66% respectively.

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Limitations of study

We did not confirm MRSA status by detecting mecA gene due to financial constrains. Susceptibility to Teicoplanin is also not done due to non-availability of the Teicoplanin disc and Small sample size.

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