

## NASAL COLONIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG CLINICAL POSTGRADUATES IN A COASTAL KARNATAKA MEDICAL COLLEGE - PREVALENCE AND ANTIBIOGRAM PATTERN

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### ABSTRACT

**Objective:** The incidence of community-acquired and hospital-acquired *Staphylococcus aureus* infections has been rising with increasing emergence of drug-resistant strains called methicillin-resistant *S. aureus* (MRSA). The prime objective of the study was to ascertain the prevalence of carriage rate of MRSA among the clinical postgraduate students of a medical college of Coastal Karnataka, India.

**Methods:** A total of 133 clinical postgraduates have participated in the present study. Swabs from both anterior nares were collected and processed for *S. aureus* from the consenting participants after giving a set of questionnaires. The isolated strains of *S. aureus* were screened for methicillin resistance by the modified Kirby-Bauer method using cefoxitin (30 µg) disc. Antibiotic susceptibility testing for all isolates of *S. aureus* was also done against other antibiotics. E test was used to know vancomycin resistance for MRSA.

**Results:** The numbers of *S. aureus* isolated of 133 participants were 22 (16.5%). Of 22 isolates of *S. aureus*, 12 were MRSA (54.5%). The MRSA carriage rate of clinical postgraduates of a medical college from Coastal Karnataka, India, was 9%. All the MRSA isolates were susceptible to vancomycin and teicoplanin. Contact with chronic patients and consumption of antibiotics in past 6 months were found to be statistically significant as the risk factor associated with the acquisition of *S. aureus*/MRSA carriage status among the participants. No vancomycin-resistant *S. aureus* was reported from the present study.

**Conclusion:** The *S. aureus* and MRSA nasal carriage rates of clinical postgraduates recorded in this study were significantly high when compared with the general population. This could be due to their continuous exposure to patients in clinical settings. It is desirable that these students must receive sufficient knowledge regarding control measures to avoid the spread of MRSA infection in hospitals.

**Keywords:** *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, Nasal carriage, Clinical postgraduates.

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### INTRODUCTION

*Staphylococcus aureus*, in particular, methicillin-resistant *S. aureus* (MRSA) has gained prominence all over the world. There is hospital-acquired MRSA (HA-MRSA) as well as community-acquired MRSA (CA-MRSA) strains and infections. MRSA nosocomial infection (NI) outbreaks and prevalence among various populations have been well reported in literature particularly for developed countries. MRSA strains are resistant to a cluster of antibiotics, and because of the same reason, the treatment of infections due to this organism is always a challenge, more so when the critically ill patients are infected. Hospital infection due to MRSA is a known cause of increased hospital stay, cost, morbidity, and mortality especially among the critically ill. Asymptomatically colonized patients, and health-care providers are the primary sources of MRSA in the hospital environment, with the latter being more commonly notified as links in the transmission of MRSA between patients.

*S. aureus* is one of the most famous human bugs, capable of causing a wide range of infections. Even though primary infections are not familiar, a great deal of the virulence occurs through cross-infection in hospitals and other institutional settings. Whereas, individuals with good health have a small risk of contracting an invasive infection caused by *S. aureus*, whereas they can be carriers of the organism [1]. Because its primary habitat is moist squamous epithelium of the anterior nares, most invasive *S. aureus* infections are believed to arise from the nasal carriage [2]. The overall incidence of CA and HA *S. aureus* infections has been rising with increasing emergence of

drug-resistant strains called as MRSA [3]. Earlier limited to hospitals, MRSA infections have been increasingly reported in the community nowadays [4]. One of the recent meta-analysis of 27 studies of the prevalence of CA-MRSA among hospital patients that used clinical specimens, conducted at the time of admission, yielded a prevalence of MRSA of 30.2% [5]. A diverse of studies have examined community prevalence of nasal carriage of *S. aureus* in various subpopulations, such as adult outpatients, health care workers, college students, and injection drug users [6]. The overall prevalence of *S. aureus* ranges from 20% to 45%, with an estimate of MRSA colonization from 10 community surveillance studies was 1.3% [5]. These data are quite useful to clinicians when trying to decide the likelihood that a given patient has a Staphylococcal infection and, if so, whether antibiotic coverage should be provided for resistant strains. Exposure to microbes is an inherent risk of working in patient care settings. Given its increasing incidence in the general population, acquisition of MRSA is a particular concern for healthcare workers [7,8].

The medical course pattern varies from country to country. In a study of university students of Brazil, the nasal carriage of *S. aureus* was 40.8% (102/250). Of the isolates, six isolates (5.8%) were methicillin-resistant and carried the *mecA* gene suggestive of CA-MRSA in Brazil [9]. The study including medical students of Taiwanese University revealed overall *S. aureus* and MRSA carriage rate of 19.3% and 2.2%, respectively, without any significant difference between the pre-clinical and clinical students regarding nasal carriage of *S. aureus* and MRSA [10]. Another cross-sectional study enrolling the whole

medical student population (n=387) from Colombia, showed the carriage rates MSSA and MRSA of 25% and 1.6%, respectively [11]. Another cross-sectional study from the medical students of Texas University including 203 participants concluded that 60 (29.6%) were *S. aureus* carriers and 15 (7.4%) were MRSA, respectively. Hospitalization in the past 12 months and recent skin infection were significantly associated with the risk of being an MRSA carrier [12]. In one of the studies at University students of Malaysia comprised 209 medical students both pre and clinical showed 21(10%) *S. aureus* without any MRSA as nasal carriage but not associated with any risk factor significantly [13]. A cross-sectional study involving 450 medical students of Winston-Salem, North Carolina revealed 29% of *S. aureus* carriage rate and 2% of MRSA. Older male volunteers suffering from persistent sinusitis and not taking antibiotics were at higher risk for carrying *S. aureus* [6]. Literature survey also revealed a US-based study where 182 students of 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> years of medical course with 34% and 2.7% of *S. aureus* and MRSA carriage, respectively, without an occupational hazard of patient care exposure in 3<sup>rd</sup>-year medical students [14].

Postgraduate students with the clinical specialization in medical institutions comprise a unique population at risk for MRSA acquisition as they are always in touch with patients in the hospital and clinics. This study has been designed to see the nasal carriage status among the Clinical Postgraduates of our institution. At the same time, this study might help us to highlight any significant relation, if present, between the specific clinical departments such as surgery, medicine and obstetrics and the carriage of the organism among the Clinical Postgraduates of our institution.

## METHODS

The proposed study was conducted in the microbiology diagnostic laboratory of a medical college of Coastal Karnataka, India.

### Study design

The said work was a type of cross-sectional study. A sample size of 168 was calculated considering the power of 80%, the confidence level of 95%, and a relative precision 6%, and prevalence of nasal carriage of MRSA to be 21%, and a non-response rate of 10% [6].

**Table 1: Gender-wise distribution of participants, carriage status of *S. aureus* and MRSA**

Gender	Number of participants(%)	<i>S. aureus</i> carriers(%)	MRSA carriers (%)
Male	80 (60.15)	16 (72.73)	6 (50)
Female	53 (39.85)	6 (27.27)	6 (50)
Total	133	22	12

*S. aureus*: *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*

**Table 2: Participants' parameters: Department and PG year wise distributions**

Department	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year	Total numbers (%)
Orthopedics	2	4	3	9 (6.8)
E.N.T.	5	2	4	11 (8.3)
Dermatology	1	0	2	3 (2.3)
Medicine	12	12	10	34 (25.56)
Surgery	4	3	3	10 (7.5)
Ophthalmology	4	4	5	13 (9.8)
Pediatrics	6	9	6	21 (15.78)
O.B.G.	2	1	1	4 (3)
Audiology	9	0	0	9 (6.8)
Psychiatry	0	0	1	1 (0.8)
Anesthesia	2	3	3	8 (6)
Physiotherapy	3	4	0	7 (5.3)
Radiology	1	1	1	3 (2.3)
Total n=133	51 (38.35)	43 (32.33)	39 (29.32)	133

The formula used was

$$N=4pq/d^2$$

Where, p is prevalence, q is 1-p, then d is relative precision

All consenting clinical postgraduates were randomly recruited into the study. The study proposal was submitted to the Ethical Review Committee of the Institution for approval. The age, sex, lifestyle, current skin infection/other illness, particulars of clinical specialty and additional relevant information about the participants were obtained in a pro forma designed for this purpose. The proposed study was carried out between June 1, and August 31, 2015. Of 241 clinical postgraduates, 133 did participate in the study. Swabs from both anterior nares of consenting persons were taken with a sterile swab stick moistened with sterile physiological saline. Processing of the samples was done immediately within 2 h after collection. In case of delay, more than 2 h the swabs were stored at 4°C for maximum 24 h. The swabs were inoculated onto Mannitol Salt Agar (MSA), and the inoculated MSA was incubated at 37°C for 18–24 h. The growth of the organism was identified as *S. aureus* using standard tests, such as colony morphology, Gram stain, catalase test, and coagulase test [15]. The isolated strains of *S. aureus* were screened for methicillin resistance by the modified Kirby-Bauer method using cefoxitin (30 µg) disc on Mueller-Hinton agar (MHA) [16]. The MHA on which cefoxitin disc was placed was incubated aerobically at 37°C for 18 h. Isolates with inhibition zone diameter ≤21 mm around cefoxitin disc were considered as MRSA strains [17]. Screening for vancomycin susceptibility was also done by the modified Kirby-Bauer method using vancomycin disc (30 µg) on MHA incubated at 37°C for 24 h. Vancomycin-resistant was determined MIC determination by E test. Antibiotic sensitivity to all *S. aureus* isolates against other antibiotics such as penicillin, amoxicillin/clavulanic acid, erythromycin, clindamycin, chloramphenicol, cotrimoxazole, ceftriaxone, gentamycin, linezolid, teicoplanin, and ciprofloxacin were determined by the modified Kirby-Bauer method. All inoculum on MHA was 4–6 h growth of pure isolates in Mueller-Hinton Broth, with the density equivalent to a 0.5 McFarland turbidity standard. Controls strains such as *S. aureus* ATCC 25923, MRSA ATCC 29213, and MSSA ATCC 33591 were used. Antibiotic discs were procured from HiMedia Laboratories Pvt., Ltd., India. Antibiotic sensitivity testing and interpretation of the result were done according to CLSI guidelines [18].

### Statistical analysis

Results were compelled, tabulated and all data were subjected to the statistical package Statistical Package for the Social Sciences version 17.0. The outcomes were presented in the form of tables and graphs. Associations of different factors were done using Chi-square test. p-value <0.005 was considered as significant.

**RESULTS**

Of 241, a total of 133 clinical postgraduates participated in the said study. Among the 133 participants, those were screened for nasal carriage of *S. aureus*. 80 (60.15%) were males, and 53 (39.85%) were females (Table 1). The department wise and year wise categorization of the participants were shown in Table 2. The types of organisms isolated from the anterior nares of the participants were shown in Table 3. Coagulase-negative *Staphylococcus* (CoNS) was found to be predominant 84 (63.16%) followed by *S. aureus* 22 (16.54%). Of 133, 22 participants were positive for nasal carriage of *S. aureus* giving a carriage rate of 16.54% (Fig. 1). 12 of 22 isolates of *S. aureus* turned out to be MRSA with the pace of 54.54% (Fig. 2). Overall, MRSA nasal carriage rate was 9% in this study (Fig. 3). The antibiotic susceptibility pattern of *S. aureus* was shown in Fig. 4. Usage of cefoxitin disc was found to be convenient over oxacillin disc in the determination of MRSA. Of the 22 isolates of *S. aureus*, 95.5% were resistant to penicillin, and 81.8% were resistant to ciprofloxacin whereas resistance to erythromycin was 54.55%. Eventually, all *S. aureus* strains were 100% susceptible to vancomycin, and teicoplanin and 95% sensitive to linezolid (Fig. 4). The MRSA isolate was 100% responsive to vancomycin, teicoplanin and 91.67% sensitive to linezolid (Fig. 5). Maximum numbers of MRSA were found in the postgraduates of pediatrics (4) followed by surgery (3) department (Table 4). Year wise distribution of MRSA carriers was shown in Table 5. It was observed that of 12, eight strains of MRSA were isolated from 1<sup>st</sup>-year students. The association between *S. aureus*/MRSA carriage status and the various factors was illustrated in Figs. 6 and 7. Contact with the

patient with chronic infection and consumption of antibiotic in past 6 months were found to be associated with MRSA carriage status.

**DISCUSSION**

The anterior nares of humans found to be the primary ecological niches of the *S. aureus* [19]. The carriage patterns are of three types. Approximately 20% of the individuals almost always carry one kind

**Table 3: Nature of organisms isolated from anterior nares**

Organism isolated	Number (%)
No growth	18 (13.53)
CoNS	84 (63.16)
<i>S. aureus</i>	22 (16.54)
Diphtheroids	4 (3.007)
<i>Candida</i>	5 (3.76)
Total (n=133)	133 (100)

CoNS: Coagulase-negative *Staphylococcus*, *S. aureus*: *Staphylococcus aureus*

**Table 4: Carriage status of *S. aureus* and MRSA among the postgraduates of various clinical departments**

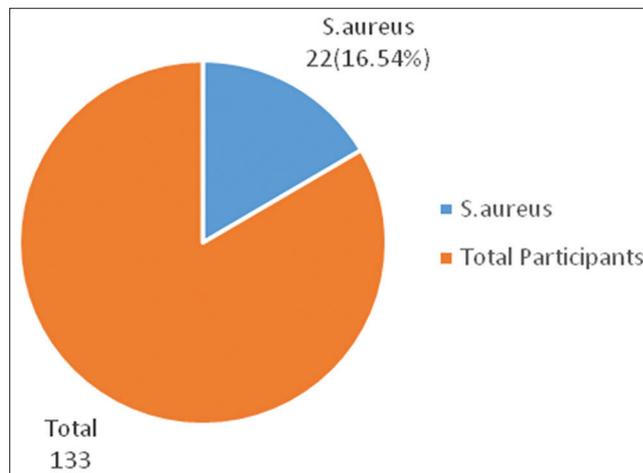
Department	<i>S. aureus</i> (%)	MRSA (%)	Others	Total
Orthopedics	4 (18.2)	1 (8.3)	5 (4.5)	9
E.N.T.	0 (0)	0 (0)	11 (9.9)	11
Dermatology	1 (4.5)	0 (0)	2 (1.8)	3
Medicine	4 (18.2)	2 (16.7)	30 (27)	34
Surgery	3 (13.6)	3 (25)	7 (6.3)	10
Ophthalmology	0 (0)	0 (0)	13 (11.7)	13
Pediatrics	6 (27.3)	4 (33.3)	15 (13.5)	21
O.B.G.	1 (4.5)	1 (8.3)	3 (2.7)	4
Audiology	1 (4.5)	1 (8.3)	8 (7.2)	9
Psychiatry	0 (0)	0 (0)	1 (0.9)	1
Anesthesia	1 (4.5)	0 (0)	7 (6.3)	8
Physiotherapy	0 (0)	0 (0)	7 (6.3)	7
Radiology	1 (4.5)	0 (0)	2 (1.8)	3
Total	22	12	111	133

*S. aureus*: *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*

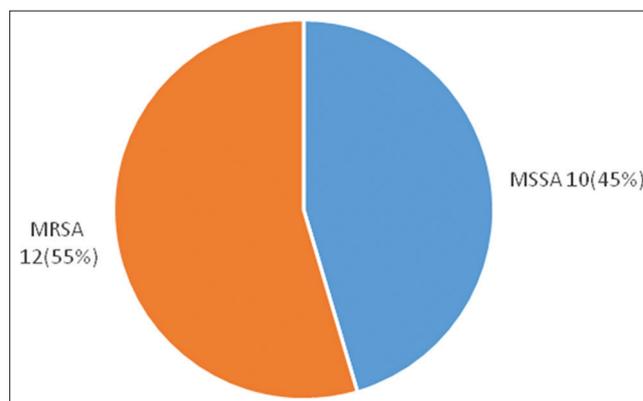
**Table 5: Carriage status of *S. aureus* and MRSA among the postgraduates in various years**

P.G.	<i>S. aureus</i> (%)	MRSA (%)	Others (%)	Total
1 <sup>st</sup>	12 (54.5)	8 (66.7)	39 (35.1)	51
2 <sup>nd</sup>	6 (27.3)	3 (25)	37 (33.3)	43
3 <sup>rd</sup>	4 (18.2)	1 (8.3)	35 (31.5)	39

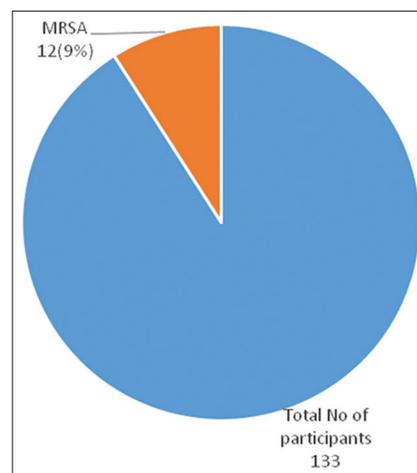
*S. aureus*: *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*



**Fig. 1: Percentage of *Staphylococcus aureus* carriers among the total participants**



**Fig. 2: Percentage of methicillin-resistant *Staphylococcus aureus* carriers among the *S. aureus* isolates**



**Fig. 3: Percentage of methicillin-resistant *Staphylococcus aureus* carriers among the total participants**

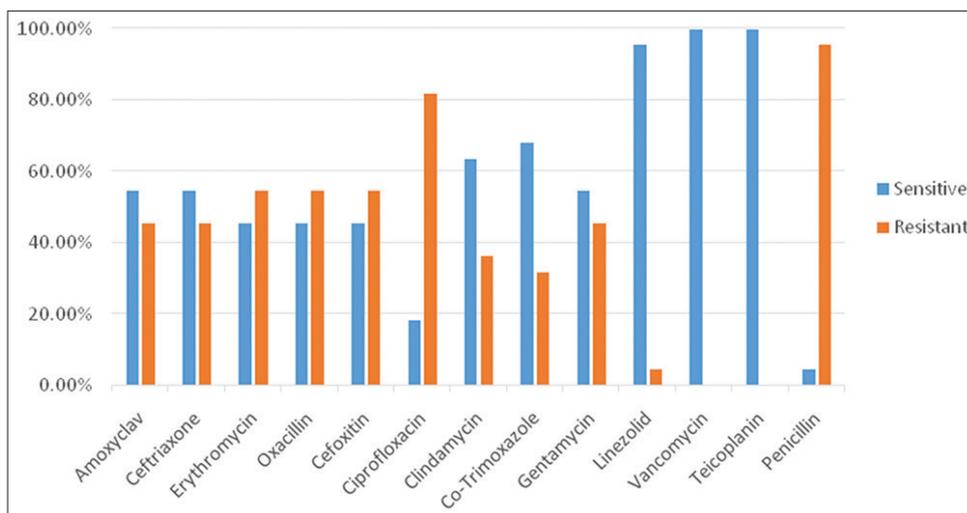


Fig. 4: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates

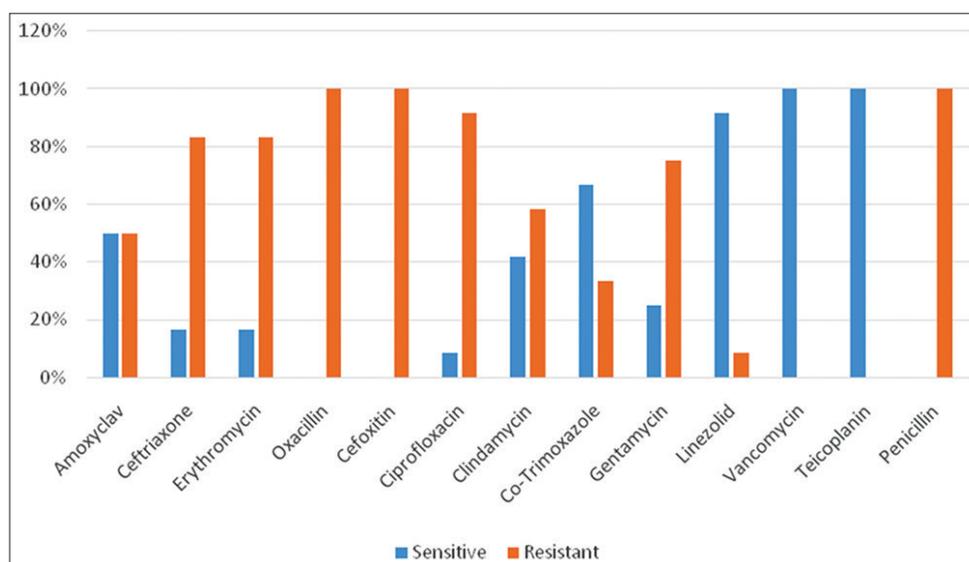


Fig. 5: Antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates

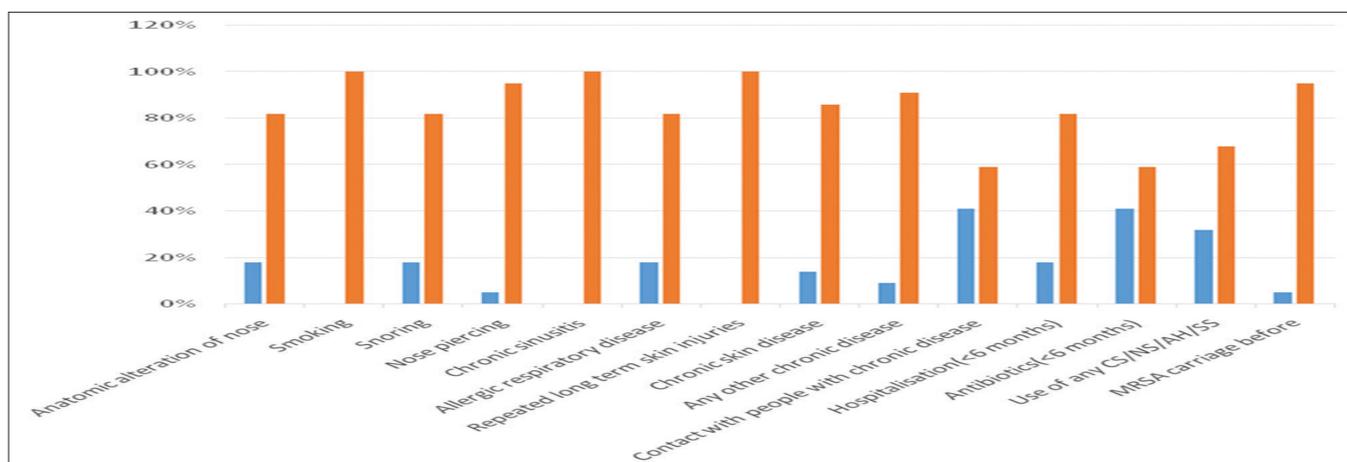


Fig. 6: Relationship between the various parameters and *Staphylococcus aureus* carriage

of strain, and they are called persistent carriers. A large proportion of the population (60%) harbors *S. aureus* intermittently, and the strains change with varying frequencies. Such persons are called

intermittent carriers. Finally, minorities of the people (20%) almost never carry *S. aureus* and they are called noncarriers. The reasons for these dissimilarities in the colonization format are unknown [20]. It is

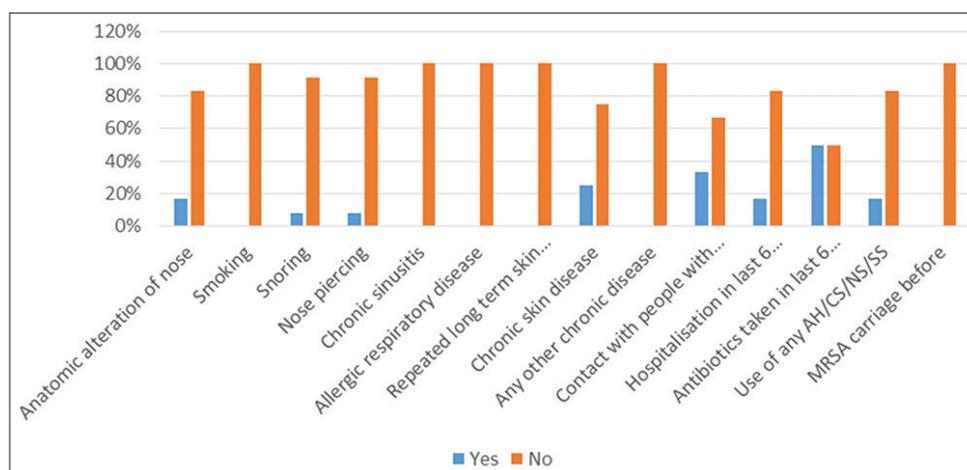


Fig. 7: Relationship between the various parameters and methicillin-resistant *Staphylococcus aureus* carriage

exciting and necessary to detect the *S. aureus* and MRSA nasal carriage rates as a part of HCWs like clinical postgraduates of medical colleges. At present, there were 241 clinical postgraduates, of which 133 had voluntarily participated in the present study representing 55.18%. It was presumed that these individuals might acquire the carriage status of *S. aureus*/MRSA during their continuous interaction with the patients and thereby may act as a potential source of infection to other patients, resulting in NIs. The prevalence of *S. aureus* and MRSA varies between institutions and geographic areas. The differences in the study design such as the sample size and the method employed for *S. aureus* and MRSA detection may account for the disparity in the carriage rate.

According to the result of the present study, the nasal carriage of *S. aureus* among clinical postgraduates in a medical college of Coastal Karnataka, India, was 16.54% (22 of 133). Of 22 isolates of *S. aureus*, 12 were MRSA (54.54%). The *S. aureus* and MRSA nasal carriage rates of clinical postgraduates recorded in this study were significantly high with the other student population, and this could be due to their continuous exposure with patients in clinical settings [21-24]. A similar kind of research was conducted in our institution past year by involving internees. It was observed that *S. aureus* carriage rate among the said internee's population was 47.3%, the overall carriage rate of MRSA was 1.4%, and among the *S. aureus* carriers, 0.6% was MRSA [25]. Compared to past year's study, the *S. aureus* carriage rate among the clinical postgraduates was remarkably low (47.3% and 16.54%), and MRSA was significantly high (0.67% and 9%), respectively. In all the 12 cases repeated isolation of the MRSA with the same antibiogram pattern concluded that the carriage status was not transient. The study also found that no significant differences in the carriage status of MRSA among the students of various surgical or nonsurgical specialties. Surprisingly, the numbers of MRSA carriers were more in 1<sup>st</sup>-year students when compared with 2<sup>nd</sup> and final year students. It was observed that among the various risk factors, contact with the chronic patients and consumption of antibiotics in past 6 months found to be statistically significant in relation with nasal carriage status of *S. aureus*/MRSA ( $p < 0.005$ ). The MRSA was 100% sensitive to vancomycin and teicoplanin. Aiming at 0% carriage rate of MRSA among the clinical postgraduates of our institution the identified MRSA carriers may be treated with mupirocin for decolonization and further screened for carriage status to check the efficacy of treatment. However, periodic screening for MRSA should be an on-going practice in the medical student population. This practice can be seen as a task because of this particular group with the highest frequency of contact with the patients and the most likely to transmit this superbug. A further advantage of treating the carriers and institution of effective hospital control policies is in the prevention of its transmission to their family members and others in the community. The most critical factor for preventing NIs is compliance of the health professionals with the sanitary and the antibacterial guidelines, for which the health professionals should be

informed about the potential consequences of the NIs, both inside and outside the hospital, and their cooperation should be sought to diminish the carriage of *S. aureus*. Simple preventive measures like hand washing before and after the patient examination, the use of sterile aprons and masks in the post-operative ward, awareness during the investigation of immunocompromised patients, and avoiding touching one's nose during work, can reduce the disease transmission rate considerably. All the HCWs should be periodically educated and trained in the maintenance of hygiene and infection control and the effects of the use or rather, the misuse of antibiotics.

#### CONCLUSION

Of 241 clinical postgraduates, 133 did participate in the present study. Swabs were collected from the consenting participants after giving a set of questionnaires. The organisms isolated were identified by standard bacteriological methods, and all the isolates of *S. aureus* were further subjected to antibiotics sensitivity testing including methicillin. The numbers of *S. aureus* isolated out of 133 participants were 22 (16.5%). Of 22 isolates of *S. aureus*, 12 were MRSA (54.5%). The MRSA carriage rate of clinical postgraduates of Kasturba Medical College, Mangalore, was 9%. Each of the MRSA isolates was sensitive to vancomycin and teicoplanin. Contact with the patients with chronic infections and consumption of antibiotic in past 6 months were found to be statistically significant as the risk factor associated with the acquisition of *S. aureus*/MRSA carriage status among the participants. There was no report of vancomycin-resistant *S. aureus* from the present study.

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#### CONFLICTS OF INTEREST

Nil.

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