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PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AND ITS SCCmec TYPE AMONG STUDENTS AT A PRIVATE DENTAL COLLEGE HOSPITAL IN CHENNAI

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ABSTRACT

Background: Since asymptomatic nasal carriage of Methicillin-resistant *S. aureus* (MRSA) is on the rise among healthcare professionals (HCPs), their prompt detection and decolonization would substantially decrease disease transmission. The study's primary goal was to identify MRSA nasal carriers among dental students/CRIs and determine their SCCmec type and their susceptibility to mupirocin. **Methods:** Anterior nasal swabs from 102 healthy dental students/ CRIs were collected aseptically and subjected to standard microbiological testing. The antibiotic susceptibility pattern of the *S. aureus* isolates was determined by Kirby Bauer disc diffusion method and m*ecA-mediated* oxacillin (methicillin) resistance was determined using the cefoxitin disc method. PCR analysis of MRSA isolates determined the presence of *mecA* and their SCCmec types. D test deduced inducible clindamycin resistance. **Results:** Of the 102 participants screened, only 8 (7.8%) carried *S. aureus.* Antibiotic susceptibility testing revealed that 5/8(62.5%) were MRSA isolates. Of which, only 2 MRSA isolates harbored *mecA,* and both belonged to the SCCmec type I. All *S. aureus* isolates were sensitive to Vancomycin, teicoplanin, and tigecycline when tested using agar screening method while 2/8 (25%) of the S. aureus isolates were D test positive and belonged to iMLS$_B$ phenotype. **Conclusion:** MRSA nasal carriers amongst HCWs/dental students are a cause of concern as they are a significant reservoir for transmission of MRSA among their co-workers as well as the community. Our results signify the need for screening for MRSA carriage and decolonization with mupirocin to substantially decrease the transmission of MRSA among dental students/patients.

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INTRODUCTION

Staphylococcus aureus has been implicated in a variety of mild and invasive infections, both community and hospital acquired infections with higher morbidity and mortality rate, lengthened hospital stays, and escalated healthcare expenses[1].With the global nasal carriage rate ranging from 10 to 35%[2], asymptomatic nasal carriage of Methicillin-resistant *S. aureus* (MRSA) amongst HCWs/dental students is a cause of concern as they are a significant reservoir for transmission of MRSA among their co-workers as well the community[3-6].

Owing to its profound antibacterial activity (binds to leucine-specific tRNA aminoacyl synthetase-inhibits protein synthesis) and clinical application, mupirocin (pseudomonic acid A (Bactroban, Smith Kline Beecham, Welwyn, GardenCity, UK) was considered to be the ideal topical agent [7]. Soon after its introduction in the UK(1985), mupirocin had been widely used in clinical practice and as a decolonizer among healthcare workers which eventually led to the emergence of mupirocin resistance in *S. aureus*[8,9].Topical mupirocin has been approved by the CDC for the decolonization of MRSA and methicillin-susceptible *S. aureus* (MSSA) in healthcare workers /patients[10]. It is of significance in effectively reducing the carriage rate of MRSA & MSSA, in patients, and in healthcare workers, with a success rate of 90% after 7 days and around 60% after an extended follow-up.

Periodic surveillance, detection, and decolonization strategies are crucial to curtail the transmission of staphylococcal infection especially, post-surgical infections, and to control nosocomial outbreaks of MRSA and its concomitant spread in the community [11]. However, the clinical consequence of the use of mupirocin for decolonization is a double-edged sword, that there exists a substantial association between its use and emergence of resistance to mupirocin [12]. Mupirocin resistance in MRSA could either be MupI (low-level/ intermediate resistance, MIC range 8-256 µg/ml) or MupR (high-level resistance, MIC ≥512 µg/ml) [13].

Since an asymptomatic MRSA nasal carrier among dental professionals would indeed promote an active transmission of the bacterium among themselves and to the patients whom they treat thereby intensifying the disease conditions, the outcome of the present study would definitely aid in assessing the MRSA nasal carriage rates, their SCCmec types and susceptibility to mupirocin of the isolates from the dental students and CRIs of the institution.

MATERIAL AND METHODS

The Institutional Ethics Committee reviewed and approved the study proposal (Ref No: SBDCH/IEC/06/2021/1). After obtaining written informed consent, a total of 102 dental students (male, n=33, age range (21-24 years), female, n=69 (age range (21-28 years)) studying Bachelor of Dental Surgery (BDS)/ CRIs at a private dental college &Hospital were recruited for the study. The following criterion was used for the selection of the study participants. Inclusion criteria: Dental students/CRIs, both genders, diabetic/non-diabetic subjects. Exclusion criteria: HCWs with a history of recent respiratory tract infection/nasal surgery, skin and soft tissue infections/rhinitis/impetigo, those who were on nasal medications/ antimicrobial therapy in the previous 2 months, immunocompromised HCWs. Anterior nasal swabs (both the nares) were collected using sterile cotton swabs and were inoculated onto nutrient broth supplemented with NaCl [14] [Fig 1]. The samples were processed using standard microbiological methods. The samples were cultured on 5% sheep blood agar plates and MacConkey agar plates [15] [Fig 2]. Identification of *S. aureus* was carried out based on microscopic morphology-Gram stain, O-F fermentation, enzyme production (catalase, oxidase, bound coagulase and free coagulase). Further species confirmation was done using mannitol fermentation i.e. yellow colored colonies on Mannitol salt agar (HiMedia Laboratories Pvt Ltd, India) [Fig 3].

Figure 1: Sample collection from the anterior nares

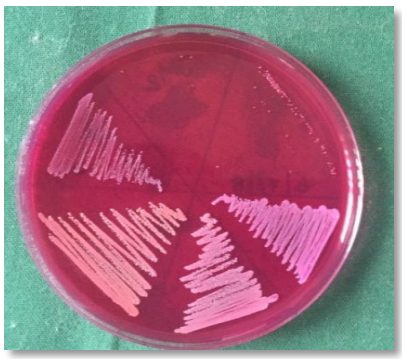

Figure 2: *S.aureus* on MacConkey Agar (lactose fermenting pink color colonies after 24 hours incubation at 37°C)

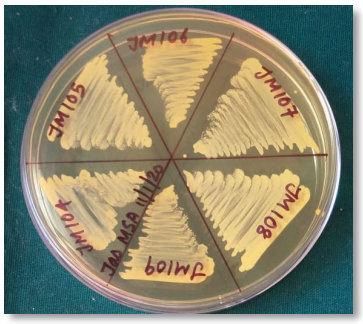

Figure 3: *S. aureus* on Mannitol Salt Agar (yellow colonies after 24 hours incubation at 37°C)

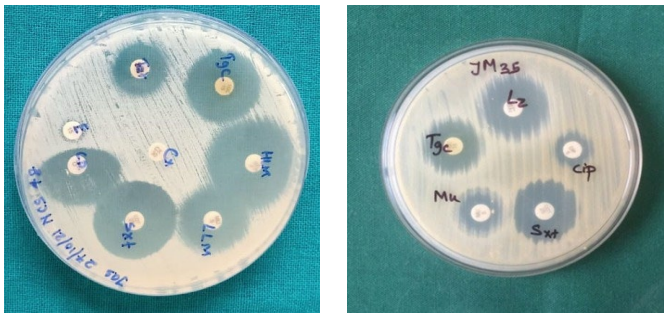

Figure 4: AST plates – MRSA isolate that is D test positive, susceptible to HLM

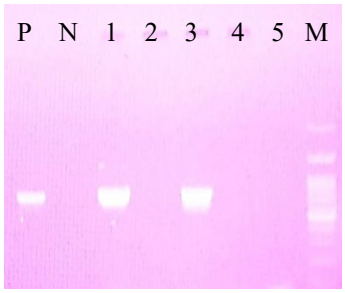

Figure 5: A representative gel picture *mecA* PCR

Lanes: P-Known *mecA* positive control, N- Negative control-no template,
lanes 1, 3: *mecA* positive, lanes 2, 4, 5: *mecA* negative, M: Molecular weight marker.

Based on the CLSI guidelines [16] the antibiotic susceptibility (AST) pattern of the *S. aureus* isolates was determined by disc diffusion method. The antibiotics tested include, erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), co-trimoxazole (25 µg), tetracycline (30 µg), tigecycline (15µg), rifampicin (5 µg) and Linezolid (30 µg). High-Level Mupirocin Discs (200 µg) were used to detect mupirocin resistance [Fig 4]. Vancomycin (6 µg/mL) agar screen method was adopted to screen for resistance to vancomycin. Detection of *mecA-mediated* oxacillin (methicillin) resistance was assessed using cefoxitin (30 µg) as the surrogate [16]. D test was employed to detect Inducible clindamycin resistance, and the appearance of the D zone towards the erythromycin disc was scored as $iMLS_B$ phenotype. *S. aureus* ATCC 25923 was included as a control [16] [Fig 4]. *mecA* and Staphylococcal Cassette Chromosome mec (SCCmec) type of the MRSA isolates were detected by PCR [17,18][Fig 5].

RESULT

Of the 102 nasal swabs collected from dental students, growth of the bacterial colonies was observed among 97(95.1%) while no growth was observed in 5(4.9%) of the nasal swab samples. Of those 102 nasal swabs samples collected from the dental students, only 8(7.8%) subjects harboured *S. aureus* in their anterior nares. All the isolates were tested resistant to benzyl penicillin and were β-lactamase producers. Table 1 represents the AST pattern of the*S. aureus* isolates and the $MIC_{50}$and MIC_{90}values. Most *S. aureus* isolates (87.5%) exhibited susceptibility to linezolid and nitrofurantoin. While 75% of the examined *S. aureus* isolates showed resistance to fluoroquinolones such as cipro^R and levo^R. Of note, one isolate was multidrug-resistant *S. aureus* i.e. resistant to multiple antibiotic classes (gentamicin, ciprofloxacin, erythromycin, co-trimoxazole). Nevertheless, this isolate was susceptible to linezolid, daptomycin, tigecycline, rifampicin, vancomycin, teicoplanin, nitrofurantoin, tetracycline, and clindamycin).

Based on the susceptibility /resistance exhibited by the *S. aureus* (n=8) isolates to cefoxitin, 5 (62.5%) were scored as MRSA, and 3(37.5%) were MSSA. Two out of 5 MRSA isolates harboured the *mecA* gene and both belonged to the SCCmec type I. All *S. aureus* isolates were sensitive to vancomycin, tigecycline, and teicoplanin when tested using agar screening method. Also, D test results showed that 2 out of 8 (25%) *S. aureus* isolates exhibited $iMLS_B$ phenotype and were tested positive for inducible clindamycin resistance. Remarkably, none of the isolates investigated demonstrated HLM resistance.

DISCUSSION

MRSA strains are notable for possessing a high transmission capability, which lengthens hospital stays and/or necessitates the use of costly treatments thereby escalating the treatment cost [19,20]. HCWs who are asymptomatic nasal carriers of MRSA pose a significant risk and are a predominant reservoir who persistently transmit MRSA among their co-workers and the community as well. Therefore, screening and decolonization is essential to curtail MRSA transmission.

In the present study, nasal carriage of S. aureus (both MRSA & MSSA) was detected among 102 dental students. The prevalence

rate of *S. aureus* among HCWs studied in Indian reports documented earlier ranged from 0% to 48% [19,21]. Of the nasal swabs collected from dental students (n=102), 8(7.9%) were *S. aureus* of which, only 5 (62.5%) were identified as MRSA using cefoxitin as the surrogate marker. Table 2 depicts the trend in the carriage rates of *S. aureus* both MRSA and MSSA reported among HCWs (only Indian studies are included) [11, 15, 19-32].

Of note, this is the first study on *S. aureus*/MRSA nasal carriage among dental students from Tamil Nadu. We report that 4.6% of the undergraduate dental students / CRIs harboured MRSA in their anterior nares. Nevertheless, another study from Karnataka reported a relatively higher carriage rate n=25/200(12.5%) of MRSA among undergraduate dental students [22]. A similar study conducted on the prevalence of the nasal carriage rate of *S. aureus* among dental students by Roberts *et al.,* (2011) [33] conducted in Seattle, Washington had shown that there is an increase in the carrier rate with increasing exposure to patients. Our results are in concurrence with the report given by TL *et al.,* (2018) [20] which showed n=9/140 (6.4%) of MRSA nasal carriage among the HCWs in the Orthopaedics department of a tertiary care centre in Karnataka. As none of the study isolates exhibited HLM resistance, E-test was not performed for the same. In concurrence with earlier studies, all the MRSA isolates of this study were found to be penicillin-resistant [19, 24, and 25]. In our study, 37.5% of the isolates tested were co-trimoxazole resistant, which is in contrast to the extremely high resistance rate 93.75% reported by Mondal *et al*, (2016) [23]. Previous reports by Radhakrishna M *et al* (2016) [24], Renushri BV *et al* (2014) [25] had documented a relatively higher resistance rates of 54% and 64.3% respectively to erythromycin among *S. aureus* isolates. Nevertheless, in our study only 37% of the isolates exhibited resistance to the drug. On the contrary, Rongpharpi *et al* (2013) [15] documented a comparatively low resistance rate of 18.6% towards erythromycin. In our study, 75% of the *S. aureus* were ciprofloxacin susceptible. This is in agreement with the report by Renushri BV *et al* (2014) [25] wherein, 71.4% of isolates exhibited ciprofloxacin susceptibility. While Rongpharpi *et al* (2013) [15] and Radhakrishna et al (2016) [24] reported a susceptibility of 82.86% and 66.7% each. Our study report that almost 87.5% of MRSA isolates were susceptible to Linezolid while Radhakrishna *et al* (2016) [24] documented that only 33.3% of MRSA isolates were susceptible for the said antibiotic. However, our results coincided with other Indian reports that exhibited 100% teicoplanin susceptibility [15, 25-27].

Limitations of the study

1. Further studies with a larger sample size are required to ascertain the nasal carriage rate of MRSA among dental students.
2. The use of mupirocin as an empirical decolonsing agent needs to be explored.

Table 1: Antibiotic susceptibility results of the *S. aureus* isolates.

Antibiotic	Susceptible n(%)	Intermediate n(%)	Resistant n(%)	MIC_{50}	MIC_{90}
Penicillin	0(0%)	0(0%)	8(100%)	≥ 0.5	≥ 0.5
Gentamicin	3(37.5%)	0(0%)	5(62.5%)	≤ 0.5	≥ 16
Ciprofloxacin	6(75.0%)	0(0%)	2(25.0%)	≥ 8	≥ 8
Levofloxacin	1(12.5%)	5(62.5%)	2(25.0%)	4	4
Erythromycin	5(62.5%)	0(0%)	3(37.5%)	≤ 0.25	≥ 8
Clindamycin	0(0%)	0(0%)	8(100%)	0.25	0.5
Linezolid	7(87.5%)	0(0%)	1(12.5%)	1	4
Daptomycin	6(75.0%)	0(0%)	2(25.0%)	1	2
Teicoplanin	8(100%)	0(0%)	0(0%)	≤ 0.5	≤ 0.5
Vancomycin	8(100%)	0(0%)	0(0%)	≤ 0.5	2
Tetracycline	6(75.0%)	0(0%)	2(25.0%)	1	≥ 16
Tigecycline	8(100%)	0(0%)	0(0%)	≤ 0.12	≤ 0.12
Nitrofurantoin	7(87.5%)	1(12.5%)	0(0%)	≤ 16	32
Rifampicin	6(75.0%)	2(25.0%)	0(0%)	≤ 0.03	2
Cotrimoxazole	5(62.5%)	0(0%)	3(37.5%)	≤ 10	≥ 320

Table 2: Indian reports on the nasal carriage of MRSA/MSSA among HCWs.

Details of the Study Participants	No of the Study Participants	MRSA/ MSSA	Carriage rate	Reference
HCWs	200	*S. aureus*	92 (50%)	Parthasarathy *et al.*, 2022 [32]
		MRSA	39 (21.66%)	
HCPs- MBBS and nursing interns, nurses, doctors, physiotherapists, Non-medical hospital staff.	200	*S. aureus*	51 (25.5%)	RD *et al.*, 2021[11].
		MRSA	13 (6.5%)	
HCWs- Orthopedic dept.	140	*S. aureus*	22 (15.7%)	T L *et al.*, 2018[20].
		MRSA	9 (6.4%)	
		MSSA	13 (9.2%)	
		VRSA		
HCWs - Dental students	n = 400 [UG (n= 200) PG (n= 200)]	Total MRSA	74 (18.5%)	Hema *et al.* (2017) [22]
		UG	25 (12.5%)	
		PG	49 (24.5%)	
HCWs	250	*S. aureus*	87	Mondal *et al.* (2016) [23]
		MRSA	16	
		MSSA	71	
		VRSA	3	
HCWs Medical; students	148	MRSA	6.1%	Radhakrishna *et al.* (2016) [24]
		MSSA	52.7%	
HCWs (Doctors, nurses, tech, nursing students, housekeeping)	200	MRSA	48%	Agarwal *et al.* (2015) [19]
		MSSA	14%	
HCWs Nursing students	119	MRSA	11.8%	Renushri *et al.* (2014) [25]
		MSSA	18.2%	
HCWs	30	MRSA	6.6%	Sharma *et al.* (2014) [26]
		MSSA	13.3%	
HCWs	315	*S. aureus*	70 (22.22%)	Rongpharpi *et al.* (2013) [15]
		MRSA	8	
		MSSA	62	
		VRSA	0	
HCWs ICU staff	200	MRSA	2.5%	Radhakrishna *et al.* (2013) [27]
		MSSA	17.5%	
HCWs (Doctors, nurses, tech, nursing students, housekeeping)	150	MRSA	14%	Malini *et al.* (2012) [28]
		MSSA	10%	
HCWs surgical staff	100	MRSA	15.4%	Vinodhkumaradithyaa *et al. (*2009) [29]
		MSSA	13%	
HCWs	57	MRSA	1(1.8%)	Mathanraj *et al.* (2009) [30]
HCWs Preclinical medical students	157	MRSA	0%	Santhosh *et al.* (2007) [21]
		MSSA	23.7%	
HCWs	150	MRSA	3.3%	Goyal *et al.* (2002) [31]
		MSSA	3.3%	

CONCLUSION

As per the WHO classification, *S. aureus*is considered as a global higher-priority pathogen that exhibits resistance to multiple antimicrobials that are used in the therapeutic management of Staphylococcal infections [33]. Our study highlights the need for MRSA screening among dental students/ CRIs professionals as they serve as plausible reservoirs of MRSA. The results of our study clearly indicate that undertaking a decolonization protocol with mupirocin would help in reducing the MRSA transmission among their co-workers, the patients and thus the community.

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FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Kesavaram Padmavathy contributed to conceptualizing, data curating, statistical analysis, reviewing, and editing the manuscript. Jebadass JasmineVinshia performed experimental work, collected data, and performed statistical analysis, textual interpretation, and drafting of the final manuscript. Baskaran Sathyapriya contributed to the investigation and supervision of the whole study. Jimson Sudha contributed to accessing resources and reviewing the manuscript. All authors read and approved the manuscript.

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