

## Original Research Article

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## Community Reservoir of Multidrug Resistant *Staphylococcus aureus* in and around Trivandrum

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### ABSTRACT

#### Keywords

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*Staphylococcus aureus* is very well adapted to colonise the human skin and produce a asymptomatic carrier state. Colonization with antibiotic resistant *S. aureus* represents both a risk factor for the colonized individual and their immediate contacts. This research study was carried out to investigate the prevalence of multidrug resistant *Staphylococcus aureus* carriers in and around Trivandrum. A total of 2361 samples were collected from healthy asymptomatic adults to isolate MRSA. Out of 166 isolated strains of *S. aureus* from the carrier samples, 58 were confirmed as MRSA strains. The carrier MRSA isolates were susceptible only to the higher antibiotics such as Linezolid and Vancomycin and showed varying degrees of resistance to the other antibiotics such as ciprofloxacin, erythromycin, co- trimoxazole, amikacin and gentamycin. The results of the present study clearly shows the healthy carriers are potential reservoirs of multidrug resistant *S. aureus* and a strict antibiotic regimen policy needs to be formulated to restrict the use of antibiotics in the community.

### Introduction

*Staphylococcus aureus* is very well adapted to colonise the human skin, and our bodies probably provide some major ecological niches for this species. We are daily exposed to the bacteria, but still only some people are carriers over longer periods of time. Carriage is asymptomatic, not harmful per se and could even to some extent be protective for a host if infected by *S. aureus* (Wertheim *et al.*, 2004, 2006 and 2008; Verkaik *et al.*, 2009 and 2011). However, carriers are at risk of

autoinfection that requires specific attention in connection to hospitalization and major surgery (Wertheim *et al.*, 2004; von Eiff *et al.*, 2001; Kluytmans *et al.*, 1995 and 1997). In addition, emergence and spread of antimicrobial resistance, such as methicillin resistant *S. aureus* (MRSA) have aggravated the situation. Danbolt (1932) suggested that the nasal “infection” was responsible for the recurrent skin infection indicating autoinfection In 1948 Moss and colleagues showed that skin carriage of *S. aureus* was dependent on nasal carriage in patients with

normal skin. In the general adult population, *S. aureus* can also commonly be found at other body sites such as the axillae (8%), chest/abdomen (15%), perineum (22%), intestine (17–31%) (Williams, 1963), and vagina (5%) (Guinan *et al.*, 1982). Pharyngeal carriage has been reported to be highly variable between populations (4–64%) (Williams, 1963) Interestingly, recent reports have shown higher carriage rates in the throat than in the nares when sampled in parallel. Also, an intestinal carriage of 20% in healthy individuals has been reported and although nasal carriage may predispose to intestinal carriage, sole intestinal carriage was also detected (Acton *et al.*, 2009). Selected studies of *S. aureus* nasal carriage in India as described by (Saxena *et al.*, 2003) revealed that 319 adults who attended well baby clinic showed 29.4%. Hence this research study was carried out to investigate the prevalence of multidrug resistant *Staphylococcus aureus* carriers in and around Trivandrum.

## Materials and Methods

A total of 2361 samples from nasal, conjunctiva, oral, ear and throat swabs from healthy individuals of both sexes between the age group of 25-50 years residing in and around Trivandrum were collected for a period from July 2014 to June 2015. The carrier screening samples were obtained from healthy people who accompanied patients to the clinical centers/ hospitals and volunteers working in these centers. All the samples were aseptically handled and were examined individually for the presence of *S. aureus* by plating them on Mannitol salt agar (HiMedia) and incubated at 37 °C for about 24 hr. The characteristic colonies were isolated and the bacterial strains were sub cultured on nutrient agar slants and stored at 4°C for further use.

The isolated strains were identified up to their species level by Gram staining and standard

biochemical tests such as catalase, urease tests. Identification of isolates was confirmed by direct-tube coagulase test with plasma. The haemolytic activity of the *S. aureus* isolates were determined by blood agar plate assay (Breneder and Janda, 1987). All strains were further tested for the production of free coagulase enzyme using tube coagulase test based on standard methods (Villanova PA, 2000) *Staphylococcus aureus* ATCC-25923 of known coagulase production was included as control strain.

The antibiotic susceptibility testing was performed at different study sites by the Kirby Bauer's disc diffusion technique<sup>5</sup> and minimum inhibitory concentration (MIC) testing, using Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI document M100-S18, 2008). All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using Cefoxitin (30 µg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. The other antibiotics used were erythromycin (15µg); gentamycin (10µg); amikacin (30µg); ciprofloxacin (5µg); co- trimoxazole (25µg); vancomycin (30µg); linezolid (30µg). Finally, the data were recorded and analysed at the completion of the study as per recommendations of the NCCLS. *S. aureus* ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing

Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18- to 24-h blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and five discs were applied on a 100mm Mueller Hinton agar plate as per CLSI guidelines. *S. aureus* ATCC

25923 was used as the quality control strain for disc diffusion.

### Results and Discussion

The prevalence of *S.aureus* and MRSA in carrier samples is given in Table 1. Totally 166 strains of *Staphylococcus aureus* were isolated from a total of 2361 carrier samples. The carrier specimen wise distribution of *S.aureus* is given in Table 1.

The overall percentage of *S.aureus* isolated from all the carrier samples such as nasal swab, conjunctival swab, oral swab, ear swab and throat swab, was found to be 7.0. The percentage of *S.aureus* isolated from nasal, conjunctiva, oral, ear and throat was 8.1, 3.0, 6.4, 3.2 and 18.9 respectively.

The reports on the methicillin resistance by these 166 isolates confirmed the presence of 58 MRSA and the remaining strains were methicillin sensitive *S. aureus* (MSSA).

The highest percentage of MRSA screened from nasal swab was 45.1. The percentage of MRSA from nasal swab, conjunctival swab, oral swab, ear swab and throat swab was found to be 45.1, 36.0, 30.7, 14.2 and 7.1 respectively.

The antibiotic resistance pattern of all these isolates is given in Table 2. All the isolates of both *S.aureus* and MRSA were highly sensitive (100%) to linezolid and vancomycin. In case of 166 strains of *S.aureus* the percentage of resistance to ciprofloxacin, erythromycin, co- trimoxazole, amikacin, gentamycin, was found to be 10.8, 32.3, 43.9, 42.7 and 27.1 respectively. In case of 58 strains of MRSA the percentage of resistance to ciprofloxacin, erythromycin, co-trimoxazole, amikacin and gentamycin, was found to be 46.5, 58.6, 53.4, 41.3 and 55.1, respectively. In case of erythromycin, co-trimoxazole and gentamycin the resistance showed by MRSA strains was found to be more than the resistance pattern of *S.aureus*.

**Table.1** Prevalence of *S. aureus* and MRSA in carrier samples

Carrier sample	No. of specimen (n=2361)	<i>S. aureus</i> (n=166=7.0%)	%	MRSA (n=58=34.9%)	MRSA %
Nasal	1057	93	8.1	42	45.1
Conjunctiva	821	25	3.0	9	36.0
Oral	201	13	6.4	4	30.7
Ear	113	7	3.2	1	14.2
Throat	169	28	18.9	2	7.1
Total	2361	166		58	

**Table.2** Antibiotic resistance pattern of 166 carrier strains of *S. aureus* and 58 MRSA

Antibiotic	Carrier <i>S. aureus</i> (n=166)	Carrier <i>S. aureus</i> %	Carrier MRSA (n=58)	Carrier MRSA %
Ciprofloxacin	18	10.8	27	46.5
Erythromycin	51	32.3	33	58.6
Co-trimoxazole	73	43.9	31	53.4
Amikacin	71	42.7	24	41.3
Gentamycin	45	27.1	32	55.1
Linezolid	0	0	0	0
Vancomycin	0	0	0	0

This study is the first report on the prevalence of multiple antibiotic resistant *S. aureus* isolated from healthy individuals in and around Trivandrum. All the carrier screening samples were obtained from healthy people who accompanied patients to the clinical centers/hospitals and volunteers working in these centers.

All the isolates of both *S.aureus* and MRSA were highly sensitive (100%) to linezolid and vancomycin. Rajadurai pandi *et al.*, (2006) and Anbumani *et al.*, (2006) also reported none of the isolates were resistant to vancomycin. The variation in the drug resistance may be well related to the type of antimicrobial agents prescribed for treating various infections in different geographical areas (Radu *et al.*, 1997).

Krishna BV *et al.*, (2004) in their study stated that out of the 116 patients with *S. aureus* infection, 18.1% had infection with methicillin-resistant strains. Clindamycin-susceptible MRSA accounted for 61.9% of cases. Among these, 46.1% patients were confirmed to have acquired the MRSA from the community, based on inclusion criteria. The community-acquired MRSA were susceptible to multiple antibiotics, as compared to nosocomial isolates.

The results of the present study clearly shows the healthy carriers are the highest reservoir of multidrug resistant bacteria as cited by Lamikanra *et al.*, 1996. Because of this fact it is imperative that a multi-centre study should be well planned to assess the prevalence of multidrug resistant *S. aureus* reservoirs in the society.

The antibiotic regimen policy needs to be formulated to restrict the use of antibiotics and also to establish systems for rapid identification of epidemics with different clads.

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