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Analysis of drug resistant *Staphyloccocus aureus* present in healthy human carriers in the community of Ambur Town, Tamil Nadu.

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ABSTRACT

The presence of antibiotic resistant varieties of *Staphylococcus aureus*, especially Methicillin Resistant *S.aureus* (MRSA) in healthy humans as carriers of the pathogen is of major concern to healthcare professionals around the world. In order to assess the level of antibiotic resistance in this organism and its molecular profile from healthy human carriers in Ambur town, Tamil Nadu, India, the present study was conducted. Kirby Bauer method, targeted PCR and MLST typing was used to determine the antibiotic resistance and molecular profile of various strains isolated from different sources. From 100 healthy contacts, one Health Care Associated MRSA (HA-MRSA) strain and one Community Acquired MRSA (CA-MRSA) strain were isolated; in addition, 13 CA-MSSA strains resistant to penicillin were also isolated from the same group. The MRSA strains were resistant to azithromycin, clindamycin, ciprofloxacin, erythromycin, rifampicin and lomefloxacin but were sensitive to linezolid. All the strains were sensitive to vancomycin. Molecular typing confirmed the presence of PVL gene in CA-MSSA and CA-MRSA strains. Both MRSA strains were SCCmec type III. MLST type of HA-MRSA strains was ST5 whereas that of CA-MRSA strain was ST1 ;all the pencillin resistant CA-MSSA strains were ST 772 type. The current study necessitates the need for additional regular screening and implementation of control, treatment and prevention measures for human carriers in order to prevent spread of antibiotic resistant varieties of *S.aureus* in the community.
Keywords: *Staphylococcus aureus;* antibiotic resistance; MRSA; community; India.

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INTRODUCTION

The global spread of antibiotic resistance in various pathogenic micro-organisms and its mechanisms of resistance has been studied in detail on a global scale [1]. Due to the increasing resistance to newer generations of antibiotics, there is higher burden on the healthcare profession to prevent the spread of these resistant pathogenic organisms.

Among the major pathogenic micro-organisms of concern is *Staphylococcus aureus*. Although a part of normal skin and nasal flora of the human body, it is also an opportunistic pathogen capable of causing range of symptoms varying from mild to life threatening that is spread primarily through nasal carriage [2]. The evolution of antibiotic resistance species of this organism, especially Methicillin Resistant *Staphylococcus aureus* (MRSA) and its variants, Health care Acquired (HA-MRSA) type and Community Acquired (CA-MRSA) type has become a major concern to health care professionals due to its almost total resistance to beta-lactam class of antibiotics and varying resistances to a considerable number of antibiotics currently in use [3]. Genetic factors such as mecA (beta-lactam resistance), Panton Valentine Leukocidin (PVL) in CA-SA strains and egr (entertoxin production) have been established to determine the resistance and virulence of these strains [4]. In addition, molecular techniques such as Multi Locus Sequence Typing (MLST) and spa gene typing have helped establish the epidemiological patterns of these resistant varieties [5,6].

Antibiotic resistance in *Staphylococcus aureus* has been widely documented in various countries in both healthcare settings and in the community [7-9]. In India, several studies have been conducted on nationwide scale as well as local area scale mainly in urban settings with large populations in both patients and the community [10-12]. Both international and national studies insist on the necessity for regularized, periodical surveillance of antibiotic resistant *S.aureus* in both health care settings and the community in order to stem the spread and prevent increase in antibiotic resistance of existing strains.

Due to the paucity of data for smaller urban and rural settings, the current study was conducted to assess the prevalence of antibiotic resistance in *S.aureus* in the community and environment to provide antibiogram data for immediate preventive and control measures, and molecular data for epidemiological assessment.

MATERIALS & METHODS

Design and Sampling

This cross sectional study was performed on healthy humans by random sampling. Nasal swabs samples were collected from healthy humans of age ≥ 18 years irrespective of sex. The entire study was carried out from January 2014 to June 2014.

The study was approved by the Internal Research Ethics Committee of Vellore Institute of Technology, Vellore. Eligibility criteria for human sampling included: (i) age ≥ 18 years, and (ii) no history of antibiotic use or hospitalization for one year prior to sampling date. Eligible persons who met the inclusion criteria and who accepted to participate in the study gave written informed consent. A well structured data collection form was used to document the following information - demographic characteristics (age, sex and area of residence) and medical history (history of antibiotic consumption and/or hospitalization during the past year and any previous documented history of infection due to *S.aureus*).

Isolates Testing

Standard methods were used for isolation, identification and confirmation were used of *S.aureus* isolates. Nasal swab samples collected through sterile swabs which were swabbed several times over the anterior nares after dipping in sterile saline. The nasal swabs were then swabbed on MSA plates. All plates were incubated at 35±2ºC for 24 hours. All the plates were inspected for growth and isolates were identified initially through colony morphology; *S.aureus* colonies are small, golden yellow colonies with yellow zone of hydrolysis around them. Further confirmation of *S.aureus* was done by performing the following tests: Gram staining (grape like clusters of Gram positive small cocci), catalase test (evolution of bubbles when colonies are mixed in 3% hydrogen peroxide on slide), plasma coagulation test (equal volumes of log phase culture and

fresh plasma are mixed in a clean tube and incubated at 35±2ºC overnight, *S.aureus* causes plasma coagulation resulting in clumping of plasma at the bottom of the tube) and hemolysis test using Blood Agar (Himedia, India) (test colonies are streak plated on blood agar plates and incubated overnight at 35±2ºC; *S.aureus* colonies appear golden yellow and have clear zone of beta hydrolysis around them).

Primary antibiotic susceptibility testing was performed using Kirby– Bauer disk diffusion method on Mueller–Hinton agar (Himedia, India) [Bauer and Kirby] using following antibiotics disc obtained from Himedia, India: Penicillin 10µg; and Cefoxitin 30µg. Temperature was maintained at 35±2ºC with an incubation period of 24 hours to accurately determine resistance. Isolates that were resistant to cefoxitin are considered as MRSA (as per CLSI guidelines 2012) and resistance to more than one antibiotic are considered Multi Drug Resistant (MDR) [13]. Secondary antibiotic susceptibility testing was performed using the previous method using multi-antibiotic disc sets Dodeca Staphylococci 1 and 2 (Himedia, India). Minimum Inhibitory Concentration (MIC) testing to determine inhibitory concentrations of Oxacillin and Vancomycin for the resistant strains was performed by the previous method using Ezy MIC Oxa-Van strips (Himedia, India). Strip concentration of oxacillin ranged from 0.064 – 8.0 µg/ml; vancomycin concentration ranged from 0.19 – 16.0 µg/ml. The interception of the inhibitory zone with the reading on the strip was determined to be the MIC for the strain. The ranges for determing resistance were – for oxacillin: ≤2 (Sensitive), ≥4 (Resistant); for vancomycin: ≤2 (Sensitive), 4-8 (Intermediate), ≥16 (Resistant).

Molecular Analysis

To perform molecular assessment of the resistant strains, DNA templates were isolated from freshly cultured strains using microwave lysis method [14]. Presence of mecA, femA and PVL was verified by multiplex PCR method [15]; *S.aureus* ATCC 25923 was used as positive control. Absence of mecA gene means that strain is Methicillin Sensitive *S.aureus* (MSSA). Determination of SCCmec type was done by multiplex PCR method as described previously [16]. Multi Locus Sequence Typing (MLST) of seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi and yqiL) was carried out as described previously [17]. MLST web site (http://saureus.mlst.net/) was used to assign allelic profiles and sequence types (ST). eBurst algorithm, available on the same website was used for phylogenetic analysis.

RESULTS AND DISCUSSION

Sampling

In this study, nasal swab specimens were collected from 100 healthy persons enrolled in this study. The mean ± standard deviation age of human participants was 43.15 ± 15.77 years (range=18-80 years); 54 persons (54%) enrolled in the study were female; and, 40 persons (40%) resided in rural areas.

Antibiotic Susceptibility

Table 1. Susceptibility of *S.aureus* strains to antibiotics tested by demographic characteristics of the study population and region

Variable	Susceptible to All Antibiotics	Resistant to One Antibiotic	Multi-Drug Resistant
Sex			
Male	36	9	1
Female	49	4	1
Age			
<40 years	36	6	0
≥40 years	49	7	2
Residential Region			
Urban	51	8	2
Rural	34	5	0

Isolates testing revealed the presence of 2 MRSA strains and 13 pencillin resistant MSSA strains. MRSA strains were present only in urban residents whereas penicillin resistant MSSA strains were primarily from rural areas. MRSA strains were present primarily in persons over 40 years of age. Penicillin resistant MSSA strains were present equally in both age groups. This is shown in Table 1.

Table 2 shows secondary antibiotic testing using multi antibiotic disc sets which revealed that all the strains were resistant to penicillin. Among the MSSA resistant strains, sensitivity was observed towards cefoxitin, cotrimoxazole, azithromycin, ofloxacin, nitrofurantoin, rifampicin and ampicillin/sulbactam and also towards gatifloxacin, and clarithromycin as well as clindamycin and linezolid ; high resistance was observed towards penicillin G; intermediate sensitivity for all the strains was observed towards lomefloxacin, erythromcin and tigecycline. All the strains were sensitive to vancomycin, teicoplanin and gentamycin. MRSA strains were resistant to several antibiotics including azithromycin, clindamycin, erythromycin, clarithromycin, moxifloxacin, rifampicin and lomefloxacin. However, they were sensitive to linezolid and vancomycin and had intermediate resistance to gatifloxacin.

Table 2. Secondary antibiotic susceptibility pattern of resistant *S.aureus* isolated from healthy human carriers

Antibiotic	Healthy Contacts with Resistant Strains (total=15)		
	Susceptible	Resistant	Intermediate
Cefoxitin	13	2	0
Vancomycin	15	0	0
Cotrimoxazole	14	1	0
Azithromycin	14	1	0
Ciprofloxacin	10	2	3
Gatifloxacin	12	1	2
Ofloxacin	14	0	1
Clindamycin	12	1	2
Penicillin G	0	15	0
Erythromycin	7	2	6
Clarithromycin	13	1	1
Linezolid	12	3	0
Nitrofurantoin	13	1	1
Teicoplanin	15	0	0
Tigecycline	9	0	6
Gentamycin	15	0	0
Rifampicin	14	1	0
Lomefloxacin	5	5	5
Norfloxacin	9	3	3
Novobiocin	12	0	3
Pristinomycin	11	3	1
Ampicillin/ Sulbactam	14	1	0
Piperacillin/ Tazobactam	12	3	0
Moxifloxacin	10	3	2

MIC testing showed that all the MSSA strains were resistant to Oxacillin whereas MRSA strains were resistant; however, all the strains were sensitive to vancomycin.

Antibiotic susceptibility profiles of our study have differences to those published in other studies. However, the MRSA strains in our study are sensitive to linezolid and vancomycin unlike other studies which report MRSA strains resistant to these two antibiotics. This may be due to the lack of exposure of the strains to these two antibiotics, making them sensitive instead of resistant. There are multiple variations in antibiotic susceptibility patterns in drug resistant *S.aureus* strains around the world, as noted in a previous study [18].

Previous studies have reported 18% of total S.aureus strains in healthy carriers to be CA-MRSA in cities such as Delhi [19] and upto 50% in Lucknow [20]. However, due to the smaller sampling size as well as the semi-urban rural setup of Ambur and lesser population size, the exposure to MRSA strains may be much lesser as compared to those of densely populated cities.

Molecular Profile

Molecular testing using multiplex PCR showed that 2 of the 15 antibiotic resistant strains were MRSA. All the strains with the exception of one human strain were CA-SA type. SCCmec typing revealed that the two MRSA strains were SCCmec type III.

MLST typing of the 15 antibiotic resistant strains revealed that the HA-MRSA strain had the allelic profile 1,4,1,4,12,1,10 indicating ST5 type; the CA-MRSA strain had allelic profile 1,4,1,4,12,1,10 indicating ST1 type; and, all the penicillin only resistant CA-MSSA strains all had same allelic profiles which is 2,2,2,2,6,3,1 indicating ST772 type. These are represented in Table 3.

Table 3. Molecular profiles of antibiotic resistant *S.aureus* strains isolated from healthy human carriers.

Strain	MRSA	PVL	SCCmec Type	ST Type
H1	Positive	Negative	III	5
H2	Positive	Positive	III	1
H3 - H15	Negative	Positive	-	772

Foot Note:
1. MRSA is indicated by presence of SCCmec gene
2. CA type is indicated by presence of PVL gene
3. H1-H15 – Resistant Strains

The global spread of ST5 [21] and ST1 [22] strains in India has been documented previously and the results of this study are concurrent with the reports. The increased number of PVL positive ST772 type MSSA with high penicillin resistance in human samples is also similar to other studies which report the growth in spread of ST772 strains worldwide especially from Asian countries [23]. The lack of variance in the ST types may be due to the rapid spread of the mentioned types throughout the country, especially with regards to ST772 type.

CONCLUSION

From the present study, we conclude that although the MRSA prevalence in the population is low, the high numbers of PVL positive CA-MSSA strains resistant to pencillin in healthy individuals is a cause for concern which needs to be addressed without delay. This necessitates increased frequency of surveillance of the community and monitored treatment regimens of individuals carrying antibiotic resistance strains in order to prevent the spread of the same in the community and to other populations.

Ethical Approval: This study was approved by the Internal Research Ethics Committee, Vellore Institute of Technology (Vellore, Tamil Nadu, India).

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