

## Epidemiological survey of methicillin resistant *Staphylococcus aureus* in the community and hospital, Gannavaram, Andhra Pradesh, South India

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### Summary

Methicillin Resistant *Staphylococcus aureus* (MRSA) has emerged as a major hospital pathogen. However studies from India between 1994-2001, reported the incidence of MRSA between 32.8%-51.6%. In recent years, there have been several reports of community-associated MRSA (CA-MRSA) infections throughout the world. The present study was aimed at the epidemiological survey of methicillin resistant *S. aureus* in the community and hospital. Samples were collected from hospital (202 samples from staff and patients, 50 samples from operation theatres and ICU) and environmental groups (Swabs from nose and axilla were collected from 70 children and 56 adults). The samples were tested for the presence MRSA. *Staphylococci* was isolated from 55 (30 from children and 25 from adults) samples from community group where as in 37 samples collected from the various groups of people in the hospital and none from the environment i.e. operation theater and ICU. The *S. aureus* isolates were studied for methicillin resistance by testing the sensitivity of the isolate to Oxacillin. Of the 26 isolates tested of *S. aureus* from the community (11 from children and 15 from adults), no MRSA was found. In the hospital, out of the 15 *S. aureus* isolates, five (33.3%) were confirmed as MRSA. Thus this study shows a prevalence of 33.3% of MRSA in hospital specimens collected from patients, and no carrier state among hospital staff or in the environments. The specimen collected from the community did not show any MRSA. It is possible that CA- MRSA has not yet established in the community studied in the present work at the present time. Further surveillance studies are necessary to continuously monitor the presence of CA-MRSA to alert the clinicians to prevent the serious complications.

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**Keywords:** Methicillin Resistant *Staphylococcus aureus*; Community-associated MRSA, Community and hospital; Prevalence; India.

### 1. Introduction

*Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections<sup>1</sup>. The organism has a differential ability to spread and cause outbreaks in hospitals<sup>2</sup>. Infections caused by *S.*

*aureus* used to respond to  $\beta$ -lactam and related group of antibiotics. However, due to development of methicillin resistance amongst *S. aureus* isolates (MRSA), treatment of these infections has become

**Table 1.** Number of *Staphylococcus aureus* isolates isolated from Hospital staff, patients and environment samples.

Hospital Staff and Patients	Positive for <i>Staphylococcus</i>	Number of Coagulase +ve <i>Staphylococcus</i>
Medical and Paramedical staff (78)	10	2
Patients in ICU (15)	05	5
Out-patients (53)	05	1
In-patients (43)	15	6
Paramedical students (13)	02	1
Operation theatres (30)	Nil	-
ICU (20)	Nil	-
Total (252)	37	15

problematic. Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, carriage of MRSA in nose are few important risk factors for MRSA acquisition<sup>3</sup>. In recent years, there have been several reports of community-associated MRSA (CA-MRSA) infections throughout the world, including several outbreaks in the United States. Most of these outbreaks have been associated with a single-clone strain. Transmission has occurred by close physical contact in situations involving children in day-care centers, children and adults on Indian reservations, athletes, military personnel, correctional facilities, and men having sex with men of concern, these patients are otherwise healthy individuals with no known risk factors for MRSA acquisition<sup>4</sup>.

The prevalence of MRSA infections is increasing in the community. Recent investigations have revealed several characteristics that differentiate CA-MRSA from health care-associated MRSA (HA-MRSA) strains. Community isolates tend to be susceptible to a variety of non- $\beta$ -lactam antibiotics, whereas HA-MRSA are typically resistant to multiple antibiotics. Other differences are that genotypes of community isolates are not the same as those of health care derived isolates, community strains harbor a novel methicillin resistance cassette gene element not identified to date among strains that are endemic to health care setting, and community isolates occur in patients lacking typical risk factors for MRSA. Finally, community isolates are more likely than health care-derived isolates to encode putative virulence factors, such as pantone-valentine leucocidin, a cytotoxin virulence factor that has been associated with severe pneumonia in children and with skin and soft tissue infections in adults<sup>5</sup>.

The center for disease control and prevention (CDC) has established criteria to distinguish CA-MRSA from HA-MRSA isolates. According to these criteria, the diagnosis of CA-MRSA must be made in an outpatient setting or by culture showing MRSA within 48 hours after admission to the hospital. The patient must not have experienced any of the following during the year before infection: skilled nursing facility, or hospice; dialysis; or surgery. In addition, the patient must be without permanent

indwelling catheters or medical devices that pass through the skin into the body<sup>6</sup>. Although CA-MRSA strains have been recognized as a leading cause of skin and soft tissue infections<sup>7</sup>, especially in patients with no established healthcare risk factors<sup>8</sup>, they also cause severe invasive infections<sup>9</sup>. Recent reports based on genotypic evidence have suggested that CA-MRSA is likely spreading within hospitals as well, blurring the line between CA-MRSA and HA-MRSA infections<sup>10</sup>. The CA-MRSA is a growing public health concern that has been associated with pediatric fatalities. It is hypothesized that the evolution of CA-MRSA is a recent event due to the acquisition of mec DNA by previously methicillin-susceptible strains that circulated in the community. In India, epidemiological surveys for CA and HA-MRSA were lacking. The present study was aimed at the epidemiological survey of methicillin resistant *S. aureus* in the community and hospital in the Gannavaram, Southern part of India.

## 2. Methods

### 2.1. Study group and samples

In our study the samples were collected from two environmental groups 1) Hospital and 2) Community. The number of samples to be included in the study was determined by calculating the power (0.80) of the samples size. In total, 382 samples were collected. Number of samples in each sub group was shown in Figure-1.

1) In the Hospital the samples were collected from A) Staff and patients: i) Medical and paramedical person (Doctors, Nurses and Lab Technicians), ii) Patients in Intensive Care Unit, Out-patients and In-patients and iii) Paramedical students.

B) Environment: i) Operation Theatres: samples were collected from different areas of operation Theatres (General surgery, Orthopaedics, Ophthalmology, E.N.T, Gynaecology and Emergency) i.e. from wall, floor, operation table, microscope and instrument Trolley ii) Intensive care unit (ICU): Samples were collected from different areas in ICU i.e. from patient's bed, A.C duct, wall, floor, and gas pipe line in Dr. Pinnamaneni Siddhartha Institute of Medical

**Table 2.** Number of Coagulase positive and negative Staphylococcal isolates from community samples.

Community	Coagulase Positive
Healthy school children	11
Healthy adults	15
	26

Sciences and research Foundation, Chinnaoutpalli, Gannavaram Mandal, Krishna dist- 521286, A.P.

2) In the community, samples were collected from two categories of people

A) Childrens: This group comprised of children of age group 5 to 15 years residing in hostels (Social welfare SC- Boys, Gannavaram, Krishna Dist- A.P, South India). They were from a low-socio economic status without any ailments and B) Adults: This group comprised of adults of all age groups residing in slum areas in village of Gannavaram, Andhra Pradesh, South India.

Sterile cotton swabs were used for collection of samples and the swabs were taken from nose and axilla.

1. Nasal swab: A sterile cotton wool swab was passed into the anterior nares of both the nostrils and rotated in both directions.

2. Axillary swab: A separate sterile cotton swab was rotated in both the axilla.

Nose and axillary swabs were placed in separate sterile tubes, without any transport medium. The swabs were transported to the laboratory within 30 minutes for further processing.

## 2.2. Culture

The swabs were inoculated onto blood agar plates, which were divided into 4 parts, each plate with axillary and nasal swabs of two persons. These inoculated plates were incubated at 37°C for 18-24 hours.

After inoculation on to blood agar, the swabs were placed in brain heart infusion broth (BHI) with 7.5% sodium chloride, which was incubated at 37°C for 18-24 hours. Inoculated BHI broth was sub cultured onto blood agar plates. From these blood agar plates, the colonies which were opaque, circular, pigmented with β hemolysis were identified as *S. aureus* by the Grams staining, Catalase and Coagulase (Slide and Tube) test<sup>11</sup>. Adequate controls were put-up at every stage.

## 2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed for various antibiotics by Kirby-Bauer disc diffusion technique with quality control strains *S. aureus* ATCC

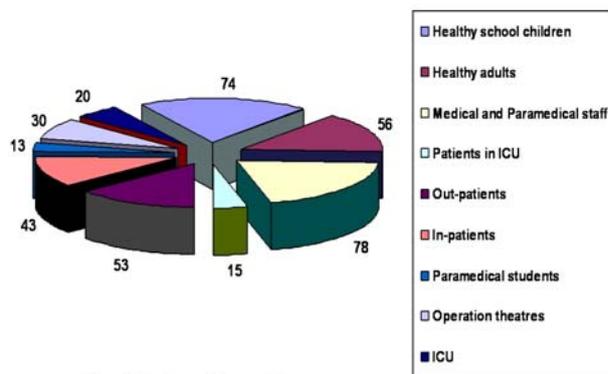


Figure 1: Break up of the samples

25923 according to the guidelines of National Committee for Clinical Laboratory Standards<sup>12</sup>, for the following antibiotics (Penicillin, Oxacillin, Gentamicin, Erythromycin, Vancomycin, Cotrimoxazole). Bacterial suspensions matching 0.5 McFarland turbidity standard were inoculated on Muller-Hinton agar containing 4% NaCl and 6µg/ml oxacillin. Isolates showing visible growth after full 24 hours incubation at 33-35°C were identified as MRSA. Oxford strains of *S. aureus* NCTC 6571 sensitive to methicillin and *S. aureus* NCTC 12493 resistant to methicillin were used as control organisms. Final identification of MRSA was made on detection of *mecA* gene by real-time PCR<sup>13</sup>.

## 3. Results and discussion

*Staphylococcus aureus* was isolated from 37 samples collected from various groups of people in the hospital and none from the environment i.e. operation theater and ICU. Out of the 37 isolates from hospital staff and patients, 15 were coagulase positive and 22 were negative (Table-1). Similarly, *Staphylococci* were isolated in 55 samples collected from the community (30 children and 25 adults). Of these, 26 were coagulase positive (COPS) and 29 were coagulase negative (CONS) (Table-2).

The *S. aureus* isolates were studied for methicillin resistance by testing the sensitivity of the isolate to Oxacillin, simultaneously with other groups of antibiotics. Of the 26 COPS from the community, no MRSA was found and in the hospital, out of the 15 COPS isolates, five were positive for methicillin resistance. The total number of MRSA isolated along with their susceptibility pattern to various antibiotics is given in the following tables (Table 3 and 4) and figure (Figure 2).

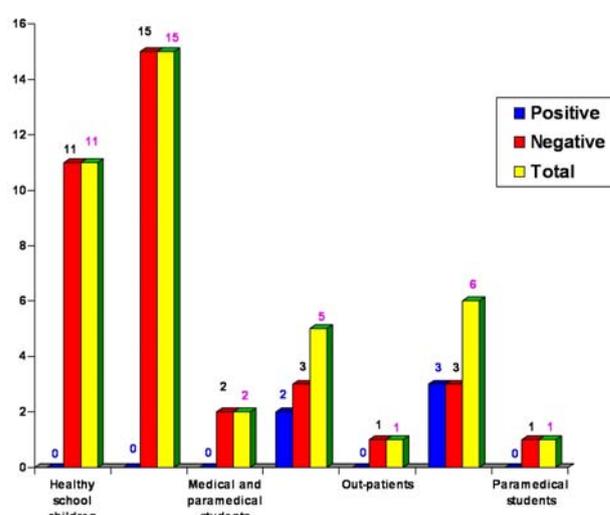
Methicillin Resistant *S. aureus* has emerged as a major hospital pathogen in various hospitals in Europe and America in 80's and continued to be so in

**Table 3.** Susceptibility pattern of the coagulase positive *Staphylococcus aureus* isolated from community to various antibiotics.

Antibiotics	Resistant %	Intermediate%	Sensitive %	Total
Penicillin	76	-	24	26
Oxacillin	-	-	100	26
Erythromycin	-	11	89	26
Cotrimaxzole	62	-	38	26
Gentamicin	-	-	100	26
Vancomycin	-	-	100	26

90's. The prevalence of MRSA has varied from hospital to hospital in various countries. About 40% of *S. aureus* infections acquired in large US hospitals are methicillin-resistant<sup>14</sup>. Prevalence is constantly soaring in many countries, and in some hospitals, more than half of all *S. aureus* disease isolates are MRSA<sup>15</sup>. In many American and European hospitals, the percentage of MRSA has ranged from 29% to 35% of all clinical isolates. Studies show that the epidemiology of MRSA over different parts of India is not uniform. Some studies have reported comparable prevalence: 38.56% in Delhi<sup>16</sup>, 31.1% in a multi center study in Tamilnadu<sup>17</sup>, and 39.50% in South Gujarat<sup>18</sup>, 38.44% in a tertiary care hospital from North India<sup>19</sup>. In contrast, other studies have reported entirely different prevalence: 24% in Vellore<sup>20</sup>, 80.89% in Indore<sup>21</sup>, 52.9% in Assam<sup>22</sup>, 19.56% in Nagpur<sup>23</sup> and 24% in Chandhigarh<sup>24</sup>. Although it's extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expansion and drug pressure in community. In the present study we have found an incidence of 33% HA-MRSA and no CA-MRSA. This figure need not indicate an absolute idea about the possible incidence in our hospital setup. Factors like this being a relatively new hospital that is only three years old since its inception, low admission rate of complicated chronically infected old patients and relatively lower occupancy rate in the ICUs have to be kept in the mind. These are the conditions well identified as favorable factors for increased incidence of MRSA in a hospital setup. A repeat study similar to this after another 2-3 years is likely to show higher rates of incidence provided no intense measures to control the rates are instituted.

Various studies indicate carrier state of CA-MRSA in various communities. In a study in Finland, 21% were found to be CA-MRSA from a total of 526 patients<sup>25</sup>. A meta-analysis applied to various types



**Fig 2.** Number of *Staphylococcus aureus* strains identified as MRSA from different groups

of CA-MRSA publications yielded several sets of key statistics. In nine studies where researchers obtained culture samples before making risk assessments, the pooled MRSA colonization rate was 2.1% (among 4825 patients). Another revealing finding was that nearly one half of patients with CA-MRSA had one or more risk factors for HA-MRSA, and among the remaining 3525 patients the colonization rate of CA-MRSA strains dropped to only 0.2%. Patients from whom samples were obtained in a health care facility were 2.4 times more likely to carry MRSA than community members cultured outside of a health care setting (95% CI 1.56-3.53). Finally, 17.8% of household contacts of patients colonized with HA-MRSA were found to also carry the index strain<sup>26</sup>. To date, the true incidence of CA-MRSA is unknown, since most studies have characterized this organism in a relatively small group of patients over a short, fixed

**Table 4.** Susceptibility pattern of the coagulase positive *Staphylococcus aureus* to isolate from hospital various antibiotics.

Drug (Discs)	Resistant %	Intermediate%	Sensitive %	Total
Penicillin	86	-	14	15
Oxacillin	33	-	67	15
Erythromycin	-	-	100	15
Cotrimoxazole	53	-	47	15
Gentamicin	-	-	100	15
Vancomycin	-	-	100	15

time interval. In our present study we did not find a single isolate of CA-MRSA among the 130 people from community. It is possible, till the time of the study; CA-MRSA has not set its footing in this community. However sample size could have influenced the outcome in our study. The risk factors for HA-MRSA were not present in any of the people in the present study.

The paramedical students were considered separately since they form an intermediary group of short exposure to hospital environment, but even they did not show any positive for MRSA. During the course of study, we have thought it is not out of place to know the relative sensitivity pattern against other useful anti *Staphylococcal* antibiotics. All the hospital isolates of *Staphylococcus aureus* were sensitive to Erythromycin where as 11% of *Staphylococcus aureus* from community showed intermediate sensitivity pattern. Similarly 38% of community isolates were sensitive to Cotrimoxazole, but only 47% of hospital isolates were sensitive. Whether they are clonally related or not was not looked for. Molecular analysis either by PFGE or Ribotyping would indicate similarity or other wise of their origin. Penicillin sensitivity of the isolates was also looked for and observed that 14% of the hospital isolates and 24% of community isolates were sensitive to Penicillin. Both the figures are higher than what is generally expected. It is possible that the reduced usage of Penicillin and dependence on other groups of antibiotic to treat many infections in general possibly has contributed to this rather unexpected result.

Thus this study shows a prevalence of 33.3% of MRSA in hospital specimens collected from patients, and no carrier's state among hospital staff or in the environments. The specimen collected from the community did not show any MRSA. In this study, we have observed that CA- MRSA has not yet established in the community studied and it may not indicate the true absence of these strains in the community. Close observations by repeat studies will be of a value since knowledge about incidence of CA-MRSA will be useful in managing some cases of community acquired Staphylococcal infections either generalized or localized infections.

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