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***RESEARCH ARTICLE***

# Incidence, Prevelance and Management of Methicillin-Resistant *Staphylococcus aureus* in Chennai

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## ABSTRACT:
Resistant strains of *Staphylococcus aureus* produces an enzyme called β-lactamase which inactivates β-lactam ring present in antibiotics. Methicillin Resistant *Staphylococcus aureus* (MRSA) infections occur in people especially working in hospitals, patients with cut or open wounds and are resistant to many type of antibiotics that are used to treat *Staphylococcus* diseases. MRSA began as a hospital acquired MRSA (HA-MRSA) and has become community associated MRSA (CA-MRSA). The aim of the study was to screen the percentage of MRSA and their antibiotic susceptibility in Chennai. The study was conducted with samples collected from hospitals and public places. Isolation of *Staphylococcus aureus* was carried out by culturing it in the Mannitol Salt Agar (MSA). Golden yellow coloured colonies were selected and identified by Gram's staining and biochemical tests .Antibiogram was performed to screen out MRSA and identify the drug choice of susceptibility. Among the 19 samples screened, growth percentage of MRSA was found to be 63.1% (12) and it was found to be susceptible to Ciproflaxacin (24.4mm), Gentamicin (18.8mm), Vancomycin (16.5mm), Doxy cycline hydrochloride (16.2mm), intermediate to Erythromycin (17 mm), Co-trimoxazole (11mm) and also resistant to Cefoxitin, Penicillin. From this study, we concluded that the surveillance of MRSA more prone in and around Chennai with higher frequency of isolation in hospitals. Among the isolates, more strains were susceptible to Gentamicin and it was found to be the drug of choice.

**KEYWORDS:** *Staphylococcus aureus*, Resistance, Mannitol Salt Agar, Antibiotics, Antibiogram

## INTRODUCTION:
Methicillin Resistant *Staphylococcus aureus* (MRSA) is a kind of *Staphylococcus aureus* which are resistant to different classes antibiotics includes penicillin, methicillin , amoxicillin, oxacillin and others[1]. MRSA is most commonly found in hospitals and health care people. Now it appears in general community causing infections in healthy people and is known as Community Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA)[2, 3].

MRSA bacteria are usually spread through skin-to-skin contact with someone who has an MRSA infection/carrier and spread through fomites. Those most common risk factors associated with MRSA infection are existence open wound, diabetes, immunodeficiency, catheterization, intravenous drip and surgery. MRSA continues to be the leading cause of nosocomial infections accounting for 20% to 70% incidence in India[4]. In the community setting, incidence of MRSA causing skin and soft tissue infections are increasing (10–20%). CA-MRSA has been recognized as a substantial public health threat owing to their high virulence and spread in the community. The wide spreading of CA-MRSA clones in both the community and hospitals, coupled with their ability to carry virulence genes as well as express resistance to multiple drugposes a significant challenge to therapeutic

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management and infection control[2]. Evidence from the literature shows that active screening for MRSA and subsequent control measures lead to areduction in the incidence of MRSA infections. Screening is the only strategy with proven efficacy in high endemic settings[5] [6].In India, there is lack of data on the epidemiological aspects of *S. aureus*in respect to community associated MRSA. The primary objective of this study was to determine the prevalence of Community acquired MRSA by collecting swab sample from different places.

## MATERIALS AND METHODS:

### Sample Collection:

Swabs were prepared by using absorbent cotton and allowed to sterile in a screw cap tube. Sterilized swabs were used to collect samples from skin, hospital beds, bus stand and railway station. The collected swab samples were inoculated in peptone water or directly streaked in selective medium and allowed to incubate at 37C for 24-48 hours.

### Microbiological examination:

The primary cultures was done with Mannitol Salt Agar (MSA) (HiMedia) plates and incubated at 37°C for 24hrs. After 24hrs, various colonies were distinguished based upon colony morphology and colour. Single yellow colour colony from each MSA plate was then transferred into a sterile Nutrient agar slant and taken for further test.

### Identification of organisms:

Gram's staining for each colony was performed to identify the colony morphology and to confirm the presence of *Staphylococcus aureus*. The gram-positive colonies were further identified using biochemical tests such as Slide Coagulase Test using Plasma, Sugar Fermentation Test, DNase Test and β Haemolysis on Sheep Blood Agar. These tests were done according to the standard procedure [7].

### Screening for MRSA:

The antibiotic susceptibility test was carried out by the standard Kirby-Bauer disc diffusion method[8]. The various antibiotic discs used were penicillin (10units), gentamicin (10µg), cefoxitin (30µg), erythromycin (15µg), doxycycline hydrochloride (30 µg), co-trimoxazole (1.25/23.75 µg), vancomycin (15µg), methicillin (5 µg) and ciprofloxacin (5µg). The discs were placed on Muller Hinton agar plates previously streaked with gram positive cultures of *S. aureus* and incubated at 37°C for 24-48 hrs and the zones of inhibition were measured[9-12].

### Statistical Analysis:

The collected data were tabulated and statistically analysedby usingGraphPad Prism.

## RESULTS AND DISCUSSION:

The colonies were grown in MSA medium and were found to be circular, concave, mucoidal and yellow in colour. (Fig.1).Among 19 samples collected from hospital and public places, 12 samples showed positive for *S. aureus*which is tabulated (Table 1). Total percentage of *Staphylococcus aureus* growth – 63.1%.

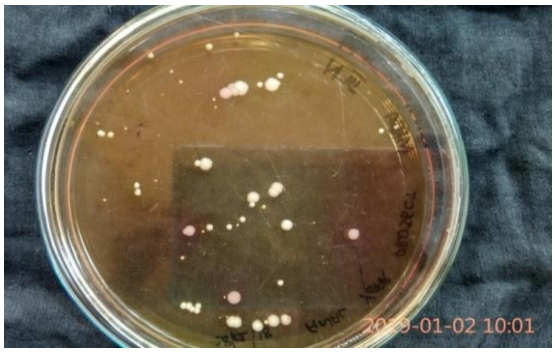


**Fig. 1:Growth of *Staphylococcus aureus* in MSA**

**Table 1:Types sample and its positivity**

| S. No. | Type of sample | Total no. sample screened | No. of positive sample |
|---|---|---|---|
| 1 | Bed swab samples (Hospital) | 10 | 7 |
| 2 | Skin swab samplse | 5 | 3 |
| 3 | Swab sample (Bus stand and railway station) | 4 | 2 |

### Identification of organisms:

All the strains were screened for *S. aureus*. From that we found the presence of gram positive cocci arranged in clusters. In the sugar fermentation test, we have found the fermenting of glucose and maltose by change of colour from blue to yellow without producing gas in Durham tube. This concludes that the organism which produces acid does not produce gas. A portion of the isolated colony was emulsified with a sterile loop into the saline in order to make thick suspensions. A drop of plasma was added to one of the suspensions and mixed gently.Clumping of the organisms were observed within 10 seconds.This confirms the production of coagulase enzyme. Thus we conclude the presence of *Staphylococcus aureus*.

The isolated colonies were inoculated in blood agar medium. In blood agar medium, beta haemolysis production was tested. When the organism grows, it produces heamolysin. Haemolysins are enzymatic proteins that can cause lysis of RBC by membrane destruction. This heamolysin gets diffused in the agar medium and attacks RBC cells that produces necrotic effect on RBC. This causes lysis of RBC producing clear zone around the organism (heamolysis). Theorganism that grows in the medium produces DNase. DNaseis extracellular endonucleases that cleave DNA and release free nucleotides and phosphate. When

the DNA is broken down, it no longer binds to the methyl green. **Green color fades and the colony is surrounded by a colorless zone.** The change of colour from green to colourless around the line indicates that there is degradation of DNA. Thus we concluded the presence of *Staphylococcus aureus.*

**Antibiogram test:**

The antibiotic susceptibility test was carried out by Kirby-Bauer disc diffusion method (Fig2) by using 9 antibiotic discs such as Co-trimoxazole, Erythromycin, Doxycycline hydrochloride, Gentamicin,Vancomycin, Ciprofloxacin, Cefoxitin, Methicillin and Penicillin in lawn culture of *Staphylococcus aureus* which were isolated from clinical sample. The zone of inhibition was measured and tabulated (Table 2). From this test, we have concluded that *Staphylococcus aureus* was resistant to Methicillin, Cefoxitin and Penicillin and sensitive to Co-trimoxazole, Erythromycin, Doxycycline hydrochloride, Gentamicin,Vancomycin and Ciprofloxacin. Among which Gentamicin showed more sensitivity against *Staphylococcus aureus*(Fig.3)

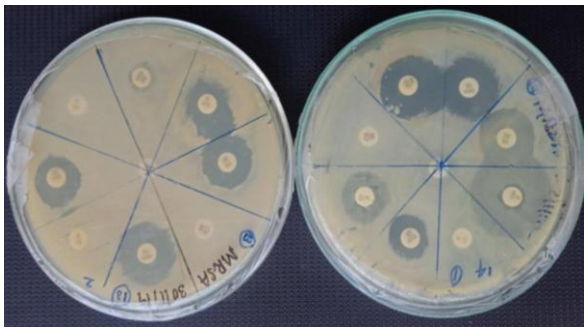

**Fig. 2: Antibiotic Susceptibility Test on MHA-shows zone of inhibition around the disc**

**Table 2: Antibiogram results of *S.aureus against* different antibiotics**

| S. No. | Antibiotics | Sensitive | Intermediate | Resistance |
|---|---|---|---|---|
| 1. | Methicillin | Nil | Nil | 12 |
| 2. | Co-trimoxazole | 7 | 3 | 2 |
| 3. | Erythromycin | 3 | 8 | 1 |
| 4. | Cefoxitin | Nil | Nil | 12 |
| 5. | Doxycycline hydrochloride | 7 | 5 | Nil |
| 6. | Gentamicin | 12 | Nil | Nil |
| 7. | Vancomycin | 8 | 2 | 2 |
| 8. | Ciprofloxacin | 8 | 3 | 1 |
| 9. | Penicillin | Nil | Nil | 12 |

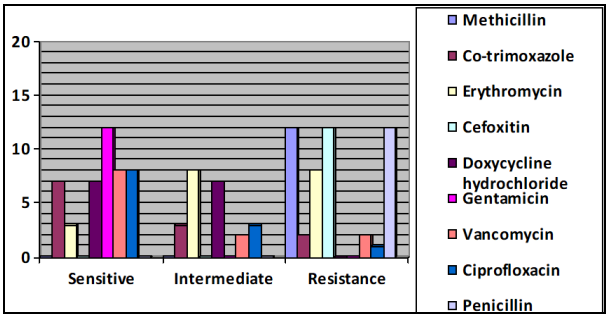


**Fig.3: Antibiotic sensitive pattern of *S.aureus***

The screening of CM-MRSA in and around Chennai by collecting swab sample from different places.The samples collected from both animate (skin swab) and inanimate objects (Swab from hospital beds and Bus stand and railway station). The skin swab sample collected form normal individual without any illness. The test results shown that there is strong evidence in the existence and survival of MRSA in hospital atmosphere and in community.Out of 12positive samples, hospital sample shown higher rate MRSA positivity (58.3%) than skin sample (25%) and bus stand and railway sation(16.7%). In antibigrm test the strains are found to be susceptible to Ciproflaxacin (24.4mm), Gentamicin (18.8mm), Vancomycin (16.5mm), Doxy cycline hydrochloride (16.2mm), intermediate to Erythromycin (17 mm), Co-trimoxazole (11mm) and also resistant to Cefoxitin, Penicillin.

This prevalence MRSA report is compared to the study conducted in other settings in India such as Bangalore and western Tamil Nadu[13,14].They found MRSA in community acquired infections was 66.9% and hospital acquired was 58.3%, this report more or less similar to our findings.The reason being that there is no previous epidemiological data on MRSA in that geographical zone. So there must be an effective periodic surveillancereport of MRSA strains needs to be done and implement infection control and preventive measures.

## CONCLUSION:

Out of 19 swab samples screened for MRSA collected from hospitals and public places, 12 samples showed the growth of *Staphylococcus*. All the 12 samples showed resistance to Methicillin (63.1%).From this study, we concluded that the pyogenic and other infection caused by *Staphylococcus aureus*is difficult to treat by using Methicillin, Cefoxitin and Penicillin.Moreover, we also found that most of the MRSA were sensitive to Gentamicin, while some strains showed sensitivity to Ciprofloxacin and Vancomycin.So instead of Methicillin, Gentamicin can be used as a drug of choice for Staphylococcalinfections.

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