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Medical Science

# "PREVALENCE AND ANTIBIOGRAM OF MRSA IN TERTIARY HOSPITAL"

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**ABSTRACT** **Background: Methicillin-resistant *Staphylococcus aureus* (MRSA)** is a significant health threat and public burden worldwide. MRSA is a major nosocomial pathogen that cause severe morbidity and mortality. MRSA strains are endemic in many American and European hospitals and accounts for 29% - 35% of all clinical isolates. The evolution of new genetically distinct hospital-acquired, community-acquired and livestock-acquired MRSA and extended resistance to other non-β-lactams including vancomycin has only amplified the crisis. Therefore, to provide a clear and comprehensive understanding of MRSA infection, this study evaluated the prevalence of MRSA in hospitalized patients in a Tertiary Care Hospital, Jammu and compare the efficacy of different phenotypic methods of detection of MRSA. **Method:** The retrospective study was conducted among 1506 patients recruited from Government Medical College and hospital. The isolation of *S. aureus* was based on culture and biochemical profiles. The detection of MRSA was done using phenotypic methods such as Oxacillin disc diffusion method, Oxacillin screen agar method and Cefoxitin disc diffusion method. Data were analyzed using Excel. **Result:** Out of 1506 samples, 360 (23.9%) samples showed bacterial growth from which 295 (84.15%) were Gram negative bacteria while rest of 65 (15.85%) were Gram positive bacteria. In 65 Gram positive isolates42 were *Staphylococcus aureus* and 11, 9 and 3 were *Enterococcus, Streptococcus* and *Coagulase Negative Staphylococcus (CONS),* respectively. Patients <15 years of age were more likely to be colonized by *Staphylococcus aureus* compared to patients above 55 years. Females (57.1%) are more prone to *Staphylococcal infection* than males (42.9%).The proportion of MRSA among the isolates was24 (57.1%) and methicillin-sensitive *Staphylococcus aureus* (MSSA) was18(42.9%).The Gram positive isolates were mostly from pus (22) followed by urine (13), blood (12), CSF (4) and miscellaneous (14).Antimicrobial susceptibility test showed that 100% of MRSA strains were susceptible to vancomycin, linezolid, and teicoplanin, 75% susceptibility of azithromycin and clindamycin and in aminoglycosides amikacin was more sensitive (54.1%) while quinolones showed high resistance pattern so as oxacillin, cefoxitin and cephalexin. **Conclusion:** This study reported that there is high prevalence of MRSA in the hospital especially in pediatric wards and surgery wards which demands serious concerns over hospital infection control practices. Isolates were not resistant to vancomycin but its unique ability to acquire and transfer antibiotic resistance calls for urgent and well-coordinated surveillance program to combat this situation.

**KEYWORDS :**

## INTRODUCTION

*Methicillin-resistant Staphylococcus aureus* (MRSA) is a significant nosocomial pathogen globally, often leading to severe infections. It is resistant to multiple antibiotics, making treatment difficult and increasing hospital costs. Initially a hospital-acquired infection, MRSA has now also emerged as a community-acquired pathogen. In India, hospital-acquired MRSA is a common cause of wound infections and shows a high degree of resistance to antibiotics. This study investigates the prevalence of MRSA in a tertiary hospital and compares the effectiveness of phenotypic detection methods.

## LITERATURE REVIEW

*Staphylococcus aureus* was first identified in the 1880s and was treated with Penicillin, an antibiotic discovered in 1941. It was only one year after an Oxfordshire constable, Albert Alexander, became the first recipient of Penicillin, that Rammelkamp reported the identification of isolates of *Staphylococcus aureus* resistant to this miracle drug.[44] Infections caused by penicillin-resistant *Staphylococcus aureus* were initially limited to hospitalized patients and were only later detected in the community, where they eventually became common.[12]

Overuse of this antibiotic led to a resistance of penicillin by the 1960s .[18] The antibiotic Methicillin, a semi-synthetic Penicillin, was then developed and used for the treatment of *staphylococcus aureus* infections .[18] Once again, MRSA mutated and developed a particular strain of *Staphylococcus aureus* that is resistant to many other antibiotics.

The first reported case of MRSA occurred in 1961. This case was documented in a hospital in the United Kingdom. The United States saw its first case of MRSA in 1968, also occurring in a hospital setting. The presence of this strain of *Staphylococcus aureus* was named Hospital-Acquired MRSA (HA-MRSA). This infection became prevalent in hospitals all over the world, usually showing resistance to multiple antibiotics.[9] Up until 1980, MRSA was only found in health care facilities, hence the name HA-MRSA. In 1980, however, a MRSA case was reported outside of the hospital setting in the United States. Throughout the 1990s, and still today, MRSA has developed into a widespread problem in communities throughout the country The name Community-Acquired MRSA (CA-MRSA) is given to cases that are not obtained in hospitals .[8] Infections caused by CA-MRSA have been reported in multiple states across the US and throughout many countries.[14] The most common sites of infection for both HA-MRSA and CA-MRSA are the skin and soft tissues.

In India, MRSA is more in hospital population (patient and staff) as carrier than in the community.[17] And one of the common causes of hospital-acquired wound infections either after accidental injury or surgery and these strains generally show multiple drug resistance, which limits treatment possibilities.[9]

Methicillin-resistant *Staphylococcus aureus* (MRSA) is cross-transmitted in hospital settings, and has a high impact not only on patient morbidity and mortality but also on hospitalization costs. Worldwide, it has been endemic in many healthcare facilities since the 1990s.[7] MRSA remains a major pathogen in nosocomial infections in developing countries.[12] Phenotypic detection of MRSA has been problematic ever since its discovery in the early 1960s. The emergence of low-level-resistant MRSA clones acquired in the community has only added to these difficulties.

Recent investigations suggest that disk diffusion using Cefoxitin is superior to most previously recommended phenotypic methods, including Oxacillin disk diffusion and Oxacillin screen agar testing.[15] In 2005, the Clinical and Laboratory Standards Institute (CLSI) published zone diameter breakpoint guidelines for Cefoxitin (

**CLSI,2005)**. The CLSI M100-S15 document stipulates an incubation time of 24 h unless the isolate has a zone diameter of ≤19 mm (i.e., resistant), in which case it can be reported after 18 h of incubation. For Methicillin and Oxacillin, incubation temperature is known to affect the test results.[13] For Oxacillin, a maximum of 35°C for testing of staphylococci is specified by CLSI. For Cefoxitin, high accuracy has been found using standard incubation temperatures, i.e., 35 to 37°C.[6& 15] In a recent investigation, zone diameters obtained on IsoSensitest agar with semi confluent inoculum were dependent on whether the plates were incubated at 35°C or 36°C . The 10-µg Cefoxitin disk has been shown to be superior to the 30-µg disk with IsoSensitest agar and semiconfluent growth.[16]

## MATERIAL AND METHODS

### Study Design And Area

The present study was a retrospective study which was carried for a period of 4 months in the Department of Microbiology, Govt. Medical college and hospital.

### Mannitol Salt Agar:

1% mannitol,7.5% NaCl & 0.0025% phenol red is added to nutrient agar. *Staphylococcus aureus* ferments mannitol and thus produce yellow coloured colonies.

## MRSA DETECTION USING PHENOTYPIC METHODS

### Oxacillin disc diffusion method

A direct colony suspension of *Staphylococcus* aureus isolate will prepared to 0.5 Mcfarland standard and plated on Mueller Hinton agar. An oxacillin (1µg) disc will placed on the surface and incubate at 37°C for 24 hours. Oxacillin disc is more resistant to degradation in storage and more likely to detect hetero resistant strains. Zone diameter of less than 10 mm is considered as oxacillin resistant and greater than 13 mm considered as oxacillin sensitive.

### Oxacillin screen agar method

Mueller-Hinton Agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin was prepared. Plates were inoculated with 10 µL of 0.5 McFarland suspension of the isolate by streaking in one quadrant and incubated at 35C for 24 hours. Plates were observed carefully in transmitted light for any growth. Any growth after 24 hours was considered oxacillin resistant.

### Cefoxitin Disc Diffusion Method

All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc. A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37°C for 24 hours and zone diameters were measured. An inhibition zone diameter of less than 19 mm was reported as cefoxitin resistant and greater than 19 mm was considered as cefoxitin sensitive.

## RESULT

The present study was conducted on a total of 42 isolates of *Staphylococcus aureus* which were isolated from various clinical specimens received in the department of Microbiology, Govt. Medical College and Hospital for bacteriological processing.

### Outcome Of Samples Received From Indoor Patients

Out of 1506 different samples received from IPD patients 360 (23.9%) showed bacterial growth while 1146 samples were negative for any growth. (Table 1)

**Table No. 1: Outcome Of Samples**

| Samples | Number | %age |
|---|---|---|
| Positive | 360 | 23.9% |
| Negative | 1146 | 76.1% |
| Total | 1506 | 100% |

**Table No.2 : Outcome Of Samples In Reference To Isolates**

| SAMPLES | Sample showing growth | | | Sample showing no growth |
|---|---|---|---|---|
| | No. of Gram Positive isolates | No. of Gram Negative isolates | Total number | |
| CSF | 4 | 21 | 25 | 76 |
| Pus | 22 | 33 | 55 | 99 |
| Urine | 13 | 138 | 151 | 516 |
| Blood | 12 | 20 | 32 | 359 |
| Miscellaneous | 14 | 83 | 97 | 96 |
| TOTAL | 65 | 295 | 360 | 1146 |

### Laboratory Outcome Of Samples In Reference To Isolates

Table 2 showed laboratory outcome of samples in reference to isolates. Majority of isolates amongst positive samples were gram negative (84.15%) while rest were gram positive in nature (15.85%). No growth was observed in 1042/1370 (76.1%) samples. Maximum no. of gram negative isolates(124/276:44.93%) were found in urine and in case of gram positive isolates pus samples showed maximum positivity (18/52:34.62%).

### Distribution Of Gram Positive Isolates

Table 3 showed distribution of gram positive isolates .Out of 65 gram positive isolates 42 were *Staphylococcus aureus* and 11 , 9 and 3 were *Enterococcus, Streptococcus* and Coagulase negative *Staphylococcus* (CONS), respectively.

**Table No.3 : Distribution Of Gram Positive Isolates**

| GRAM +VE ISOLATES | NO. | PERCENTAGE |
|---|---|---|
| Enterococcus | 11 | 16.9% |
| Streptococcus | 9 | 13.9% |
| Staphylococcus aureus | 42 | 64.6% |
| CONS | 3 | 4.6% |
| TOTAL | 65 | 100% |

### Age Distribution (n = 42) In Reference To *Staphylococcus Aureus* Positive Samples

Table 4 showed that maximum numbers of *Staphylococcus aureus* positive samples were found in age group of less than 5 years i.e. 26.2% followed by 21.4% in ages more than 55 years age group . Age group between 36 – 45 years were least infected by *Staphylococcus aureus*

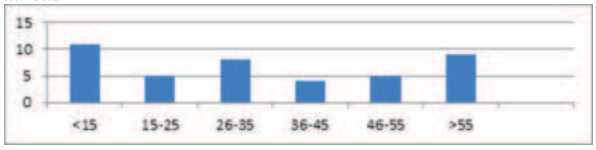


**Fig 4:** Graph depicting distribution of *Staphylococcus aureus* age wise Gender distribution ( n = 42)

Table 5 shows Gender distribution of isolates *Staphylococcus aureus* showed that females were more infected than males.

**Table No 5. : Gender Wise Distribution Of *Staphylococcus Aureus***

| SEX | NO. | PERCENTAGE |
|---|---|---|
| Male | 18 | 42.9% |
| Female | 24 | 57.1% |
| TOTAL | 42 | 100% |

### Status Of *Staphylococcus Aureus* In Reference To Oxacillin Disc Diffusion Test

Table 6 showed that 47.6% of *Staphylococcus aureus* were Methicillin resistant by oxacillin disc diffusion test and 52.4% were sensitive to Methicillin.

**Table No.6 : *Staphylococcus Aureus* In Reference To Oxacillin Disc Diffusion Test**

| ISOLATE | NO. | PERCENTAGE |
|---|---|---|
| MRSA | 20 | 47.6% |
| MSSA | 22 | 52.4% |
| Total | 42 | 100% |

**Table No. 7 : Status Of *Staphylococcus Aureus* In Reference To Oxacillin Screen Agar**

| ISOLATE | NO. | PERCENTAGE |
|---|---|---|
| MRSA | 22 | 52.4% |
| MSSA | 20 | 47.6% |
| Total | 42 | 100% |

**Table No. 8 : Status Of *Staphylococcus Aureus* In Reference To Cefoxitin Disc Diffusion Test**

| ISOLATE | NO. | PERCENTAGE |
|---|---|---|
| MRSA | 24 | 57.1% |
| MSSA | 18 | 42.9% |
| Total | 42 | 100% |

**Comparative table showing % positivity for MRSA using three**

**different methods**

Below Table No. 9 shows comparison showing % positivity for MRSA using three different methods. Cefoxitin disc diffusion method showed the highest positivity (57.1%) followed by Oxacillin screen agar (52.4%). Oxacillin disc diffusion test was observed to be least sensitive test.

**Table No. 9: Comparison Of Oxacillin Disc Diffusion On Oxacillin Screen Agar & Cefoxitin Disc Diffusion Method.**

| METHOD | No. of MRSA Isolates | PERCENTAGE |
|---|---|---|
| Oxacillin disc diffusion | 20/42 | 47.6% |
| Oxacillin screen agar | 22/42 | 52.4% |
| Cefoxitin disc diffusion | 24/42 | 57.1% |

**Distribution Of MRSA Sample Wise**

Of 24 MRSA isolates, maximum were from pus samples (10/24 :41.6%) while blood samples showed isolation rate of 25%

**Table No.10 : Chart Depicting Distribution Of MRSA Sample Wise**

| SAMPLE | NO. | PERCENTAGE |
|---|---|---|
| Pus | 10 | 41.6% |
| Blood | 6 | 25% |
| Urine | 4 | 16.7% |
| Miscellaneous | 4 | 16.7% |
| Total | 24 | 100% |

## DISCUSSION

The present study was undertaken to know the prevalence of MRSA in hospitalized patients in a Tertiary Care Hospital. A total of 1506 different samples were received from IPD patients. Of these, 360 showed bacterial growth while 1146 samples were negative for any growth.

Of these 360 isolates 295 were gram negative while 65 were gram positive in nature. Amongst the 65 gram positive isolates, 42 were *Staphylococcus aureus,* which formed the study group.

Of the 42 *Staphylococcus aureus* isolates, maximum were from patients of age group less than 5 years followed by age group >55 years . Gender distribution of the patients with *a Staphylococcus aureus* positive growth indicated higher prevalence in female patients (57.1%) as compared to male patients (42.9%).

For the detection of Methicillin resistance in these isolates, three different methods were used to look for Methicillin resistance.

It was observed that maximum number of MRSA strains (57.1%) were identified using Cefoxitin disc diffusion test and least sensitive method was found to be Oxacillin disc diffusion method which detected only (47.6%) of isolates as MRSA. With Oxacillin screen agar test 52.8% of isolates were labelled as MRSA in nature, making it more sensitive method than Oxacillin disc diffusion method

Similar findings have been reported by some other authors who suggested disk diffusion using Cefoxitin to be superior to most previously recommended phenotypic methods including Oxacillin disk diffusion and oxacillin screen agar testing.[16] For Methicillin and Oxacillin, incubation temperature is known to affect the test results.[12] For Oxacillin, a maximum of 35°C for testing of staphylococci is specified by CLSI. For Cefoxitin, high accuracy has been found using standard incubation temperatures, i.e., 35 to 37°C.[1 & 6] but one study showed relatively low sensitivity at 37°C.[13]

Our study showed the prevalence rate of 57.1% of MRSA in hospitalized patients. Review of literature shows considerable variation in the prevalence rate of MRSA in different studies. Different studies had shown prevalence rate of MRSA ranging from 25% to 60% .[10] Maximum number of MRSA isolates was from pus samples which showed 40% of MRSA positivity, followed by blood and urine samples with positivity of 25% and 20% respectively.

Paediatric ward had the maximum MRSA positive samples with 33.3% prevalence rate and was closely followed by surgical & orthopaedics ward (25% & 16.7%, respectively). Antimicrobial susceptibility testing showed very low susceptibility to some of the beta-lactam drugs, stressing upon the need of detection of MRSA in the laboratories for proper guidance of treatment options in these infections. Higher resistance was observed against Cephalosporins, Quinolones and Gentamicin .

## CONCLUSION

The present study briefly illustrates the high prevalence of MRSA infection in Tertiary hospital. Widespread of MRSA in the community is considered a public health threat. The development of multi-drug resistance is the main obstacle in the treatment of MRSA infection. Out of 1506 different samples received from IPD patients 360 showed bacterial growth while 1146 samples were negative for any growth. Out of 360 positive samples 42 were *Staphylococcus aureus*. Out of these 42 *Staphylococcus aureus,* 24 were found to be resistant to Methicillin and were reported as MRSA. Cefoxitin disk diffusion test is better method to detect MRSA rather than Oxacillin disk diffusion test and Oxacillin Screen Agar Test. Treatment options for MRSA are limited and less effective than options available for susceptible *Staphylococcus. aureus* infections and result in higher morbidity and mortality. Continuous monitoring of the antibiotic sensitivity is the key for MRSA infection treatment. Strict antibiotic prescription policies should be enforced to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens.

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