**Original article**

# Study of Methicillin-resistant *Staphylococcus aureus* in indoor patients of a tertiary care hospital in North India

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**Abstract**

**Background:** Methicillin-resistant *S. aureus*(MRSA), is one of the most common cause of nosocomial pathogen responsible for causing variety of human infections that may range from minor skin disease to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drugs resistant organism is becoming more common. Prevalence of MRSA varies from one setting to other, therefore a study was planned to know prevalence and antibiotic susceptibility pattern of MRSA isolate in our hospital. To know the prevalence and antibiotic susceptibility pattern of MRSA isolates in indoor patients**.**

**Materials and methods:** Detection and antibiotic susceptibility pattern of MRSA strains in *S. aureus*isolates from various clinicalspecimenssentfromdifferentdepartmentsofthehospitalwasdoneforaperiodofoneyear.

**Results**: Out of a total of 100 *S. aureus*isolates 31(31%), were found to be MRSA, detection MRSA was found be 100% by cefoxitin disc diffusion method and E test compared to oxacillin method (93.5%). Prevalence of MRSA strains was almost equal in male and females, but was more common in middle age group patients, no case was found in <10 years age group. In clinical samples, prevalence of MRSA was highest in urine samples (41.2%) followed by sputum (33.3%) and pus (28.9%). On comparing different departments of the hospital it was found to be most prevalent in medicine (41.7%) followed by surgery (38.1%), obs. &gyne (35.7%), TB & chest (33.3), ENT (23.8%) and orthopaedics (20%). Multi-drug resistance was seen in 75% of MRSA isolates compared to around 30% in MSSA isolates. All isolates were found to be vancomycin sensitive.

**Conclusion:** Multi-drug resistant MRSA is prevalent in our hospital setting and regular monitoring is needed to keep its prevalence controlled in future.

## Introduction

Emergence of multi-drug resistance (MDR) in bacterial isolates is the most troubling aspect in current medical microbiology. MDR has emerged in almost every genus and species of commonly isolated aerobic bacteria. Methicilliin-Resistant *Staphylococ-cusaureus*(MRSA) is an MDR strain of *Staphylococcus aureus,* resistant to penicillins, cephalosporins, carbapenems and macrolides. Methicillin was first introduced in 1959 to treat *S.aureus*infections resistant to penicillin.[1] First case of MRSA in humans was reported in England. [2] Since then it has emerged as a major cause of hospital acquired infections worldwide.[3] As, a recent study by Klevens et al. showed that deaths from MRSA infections in the U.S. have eclipsed the number of deaths caused by HIV/AIDS on an annual basis. These investigators estimated that MRSA caused 94,000 invasive infections and over 18,000 deaths in 2005.[4] Many MRSA isolates are sensitive to noly

glycopeptides and even decreases susceptibility to them is emerging.[5] The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence, and important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers is the predominant mode for patient-to-patient transmission.[6] Prevalence of MRSA varies from hospital to hospital. Therefore, we planned a study to know the prevalence and antimicrobial susceptibility pattern of MRSA in indoor patients of our hospital- an upcoming tertiary care hospital of North-India.

**Materials and methods**

A prospective study from 1st January 2011 to 31st December 2011 was conducted in the Department of Microbiology of a tertiary care hospital in North- India. A total of one hundred *S. aureus*isolates from clinical samples from indoor patients admitted in different departments of the hospital, were subjected to MRSA screening, using conventional microbiological methods. Specimens included pus, sputum, genital specimen (high vaginal swab, semen, and urethral discharge), urine, devices (urinary catheter, central venous line), blood, body fluids. All specimens were handled and processed aseptically. The standard microbiological methods were followed in this study during culture and antibiotic sensitivity test following universal precaution All isolates were identified by conventional methods including colony morphology, Gram staining, catalase test, coagulase test (tube & slide) &DNase test.[7]

All the confirmed *S. aureus*strains were subsequently tested for methicillin resistancebased on Kirby-Bauer disk diffusion method using oxacillin discs (1µg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin resistant if the zone of inhibition was 12 mm orless.

Cefoxitin is reported to be more sensitive in detection MRSA strains, therefore all suspected MRSA strains were cross checked by Cefoxitin disc diffusion test, using 30 µ g disc. An inhibition zone of ≤21 mm was taken as MRSA.[8] E test for detection of MIC for oxacillin of MRSA isolates was also performed using Hi comb strips (Himedia, Mumbai). Further, the antibiotic susceptibility pattern of methicillin resistant *S. aureus*strains was determined on the day of their isolation by the modified Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. The antibiotics used were penicillin-G (10 unit); ampicillin (10 µ g); cloxacillin (30µ g); cephalexin (30µ g); cephotaxime (30µ g); erythromycin (15µ g); gentamycin (10µ g); amikacin (30µ g); netillin (30µ g); ciprofloxacin (5µg); ofloxacin (5µ g); norfloxacin (10µg); co-trimoxazole (25µ g); vancomycin (30µ g); linezolid (30µ g). Finally, the data were recorded and analyzed at the completion of the study as per recommendations of the NCCLS.[8] *S. aureus*ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing. Chi-square test was used to calculate p value, while comparing various parameters between MRSA and MSSA. $P<0.05$ was taken as statisticallysignificant.

**Results**

Out of a total 100 isolates of S. aureus 31 were found to be MRSA strains. Male female distribution of these isolates was 1:1.1. Maximum number of isolates were found in 31-40 years age group (32.2%) followed by 21-30 (25.8%), 41-50 (19.3%), 51-60 (16.1%) and 3.2% in 11-20 and 61-70 years age group (Table 1). Maximum number of MRSA isolates were from pus 23 (74.2%) followed by urine 7 (22.6%) and only 1 (3.2%) from sputum and blood each. Prevalence of MRSA isolates was maximum in urine (41.2%) followed by sputum (33.3%) and pus (29.5%) [Table 2]. While examining ward wise distribution of MRSA isolates, maximum isolates were obtained from the department of ENT (23.8%) followed by surgery (25.9%) medicine and Obs&Gyne (16.1% each) and only one case (3.2%) from orthopaedics, dermatology and TB & chest each. Prevalence of MRSA was highest in isolates from the departments of medicine (41.7%0 followed by surgery (38.1%), obs. & gyn. (35.7%), TB & chest and dermatology (33.3% each), ENT (23.8%) and orthopaedics (20%). (Table 3). Out of 100 *S. aureus*isolates, 29 were MRSA and 71 were MSSA by disc diffusion using oxacillin (1µg) disc, 31 wereresistant tocefoxitin (30µg) disc diffusion test and in these 31 isolates E-test (Hi comb MIC test) done to know the MIC value for oxacillin. MIC range for oxacillin was between 2µ g/ml to 256µ g/ml for MRSA strains. Majority of MRSA isolates (83.9%) have MIC in range of 16- 256 µ g/ml (Table. 4). The sensitivity and specificity of these two phenotypic tests was compared with E-test. Sensitivity of Oxacillin disc diffusion test was found to be only 93.5% compared to cent percent in rest of two methods. (Table 5)

The susceptibility pattern of antibiotics showed that all MRSA isolates were significantly less sensitivity to antibiotics as compared to MSSA. The value were statistically significant as P-value was <0.05 for every antibiotics. Vancomycin was 100% sensitive in both MSSA as well as MRSA. Out of 31 MRSA isolates, 12(67.7%) were sensitive to amikacin, followed by 14(45.2%) to gentamicin, 11(35.5%) to ciprofloxacin, 10(32.3%) to Ceftazidime and 9(29%) to erythromycin. Whereas, out of 69 MSSA isolates 62(89.9%) were sensitive to amikacin followed by 58(84.1%) to erythromycin, 54(78.3%) to ciprofloxacin, 52(75.3%) to ceftadizime and 44(66.7%) to gentamicin (Table6).

**Table 1: Age and gender distribution of MRSA isolate**

| Age group (years) | Total No. of MRSA N (%) | Male | Female | Male Female ratio |
|---|---|---|---|---|
| **0-10** | 0 | 0 | 0 | 0 |
| **11-20** | 1 (3.2) | 1 | 0 | 1 |
| **21-30** | 8 (25.8) | 2 | 6 | 1:3 |
| **31-40** | 10 (32.3) | 5 | 5 | 1 |
| **41-50** | 6 (19.3) | 2 | 4 | 1:2 |
| **51-60** | 5 (16.2) | 4 | 1 | 4 |
| **61-70** | 1 (3.2) | 1 | 0 | 1 |
| **Total** | **31 (100)** | **15** | **16** | **1:1.1** |

**Table 2: Prevalence of MRSA isolates from different clinical samples**

| Clinical samples | No. of *S. aureus* (n=100) | No. of MRSA (n=31) | Prevalence of MRSA |
|---|---|---|---|
| **Pus** | 78 | 23 (74.2) | 29.5 |
| **Urine** | 17 | 7 (22.6) | 41.2 |
| **Sputum** | 3 | 1 (3.2) | 33.3 |
| **Blood** | 2 | 0 | 0 |

**Table 3: Prevalence of *S. aureus* & MRSA isolates in different departments**

| Department | Total No. of *S. aureus* | No. of MRSA N (%) | Prevalence of MRSA (%) |
|---|---|---|---|
| **ENT** | 42 | 10 (32.3) | 23.8 |
| **Surgery** | 21 | 8 (25.9) | 38.1 |
| **Medicine** | 12 | 5 (16.1) | 41.7 |
| **Gynecology** | 14 | 5 (16.1) | 35.7 |
| **TB chest** | 3 | 1 (3.2) | 33.3 |
| **Orthopaedics** | 5 | 1 (3.2) | 20 |
| **Dermatology** | 3 | 1 (3.2) | 33.3 |
| **Total** | 100 | 31 (100) | 31 |

**Table 4: Oxacillin MIC range in MRSA isolates**

| Oxacillin MIC value (µg/ml) | MRSA isolate (n=31) N (%) |
|---|---|
| 4 | 2(6.5%) |
| 8 | 4(12.9%) |
| 16 | 6(19.4%) |
| 32 | 5(16.1%) |
| 64 | 3(9.7%) |
| 128 | 5(16.1%) |
| 256 | 6(19.4%) |

**Table 5: Comparison of two phenotypic methods with E-test (Hi comb MIC test) for detection of MRSA**

| Test methods | Detected as MRSA | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| **Oxacillin (1µg) disc diffusion** | 29 | 93.54% | 100% |
| **Cefoxitin (30µg) disc diffusion** | 31 | 100% | 100% |
| **E-test (Hi Comb MIC test)** | 31 | 100% | 100% |

**Table 6: Comparative analysis of antibiotic susceptibility pattern of MRSA and MSSA isolates**

| Name of antibiotics | MRSA (n=31) N (%) | MSSA(n=69) N (%) | p value |
|---|---|---|---|
| **Amikacin ( 30µg)** | 21(67.7%) | 62(89.9%) | 0.003* |
| **Gentamycin (10µg)** | 14(45.2%) | 46(66.7%) | 0.021* |
| **Ciprofloxacin (5µg)** | 11(35.3%) | 54(78.3%) | <0.001* |
| **Ceftazidime ( 30µg)** | 10(32.3%) | 52(75.3%) | <0.001* |
| **Erythromycin (15µg)** | 9(29%) | 58(84.1%) | <0.001* |
| **Vancomycin (30µg)** | 31(100%) | 69(100%) | NA |

**Discussion**

In this study, we isolated 31(31%) MRSA out of 100 *S.aureus*isolates from various clinical specimens from patients admitted in different departments of our hospital. Out of 31 MRSA isolates, 15 were from male and 16 from female cases, so it can be inferred that, there is no gender predilection in acquisition of infection by an MRSA isolate. The prevalencerate

of MRSA was found to be 31% in our study, which is in accordance with the findings of studies from Anbumani N et al from Chennai (31%)[9] and Mehta AA et al from Mumbai (31.8%)[10] where as few studies from India has reported high prevalence rate of MRSA as compared to this study such as 46% by Arora S et al from Amritsar [11], 48.72% by Deepa S et al from Mysore, South India[12], 51.6% by Vidhani S et al from New Delhi[13], 54.85% by Anupurba S et al from Banaras Hindu University[6]. In another study in Nagpur the rate of MRSA was 19.5%[14] which is also low compare to this study. However, in another study from Indore, the rate of MRSA was very high 80.8%[15] as compared to this study. Above studies clearly shows that prevalence of MRSA varies from one setting to other and our hospital being a newer one, the prevalence rate has been found at lower levels and it might increase withtime.

In the present study the prevalence rate of MRSA was higher in age group of (31-40) year (32.25%) and no MRSA strains were seen in as (0-10) years. Majority of MRSA strains were obtained from medicine ward (41.7%), followed by surgery (38.1%) obs&gyne (35.7%), TB chest (33.3%), dermatology (33.3%), ENT (23.8%) and orthopaedics (20%). However, no inference can be drawn from these values, due to low sample size.

In the present study, comparison of two phenotypic methods proved that cefoxitin (30µg) disc diffusion method is better than oxacillin (1µg) disc diffusion method in screening of MRSA strains. Currently use of cefoxitin discs is recommended for better detection of MRSA strains in clinical specimens.[16] Another significant finding of this study hasbeen that, all MRSA isolates were significantly less sensitive to antibiotics, as compared to MSSA and this difference was found to be statistically significant (p<.0.001-.03), similar findings have been reported previously by other workers.[6,9,10,11,13-15] However, all *S. aureus*isolates were sensitive to vancomycin. Therefore, in our study the prevalence and vancomycin sensitivity pattern are in accordance with the previous studies from other places in India.

**Conclusion**

Methicillin-resistant *S. aureus*is one of the most common causes of nosocomial pathogen responsible for causing variety of human infections that may range from minor skin disease to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drug resistant organism is becoming more common.

In India, prevalence and incidence of MRSA from different studies has been in the range of 6.9% to 87%. In our study, prevalence rate of 31% in indoor patients has been found; however, a larger study is needed to confirm findings of our study. MRSA is a multidrug resistant organism, therefore AST of such isolates become important and our study shows high level of muti-drug resistance in nearly 75% of MRSA isolates. Moreover, screening of every *S. aureus*isolate by cefoxitin disc diffusion test is necessary for the early detection, treatment, prevention and control of MRSA strains in hospital environment. Nevertheless, regular monitoring of in hospital environment, personnel and patients for MRSA strains, should be done to keep this notorious pathogen undercheck.

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