PREVALENCE AND RESISTANCE PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL IN CENTRAL INDIA
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ABSTRACT: Methicillin resistant Staphylococcus aureus (MRSA) strains emerged soon after the introduction of methicillin into clinical practice. In addition to being a nosocomial pathogen, MRSA has become a community pathogen. This study reports the prevalence and antibiotic susceptibility pattern of MRSA in tertiary care hospital in central India. MATERIAL AND METHODS: A total of 1042 clinical specimens were collected and strains identified using conventional microbiological methods. Subsequently the antibiotic sensitivity test was performed for the confirmed MRSA isolates using cefoxitin disc diffusion method. RESULTS: Out of 109 strains of S. aureus isolated, 70(64.22%) were found to be methicillin resistant. Amongst all MRSA, 65(92.85%) were resistant to penicillin, oxacillin and gentamicin, 40(57.14%) were resistant to erythromycin, 3(4.28%) clindamycin resistant, 54(77.14%) tobramycin and 60(85.71%) amikacin resistant. However, all the MRSA strains were sensitive to vancomycin. CONCLUSION: The determination of prevalence and antibiotic sensitivity pattern of MRSA will help the treating clinicians effectively and preventing irrational use of antibiotics.

KEYWORDS: MRSA, antibiotic resistance pattern.

INTRODUCTION: Staphylococcus aureus is one of the most important pathogenic species among the genus Staphylococci. It has overcome most of the therapeutic agents that have been developed in the recent years and hence the antimicrobial chemotherapy for this species has always been empirical. The most notable example of this phenomenon was the emergence of methicillin resistant Staphylococcus aureus (MRSA), which was reported just one year after the launch of methicillin. Methicillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the mecA gene that permits the organism to grow and divide in the presence of methicillin and other beta-lactam antibiotics. The mecA gene is located on a mobile genetic element called staphylococcal chromosome cassette (SCCmec).

Since its first report in 1961, the strain has been progressively causing increased mortality, morbidity, and health care costs with skin and soft tissue infections, ventilator-associated pneumonia, catheter associated bacteremia, and many other infections in hospitals and communities. First detected in hospitals, methicillin resistance is now increasingly recognized in the community. Health care associated MRSA is a major cause of nosocomial infections worldwide, with significant attributable morbidity and mortality in addition to profound healthcare costs. 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.

Methicillin resistant S.aureus (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India to 50 per cent in South India. Community acquired MRSA
CAMRSA has been increasingly reported from India. Hence, the determination of prevalence and antibiotic sensitivity pattern of MRSA will help the treating clinicians effectively and preventing irrational use of antibiotics.

**MATERIAL AND METHODS:**

**Isolation and Identification of Staphylococci from Clinical Specimens:** Confirmation of the strains was done using standard microbiological procedures like catalase, slide and tube coagulase, and growth on Mannitol salt agar etc. S.aureus ATCC 25923 (mecA negative) and ATCC 43300 (mecA positive) were used for the quality control of all the tests.

**Antibiotic Susceptibility Testing** It was performed by Kirby–Bauer disc diffusion method for the following antibiotics procured from Hi-Media Laboratories Pvt. Ltd. according to Clinical and Laboratory Standards Institute (CLSI) guidelines:

- Penicillin 10U
- Oxacillin 1ug
- Ampicillin 10ug
- Tetracyclin 30ug
- Amikacin 30ug
- Erythromycin 15ug
- Trimethoprim/sulphamethoxazole 1.25ug/23.75ug
- Ciprofloxacin 5ug
- Tobramycin 10ug
- Netilin 30ug
- Chloramphenicol 30ug
- Linezolid 30ug
- Rifampicin 5ug
- Norfloxacin 10ug
- Nitrofurantoin 300ug
- Clindamycin 2ug
- Gentamicin 10ug

Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18- to 24-h blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and five discs were applied on a 100mm Mueller Hinton agar plate as per CLSI guidelines. S.aureus ATCC 25923 was used as the quality control strain for disc diffusion. After 24 hours of incubation at 35 °C, zone sizes were interpreted according to CLSI guidelines. Minimum inhibitory concentration of Vancomycin was determined. MIC interpretive Criteria (ug/ml) Sensitive <2, Intermediate 4-8, Resistant >/=16 according to CLSI guidelines.

All the confirmed S. aureus strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using cefoxitin discs (30μg). The isolates were considered methicillin resistant if the zone of inhibition was 21 mm or less.

**RESULTS:** The prevalence of MRSA was significantly different among various clinical specimens (p <0.001) and was found that 35.7% of these isolates were from throat swabs, followed by pus (33.6%) and 9.1% from wound swabs followed by rest of the samples. (Table 1)

Out of 109 S.aureus, 70 were MRSA and the remaining strains 39 were considered as methicillin sensitive S.aureus (MSSA). Amongst all MRSA, 65(92.85%) were resistant to penicillin, oxacillin and gentamicin, 40(57.14%) were resistant to erythromycin, 3(4.28%) clindamycin resistant, 54 (77.14%) tobramycin resistant, 60(85.71%) amikacin resistant whereas all strains were sensitive to ciprofloxacin, tetracyclin, linezolid and vancomycin. (Table 2)

**DISCUSSION:** MRSA is a major nosocomial pathogen causing significant morbidity and mortality. The 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. In this study, the prevalence of MRSA was 6.71% while, other studies such as in Lalitha et al 1988 has showed prevalence rate as 6.9%, and Chawla et al as 10.7% in 2008. As high as 35.7% of MRSA strains were obtained from throat swabs and 33.6% of strains were obtained...
from pus among clinical isolates. High isolation of MRSA in throat swab may suggest their colonization as in case of carriers. A study done by Ringberg H et al\textsuperscript{18} reported 33% MRSA in throat samples, they have concluded that throat is an important reservoir for MRSA and that samples taken from the throat should be included in screening patients for MRSA.

Similarly Dominik Mertz et al\textsuperscript{19} stated that colonization of the throat but not of the nares may be selectively colonized and escape routine screening programs. Unrecognized carriers may spread MRSA and render infection control programs futile. The addition of the throat cultures significantly increased the sensitivity of screening by 25.7%. Overall, 37.1% of subjects had nasal carriage and 12.8% were solely throat carriers. Similar observation was made by Mehta, who in his study on control of MRSA in a tertiary care center, had reported an isolation rate of 33% from pus and wound swabs.\textsuperscript{20} However, Qureshi from Pakistan reported a high isolation rate of up to 83% MRSA from pus.\textsuperscript{3}

Among 70 MRSA isolates, 65(92.85%) were resistant to penicillin, oxacillin and gentamicin, 40(57.14%) were resistant to erythromycin, 3(4.28%) clindamycin resistant, 54(77.14%) tobramycin resistant and 60(85.71%) amikacin resistant.

Although MRSA from clinical specimens showed higher susceptibility to individual antibiotics when compared with others, we obtained high percentage of multidrug resistant MRSA from these specimens. Majumder from Assam had reported 23.2% of the MRSA isolated from clinical specimens to be multidrug resistant.\textsuperscript{21}

Anupurba from Uttar Pradesh had reported a higher percentage of multidrug resistant MRSA.\textsuperscript{22} In this study, all MRSA strains were sensitive to ciprofloxacin, tetracycline, linezolid and vancomycin. Vancomycin continues to be the drug of choice for treating most MRSA infections caused by multi-drug resistant strains. But reports of emergence of low level Vancomycin has also been reported in MRSA strains.\textsuperscript{23}

De-escalation of vancomycin to β-lactams should be encouraged in all cases of MSSA. With MRSA isolates being widespread, it is imperative that treating physicians de-escalate to β-lactams once the culture sensitivity results reveal a MSSA isolate. Preservation of glycopeptides and linezolid for use only in MRSA cases should be encouraged.\textsuperscript{24}

To conclude, MRSA remains a major threat to health care modalities and the most effective way to prevent therapeutic crisis due to MRSA infection is to do continuous surveillance of the resistance profiles of Staphylococcus aureus isolates to formulate antibiotic policies and effective infection control program.

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| Samples         | Number | Percentage |
|-----------------|--------|------------|
| Throat swab     | 25     | 35.7       |
| Pus             | 24     | 33.6       |
| Blood           | 7      | 10.1       |
| Wound swab      | 6      | 9.1        |
| Urine           | 5      | 7.1        |
| Conjunctival swab | 3      | 4.2        |

Table 1: Distribution of samples among MRSA isolates (n=70)

| Antibiotic       | Resistance | Percentage |
|------------------|------------|------------|
| Cefoxitin        | 70         | 100        |
| Penicilin        | 70         | 100        |
| Oxacillin        | 70         | 100        |
| Ciprofloxacin    | 0          | 0          |
| Erythromycin     | 40         | 57.14      |
| Clindamycin      | 3          | 4.28       |
| Linezolid        | 0          | 0          |
| Tetracycllin     | 0          | 0          |
| Tobramycin       | 54         | 77.14      |
| Amikacin         | 60         | 85.71      |
| Gentamicin       | 70         | 100        |
| Co-trimoxazole   | 10         | 14.28      |
| Norfloxacin      | 12         | 17.14      |
| Nitrofurantoin   | 10         | 14.28      |
| Vancomycin       | 0          | 0          |
| Ampicillin       | 15         | 21.42      |
| Netilin          | 20         | 28.57      |
| Chloramphenicol  | 9          | 12.85      |
| Rifampicin       | 10         | 14.28      |

Table 2: Antibiotic resistance profile in MRSA isolates (n=70)
