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Original Research Article

Antibiotic Resistance Pattern in MRSA: Study in Central India

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Conflict of interest: Nil

Abstract

Introduction:- Infections caused by MRSA are worldwide, detecting the *mec*A gene or its product by PCR is recognized as a gold standard for detection of MRSA. In resource limited clinical settings phenotypic method which is simple, rapid, accurate and cost effective is required. Cefoxitin disc diffusion is considered as surrogate marker for *mecA* gene, and could be considered as gold standard for MR isolates. MRSA infection is of concern because it is resistant to a number of widely used antibiotics. Treatment options for MRSA are limited and less effective, than options available for susceptible *S. aureus* infections leading to increased morbidity and mortality in hospitalized patients. To control MRSA in hospitals, correct antibiotic treatment in infected patients is required and prevent their spread.

Object: This study is conducted to know the resistance pattern of various antibiotics in Methicillin-resistant *Staphylococcus aureus*(MRSA)

Material and Methods:

Type of study: Cross-sectional prospective analytical study

Study time: November 2012 to April 2014

Sample size: Total of 174 *S. aureus* isolated from non-repetitive clinical samples from IPD and OPD of tertiary care hospital in Bhopal.

Result: 174 *Staphylococcus aureus* strains isolated from the non-repetitive clinical samples were processed for MRSA identification. Out of 174 *S. aureus* isolates 69(39.65%) were found to be MRSA by cefoxitin disc diffusion test and rest 105 strains were MSSA. Among MRSA more than 70% resistance is for Ampicillin and Erythromycin and low resistance was for Netilmycin 27.53%, Doxycycline 24.63% and 5.79% for Linezolid and no resistance for Teicoplanin and Vancomycin.

Discussion: Multidrug resistance among *S. aureus* is a potential threat for the health care settings. Prolonged hospitalization and antibiotic therapy especially with β-lactam antibiotics predispose patients to the acquisition of MDR. To control and prevent the spread of MRSA in hospitals, correct antibiotic treatment in infected patients is required and ad mistered.

Keywords: MRSA(Methicillin resistant *Staphylococcus aureus*), MSSA(Methicillin resistant *Staphylococcus aureus*).

Introduction

Methicillin resistance is mediated by *mec*A gene which encodes an altered Penicillin Binding Protein (PBP) called PBP 2a. These PBP 2a exhibit very low affinity for Methicillin and other β lactam drugs. Methicillin resistance requires the presence of the chromosomally localized *mecA* gene. [1] SCC*mec* carries *mec*A gene responsible for Methicillin resistance. But *mec*A response to β- lactam antibiotics is regulated by *mec*I and *mec*R1. The gene *mec* A is carried on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC *mec*) [2] SCC*mec* typing is useful epidemiological tool for MRSA. [3,4]

Thus, resistance to Methicillin confers resistance to all β-lactam agents, including Cephalosporins. These strains may appear to be susceptible to cephalosporins on disc-sensitivity testing, but there usually is a significant population of microbes that is resistant to cephalosporins. Cephalosporin resistance may emerge during therapy.

There are some non *mec*A mediated mechanisms for expression of MRSA explaining the heterogenicity e.g There are about 20 accessory determinants (*fem*ABC, *fhm*B etc.) which are required for the expression of Methicillin resistance. Any alteration in these elements decreases the expression of Methicillin resistance in spite of the fact that PBP2a is present. The *fem* genes which play a role in cross-linking peptidoglycan strands contributing to the heterogeneity of expression of Methicillin resistance.[5]

The first MRSA was reported in United Kingdom in 1961,shortly after Methicillin was introduced into clinical practice. Seven years later, the resistant strains had become widespread in Japan, Europe and Australia.[6]

At the laboratory level, detection of MRSA by routine antibiotic susceptibility test must be done at the earliest for the better outcome of patients, to contain the infection and prevent their spread in this geographical area, those drugs which are not commonly prescribed by the clinicians might be a good alternative for MRSA in this area.

Material and Methods

This cross sectional prospective analytical study was carried out during November 2012 to April 2014 in the Department of Microbiology, People's College of Medical Sciences and Research Centre, Bhopal. A total of 174 *S. aureus* isolated from non-repetitive clinical samples from IPD and OPD of People's Hospital were included in study after Institutional Ethics Committee (IEC) approval. MRSA detected by Cefoxitin disc diffusion test.

Cefoxitin Disc diffusion test [7]

It was done using Cefoxitin (30µg) antibiotic disc. Inoculum of test isolate was prepared and incubated for 2 -3 hours. The turbidity after incubation was matched to 0.5 McFarland standard. After the standardization of the inoculum, a freshly prepared, dried MHA plate was inoculated for lawn culture using a sterile cotton swab stick. Cefoxitin 30µg disc was placed in the center and the plate was incubated aerobically at 35ºC ± 2ºC for 24 hours. The zone size was measured in reflected light and was interpreted as Resistant ≤ 21mm and Sensitive ≥ 22 mm as per CLSI guidelines.(Fig: 1 & 2)

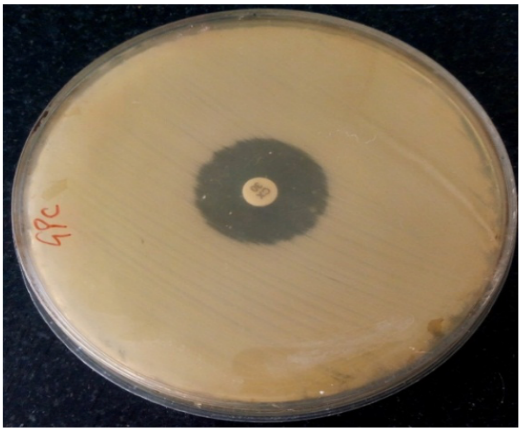


Fig:1 Cefoxitin Sensitive Isolate

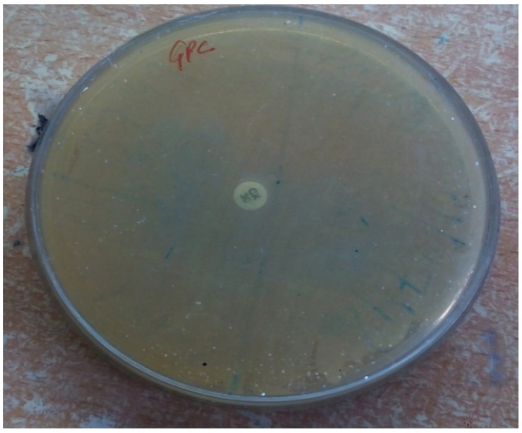


Fig:2 Cefoxitin Resistant Isolate

Two standard strains, one MSSA ATCC (29213) and one MRSA ATCC (43300) were included in each batch of testing by different method.

Antibiotic Susceptibility Test (AST) [8]

Routine antibiotic susceptibility was done by Kirby-Bauer`s disc diffusion method The panel of antibiotic discs used were obtained from HIMEDIA.

Method

Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4 ml of nutrient broth and incubated for 2-3hours. The turbidity after incubation was matched to 0.5 McFarland standard (Contains 10^8CFU/ml). Using a sterile swab, inoculate a freshly prepared, dried Mueller-Hinton-Agar (MHA) plate. Remove excess fluid by pressing and rotating the swab against side of the tube. Streak the swab evenly over the surface of the medium in 3 directions, rotating the Plate approximately 60º to ensure even distribution. With the petri dish lid in place, allow 3-5 min for the surface of agar to dry. Using a sterile forcep place antibiotic disc on the inoculated plate and incubated it aerobically at 35ºC for 16-18 hours.

Control strains: control strain is used to test the performance of the method.

Staphylococcus aureus ATCC 25923

Interpretation: Examine the control and test plates to ensure the growth is confluent. Use a ruler on the underside of the plate to measure the diameter of each zone of inhibition in mm. Once the zone sizes are recorded, they are interpreted as sensitive or resistant as per CLSI guidelines 2013 [9].

Vancomycin susceptibility test [10]

Vancomycin susceptibility testing was done by Himedia Oxacillin- Vancomycin Ezy MIC strip.

Material required: Ezy MIC- Oxacillin-Vancomycin (EM-063) MHA with 2% NaCl 0.5 McFarland standard

Method: Prepare plate with suitable Muller Hinton Agar with 2% NaCl. Dip a sterile non-toxic cotton swab in to a standardized inoculum (0.5 McFarland) and streak the entire agar plate. Then apply Ezy MIC Oxacillin-Vancomycin strip as per manufacturer's instruction. Incubate the plates at 35ºC ± 2ºC for 24 hours for ORSA and 48 hours for VISA strain. Read the plates only when sufficient growth is seen and MIC where the ellipse intersects the MIC scale on the strip as per manufacturer's instruction.

Control: *S. aureus* (MSSA) ATCC 29213 and *S. aureus* (MRSA) ATCC 43300

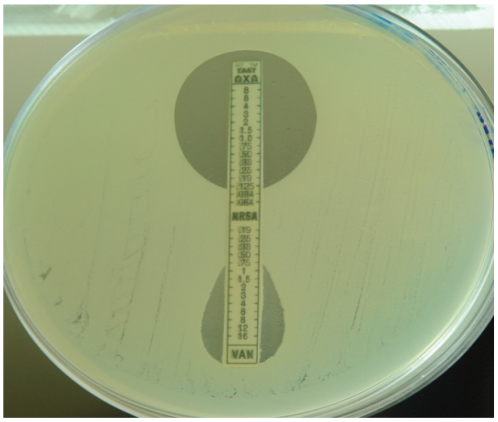


Figure 16: Oxacillin sensitive, Vancomycin sensitive

Figure 17: Oxacillin resistant, Vancomycin sensitive

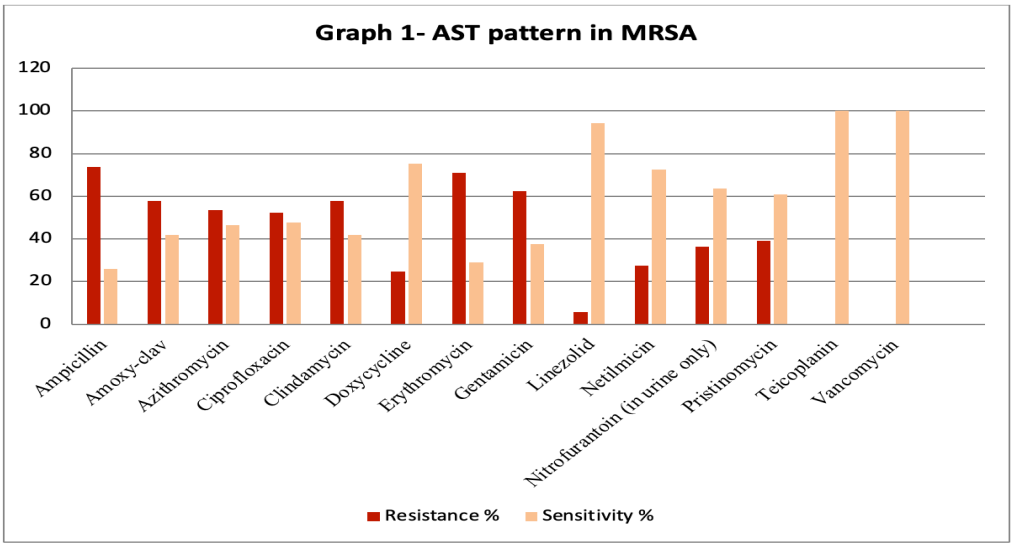
Result

A total of 174 *Staphylococcus aureus* strains isolated from the non-repetitive clinical samples were included and processed for MRSA identification. Out of 174 *S. aureus* isolates 69(39.65%) were found to be MRSA by cefoxitin disc diffusion test and rest 105 strains were MSSA. The MRSA and MSSA strains were subjected to all such Antibiotics like Ampicillin(10µg), Amoxycillin- clavulanic acid (20/10µg), Azithromycin (15µg), Ciprofloxacin (10µg), Clindamycin (2µg), Doxycycline (30µg), Erythromycin (15µg), Gentamicin (10µg), Linezolid (30µg), Netilmicin(30µg), Nitrofurantoin(300µg) (in urine only) Pristinomycin(15µg) Teicoplanin(30 µg) and Vancomycin.

Maximum resistance in our setting was with antibiotic Ampicillin 73.91% and Erythromycin 71.01% while low resistance was for Netilmycin 27.53%, Doxycycline 24.63% and 5.79% for Linezolid and no resistance for Teicoplanin and Vancomycin. As depicted in Graph (1). Table 1 depicts antibiotic resistance pattern in MRSA.

Table 1: Antibiotic Resistance pattern in MRSA

Antibiotic	n = 69	Resistance %
Ampicillin	51	73.91%
Amoxycillin-clavulanic acid	40	57.97%
Azithromycin	37	53.62%
Ciprofloxacin	36	52.17%
Clindamycin	40	57.97%
Doxycycline	17	24.63%
Erythromycin	49	71.01%
Gentamicin	43	62.31%
Linezolid	4	5.79%
Netilmicin	19	27.53%
Nitrofurantoin (in urine only)	4	36.36%
Pristinomycin	27	39.13%
Teicoplanin	0	0.00%
Vancomycin	0	0.00%

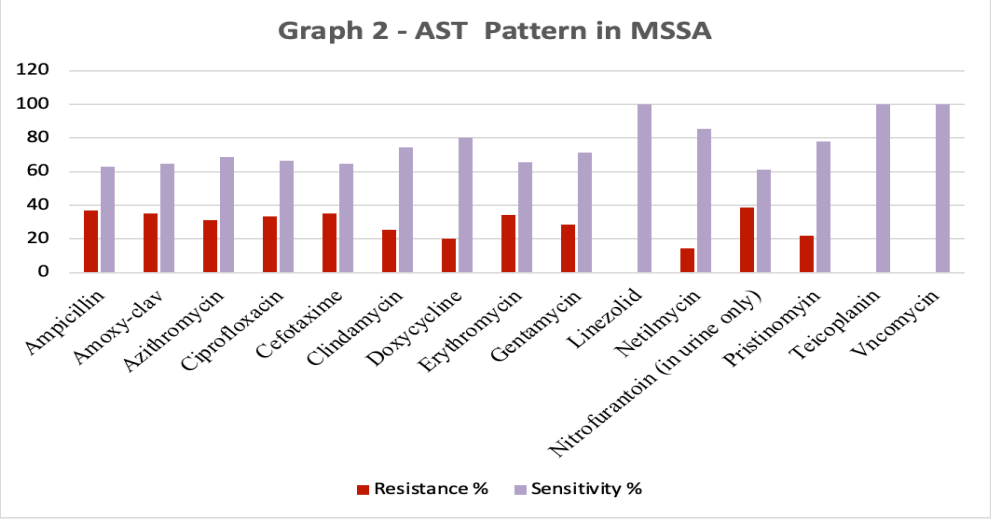


Graph 1: Showing Sensitivity and Resistance Pattern among Methicillin resistant Staphylococcal aureus

Maximum resistance shown in MSSA was with Ampicillin 37.14%, Amoxy-clav and Ciprofloxacin 35.23%, while no resistance was shown for Linezolid, Teicoplanin and Vancomycin as depicted in Graph (2). Table-2 shows antibiotic resistance pattern in MSSA.

Table 2: Antibiotic Resistance Pattern in MSSA

Antibiotic	n = 105	Resistance %
Ampicillin	39	37.14%
Amoxycillin-clavulanic acid	37	35.23%
Azithromycin	33	31.42%
Ciprofloxacin	35	33.33%
Clindamycin	27	25.71%
Doxycycline	21	20.00%
Erythromycin	36	34.28%
Gentamicin	30	28.57%
Linezolid	0	0.00%
Netilmicin	15	14.28%
Nitrofurantoin (in urine only)	7	38.88%
Pristinomycin	23	21.90%
Teicoplanin	0	0.00%
Vancomycin	0	0.00%



Graph 2: Showing sensitivity and resistance pattern in Methicillin sensitive Staphylococcus aureus

Discussion

Testing of Methicillin Resistance in *S. aureus*, has been a challenge for clinical laboratories in recent years. Several studies have been showed that detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories [11]. Some genes may be silent or non-functional hence organism is unable to express resistance but may be over diagnosed by genotypic method, hence molecular method cannot be specific for few resistant mechanism when majority of susceptibility testing is by phenotypic method. [12]

There are some non *mec*A mediated mechanisms for expression of MRSA explaining the heterogenicity. Any alteration in these elements decreases the expression of Methicillin resistance in spite of the fact that PBP2a is present. The fem genes which play a role in cross- linking peptidoglycan strands contributing to the heterogeneity of expression of Methicillin resistance. [5]

However, most laboratories especially in developing countries are not in position to perform molecular methods. Limitations with genotypic method can overcome by using phenotypic methods as well. In various study results of Cefoxitin disc diffusion test are in concordance with the PCR for *mec*A gene. Thus, the test can be an alternative to PCR for detection of MRSA in resource constraint settings. [13,14] Cefoxitin disc diffusion is considered as surrogate marker for *mecA* gene. [15]

In this study the resistance pattern for MRSA and MSSA were detected.

Among MRSA isolates Resistance was as observed 73.91% to Ampicillin, 71.01% to Erythromycin, 62.31% to Gentamicin, 57.97% to Amoxycillin-clavulanic acid, 57.97% to Clindamycin, 53.62% to Azithromycin, 52.17% to Ciprofloxacin, 39.13% to Pristinomycin, 27.53% to Netilmicin, 24.63% to Doxycycline, 5.79% to Linezolid. There is no resistance for Teicoplanin & Vancomycin.(Table-1,graph-1)

Other studies like Pramodini et al find 85.00% resistance to Ampicillin, 80.00% to Erythromycin & 30.00% to Ciprofloxacin. In Mohanasundaram et al [16] 88.00% resistance to gentamycin, 85.00% Erythromycin, 97.00% Ciprofloxacin, 30.00% Netilmicin. In Karami et al [17] 97.00% resistance to Erythromycin and Clindamycin and 95.28% Gentamicin.

In present study there is high resistance for Ampicillin, Erythomycin, Gentamicin, Amoxy-clav, Clindamycin, Azithromycin and Ciprofloxacin because of its frequent use in the wards as prescribed. While Netilmycin and Doxycycline show less resistance as compared to other studies because in this geographical area, these drugs are not commonly prescribed by the clinicians. So it might be a good alternative for MRSA in this area.

Owing to the increasing numbers of infections caused by multiresistant MRSA, the Linezolid & the Glycopeptides- Vancomycin and Teicoplanin have become the drugs of choice for treatment of staphylococcal nosocomial infections.

Among MSSA isolates, 37.14% to Ampicillin, 35.23% to Amoxycillin clavulanic acid, 35.23% to Ceftriaxone, 34.28% to Erythromycin, 33.33% to Ciprofloxacin, 28.57% to Gentamicin, 25.71% to Clindamycin, 31.42% to Azithromycin, 21.90% to Pristinomycin,14.28% to Netilmicin, 20.00% to Doxycycline. There is no resistance for Linezolid, Teicoplanin and Vancomycin.(Table 2, graph-2)

The emergence of Methicillin resistant strains due to a different resistance mechanism also contain insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. [18] The overall prevalence of MRSA isolation has gradually increased over a period of time in India from 12.00% in 1992 to 81.00% in 1999. [19] In the present study, 39.65% of *S. aureus* were MRSA. Recent studies during 2011 from various parts of India report that, the overall prevalence of MRSA ranges from 24.00% to 78.00%. [20] We also observed that the antibiotic resistance among MRSA was higher than MSSA.

Multidrug resistance among *S. aureus* is a potential threat for the health care settings. Prolonged hospitalization and antibiotic therapy especially with β-lactam antibiotics predispose patients to the acquisition of MDR. Hospital acquired MDRS are usually associated with increased expression of multiple antibiotic resistance genes, including those coding for Aminoglycoside resistance.

At the laboratory level, detection of MRSA by routine antibiotic susceptibility test must be done at the earliest for the better outcome of patients, to contain the infection and prevent their spread.

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