

Original Research Article

To determine antibiotic susceptibility pattern along with Methicillin Resistance in the isolated *Staphylococcus aureus* – A study in Fathima Hospital

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Abstract

Background: Over the last four decades Methicillin Resistant *Staphylococcus aureus* (MRSA) has spread throughout the world and has become highly endemic in many geographical areas.

Materials and methods: Methicillin resistance was determined by 2 methods: Disk diffusion method using Oxacillin 1µg disk and MIC HiComb strips.

Results: Out of 170 isolates 105 strains were Coagulase Positive and 65 strains Coagulase Negative Staphylococci. Most effective markers were Thermonuclease test and growth on high salt agar. 89 (84.76%) of the 105 isolates showed resistance to Penicillin, 54 (51.42%) to Amyoxyclav, 81 (77.14%) to Cefdinir, 61 (58.0%) to Cefepime, 86 (81.92%) to Gentamicin, 44 (41.9%) to Clindamycin, 40 (38.0%) to Amikacin, 82 (78.0%) to Erythromycin, 51 (48.57%) to Cotrimoxazole and 69 (65.71%) to Ofloxacin. Maximum resistance was seen for Penicillin and least to Amikacin. Oxacillin Disc diffusion method: Among 105 isolates 48 (45.7%) were susceptible to Oxacillin, 9 (8.57%) showed intermediate sensitivity and 48 (45.7%) were resistant to Oxacillin. MIC Determination by MIC HiComb strips: Among 105 isolates 59 (56.1%) showed MIC ≤ 2 µg indicating susceptible strains and 46 (43.8%) isolates showed MIC 4 ≥ µg indicating Methicillin resistance.

Conclusion: The antimicrobial resistance pattern in the present study gives serious reason for concern because majority of the strains are highly resistant to commonly available antibiotics. Surveillance studies should be carried out in every geographical area to detect the prevalence of MRSA strains and

appropriate infection control measures should be performed. In conclusion, considering the increasing occurrence of MRSA infections, highly reliable, accurate and rapid testing for Methicillin Resistance is essential for both antibiotic therapy and infection control regimens.

Key words

Antibiotic susceptibility pattern, Methicillin resistance, Antimicrobial resistance, *Staphylococcus aureus*.

Introduction

Since ages mankind is fighting an undeclared war against microorganisms for their survival. These microorganisms are causing untold miseries to human beings. One of the commonest yet notorious among them is *Staphylococcus aureus* [1-7].

Over the last four decades Methicillin Resistant *Staphylococcus aureus* (MRSA) has spread throughout the world and has become highly endemic in many geographical areas. HA-MRSA strains are most significant human pathogens among nosocomial infections and have been recognized as one of major challenges in control of hospital infections. These multidrug resistant isolates act as reservoir for the drug resistant gene. Recent emergence of Vancomycin Resistant *Staphylococcus aureus* (VRSA) strains has still further complicated the diagnosis and treatment of *Staphylococcus aureus* [8-17].

Clinicians must be aware that prevalence and antibiograms vary widely among various populations and must become familiar with patterns in their own community in order to select appropriate antibiotics for treatment. Surveillance of antimicrobial susceptibility along with aggregation of institutional antibiograms is critical for developing strategies to control increasing antimicrobial resistance and monitoring resistance trends in a population [18-26].

Therefore appropriate approach in rapidly and accurately identifying *Staphylococcus aureus* isolates along with choosing correct initial empirical antibiotics minimizes likelihood of

promoting resistance to existing antibiotics and emergence of resistance to newer antibiotics.

Materials and methods

The specimens for the present study were collected from patients attending to Fatima Institute of Medical Sciences Hospital, Kadapa over a period from April 2014 to September 2014. Various specimens – Pus, Wound Swab, Sputum, Throat Swab, Blood and Urine – were collected from patients of all ages. Out of 170 samples collected *Staphylococcus aureus* was isolated from 105 samples by Tube Coagulase Test.

Collection of Samples

Wound Swabs, pus, throat swabs, sputum, blood and urine are the samples collected under Aseptic conditions. Direct smears were made from samples and stained by Gram's stain to look for Gram Positive Cocci in singles, short chains, pairs or clusters. Samples were then inoculated onto Nutrient agar, Blood Agar and MacConkey's agar. Presumptive identification of staphylococcal colonies was done by colony morphology, Gram's stain and Catalase test.

Nutrient agar: Golden yellow colonies, butyrous, smooth, opaque, convex and 1 mm in size.

Blood agar: Hemolytic/ Non-Hemolytic, smooth, low convex, glistening and opaque colonies

MacConkeys agar: Fine Lactose fermenting colonies were observed.

Gram's stain was done on smears made from colonies morphologically resembling staphylococcus colonies – Violet colored

spherical cocci arranged in clusters resembling *Staphylococcus* were identified. Colonies were subcultured in Nutrient Broth for further study, incubated for 4 - 6 hours at 37°C. Tube coagulase test was done.

Tube Coagulase Test

Plasma was diluted in the proportion of 1 in 6 with normal saline. 0.5 ml of plasma was added to 2 sterile test tubes. 0.5 ml of overnight broth culture was added to one tube and incubated at 37°C. The other tube of diluted plasma was kept as control without addition of culture. Tubes were examined for coagulation at 1, 3, 6 and 24 hours. Plasma is converted to a stiff gel [27].

Grading

- A) 4+: Coagulum remains in place even when tube is inverted.
- B) 3+: Large clot
- C) 2+: Small organized clot
- D) 1+: Small unorganized clot

Antibiogram was done using following antibiotics

Modified Kirby-Bauer Disk Diffusion method was done as per CLSI standards.

Penicillin, Amoxycylav, Cefdinir, Cefepime, Gentamicin, Erythromycin, Clindamycin, Cotrimoxazole, Amikacin and Ofloxacin antibiotic discs were available from Himedia laboratories.

Methicillin Resistance was tested by

- Disk diffusion method using Oxacillin 1 µg disk.
- MIC HiComb strips

Susceptibility to Vancomycin was tested using MIC HiComb Strips

Note: All tests were standardized using *Staphylococcus aureus* NCTC 6571 strain. Turbidity was standardized by comparing with McFarland Tube 1 (0.5)

Antibiogram: Antibiotic sensitivity of the isolates was tested using Modified Kirby – Bauer disc diffusion method.

Methicillin Resistance was determined by 2 methods

Mueller-Hinton High Salt Agar having 5% sodium chloride was prepared. Staphylococcal broth cultures were inoculated into the media and oxacillin 1µg disc is placed on it. The plate was incubated at 32°C for 48 hours.

Interpretation: CLSI Standards

- Susceptible: ≥ 13 mm
- Intermediate: 11-12 mm
- Resistant: ≤ 10 mm

Minimum Inhibitory Concentrations were estimated using Himedia MIC HiComb strips. Mueller-Hinton agar was used. MIC HiComb strips were placed on the agar surface with the MIC scale facing upwards.

Interpretation

Zone of inhibition will be in the form of an ellipse. MIC value is where the zones convene with the comb like projections of the strips (not the handle). Each comb represents an antibiotic disk of different concentration [28-30].

Vancomycin susceptibility testing

It was done as described for methicillin using MIC HiComb strips.

Interprétation: CLSI Standards

- Susceptible – MIC ≤ 4 µg
- Intermediate – MIC 8-16 µg
- Resistant – MIC ≥ 32 µg

Results

Over a period of six months, 170 *Staphylococcus* strains were isolated from various samples based on Gram's Stain and Colony Morphology (Table – 1).

Distribution of 105 isolates of *Staphylococcus aureus* among various samples collected

Out of 105 *Staphylococcus aureus* strains isolated, 40 (38.09%) were from Wound swabs, 31 (29.5%) from Pus, 15 (14.2%) from Blood, 7

(6.6%) from Throat swab, 6 (5.7%) from Sputum and 6 (5.7%) from Urine.

Resistance Pattern of 105 *Staphylococcus aureus* isolates

89 (84.76%) of the 105 isolates showed resistance to Penicillin, 54 (51.42%) to Amyoxyclav, 81 (77.14%) to Cefdinir, 61 (58.0%) to Cefepime, 86 (81.92%) to

Gentamycin, 44 (41.9%) to Clindamycin, 40 (38.0%) to Amikacin, 82 (78.0%) to Erythromycin, 51(48.57%) to Cotrimoxazole and 69 (65.71%) to Ofloxacin. Maximum resistance was seen for Penicillin and least to Amikacin.

Detection of MRSA

MRSA was detected by 2 methods as per **Table – 2** and **Table – 3**.

Table - 1: Total number of samples processed.

Total number of specimens	Coagulase positive <i>Staphylococcus</i>	Coagulase negative <i>Staphylococcus</i>
170	105	65

Table - 2: Oxacillin Disc diffusion method.

Method	Susceptible $\geq 13\text{mm}$	Intermediate 11 – 12mm	Resistance $\leq 10\text{mm}$
Disc diffusion method	48/105 45.7%	9/105 8.57%	48/105 45.7%

Table - 3: MIC Determination by MIC HiComb strips.

Method	Susceptible $\leq 2\mu\text{g}$	Resistant $>4\mu\text{g}$
MIC comb strip	59/105 56.1%	46/105 43.8%

Among 105 isolates 59 (56.1%) showed MIC $\leq 2\mu\text{g}$ indicating susceptible strains and 46 (43.8%) isolates showed MIC $4 \geq \mu\text{g}$ indicating Methicillin resistance. MIC HiComb Strip method was more sensitive in isolating MRSA strains than oxacillin disc diffusion method (which detected false positives) as per **Table – 4**.

Distribution of MRSA among the 105 specimens collected

Maximum number of MRSA strains were isolated from Wound swabs at 23 (57.5%) followed by Throat swabs – 57.1%, Pus 38.7%, Sputum 33.3%, Blood 26.6%, and Urine 16.6% (**Table – 5**).

Resistance pattern among the 46 MRSA isolates

All 46 (100%) isolates were resistant to Penicillin, 40 (86.95%) to Amoxyclav, 43 (93.47%) to Cefdinir, 42 (91.30%) to Cefepime, 41 (89.13%) to Gentamicin, 40 (86.95%) to Erythromycin, 18 (39.13%) to Clindamycin, 22 (47.80%) to Amikacin, 35 (76.0%) to Cotrimoxazole and 38 (82.60%) were resistant to Ofloxacin.

MIC's for Vancomycin among 46 MRSA strains using MIC HiComb strip

All isolates were sensitive (MIC $\leq 4\mu\text{g/ml}$) to Vancomycin. Maximum resistance among MRSA isolates was seen towards Penicillin and least resistance towards Clindamycin apart from Vancomycin to which all strains were susceptible.

Table - 4: Comparison of Disc diffusion method with MIC HiComb Strip method.

Method	Resistant isolates	Percentage
Disc diffusion method	48/105	45.7%
MIC HiComb method	46/105	43.8%

Table - 5: Percentage of MRSA among 105 isolates of *Staphylococcus aureus*.

No. of isolates	MRSA	Percentage
105	46	43.8%

Conclusion

Majority of the *Staphylococcus aureus* strains were obtained from Wound swabs and Pus. Antimicrobial susceptibility testing for 105 isolates showed maximum resistance to Penicillin (84.76%), followed by Gentamicin (81.92%). Maximum sensitivity was seen towards Amikacin. 43.8% of the 105 isolates were MRSA strains, identified by MIC HiComb strip method. Majority of the MRSA strains were isolated from Wound swabs (57.5%) and least from Urine (16.6%). Multidrug resistance was seen among MRSA strains with maximum resistance to Penicillin (100%) and least resistance to Clindamycin (39.13%). All the MRSA isolates were sensitive to Vancomycin.

The antimicrobial resistance pattern in the present study gives serious reason for concern because majority of the strains are highly resistant to commonly available antibiotics. Surveillance studies should be carried out in every geographical area to detect the prevalence of MRSA strains and appropriate infection control measures should be performed. In conclusion, considering the increasing occurrence of MRSA infections, highly reliable, accurate and rapid testing for Methicillin Resistance is essential for both antibiotic therapy and infection control regimens.

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