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Original Research Article

Microbiological Study of MRSA IsolatedFrom Wound Samples

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Abstract:

Background: Staphylococcus aureus present on skin and nasal passage, enter through the cuts or invasive procedures. It is responsible for a number of infections such as wound infections, deep infections that spreads from skin to cause bacteremia with or without endocarditis. It may also involve bone, joints, deep organs and tissues.

Material and Methods: Total 150 isolates of MRSA collected from wound samples were characterize by different biochemical tests. Strains were tested with Mannitol salt agar out of 150 strains 145 strains were positive (96.6%), DNase test show's 143 wre positive (95.3%) out of 150 strains, Phosphatase test show's 146 (97.6%) strains were positiveout of 150 strains, Gelatin hydrolysis show's135 (90%) were positive out of 150 strains and Urease test show's 142 (94.6%) were urease positive and 8 (5.4%) were negative.

Conclusion: Staphylococcus species is a major concern for the medical community. In the past, patients were commonly treated with various Pencillin, Clindamycin Erythromycin and /or Gentamycin for Staphylococcal infections. However, owing to many factors, including the extensive use of these antibiotics *Staphylococci* have developed resistance.

Keywords: Antibiotic susceptibility pattern was available for 150 iosolates. out of this pencillin resistance to pencillin was (100%) followed by Erythromycin (51.3%), Tetracyclin (44.6%), Gentamycin (24%), Chloramphenicol (3.3%), Rifampicin (7.3%), Teicoplanin 0% and Vancomycin 0% were found.

Introduction

Gram positive cocci, particularly *Staphylococci aureus are* most frequently isolated in the Microbiology laboratory second only to Enterobacteriaceae. Methicillin resistant *Staphylococcus aureus (MRSA)* is a strain of *Staphylococci* that has become resistant to the antibiotic Methicillin. *Staphylococcus aureus* present on skin and nasal passage, enter through the cuts or invasive procedures. It is responsible for a number of infectionssuch as wound infections, deep infections that spreads from skin to cause bacteremia with or without endocarditis. It may also involve bone, joints, deep organs and tissues. Methicillin resistant *Staphylococcus aureus* (MRSA) is increasingly being reported as multi-drug resistant with high resistance to macrolides (Erythromycin, Clarithromycin) and lincosamides (Clindamycin, Lincomycin). Rapid and accurate diagnosis of MRSA is important for proper management, prevention of transmission and to start correcting treatment. [1] In the present study characterization of MRSA was done by using biochemical tests. Their antibiotic susceptibility pattern was also studied. Different methods for the detection of MRSA like Oxacillin disc diffusion and Cefoxitin disc diffusion, MIC of Oxacillin by agar dilution and broth dilution were done and compared. MRSA is an important cause of nosocomial pathogen and it continuesto be a cause of significant morbidity and mortality [2] . It causes nosocomialand community acquired infections. Infected and colonized patients providethe primary reservoir and transmission is mainly through hospital staff. MRSA present on skin and nasal passage, enter through the cuts or invasive procedures. It is responsible for a number of infections such wound infections.It can also be a causative agent of deep infection that spreads from skin to cause bacteremia with or without endocarditis. It may also involve bone,joints, deep organs and tissues. The risk factors which contribute to MRSAare excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalization [3]. The incidence of MRSA has been on the rise for the past 20years [4]. It has undergone rapid evolutionary changes and epidemiological expansion, and it has spread beyond the confines of health carefacilities [5].

Objectives

To Characterize 150 MRSA isolates from wound samples.

To Study their antibiotic susceptibility patterns

To detect MRSA by Oxacillin disc diffusion, Cefoxitin disc diffusion and resistance to Oxacillin by the MIC method. [6]

Materials and Methods

The study was conducted in the department of Microbiology at Indira gandhi Institute of Medical Sciences, Patna Bihar. 150 consequtive MRSA strains as identified by Cefoxitin disc diffusion test were further characterised. The isolates were obtained from the following clinical samples.

Wounds swabs and aspirates: 142 Swabs received in duplicate, and aspiratesreceived in sterile containers in the lab. Blood(Bacteremia): 8 blood samples received in Brain Heart Infusion Broth (BHI) bottles.

Wound swabs and aspirates received in sterile containers were immediately processed. If there was any delay in processing the samples were then kept in refrigerator. Samples were plated on 5% sheep blood agar, Mc. Conkey agar and Thyoglycolates respectively. Plates were incubated t at 37°C. After overnight incubation colony morphology and hemolysis was observed. Grams stain was done on white colony showing lysis on blood agar. Colonies showing Gram positive cocci arranged in clusters were subjected to coagulase test by slide and tube coagulase . Coagulase positive strains were subjected to antibiotic susceptibility by the modified Kirby Bauer method and isolates showing methicillin resistance in the screening by Cefoxitin disc diffusion (MRSA) were included in study.

Inclusion Criteria: *Staphylococcus aureus* resistant toCefoxitin (<20).

Exclusion Criteria: Organisms other than MRSA Colonies were inoculated on 5% sheep blood agar and incubated overnight in a 5% CO2 atmosphere. A narrow zone of clearing around the colonies was looked for. Any strains showing clearing around the colonies was considered hemolytic and no clearing was considered non-hemolytic.

Performance of test: MIC by microbroth dilution for above stated antibioticswere performed in a microtitre dilution plate having 96 wells. Controls wells were one for viability (growth) control which had in it 100 µl each of sterile Mueller Hinton Broth (MHB) and test organism. Other was sterility control well with 100 µl of Mueller Hinton Broth only. All other wells were loaded with 100 µl of MHB, 90 µl appropriate antimicrobial dilutions and finally 10 µl of standardized inoculum (adjusted to 0.5 MacFarland standard).

Reading: Reading was done by a parabolic mangnifying mirror and tray stand that allows clear visual inspection of the under slides of the titre plates. The growth control well was examined for the organism viability and MIC for the control strain was confirmed. Then, the MIC of the test organism was recorded.

Results

All isolates subjected to MSA, 5were negative, 145were positive.

Table 1:

MSA POSITIVE	**145**
MSA NEGATIVE	**05**
TOTAL	**150**

DNase test

Table 2:

DNase POSITIVE	**143**
DNase NEGATIVE	**07**
TOTAL	**150**

All isolates subjected to DNase test, 7 were negative, 143 were positive.

Table 3:

Phosphatase POSITIVE	**146**
Phosphatase NEGATIVE	**04**
TOTAL	**150**

All isolates subjected to phosphatase, 4 were negative,146 were positive.

Gealatin Liquefaction Test

Table 4:

Gelatinase POSITIVE	**135**
Gelatinase NEGATIVE	**15**
TOTAL	**150**

All isolates subjected to Gelatin hydrolysis test, 15were negative,135werepositive.

Table 5:

Urease POSITIVE	**142**
Urease NEGATIVE	**08**
TOTAL	**150**

All isolates subjected to urease production, 8 were negative, 142 were positive.

Detection of MRSA was done with cefoxitin disc diffusion test. Oxacillin disc diffusion compared with cefoxitin. By oxacillin disc diffusion test 137 (97%) isolates were detected as MRSA out of 141(MRSA by MIC) isolates.

All the strains which were detected as MRSA by MIC method were also resistant by the Cefoxitin disc. However, 9 strains detected as sensitive by MICmethod were resistant by the Cefoxitin disc diffusion

Table 6:

TESTS	Detectedas MRSA	Detected as MSSA	total
Oxacillin disc	**137**	**13**	**150**
Cefoxitin disc	**150**	**0**	**150**
MIC of oxacillin	**141**	**9**	**150**
Oxaciilin screening agar	**128**	**22**	**150**

Detection inducible clindamycin resistance

Out of 150 isolates 66 were Ethromycin resistant and Clindamycin susceptible. These isolates were subjected to detection of inducible Cindamycin resistance by D test, 17mm(edge-edge) distance was kept between Erythromycin(15mcg) and Clindamycin (2mcg)discs. All 66 isolates were positive by D test.

Table 7:

Test	Total	
Inducible Clindamycin resistance detected	66	66
No inducible Clindamycin resistancedetected	00	66

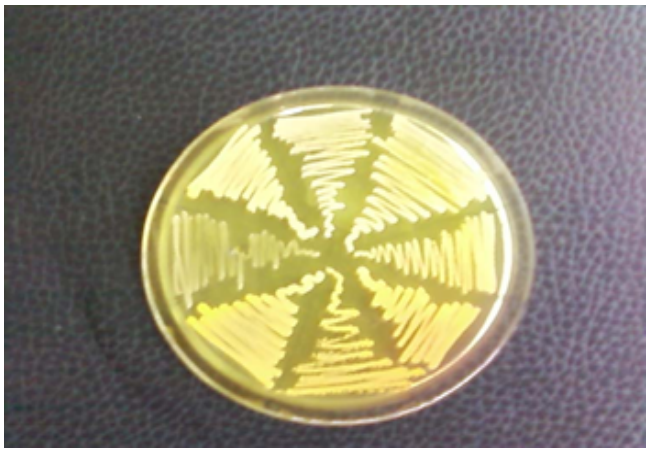
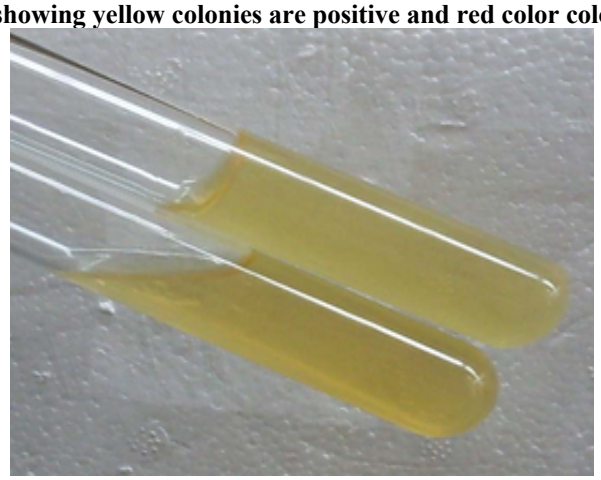
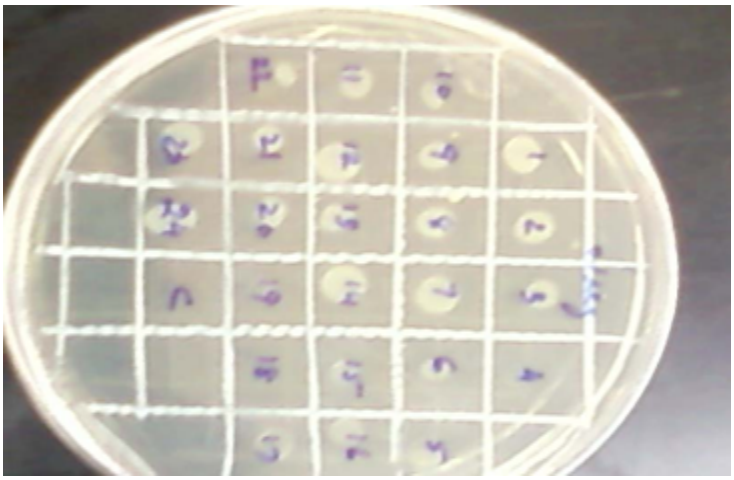


Figure 1: Msa showing yellow colonies are positive and red color colonies are negative



Gelatine liquefaction test showing gelatine liquefaction negative(top) and gelatine liquefaction positive (bottom)



Mic by agar dilution showing high resistance.

Discussion

Staphylococcus aureus is a major pathogen responsible for nosocomial and community acquired infection and MRSA has emerged as a major nosocomial pathogen and an increasingly frequent cause of community acquired infections that cause significant morbidity and mortality. Increased report of multidrug resistance in MRSA is a major concern in treating patients with MRSA. The various tests done for the characterization of this important pathogen are discussed below. [7]

Haemolysis:

138(92%) were strains showing haemolysis and 12 strains(8%) did not show haemolysis on 5% sheep blood agar. Beta lytic type of haemolysis was noted just under the colonies which were enhanced in an atmosphere of CO2. The zone of haemolysis on sheep blood are narrow and hazy and many CONS species may also produce lysis. Hence hemolysis alone it not useful assigninga species. [8]

Pigment Production

Pigment production was observed in 98% of our strains and helps in differentiation of *Staphylococcus* species but is not confirmatory. *Staphylococcus aureus* produce golden yellow pigment and *coagulase negative Staphylococcus* produce white colour pigmentation on nutrient agar or MHA. [9]

Coagulase Test

Coagulase test was helpful in identifying species as coagulase positive or negative and established them into two broad groups. Isolates showing coagulase positive were included in the study. There are other strains which give coagulase positive are *Staphylococcus intermedius* and *S.hyicus* which aremainly seen in animals, so coagulase test can be used to identify the pathogenic *Staph.aureus* in human. Though not 100% accurate, tube coagulase is still a clinically valuable test, particularly in experienced hands, because of its low cost and simplicity.

Mannitol Salt Agar

96.6% of the strains were positive and 3.4% were negative in the present study. Zheolin Ham, described mannitol salt agar specificity to be 99.6% and sensitivity 76.5 at 24 hrs incubation and after 48 hrs incubation sensitivity 95.8% and sensitivity 84.3%. MSA can also be used as a selective medium for detection of MRSA by adding Oxacillin or Cefoxitin to the medium.

Urease Test

In our study 142 (94.6%) were urease positive and 8 (5.4%) were negative. E.

E. Udo, N. Al-Sweih et al [10], report that most of the urease-positive isolates were from wound or skin samples, which are sources usually associated with CA- MRSA.)

DNase Test

In this study 143 (95.3%) were positive and 7 (4.7%) were negative. Deoxyribonuclease (DNase) plates can be used to screen isolates but, as variousamounts of DNase are produced by *CONS* also, positives should be confirmed with an additional test. Heat-stable nuclease tests can be used to identify *S. aureus*, although some rare coagulase-negative species can be positive. [11] Therefore DNAase test may not be a confirmatory for identification S*taph. aureus.*

Phosphatase Test

In this study 146 (97.6%) were positive and 4 (3.4%) were negative. Originally the assay of this enzyme was used for separating *Staphylococcus aureus* from other *Staphylococci*, as it was thought that only the former possessed phosphatase activity. Later it was shown that a significant percentage of non-*Saureus staphylococci* also carry this enzyme. Despite this observation the assay of phosphatase activity

continues to be used for identification of *Staphylococci.* All the strains which were detected as MRSA by MIC method were alsoresistant by the Cefoxitin disc. However, 9 strains detected as sensitive by MICmethod were resistant by the Cefoxitin disc diffusion. Detection of Oxacillin resistance is complicated because different populations of *staphylococci* express different levels of resistance. To accurately characterize these 9 strains as MSSA/MRSA [12], the detection of the mecA gene by molecular methods have tobe undertaken as molecular methods are considered to be a "gold standard" for the diagnosis of Oxacillin resistance. However these methods are expensive for many of our laboratories and the use of rapid and accurate phenotypic tests has become an alternative [13] . Laboratories using disk diffusion as their primary test for *staphylococci*, the substitution of a Cefoxitin disk for an Oxacillin disk will result in an easier-to- read test and provide equivalent detection (sensitivity and specificity) of Oxacillin resistance in *S. aureus* and equal sensitivity but improved specificity in CoNS [14].

Conclusion

Staphylococcus species is a major concern for the medical community. In the past, patients were commonly treated with various Pencillin, Clindamycin Erythromycin and /or Gentamycin for *Staphylococcal* infections. However,owing to many factors, including the extensive use of these antibiotics *Staphylococci* have developed resistance.

MRSA is emerged as a major nosocomial pathogen as well as community associated pathogen. Increased resistance to a number of antibiotics and acquired inducible resistance to Clindamycin has been reported. The present study also shows increasing resistance to various antibiotics.

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