



**ISOLATION AND SCREENING OF BETA-LACTAMASE PRODUCING
STAPHYLOCOCCUS AUREUS (MRSA) FROM CLINICAL SAMPLES**

Ramakrishna D.*

Department of Studies and Research in Microbiology, Government College, Gulbarga, Karnataka- 585105, India.

***Author for Correspondence: Dr. Ramakrishna D.**

Department of Studies and Research in Microbiology, Government College, Gulbarga, Karnataka- 585105, India.

Article Received on 23/12/2015

Article Revised on 12/01/2016

Article Accepted on 02/02/2016

ABSTRACT

Methicillin resistance *Staphylococcus* is pathogenic and is an important nosocomial organism. A study to investigate the multidrug resistance of *Staphylococcus aureus* to conventional antibiotics being frequently used in these 8 often 12 isolates that produced beta lactamase were resistant to all the β -lactam antibiotics used in this studies. This revealed that the resistance is purely plasmid based since β -lactamase production is plasmid based. This contributes a lot to the level of multiple drug resistance as about 88% of the isolates show resistance to more than five antibiotics. The resistance however might suggest the role of *mecA* gene in the isolates.

KEYWORDS: *Staphylococcus*, *Staphylococcus aureus*, *mecA*.

INTRODUCTION

Staphylococcus aureus is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. Infection rate from *S. aureus* is high the recent increased recognition of community acquired infections has important clinical and pharmacological implications for the health care provider. Urgent control measures should be taken to combat the renowned aetiology of both nosocomial and community acquired infections. In recent years, many isolates of *Staphylococcus aureus* have evolved resistance to both synthesis and traditional antimicrobial chemotherapy and their prevalence outside the hospital is of potential epidemiological treat, Lowry FD (1998), Komoloafe AO Aegoke AA (2008).

The study on epidemiological studies on multidrug resistance strains of *S. aureus* as hospital acquired infection in these districts was undertaking the all aspects of Staph. Infection as mention above. Antibiotic resistance is a direct consequence of antibiotic use. Both continue to isolate despite many cells for moderation of antibiotic use. Much has been written on antibiotic policies and others control measures. Despite the lack of properly controlled studies, which would be very difficult to perform, there is no doubt that polices can be efficacious in reducing costs and levels of use without being detrimental to patient care. However, may barriers and each institution will need to adapt these measures to their own needs. Timely information and education are crucial and efforts certainly need to be continuous to maintain effect.

MATERIALS AND METHODS

Sample collection

Collection from the different labs of Gulbarga and Raichur districts weekly once. The samples from the patient's swabs from pus, wound infections and then swabs are directly put into the sterile test tubes containing nutrient broth to prevent drying of swabs. Then inoculated on the plates containing different media namely nutrient agar, mannitol salt agar and were incubated at 37°C for about 24 hrs. The organisms on the positive plates indicates golden yellow coloured colonies on nutrient agar and yellow coloured colonies on mannitol salt agar media, then were gram staining catalase test and coagulase test were carried out on the gram positive cocci in cluster. The organism was characterized using the criteria of Cowan and Steel (2004).

Antimicrobial susceptibility pattern

Totally all isolates are subjected to test antimicrobial susceptibility test. Our objective to study resistance to the commonly used conventional antibiotics in these areas. Penicillin, ampicillin, amoxicillin, gentamycin, tetracycline, erythromycin, methicillin and vancomycin the disc diffusion method for in vitro antibiotics susceptibility test Kirby – Bauer (1996) was used in the study.

Beta lactamase test

Based on the resistance of the *Staphylococcus aureus* isolates to penicillin and others beta lactam antibiotics used, beta lactamase test was carried out using the method of (Odugbemi et al., 1997) Strips of starch paper about 4.7 cm were cut and sterilized using 70% ethanol,

the strips were soaked for 10 min. in benzyl penicillin dissolved in phosphate buffer with 100,000 units. The cut strips were then spread evenly on petri dishes and about 18-24 hrs old cultures grown on nutrient agar were inoculated on the surface of the test paper and spread over an area 2-3 mm. The petri dishes were incubated at 37°C for 30 min then Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turns uniformly black within 30 sec. of application colonies with decolorized zones are positive for beta-lactamase but colonies with black background show beta lactamase negative. The result was read within 5 min because of the time extended further the black background indicative of negative will start decolorizing thus giving a false positive result.

RESULTS AND DISCUSSION

A total of 200 clinical samples were collected for the study from different hospitals and laboratories of Gulbarga and Raichur districts. 60 of *Staphylococcus aureus* they show coagulase, catalase positive. Maximum of 20 samples were obtained from the age group (11-20 years) followed by the age groups 31-40 years, (18 samples). From the age groups of 1-10, 41-50 and 51-60 years (Figure 1). Four and five samples were obtained respectively.

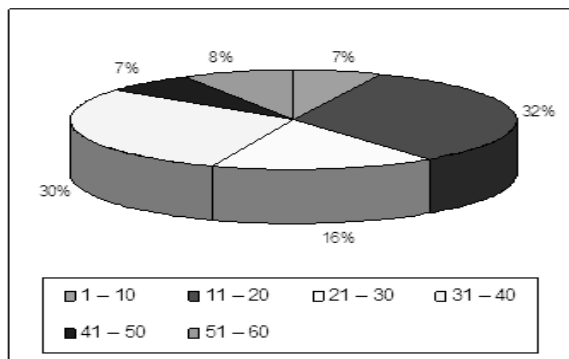


Fig. 1: Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among different age groups of samples.

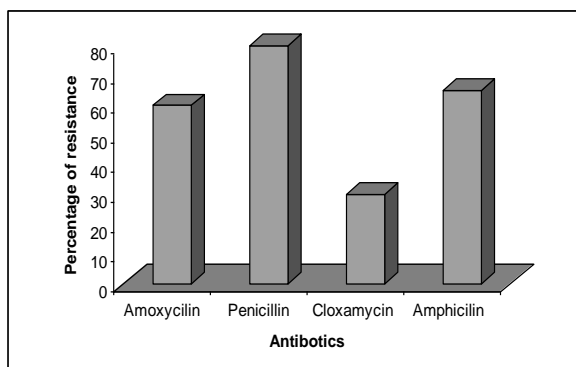


Fig. 2: Percentage of the isolates to the beta-lactam.

Antibiotics

The multidrug resistance pattern of methicillin resistance *Staphylococcus aureus* isolated from clinical specimens.

Samples were found to be high resistance variable. All the MRSA strains (96.0%) screened from clinical specimens were resistant (Fig. 2) to penicillin, ampicillin 90%, amoxicillin 85%, gentamycin 55%, streptomycin 65%, tetracycline 64%, erythromycin 72%, methicillin 19% and vancomycin 25%. However, MRSA strains are sensitivity to vancomycin & 25% high percentage of intermediate resistance was recorded against erythromycin, streptomycin and ciprofloxacin. Based on the resistance pattern of the isolates against the beta-lactam antibiotics (Fig. 2), 8 of the 12 isolates tested for beta-lactamase were the for the production of the enzymes.

CONCLUSION

Staphylococcus aureus is a major noscominal pathogen causing significant morbidity and mortality.^[8] The important reservoirs of MRSA in hospitals are infected or colonised patients and transient carriage and hands of health care workers is the predominant mode for patient to patient transmission.

8 often 12 isolates that produced beta lactamase were resistant to all the beta-lactam antibiotics used in this studies. This revealed that the resistance is purely plasmid based since beta-lactamase production is plasmid based. This contributes a lot to the level of multiple drug resistance as about 88% of the isolates show. resistance to more than five antibiotics. The resistance however might suggest the role of *mecA* gene in the isolates. (Murakami K, Minamide et al., 2004).

The upsurge in the antibiotic resistance noticed in this study is in agreement with an earlier report. Where antibiotic abuse and high prevalence of self medication with antibiotics were identified as being responsible for the selection of antibiotic resistant bacterial strains.

Prevention of MRSA infection in patients should be an achievable goal by taking appropriate control measures. It can be considered that in the present study the prevalence of MRSA is very high, the patients in the present study have to be handled carefully and there is every possibility for the spread of MRSA by the carriers. Hence, it is suggested that the measures to control MRSA is an immediate need.

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