Antibiotic resistance and biofilm production among the isolates of *Staphylococcus aureus* isolated from clinical samples in Salem, Tamil nadu, India

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***Abstract*:** This present study was evaluates the antibiotic susceptibility pattern of *Staphylococcus aureus* isolates from clinical sample in around Salem area, Tamilnadu, India. A total of 25 clinical samples of pus, sputum, urine and blood was collected and subjected to isolation of as *Staphylococcus aureus* using selective media and biochemical tests. Disc agar diffusion method was used for the antibiotics resistance test. Our findings showed that 64% of the isolates were resistance to vancomycin and 56% for oxacillin followed by Tetracycline and Gentamycin (44%). The isolates were 100% sensitive to neomycin and cefoxidime. Presently 13 types of antibiotic resistance patterns were observed, among them, TE, NV, VA pattern was frequently observed in 20% of isolates. Furthermore, 56% biofilm producers were carried out by CRA method, which isolates had highest antibiotic resistance especially vancomycin and oxacillin. The early identification and select efficient control protocol against biofilm forming VRSA can be one of the important steps towards the prevention of the most serious nosocomial infections.

Keywords:- *S.aureus*, VRSA, Oxacillin, Biofilm

I. INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that lives on the skin, mouth and upper respiratory system as a commensal, making it a peril factor for opportunistic and nosocomial infections. Resistance to commonly used antimicrobial drugs is frequently inhibited with *S.aureus*. Some of the mechanisms in resistance include; inactivation of antibiotics by the enzymes, decline affinity for the antibiotics caused by conversion of the target, efflux pumps, and trapping of the antibiotics (Wilfred *et al*., 2018).

The carriage of *Staphylococcus aureus* is an important source of nosocomial infection and community acquired infections; especially methicillin resistant *Staphylococcus aureus* (MRSA) are easily be spread from patient to patient through the hands of the staff and can lead to frequent epidemics (Cennet *et al*., 2016). Antimicrobial therapy is essential for the management of staphylococcal infections in patients. The types and percentage of antibiotic resistance was varying because of geographical variation in the antibiogram of the isolates. This phenomenon may be occurring due to variation in drug pressure and exposure (Archana *et al*., 2017).

Very few reports only available in Tamil Nadu state recording the antibiotic resistance of *S.aureus*, especially in biofilm producing *S.aureus*. Taking this into consideration, it is aimed to retrospectively determine the antibiogram of the *Staphylococcus aureus* isolated from various clinical samples from the Salem area, Tamil Nadu and also determine the association between biofilm and antibiotic resistance character.

II. MATERIALS AND METHOD

ISOLATION OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS (S.aureus)*

The clinical samples of pus, sputum, urine and blood were collected from Salem area and which were subjected to identification *S.aureus* through, standard conventional microbiological methods. These are identified by conducting catalase test, slide and tube coagulase test and mannitol fermentation test.

ISOLATION OF ANTIBIOTIC RESISTANCE PATTERNS

Antibiotic susceptibility testing was done by using Kirby Baur disk diffusion method as per CLSI (2014) guidelines. The antibiotics were selected according to the clinical samples and recommended by CLSI. Following antibiotics (Himedia, India) were used for the determination of the antibiotic resistance profile, including Ciprofloxacin (5µg), Ceftazidime (30µg), Erythromycin (15µg), Gentamycin (10µg), Oxacillin (1µg), Penicillin (10µg), Rifampin (30µg), Tetracycline (30µg), Levofloxacin (5µg), Neomycin (30µg), Chloramphenicol (30µg), Imipenem(30 µg) , Co-trimoxazole (20µg), Vancomycin (30µg), Cefoxime (30µg), Ceftrizone (30µg), Amoxiclove (30 µg), Amoxicillin (30µg), Novabiocin (30µg) and Norfloxacine (10µg).

ISOLATION OF BIOFILM PRODUCING ISOLATES

The biofilm producing isolates were screened by Congo Red Agar (CRA) method. Briefly, the Brain Heart Infusion (BHI) Broth (37g/l) supplemented with sucrose (50 g/l), agar (10 g/l) and Congo red dye (0.8 g/l) was used. The bacterial cells of isolates were streaked on the agar media and incubated aerobically at 37°C for 24 h. The colonies that were black with dark consistency were considered strong slime producers whereas pink colonies were slime non-producers (Freeman *et al*., 1989).

III. RESULTS AND DISCUSSION

A total of 25 isolates of *S. aureus* were collected from various clinical specimens and identified by standard tests. The Figure 1 depicts the source of 25 *S. aureus* isolates. The majority of the isolates were obtained from pus and sputum sources. All isolates were subjected to antibiotic susceptibility test 20 different antibiotics. Among them Vancomycin (64%) showed the highest resistance and second most was Oxicillin (52%). The isolates of vancomycin resistant *S. aureus* have emerged in many parts of the India, such as the West Bengal (Subhankari *et al*., 2011), Uttar Pradesh (Ruchi *et al*., 2016), New Delhi (Anjali *et al*., 2017). Those reports of resistance degree varied because of antibiotic resistance in relation to different geographical regions.

These varying levels of vancomycin resistance have led to increasing concern about its therapeutic efficacy in severe staphylococcal infections. While the determination of the antimicrobial susceptibility is critical for a perfect therapy, for epidemiological purposes and for infection control measures the treatment of the *S. aureus* infections have become critical because of the emergence of resistance to methicillin, vancomycin and other antibiotics (Olajuyigbe *et al*., 2017).

In this study, VRSA isolates had highest rate of antibiotic resistance (32%) then VSSA isolates (23.5%) including Oxicillin and Erythromycin. This was agreed with Ghoniem *et al*., 2014. They were indicating that VRSA tends to be multi-drug resistant against a large number of currently available anti-microbial including ciprofloxacin, gentamicin, levofloxacin, tetracycline (33.3%). The lowest percentage of resistance was against to Rifampicin and imipenem (4%). Among the 20 antibiotics, Neomycin and cefoxidime were sensitive to 100% of isolates (Figure 2). Presently 13 types of antibiotic resistance patterns were observed, among them, TE, NV, VA pattern was frequently observed in 20% of isolates (Table 1).

Aminoglycosides are potent, broad spectrum antibiotics, especially gentamycin and tobramycin were mostly used for the treatment of MRSA and Staphylococci endocarditis. Although, increased the resistance to these drugs have been reported from many countries. In India, few data only available about the aminoglycosides resistance isolates of *S.aureus*. From India, previous study of Gata and Qazi was observed that aminoglycosides antibiotics of gentamycin and tobramycin resistance isolates were from clinical samples.

In case of source wise, pus isolates were showed highest antibiotic resistance (36%), especially, Oxicillin and Erythromycin (80%) were showed highest resistance and lowest resistance was in sputum sample's isolates (21%). Antibiotic resistance represents a distinguished characteristic of clinically relevant biofilm infections. The biofilm producing isolates had multiple mechanisms responsible for the decreased susceptibility of biofilms to antibiotics (Jeetendra *et al*., 2013).

In the present 56% of biofilm producers were observed with CRA method and most of the biofilm producing isolates had highest drug resistance than non biofilm producers.. This was correlated with previous studies of Sharvari and Chitra, 2012, Ramakrishna *et al*., 2014 and Singh *et al*., 2017, they were found that staphylococci biofilm producers were more resistant to commonly used antibiotics. Presently, biofilm producing isolates were more resistant to almost all the classes of antibiotics, especially which were highest in betalactam antibiotic resistance isolates. This was concordant with Lee *et al*, 2008; they also reported that there is a strong relationship between biofilm formation and production of beta lactamases.

The conclusion of this study revealed that there is a high prevalence of vancomycin-resistant *S. aureus* (VRSA) amongst isolates from the clinical samples tested and also this study determinate the high prevalence of VRSA isolates producing biofilms in clinical staphylococcal samples. This study, showed that additional information about the status of biofilm producing clinical isolates and their association with multiple antibiotic resistances, which was persisting in the hospital environment and increase the risk of disease development in hospitalized patients. The appropriate antibiotic prescription, environmental hygiene, and improved knowledge about antibiotic policy may help to control the spread of antimicrobial-resistant microorganisms, including VRSA.

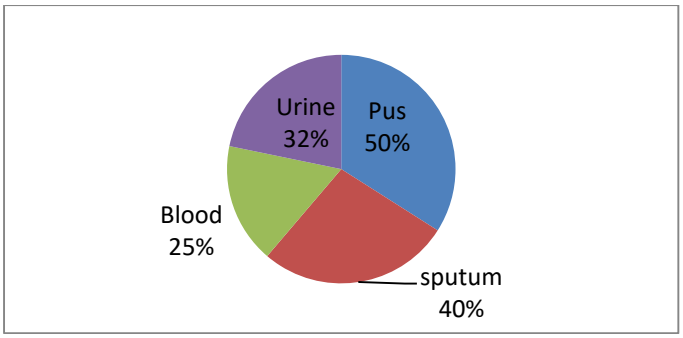

Figure1. Distributions of *S.aureus* on various sources

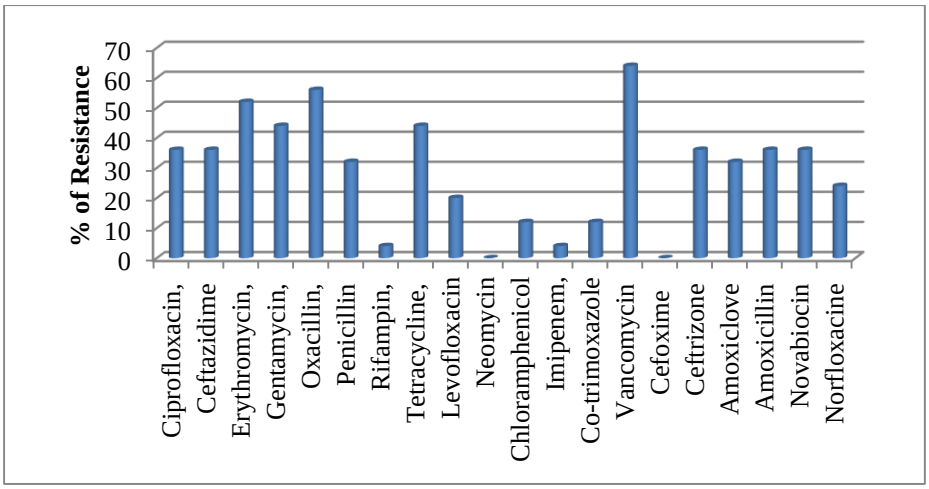

Figure2. Antibacterial resistance rates of *S.aureus*

Table 1. Antibiotic resistance patterns of *S.aureus*

S. No	Antibiotics	No. of isolates
1.	C	1
2.	TE, NV, VA	5
3.	G,E, TE, OX, VA	4
4.	COT,P, CTR, AMX, CAZ,	1
5.	G, E, LV, CTR, AMX, CAZ,VA, NX, AMC, CIP	3
6.	E, P, OX	2
7.	CTR, AMX, OX, CAZ, NX,AMC, CIP	2
8.	G, E, C, TE, P, LE, OX, NV, VA, AMC, CIP	2
9.	E, P, OX, R	1
10.	VA, CIP	1
11.	G, COT, P, CTR, AMX, OX, CAZ, NV	1
12.	G, COT, P, CTR, AMX, OX, CAZ, NV, VA, IPM,	1
13.	E, CTR, AMX, OX, CAZ,NX, AMC, CIP	1

Table 2 Association between Vancomycin resistance and biofilm producing *S.aureus*

S. No	Biofilm	VRSA (%)	VSSA (%)
1.	Biofilm positive	71.4	14.2
2.	Biofilm negative	54.5	45.4

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