

Speciation and antibiogram of *Staphylococcus* isolated in a tertiary care centre

Neelesh Naik¹, Anusuya Devi D^{2,*}, Veena Krishnamurthy³

¹Associate Professor, ²Assistant Professor, ³Professor, Dept. of Microbiology, Sri Siddhartha Medical College and Hospital, Tumkur, Karnataka, India

***Corresponding Author:**

Email: annu151983@gmail.com

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Abstract

Introduction: *Staphylococcus* have become common cause of skin and soft tissue infections. Resistance to a number of drugs have increased and methicillin resistant *Staphylococcus aureus* (MRSA) and inducible clindamycin resistance (iMLS_B) have become a major problem for the treatment of Staphylococcal infections. This study was undertaken to detect MRSA and iMLS_B and to determine the antibiotic susceptibility pattern of the isolates.

Materials and Methods: 150 isolates of *Staphylococcus* were studied for detecting the antibiotic resistance pattern and also to detect MRSA using cefoxitin disc and oxacillin E test. iMLS_B resistance among MRSA strains was detected using D test.

Results: Out of 150 isolates of *Staphylococcus*, 117(78%) isolates were of *Staphylococcus aureus* and 33(22%) isolates were of Coagulase negative *Staphylococci*. *Staphylococcus* was most sensitive to vancomycin followed by linezolid and clindamycin. Penicillin was the least sensitive antibiotic. 29 (24.7%) strains of *Staphylococcus aureus* were MRSA. Among them, 16(44.8%) were erythromycin resistant and 4(13.7%) of erythromycin resistant strains were found to be inducible clindamycin resistant.

Conclusion: Testing of all the isolates of *Staphylococcus* for antibiotic resistance and to test *Staphylococcus aureus* for MRSA and for iMLS_B resistance is important in determining the antibiotic sensitivity which will prevent treatment failure.

Keywords: Antibiogram, Methicillin resistant *Staphylococcus aureus*, Inducible clindamycin resistance.

Introduction

Staphylococcus aureus and Coagulase negative *Staphylococci* (CoNS) have become the common causes of skin and soft tissue infections. People with diabetes, cancer, tissue necrotizing pneumonia, sepsis, eczema, vascular diseases and lung diseases are at higher risk of infection by *Staphylococcus aureus*.¹ CoNS which are normally present on the skin as commensal bacteria and which were previously thought as contaminants are now being recognized as important agents causing human infections. *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* are the most commonly isolated CoNS from clinical samples.² The virulence of *Staphylococcus epidermidis* is mainly due to the production of biofilms, Bap (biofilm associated protein), PIA (polysaccharide intracellular adhesion) and toxins.³

Penicillin was the first antibiotic introduced in early 1940s for the treatment of *Staphylococcus aureus* infections. However, the effectiveness of penicillin greatly reduced within a decade due to the plasmid epidemics that spread the β -lactamase gene through the entire species of *Staphylococcus aureus*. Within a few years of the introduction of penicillinase resistant β -lactams (methicillin), methicillin resistant *Staphylococcus aureus* (MRSA) strains were identified in clinical samples which is due to the acquisition of the *mecA* gene, the determinant of a unique penicillin binding protein PBP2A which has low affinity for β -lactam antibiotics. By the 1980s, epidemic clones of MRSA acquired multidrug resistant traits and spread worldwide to become one of the most important

causative agents of hospital acquired infections.⁴ Emergence of MRSA led to widespread use of macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics resulting in a number of Staphylococcal strains acquiring resistance to MLS_B antibiotics, which was commonly due to target site modification by *erm* genes.⁵ This study was undertaken to determine antibiotic sensitivity pattern of *Staphylococci* and to detect MRSA and inducible MLS_B resistance of *Staphylococcus aureus*.

Materials and Methods

This study was done in the Department of Microbiology, Sri Siddhartha Medical College and Hospital, Tumkur between June to December 2017. A total of 150 isolates of *Staphylococcus* isolated from various clinical samples like pus, sputum, urine, vaginal swab, body fluid and aspirates were included in the study. The isolates were identified as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* based on gram stain, colony morphology and biochemical tests. The standardized Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standards Institute was used for antibiotic sensitivity testing. The discs used were penicillin (10units), amikacin (30 μ g/ml), gentamicin (10 μ g/ml), cotrimoxazole (1.25/23.75 μ g/ml), clindamycin (2 μ g/ml), linezolid (30 μ g/ml), ofloxacin (5 μ g/ml), erythromycin (15 μ g/ml) and vancomycin (30 μ g/ml). All the strains of *Staphylococcus aureus* were tested for methicillin resistance using cefoxitin (30 μ g) disc. An inhibition zone diameter of ≤ 21 mm

was reported as resistant and ≥ 22 mm was considered as sensitive.⁶

The Minimum Inhibitory Concentration (MIC) of methicillin resistant *Staphylococci* was determined by using E-test oxacillin strip on Mueller-Hinton agar plate supplemented with 2% NaCl. Methicillin susceptibility and methicillin resistance were defined as oxacillin E-test MICs of ≤ 2 $\mu\text{g/ml}$ and ≥ 4 $\mu\text{g/ml}$ respectively.^{7,8}

Detection of inducible MLS_B resistance among MRSA strains was done using double disc diffusion test (D-test). Mueller Hinton agar was inoculated with 0.5 McFarland suspension of *Staphylococcus aureus* and erythromycin (15 μg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 μg) disc. After overnight incubation at 37°C, the plates were examined to detect D shaped flattening of the zone around the clindamycin disc. MRSA strains that were positive in the D-test were considered inducible MLS_B resistant, strains that were resistant to both erythromycin and clindamycin were considered constitutive MLS_B resistant and those that were resistant to erythromycin but susceptible to clindamycin were considered MS phenotype.⁵

Result

Out of 150 isolates of *Staphylococcus*, 117 (78%) were of *Staphylococcus aureus* and 33(22%) were of CoNS. The most common CoNS was *Staphylococcus epidermidis* (57%) followed by *Staphylococcus saprophyticus* (43%). *Staphylococcus aureus* was most sensitive to vancomycin (100%) followed by linezolid (92.3%), clindamycin (81.1%), amikacin (68.3%), gentamicin (65.8%), erythromycin (58.9%), cotrimoxazole (51.2%), ofloxacin (35.8%) and penicillin (5.9%). The most sensitive antibiotic against CoNS was vancomycin (100%) followed by linezolid and clindamycin. None of the CoNS isolates were sensitive to penicillin. (Table 1)

Among *Staphylococcus aureus*, 29 (24.7%) strains were methicillin resistant by cefoxitin disc diffusion test and oxacillin E test. Oxacillin MIC of 8 $\mu\text{g/ml}$ was commonly observed among MRSA strains in the study. (Table 2) MRSA strains were most sensitive to vancomycin followed by clindamycin and linezolid. (Table 3, Graph 1)

Out of 29 isolates of MRSA, 16 (44.8%) were resistant to erythromycin. Strains of MRSA which were constitutive MLS_B were 4 (13.7%), inducible MLS_B were 8 (27.5%) and 4 (13.7%) belonged to MS phenotype. (Table 4)

Table 1: Antibiotic sensitivity pattern of *Staphylococcus*

S. No.	Organisms	P (%)	AK (%)	G (%)	Co (%)	Cd (%)	Lz (%)	Of (%)	E (%)	Va (%)
1	<i>Staphylococcus aureus</i> Total no.- 117	7 (5.9)	80 (68.3)	77 (65.8)	60 (51.2)	95 (81.1)	108 (92.3)	42 (35.8)	69 (58.9)	117 (100)
2	<i>Staphylococcus epidermidis</i> Total no.- 19	0 (0)	14 (73.6)	13 (68.4)	14 (73.6)	16 (84.2)	18 (94.7)	10 (52.6)	10 (52.6)	19 (100)
3	<i>Staphylococcus saprophyticus</i> Total no.- 14	0 (0)	10 (71.4)	09 (64.2)	08 (57.1)	14 (100)	14 (100)	08 (57.1)	09 (64.2)	14 (100)
Total 150		7 (4.6)	104 (69.3)	99 (66.6)	82 (54.6)	125 (83.3)	140 (93.3)	60 (40)	88 (58.6)	150 (100)

Table 2: MIC of oxacillin for methicillin resistant *Staphylococcus aureus* isolates

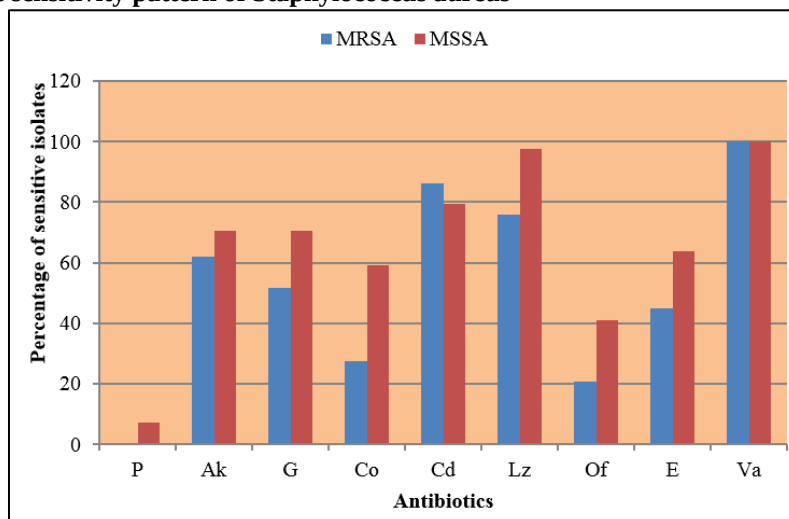
MIC value in $\mu\text{g/ml}$	No of isolates (%)
4	8 (27.5%)
6	7 (24.1%)
8	5 (17.2%)
192	2 (6.8%)
>256	7 (24.1%)
Total	29

Table 3: Antibiotic sensitivity pattern of *Staphylococcus aureus*

S. No.	MRSA/ MSSA	P (%)	AK (%)	G (%)	Co (%)	Cd (%)	Lz (%)	Of (%)	E (%)	Va (%)
1	MRSA Total no. 29	0 (0)	18 (62)	15 (51.7)	8 (27.5)	25 (86.2)	22 (75.8)	6 (20.6)	13 (44.8)	29 (100)
2	MSSA Total no. 88	7 (7.14)	62 (70.4)	62 (70.4)	52 (59)	70 (79.5)	86 (97.7)	36 (40.9)	56 (63.6)	88 (100)
Total 117		7	80	77	60	95	108	42	69	117

Table 4: Distribution of inducible clindamycin resistance among MRSA isolates

S. No.	Susceptibility pattern (Phenotype)	MRSA isolates (%)
1	Erythromycin – R, Clindamycin –R (constitutive MLS _B)	04 (25%)
2	Erythromycin – R, Clindamycin –S D test positive (inducible MLS _B)	08 (50%)
3	Erythromycin – R, Clindamycin –S D test negative (MS phenotype)	04 (25%)
Total		16

Graph 1: Antibiotic sensitivity pattern of *Staphylococcus aureus*

P-Penicillin, Ak-Amikacin, G-Gentamicin, Co-Cotrimoxazole, Cd-Clindamycin, Lz-Linezolid, Of-Ofloxacin, E-Erythromycin, Va-Vancomycin

Discussion

The isolates of *Staphylococcus* were most sensitive to vancomycin followed by linezolid and clindamycin. 58.9% of *Staphylococcus aureus* were sensitive to erythromycin, 51.2% were sensitive to cotrimoxazole and only 5.9% were sensitive to penicillin. According to a study by Krithikaa et al, sensitivity of *Staphylococcus aureus* to antibiotics in decreasing order was linezolid (91.1%), vancomycin (80%), clindamycin (68.5%), erythromycin (51.5%), cotrimoxazole (46%) and penicillin (20%).⁹ Sajjanar V et al have found CoNS to be 100% sensitive to vancomycin and linezolid. The least sensitive antibiotics to CoNS were clindamycin (50%) and erythromycin (33.3%).¹⁰ Other studies have detected more than 50% resistance to clindamycin and erythromycin.^{10,11}

29 (24.7%) isolates of *Staphylococcus aureus* were MRSA. Some studies have showed a higher incidence of MRSA. According to Giacometti et al, MRSA was seen in 54.4% of isolates.¹² Jain A in 2005 studied 97 isolates of *Staphylococcus aureus* and found that 75.26% were methicillin resistant.¹³

In the present study there were only 2 isolates of methicillin sensitive *Staphylococcus aureus* which were resistant to linezolid, but among MRSA, linezolid

resistance was seen in 7 isolates. Resistance to clindamycin, erythromycin and amikacin were 13.8%, 55.2% and 38% respectively among MRSA isolates. In a review done by Gebremariam et al, among the different studies included there were more than 70% of MRSA strains showing resistance to penicillin and erythromycin, but resistance to clindamycin and amikacin was less than 50%.¹⁴

Among the 16(55.5%) erythromycin resistant MRSA, 8(50%) were inducible clindamycin resistant, 4 (25%) were constitutive MLS_B and remaining 4 (25%) were of MS phenotype. In various other studies erythromycin resistance was found to be between 50% and 59% and inducible clindamycin resistance was found to be between 33% and 42%.^{9,15,16} Some studies have shown lower rate of inducible clindamycin resistance.^{17,18}

Conclusion

Infection with *Staphylococcus aureus* have become increasingly more difficult to treat in recent times due to the increase in resistance to commonly used antibiotics. Even the CoNS which were once considered contaminants have become resistant to a number of antibiotics. Also, the emergence and continuous increase in MRSA strains have become a major concern for the treatment of infections. Cefoxitin disc test can

routinely be used for the detection of MRSA strain by phenotypic method.⁶ Clindamycin has become a very important antibiotic in the treatment of Staphylococcal infections because of its comparative low cost, good bioavailability and because it has got better tissue penetration.¹⁵ Strains of *Staphylococcus aureus* which are resistant to clindamycin but appear to be sensitive in vitro have emerged and hence it is important to detect such resistance. In our study more than 25% of MRSA isolates which appeared to be clindamycin sensitive were actually resistant by D test. It would have resulted in treatment failure if such infections were treated by clindamycin. Hence, the accurate antibiotic sensitivity pattern of Staphylococcus along with the detection of MRSA and inducible clindamycin resistance by D test will be of immense benefit in the treatment of Staphylococcal infections.

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