Recent Trends in Sensitivity Pattern of *Staphylococcus* species

Otta Sarita¹, Bhoi Priyadarshini², Sahu Kundan Kumar³, Swain Bichitrananda⁴

¹MD (Microbiology), Associate Professor, IMS and SUM Hospital, Bhubaneswar, Odisha, India; ²MD (Microbiology), Assistant Professor, IMS and SUM Hospital, Bhubaneswar, Odisha, India; ³MD (Microbiology), Professor and Head, IMS and SUM Hospital, Bhubaneswar, Odisha, India; ⁴MD (Microbiology), Associate Professor, SLN Medical College and Hospital, Koraput, Odisha, India.

ABSTRACT

Introduction: *Staphylococcus* particularly MRSA (Methicilin resistant *Staphylococcus aureus*) and MR CoNS (Methicilin resistant Coagulase Negative *Staphylococcus*) are important cause of hospital and community acquired infections. But there are reports of resistance to anti-MRSA drugs particularly vancomycin, teicoplanin, linezolid, tigecyclin which is a cause for concern.

Aim: The present study was conducted to determine the susceptibility of *Staphylococci* and note the pattern of minimum inhibitory concentration (MIC) of the second line anti staphylococcal drugs in common clinical use.

Method: Various culture samples submitted to Department of Microbiology from Sept 2019 to May 2020 and yielding growth of *Staphylococcus* were included in the present study. Sensitivity pattern was determined by Vitek 2, Biomerieux and MIC of drugs were interpreted as per recent guidelines.

Result: 264 *Staphylococcus* were included in the study of which 39 were *Staphylococcus aureus*, 72 were *S. epidermidis* and 67 were *S. haemolyticus*. Besides these, 46, 14 and 11 strains of *S. hominis*, *S. cohnii* and *S. capitis* respectively were isolated. With cefoxitin screen method, 23 (58.9%) isolates were MRSA where as only 16 (41.03%) were MSSA. Of the *S. epidermidis* and *S. haemolyticus* isolated 98.59% and 95.01% were methicillin resistant and all other isolated CONS were methicillin resistant. MRSA isolates had higher degree of resistance in comparison to MSSA to all the antibiotics tested except quinolones and erythromycin. All the *S. aureus* isolates were 100% sensitive to linezolid, vancomycin, daptomycin, teicoplanin, rifampicin and nitrofurantoin. None of the *Staphylococcus* showed any creeping of the minimum inhibitory concentration (MIC) of vancomycin. CONS particularly *S. epidermidis* and *S. haemolyticus* had a greater degree of resistance to linezolid, daptomycin and teicoplanin than *S. aureus*. Tigecyclin a reserve drug was the most sensitive antibiotic in all the species.

Conclusion: The study highlights high degree of resistance in CONS isolates to all drugs. Linezolid a useful oral drug also showed high resistance to many CONS and few *S. aureus* strains. Rifampicin and tigecyclin are other useful drugs for *Staphylococcus* in the present study.

Keywords: *Staphylococcus spp.*, Vitek 2, Linezolid resistance, Tigecyclin, Vancomycin, MIC creep.

INTRODUCTION

Staphylococcus particularly MRSA (Methicilin resistant *Staphylococcus aureus*) and MR CONS (Methicilin resistant Coagulase Negative *Staphylococcus*) are important cause of nosocomial and community acquired infections ranging from mild localized skin and appendage infections to life threatening conditions such as sepsis, necrotizing pneumonia, septic arthritis, endocarditis, and osteomyelitis.^{1,2} CONS a normal commensal of skin and mucous membrane is recently being implicated as an important nosocomial pathogen particularly when same

strain is repeatedly isolated from same site.³ Vancomycin is the treatment of choice for serious MRSA infections.⁴ In the last few years, drugs such as linezolid, daptomycin, tigecyclin, dalbavancin, telavancin, oritavancin, ceftazidime, ceftaroline, and iclaprim have been added to the arsenal of anti-MRSA drugs.⁵ But few reports suggest resistance to even these alternative drugs which is troublesome and necessitates continuous monitoring. The present study was conducted to determine the susceptibility of *Staphylococci* to the various antibiotics and note the pattern of MIC to the second line anti staphylococcal drugs

Corresponding Author:

Dr. Sarita Otta, 1/5, Kanchanjunga Enclaves, Chandrasekharapur, Bhubaneswar, Odisha, India.

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in common clinical use i.e vancomycin (glycopeptide), daptomycin (lipopeptide), tigecycline (glycylcycline) and linezolid (oxazolidinone).

MATERIALS AND METHODS

Various culture samples (blood, wound swab, pus, urine etc) submitted to Department of Microbiology, IMS and SUM Hospital, Bhubaneswar from Sept 2019 to May 2020 were included in the present study. Blood samples were processed by automated blood culture system (bio Mérieux). All positive blood cultures and other samples were streaked on blood agar and Macconkey agar plates. The colonies thus received on the plates after overnight incubation were submitted for Gram staining. The Gram positive cocci obtained were subjected to identification and susceptibility testing by Vitek 2 (bio Mérieux) automated system. In case of *Staphylococcus* other than *S.aureus* the isolate was considered only when clinically significant. Minimum inhibitory concentration (MIC) was determined by Vitek 2 and the results were interpreted as per CLSI guidelines. (Table 1) For tigecycline interpretation was done as per European Committee On Antimicrobial Susceptibility Testing (EUCAST) guidelines. (Table 1) *Staphylococcus aureus* ATCC 29213 was employed for quality control. Being a National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited lab all procedures and chemicals were standardized and quality assured.

RESULTS

A total 264 *Staphylococcus* were isolated in the lab from non duplicate non repeat clinically significant samples during the study period. Among them 39 were *Staphylococcus aureus*, 72 were *S.epidermidis* and 67 were *S.haemolyticus*. Besides these, other less common species isolated were 46, 14 and 11 strains of *S.hominis*, *S.cohinii* and *S.capitis* respectively. With cefoxitin screen method, 23 (58.9%) isolates were MRSA where as only 16 (41.03%) were MSSA. Of the total *S.epidermidis* and *S.haemolyticus* isolated 98.59% and 95.01% were methicillin resistant. All other CONS isolated were 100% cefoxitin screen positive. Inducible clindamycin resistance was highest in *S.aureus* strains than that for CONS being 37.5 % in MRSA and 39.1% in MSSA varieties. (Table 2)

When the sensitivity pattern was looked into MRSA isolates had higher degree of resistance in comparison to MSSA to all the antibiotics tested except quinolones and erythromycin. All the *S.aureus* isolates were 100% sensitive to linezolid, vancomycin, daptomycin, teicoplanin, rifampicin and nitrofurantoin. Few MSSA isolates (4.4%) strikingly showed resistance to tigecyclin. *S.aureus* isolates showed very low

sensitivity to ciprofloxacin (13% to MSSA and 25% to MRSA), levofloxacin (13% in MSSA and 18.75% in MRSA) and erythromycin (23.08% in MSSA and 43.75% in MRSA). The MRSA isolates had additionally lower sensitivity to penicillin (13.3%). (Table 3)

CONS had a higher degree of resistance than *S.aureus* to almost all the antibiotic. Penicillin as expected is the least useful drug while vancomycin followed by tigecyclin is the most useful antibiotic. Nitrofurantoin is a good drug for all the species except *S. haemolyticus*. It is astonishing to find resistance to linezolid, teicoplanin and daptomycin in few CONS strains as these are the commonly reserved antibiotics for *Staphylococcal* strains. (Table 3)

S.epidermidis showed 100% sensitivity to vancomycin, tigecyclin and other drugs like rifampicin and nitrofurantoin. But higher degree of resistance was seen in these isolates to linezolid, teicoplanin and daptomycin in comparison to *S.aureus*. Quinolones are relatively more useful for the treatment of these strains but all other antibiotics show a higher degree of resistance than that of *S.aureus*. (Table 3)

S.haemolyticus isolates have a much higher degree of resistance to all the tested antibiotics among the different species of *Staphylococcus*. But vancomycin and tigecyclin are 100% effective in vitro against all the strains.

The MIC of all the second-line drugs were compared. In our study, none of the isolates of *Staphylococcus* showed any creeping of the MIC values to suggest heteroresistant strains of Vancomycin. In the case of linezolid, there were 7 strains of *S.epidermidis*, 1 of *S. haemolyticus*. 2 isolates of *S.cohinii* and 1 of *S.capitis* showing MIC of 8. Again 10 isolates of *S.haemolyticus* had a MIC >8. (Fig 1)

For Daptomycin, among the 4 resistant strains of *S.epidermidis*, 2 isolates each had a MIC of 2 and 4. In the case of *S.haemolyticus* there were 1 strain each with MIC of 2, 4, 8 for Daptomycin. 2 strains had MIC 4 and 8 of *S.hominis* and 1 strain of *S.capitis* had MIC of 2. (Fig 1)

There were only 2 samples each of *S.haemolyticus* and *S.hominis* with MIC > 32 for teicoplanin. For tigecycline, the majority of samples had a MIC of < 0.5. Only one sample of *S.haemolyticus* and 2 of *S.epidermidis* had a MIC of 1 and 2 respectively for tigecycline. (Fig 1)

DISCUSSION

S.aureus developed penicillin resistance soon after its introduction and followed it with methicillin resistance as well. MRSA strains are not only resistant to almost all beta-lactams but have also developed resistance to multiple other antimicrobial classes.⁶ with the increasing prevalence of MRSA, the use of vancomycin for treatment of invasive infections

has increased, along with concerns about vancomycin resistance among MRSA.⁷ CONS along with *S. aureus* is an important cause of nosocomial infection. The 30-day mortality of patients with CONS bacteraemia was up to 12.7% (20/157), particularly when associated with old age, Chronic liver and renal disease in a study.⁸ Thus knowing the recent pattern of resistance in the case of *Staphylococcus* is important not only for treatment but also to prevent the spread of infection.

In this study 39 *S. aureus* where as 225 CONS were isolated. Among the CONS most common species isolated was *S. epidermidis* and *S. haemolyticus*. *Staphylococcus epidermidis* is the most common colonizer of skin and mucous membranes and is the commonest cause of catheter-associated bloodstream infections.⁹ Other similar studies¹⁰ also noted *S. epidermidis* and *S. haemolyticus* as the most predominant species of CONS.

In the present study of all *S. aureus*, 41.03% were Methicillin-resistant while 96.4 % of CONS were methicillin-resistant. The higher rate in later is due to their penicillinase production and these are resistant to all the beta-lactams except the recently developed ceftaroline. In a study at Aligarh in India,¹¹ it was shown that 35.1% of *S. aureus* and 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. Another study¹² also found a higher rate of MR in *S. aureus* than CONS in contrast to our findings. Similar to the present study, Methicillin resistance is more common among *S. epidermidis* and *S. haemolyticus* isolates than among *S. aureus* in few other recent studies.¹³⁻¹⁵ CONS are often regarded as a harbinger of resistant genes. Probably this suggests the requirement of more stringent infection control measures.

All the species showed a high degree of resistance to penicillin. Penicillin resistance presumes resistance to all other beta-lactam drugs as well. This is probably due to the abundant production of the penicillinase enzyme. In the present study both the quinolones used had a similar degrees of sensitivity except for MRSA where ciprofloxacin had better sensitivity than levofloxacin. *S. aureus* had a much lower sensitivity to quinolones than CONS. Among the CONS *S. haemolyticus* had the highest degree of resistance to quinolones as well as for macrolides. A high degree of resistance to these drugs was noted by other studies also.^{8,16} Some recent studies suggested that CONS were highly resistant to penicillin (94.7%), oxacillin (90.7%), and erythromycin (85.3%), and more than 50% were resistant to cephalosporins, aminoglycosides and quinolones.^{17,18} But cotrimoxazole had a better sensitivity in the present study than most other studies.^{8,16} Gentamicin in our study continues to have very good sensitivity to all the species of *Staphylococcus* similar to other studies⁸ although a report¹⁶ showed higher resistance.

Linezolid, a synthetic oxazolidinone having bacteriostatic activity against MRSA is rampantly used for the treatment

of complicated skin and skin structure infections and nosocomial pneumonia.¹⁹ Present study noted that CONS particularly *S. epidermidis* and *S. haemolyticus* had a greater degree of resistance to this antibiotic than *S. aureus*. This is higher than that noted in other studies.^{8,10} Daptomycin and teicoplanin similarly had few CONS strains resistant to them. Tigecycline a reserve drug was the most sensitive antibiotic to all the species.

Rifampicin resistance is more common in *S. aureus* than that for CONS. This antibiotic can penetrate biofilms, so is the antibiotic of choice for the treatment of bone and joint infections in both chronic and acute cases.^{20,21} In the present study the resistance noted to this antibiotic was only to a few of the CONS isolates. It is a reserve drug on our part leading to such a low degree of resistance.

Reports, even in India, have described MRSA and MSSA strains with vancomycin MICs increased to the high end of the Clinical and Laboratory Standards Institute (CLSI) susceptibility range i.e MIC creep.²²⁻²⁴ This is due to clonal replacement with cells containing subpopulations of cells in the vancomycin intermediate range (VISA, MIC 4-8 µg/ml) Few studies, also show 42% of *Staphylococcus haemolyticus*, a CONS, being intermediate resistant to vancomycin.²⁵ Higher MIC of vancomycin is associated with an increased risk of treatment failure.⁶ In the present study no such heteroresistant *Staphylococcus* strains were found.

The present study carries limitations because of the short study period and taking Vitek MIC instead of actual E-test data for results. But this enlightens regarding the rising prevalence of resistance in *S. aureus* and CONS strains to the anti-A MRSA drugs and continued surveillance is a must to tackle it.

This is a lab-based study where samples were the routine cultures coming for reporting thus requiring no patient participation and raising no ethical concerns.

CONCLUSION

Rising prevalence of resistance to anti-A MRSA drugs to *S. aureus* is no new concept but the study highlights a high degree of resistance in CONS isolates to all drugs. Linezolid a useful oral drug also showed high resistance to many CONS and few *S. aureus* strains. As the CONS strains are harbourers and transmitters of resistance, this poses a challenge to antimicrobial stewardship measures in any hospital. None of the *Staphylococcal* isolates showed creeping MIC for vancomycin. Rifampicin and tigecycline are other useful drugs for *Staphylococcus*.

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Table 1: Interpretation criteria for various drugs

Organism	Vancomycin			Linezolid			Teicoplanin			Daptomycin			Tigecyclin		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S.aureus	≤2	4-8	≥16	≤4	-	≥8	≤8	16	≥32	≤1	-	-	≤ 0.5	-	≥8
S.epidermidis	≤4	8-16	≥32	≤4	-	≥8	≤8	16	≥32	≤1	-	-	≤ 0.5	-	≥8
S.haemolyticus	≤4	8-16	≥32	≤4	-	≥8	≤8	16	≥32	≤1	-	-	≤ 0.5	-	≥8
Others	≤4	8-16	≥32	≤4	-	≥8	≤8	16	≥32	≤1	-	-	≤ 0.5	-	≥8

n.b. S- Sensitive, I- Intermediate, R-Resistant

Table 2: Species distribution of various clinically significant Staphylococci

	Total	Cef pos	%	Inducible Clindamycin resistance (%)
S.aureus (MRSA)	16	16	41.03	37.5
MSSA	23	00	00	39.1
S.epidermidis	72	70	97.2	19.7
S.haemolyticus	68	66	97.06	18.9
S.hominis	46	46	100	15.2
S. lugdenensis	06	06	100	0
S.cohnii	14	14	100	0
S.capitis	11	11	100	0
S.auricularis	01	01	100	0
S.simulans	01	01	100	0
S.sciuri	01	01	100	0
S.saprophyticus	03	02	66.7	0
S.warneri	02	01	50	0

Table 3: Sensitivity pattern (in %) of the different species of Staphylococcus

	MSSA (23)	MRSA (16)	SE (72)	SH (67)	SHom (46)	SC (14)	SL (6)	Scap (11)	Others (7)
P	30.4	13.3	12.67	6.15	17.4	21.4	33.3	9.09	0
Ox	95.6	18.75	18.3	6.15	10.9	27.3	50	27.3	100
Gen	91.3	68.7	83.1	36.5	95.7	78.6	100	81.8	100
Cip	13.04	25.0	45.07	15.38	60.9	71.4	100	54.5	85.7
Le	13.04	18.75	43.66	15.38	60.9	71.4	100	54.5	85.7
E	26.08	43.75	21.13	18.97	32.6	64.3	100	45.5	28.6
CLD	60.9	56.25	52.11	34.84	60.9	64.3	100	45.5	42.9
Te	95.6	93.7	88.7	89.29	95.7	100	100	72.7	85.7
Nit	100	100	100	96.43	100	100	100	100	100
Rif	100	100	100	80.36	100	85.7	100	81.8	85.7
Cot	91.3	68.75	71.2	80.36	78.3	78.6	100	90.9	71.4
Lz	100	100	88.7	81.54	100	85.7	100	90.9	100
V	100	100	100	100	100	100	100	100	100
Dap	100	100	98.59	98.39	97.8	78.6	100	100	100
Tei	100	100	92.95	95.34	91.3	100	100	100	100
Tgc	95.6	100	100	100	100	100	100	100	100

Figure 1: The MIC of Staphylococci to various drugs.