Recent Trends in Sensitivity Pattern of
Staphylococcus species

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ABSTRACT

Introduction: Staphylococcus particularly MRSA (Methicillin resistant Staphylococcus aureus) and MR CoNS (Methicillin 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, tigecycline 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 tigecycline are other useful drugs for Staphylococcus in the present study.

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

INTRODUCTION

Staphylococcus particularly MRSA (Methicillin resistant Staphylococcus aureus) and MR CoNS (Methicillin 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, tigecycline, dalbavancin, telavancin, oritavancin, ceftobiprole, ceftaroline, and iclepram 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.
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 Maccnkey 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 Staphylococcus 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 Staphylococcus epidermidis and 67 were Staphylococcus haemolyticus. Besides these, other less common species isolated were 46, 14 and 11 strains of Staphylococcus hominis, Staphylococcus cohnii and Staphylococcus capitis respectively. With cefoxitin screen method, 23 (58.9%) isolates were MRSA where as only 16 (41.03%) were MSSA. Of the total Staphylococcus epidermidis and Staphylococcus haemolyticus isolated 98.59% and 95.01% were methicillin resistant. All other CONS isolates were 100% cefoxitin screen positive. Inducible clindamycin resistance was highest in Staphylococcus 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 Staphylococcus aureus isolates were 100% sensitive to linezolid, vancomycin, daptomycin, teicoplanin, rifampicin and nitrofurantoin. Few MSSA isolates (4.4%) strikingly showed resistance to tigecycline. Staphylococcus 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 Staphylococcus 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 Staphylococcus 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)

Staphylococcus 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 Staphylococcus 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 Staphylococcus aureus. (Table 3)

Staphylococcus 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 heterogeneous resistant strain of Vancomycin. In the case of linezolid, there were 7 strains of Staphylococcus epidermidis, 1 of Staphylococcus haemolyticus. 2 isolates of Staphylococcus cohnii and 1 of Staphylococcus capitis showing MIC of 8. Again 10 isolates of Staphylococcus haemolyticus had a MIC >8. (Fig 1)

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

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

**DISCUSSION**

Staphylococcus 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. 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 studies 10 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 penicilllinase production and these are resistant to all the beta-lactams except the recently developed ceftaroline. In a study at Aligarh in India,11 its was shown that 35.1% of S. aureus and 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. Another study 12 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.8,14 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.15,16 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 report 16 showed higher resistance.

Linezolid, a synthetic oxazolidinone having bacteriostatic activity against MRSA is rampanty 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.
ACKNOWLEDGEMENT

We acknowledge SOA University, Bhubaneswar, Odisha for their help in doing this work. The authors are also grateful to authors, editors, publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Source of support: Nil

Conflict of interest: Nil

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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

| Organism          | 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     | 72.7  | 85.7      |            |
| Nit    | 100       | 100       | 100     | 96.43   | 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       |            |
| 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       |            |
| Tgc    | 95.6      | 100       | 100     | 100     | 100       | 100     | 100   | 100       |            |
Figure 1: The MIC of Staphylococci to various drugs.

RECENT TRENDS IN SENSITIVITY OF STAPHYLOCOCCUS

Samples from Sept 2019 to May 2020
Aerobic culture- Blood agar and Macconkey agar plates
Post incubation Gram stain- Gram positive cocci
Identification and sensitivity pattern by Vitek 2

|  | MSSA | MRSA | SLPt | Shaoa | Shoa | SC | Shag | Scap | Others |
|---|------|------|------|-------|------|----|------|------|--------|
| Co | 93.3 | 68.7 | 83.1 | 96.5 | 95.7 | 78.6 | 100 | 81.8 | 100 |
| Co | 13.3 | 26.4 | 45.9 | 15.38 | 62.5 | 71.4 | 100 | 54.5 | 97.7 |
| Le | 13.3 | 18.3 | 43.6 | 15.38 | 64.5 | 71.4 | 100 | 54.5 | 87.7 |
| Ef | 26.7 | 43.7 | 52.1 | 18.97 | 52.6 | 64.3 | 100 | 45.5 | 28.6 |
| Cl | 60.9 | 56.1 | 52.1 | 34.9 | 64.3 | 64.3 | 100 | 45.5 | 4.8 |
| Ye | 95.6 | 93.7 | 88.7 | 79.3 | 95.7 | 100 | 100 | 72.3 | 95.7 |
| Ne | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Rf | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Cof | 91.3 | 68.7 | 71.2 | 38.3 | 72.3 | 72.6 | 100 | 80.9 | 71.4 |
| Le | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Oa | 98.5 | 93.9 | 98.5 | 97.8 | 97.8 | 97.8 | 100 | 100 | 100 |
| Te | 95.6 | 95.6 | 95.6 | 95.6 | 95.6 | 95.6 | 100 | 100 | 100 |
| Pg | 95.6 | 95.6 | 95.6 | 95.6 | 95.6 | 95.6 | 100 | 100 | 100 |