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Speciation and Antibiotic Susceptibility Pattern of *staphylococcus* species other than *staphylococcus aureus* Isolated from various Clinical Samples at a Tertiary Care Hospital in Rajasthan

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Abstract: **Background:** Staphylococci species other than Staphylococcus (CoNS) are increasingly recognized as significant pathogens in clinical settings, particularly among hospitalized and immunocompromised patients. Rising antimicrobial resistance has become a major concern. **Materials and Methods:** This cross-sectional study included 37 clinically significant CoNS isolates obtained from various clinical samples. Identification of isolates was done using standard microbiological methods. Antimicrobial susceptibility testing was performed by the "Vitek* 2compact automated system" (biomerieux). **Results:** Among the isolates, *Staphylococcus haemolyticus* 16(43.2%) was the most common species, followed by *Staphylococcus epidermidis* 11(29.7%) and *Staphylococcus hominis* 7(18.9%). A high level of resistance was observed to penicillin, oxacillin, indicating a high prevalence of methicillin-resistant CoNS (MRCoNS). Resistance to ciprofloxacin and erythromycin was also notable. Moderate resistance was seen for gentamicin, clindamycin, and cotrimoxazole. All isolates showed high susceptibility to vancomycin, Daptomycin, teicoplanin, and linezolid. Inducible clindamycin resistance was detected in a subset of isolates. **Conclusion:** CoNS are emerging as important multidrug-resistant pathogens. High resistance to commonly used antibiotics limits treatment options; however, glycopeptides (Teicoplanin, Vancomycin and linezolid remain effective. Routine speciation, antimicrobial surveillance and strict infection control practices are essential for better management and prevention of resistance.

Keywords: CoNS, MRCoNS, *Staphylococcus haemolyticus*, gentamicin, clindamycin, and cotrimoxazole.

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INTRODUCTION

Staphylococci are a widespread group of bacteria that belong to human and animal normal microflora [1]. Staphylococcus genus comprises of two main groups, the coagulase-negative staphylococci (CoNS) and coagulase-positive staphylococci (CoPS), which were defined according to their ability to produce the enzyme coagulase [4]. Staphylococcus species other than *Staphylococcus aureus* are normal inhabitants of the skin and mucous membranes. Staphylococcus species other than *Staphylococcus aureus* are considered opportunistic pathogens that cause various infections [1]. There are over 45 species of Staphylococcus species other than *Staphylococcus aureus.* Currently, there are 38 species of Staphylococcus species other than *Staphylococcus aureus* isolated from various human infections. The important species among them are *Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunesis. Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri, and* Staphylococci xylosus *etc*. [4,9,].

Despite their benign interaction with the host, it is now known that these species can cause critical infections, especially in immunocompromised patients, the reason why they are currently acknowledged as opportunistic pathogens and have been gaining increasing importance in the healthcare field [4,10].

Patients with CoNS infections are usually immunocompromised, with indwelling or implanted foreign bodies. CoNS play a role in bacteremia, central nervous system shunt infection, endocarditis, urinary tract infection, surgical site infections, endophthalmitis, foreign body infections and many other infections [10].

There are very few studies demonstrating the frequency and antibiotics susceptibility profile of Staphylococcus species other than *Staphylococcus aureus* at the current place of work. Hence the present study was undertaken to isolate Staphylococcus species other than *Staphylococcus aureus* from clinical samples, their speciation and antibiotic susceptibility pattern by Vitek-2 method of the isolates [4].

MATERIALS AND METHODS

Study Design and Setting

This hospital based cross-sectional study was conducted in the department of Microbiology National institute of Medical Science & Research (NIMS&R), Jaipur, Rajasthan.

Study period

The study was carried out over a period one year after obtaining approval from the institutional Ethics committee.

Ethical Consideration- The study was accredited by the ethics committee of NIMS University Rajasthan, Jaipur (Proposal no. IEC/P-843/2024)

INCLUSION CRITERIA

1. Various clinical samples including urine, pus, blood, wound swabs, Ear swabs, CSF and body fluids will be taken from various clinical departments.
2. All Age Groups and Genders
3. Patients who have given informed consent.

EXCLUSION CRITERIA

1. Sputum, stool, vaginal swab and unpaired blood culture.

Specimen Collection and Processing

All clinical specimens were collected aseptically following standard procedures and transported immediately to the microbiology laboratory for further processing.

Microscopy

Direct smears were prepared from the samples and subjected to Gram staining. Microscopic examination was performed to determine the Gram reaction and morphology of the organisms.

Culture and Identification

Specimens were inoculated onto blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18–72 hours. Identification of bacterial isolates was carried out based on colony morphology, Gram staining of colonies, and standard biochemical tests as per conventional microbiological methods.

Preliminary Identification Tests

Preliminary identification of Gram-positive cocci was performed using catalase test, coagulase test (slide coagulase and tube coagulase) where applicable.

Identification of Isolates &Antimicrobial Susceptibility Testing

Identification and Antimicrobial susceptibility testing (AST) of the confirmed staphylococcus species other than staphylococcus aureus isolates was performed using the Vitek® 2 Compact automated system (bioMérieux). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2024 and Vitek® 2 manufacturer recommendations.

RESULT

A total of 37 clinically significant coagulase-negative staphylococci (CoNS) isolates were obtained from various clinical samples. Among these, the most common species isolated was *Staphylococcus haemolyticus* 16 (43.2%), predominated followed by *Staphylococcus epidermidis 11 (29.7%), Staphylococcus hominis 7 (18.9%), Staphylococcus cohnii subspecies cohnii 1(2.7%), Staphylococcus sciuri 1(2.7%), and S. cohnii subspecies urealyticus 1(2.7%).* Both males and females were included, with a slight predominance of male patients in the study. Male patients 22 (59.4%) were more affected than females 15 (40.5%).

Table-1: Species-wise Distribution of Coagulase-Negative Staphylococci (CoNS) Isolates by Sample Type

Name of Species	Number of isolates (%)	Pus, wound swab and body fluids (%)	Blood (%)
Staphylococcus haemolyticus	16(43.2%)	2(12.5%)	14(87.5%)
Staphylococcus epidermidis	11(29.7%)	2(18.1%)	9(81.8%)
Staphylococcus hominis subsp. hominis	7(18.9%)	0	7(100%0
Staphylococcus cohnii subsp. cohnii	1 (2.7%)	0	1(100%0
Staphylococcus cohnii subsp. urealyticus	1(2.7%)	0	1(100%)
Staphylococcus sciuri	1(2.7%)	0 (100%)	0

S. heamolyticus showed high degree of resistance to penicillin (100%) followed by oxacillin(100%), erythromycin (100%), ciprofloxacin (81.2%), levofloxacin (81.2%), clindamycin (68.7%), moderate resistance towards rifampicin (43.7%), gentamycin (37.5%), Trimethoprim/ Sulfamethoxazole (37.5%) and low degree of resistance to teicoplanin(18.7%), tetracycline (18.7%), vancomycin (12.5%) and no resistance to linezolid and daptomycin.

Similarly *S. epidermidis* showed high degree of resistance towards penicillin (100%) followed by oxacillin(72.7%), levofloxacin (72.7%), ciprofloxacin (63.6%), erythromycin (63.6%), Trimethoprim/ Sulfamethoxazole (63.6%), moderate resistance towards, clindamycin (36.3%), tetracycline (27.2%) and low degree of resistance to gentamycin (18.1%), rifampicin (18.1%), and no resistance to linezolid, daptomycin, teicoplanin and vancomycin.

S. hominis subsp. hominis showed high degree of resistance towards penicillin oxacillin, erythromycin i.e. (100%), ciprofloxacin, levofloxacin, Trimethoprim / Sulfamethoxazole, clindamycin i.e. (71.4%), moderate resistance towards, rifampicin (42.8%), and low degree of resistance to Tetracycline (14.2%) and no resistance to gentamycin, linezolid, daptomycin, teicoplanin and vancomycin.

Staphylococcus cohnii subsp. Cohnii, showed high degree of resistance to penicillin, oxacillin, gentamycin, clindamycin, rifampicin i.e. (100%) and no resistance to ciprofloxacin, levofloxacin, erythromycin, linezolid, daptomycin, teicoplanin, tetracycline, Trimethoprim/ Sulfamethoxazole and vancomycin. *S. cohnii subsp. Urealyticus showed* high degree of resistance to penicillin, oxacillin, levofloxacin, ciprofloxacin, erythromycin, clindamycin, vancomycin, rifampicin, tetracycline, teicoplanin and no resistance to Trimethoprim/ Sulfamethoxazole, gentamycin, linezolid, daptomycin. Similarly, *Staphylococcus sciuri* high degree of resistance to penicillin, oxacillin, ciprofloxacin, erythromycin, clindamycin, vancomycin, rifampicin, Trimethoprim/ Sulfamethoxazole, tetracycline, and no resistance to gentamycin, linezolid, daptomycin, teicoplanin.

Table-2: Antibiotic Resistance Pattern of Coagulase-Negative Staphylococci (CoNS) Species

Antibacterial agent	S. haemolyticus (n=16) (%)	S. epidermidis (n=11)(%)	S. hominis sub sp. Hominis (n=7)(%)	S. cohnii sub sp. cohnii (n=1) (%)	S. cohnii subsp. urealyticus (n=1) (%)	S. sciuri (n=1) (%)
penicillin	100	100	100	100	100	100
oxacillin	100	72.7	100	100	100	100
gentamicin	37.5	18.1	0	100	0	0
ciprofloxacin	81.25	63.6	71.4	0	100	100
levofloxacin	81.2	72.7	71.4	0	100	0
erythromycin	100	63.6	100	0	100	100
clindamycin	68.7	36.3	71.4	100	100	100
linezolid	0	0	0	0	0	0
daptomycin	0	0	0	0	0	0
teicoplanin	18.7	0	0	0	100	0
vancomycin	12.5	0	0	0	100	100
tetracycline	18.7	27.2	14.2	0	100	100
rifampicin	43.7	18.1	42.8	100	100	100
trimethoprim/sulfamethoxazole	37.5	63.6	71.4	0	0	100

DISCUSSION

Coagulase-negative staphylococci (CoNS) are increasingly recognized as important nosocomial pathogens, particularly in immunocompromised patients and those with indwelling medical devices. Earlier considered contaminants, they are now established as significant causes of bloodstream infections, urinary tract infections, and wound infections [1].

In the present study, *Staphylococcus haemolyticus* was the most predominant isolate, followed by *Staphylococcus epidermidis* and *Staphylococcus hominis*. Similar findings have been reported in Indian studies by Usha MG *et al*. and Singh S *et al*., where *S. haemolyticus* and *S. epidermidis* were the most common CoNS species isolated from clinical samples [2,3]. However, some studies have shown *S. epidermidis* as the predominant isolate, indicating geographical variation in species distribution [4].

A high level of resistance to penicillin and oxacillin observed in the present study indicates a significant prevalence of methicillin-resistant CoNS (MRCoNS).

Similar MRCoNS prevalence ranging from 50% to 70% has been reported in previous Indian studies [3,5]. The presence of methicillin resistance is clinically important as it limits the use of β-lactam antibiotics and may facilitate transfer of resistance genes to more virulent organisms such as *Staphylococcus aureus* [6]. Resistance to ciprofloxacin and erythromycin was also found to be high in the present study. This may be due to the widespread and empirical use of these antibiotics in clinical settings. Comparable resistance patterns have been reported by Asangi SY *et al*., who observed high resistance to fluoroquinolones and macrolides among CoNS isolates [5]. Moderate resistance to gentamicin, clindamycin, and cotrimoxazole further reduces the available therapeutic options. Similar trends have been documented in other studies, suggesting increasing multidrug resistance among CoNS isolates [3,7]. All isolates in the present study were high susceptible to linezolid, vancomycin,Daptomycin and teicoplanin, which is consistent with earlier reports [5,7]. These antibiotics remain the drugs of choice for serious CoNS infections; however, they should be used judiciously to prevent the emergence of resistance. Inducible clindamycin resistance (iMLSB phenotype) detected in this study highlights the importance of performing the D-test routinely. Failure to detect inducible resistance can lead to clinical treatment failure, as reported in previous studies [8]. Overall, the findings of this study emphasize the growing problem of antimicrobial resistance among CoNS and the need for continuous surveillance, strict infection control practices, and rational antibiotic use in hospital settings [6].

CONCLUSION

Coagulase-negative staphylococci (CoNS) are important pathogens and should not be ignored as contaminants. In this study, *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* were the most common isolates. A very high level of resistance was seen to beta-lactam and macrolide antibiotics, especially penicillin and oxacillin, showing a high burden of multidrug-resistant and methicillin-resistant CoNS. This makes treatment more difficult. However, all isolates were highly sensitive to vancomycin, Daptomycin, teicoplanin, and linezolid, which remain effective treatment options. These drugs should be used carefully to prevent future resistance. The presence of inducible clindamycin resistance highlights the need for routine D-test to avoid treatment failure. Overall, proper identification of CoNS, regular monitoring of resistance patterns, good antibiotic practices, and strict infection control measures like hand hygiene are essential to manage infections and improve patient outcomes.

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