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Original Research Article

Clinico-bacteriological profile and antibiogram of *Staphylococcus epidermidis* with special emphasis on Methicillin resistance and hospital acquired infections in a tertiary care center south India

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ABSTRACT

Background: *Staphylococcus epidermidis* is a normal commensal of the skin and mucous membrane of humans and animals. Despite the growing importance of its pathogenesis especially in neonatal septicemia and device associated infections, it is still considered as insignificant isolate in the clinical practice. Hence, the present study is taken up to analyze the sources and risk factors of the isolates and to know their antibiogram along with occurrence of Methicillin resistant *S. epidermidis* (MRSE).

Materials and Methods: 150 clinically significant *S. epidermidis* isolates from various clinical specimens were considered in this study. Species identification was done by phenotypic methods. The antimicrobial susceptibility test and detection of Methicillin resistance were performed by Kirby-Bauer's disc diffusion method as per CLSI guidelines.

Results: Among 150 *S. epidermidis* isolates, 78% were recovered from hospital acquired infections. They were commonly isolated in pediatric age group (30%) and among males (60.67%). Total of 34.67% were isolated from pus samples followed by blood (25.33%). Most of the isolates were associated with multiple risk factors like hospitalization, prior antibiotic administration, foreign body in situ and ICU admission. Majority of the isolates expressed resistance towards Penicillin (93.33%), followed by Amoxicillin-Clavulanic acid (76%), Cotrimoxazole (71.33%), Fluoroquinolones (64%), Gentamicin (60%) and Erythromycin (55.33%). Resistance against Amikacin (16.67%), Tetracycline (9.3%) and Linezolid (0.67%) was low. All isolates were sensitive to Vancomycin. Inducible Clindamycin resistance was 18% and MRSE was 68%.

Conclusion: Clinical importance and emergence of drug resistance among *S. epidermidis* infections is growing with the advent of advanced medicine. This warrants the need to implement simple laboratory methods for species identification of the *S. epidermidis* and to determine the antibiotic resistant patterns on routine basis. Clinical correlation of the isolate is crucial to rule out the colonizers and contaminants.

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1. Introduction

Staphylococcus epidermidis; a Coagulase Negative Staphylococcus (CoNS), is Gram-positive cocci that occurs predominantly in 'grape-like' clusters. It is non-motile, non-spore forming and facultative anaerobe.¹

Originally, of all the staphylococci, only *Staphylococcus aureus* was considered pathogenic and all other species were grouped under CoNS and were considered nonpathogenic commensals on skin and mucous membranes of humans and other organisms.^{2,3}

* Corresponding author. E-mail address: ucumasiri@gmail.com (N. W. Nandihal). The commonest resident staphylococcal species on human skin is *S. epidermidis*. The largest populations (c.

10⁴–10⁶cfu/cm²) are found in regions of the skin with large numbers of pilosebaceous units and sweat glands and on the skin and mucous membranes surrounding openings to the body surface.¹

S. epidermidis possesses several virulence factors like Exopolysaccharide slime, Fibrinogen binding protein (Fbe), Extracellular matrix binding protein (Embp), Fatty Acid Modifying Enzyme (FAME) and Lipases.⁴It has certain adaptations such as the down-regulation of Nucleic Acid (NA), proteins and cell wall biosynthesis and biofilm formation.⁵

Host factors that often lead to infections with S. *epidermidis* include; breaches in natural mucocutaneous barriers due to surgery, trauma or inflammation, prior exposure to antibiotics and immunosuppression.³ Although host defects are clearly important in the pathogenesis, the most important factor contributing to the increasing number of nosocomial CoNS infections is the presence of indwelling prosthetic devices in both compromised and uncompromised hosts. Biofilm is a very useful and powerful factor contributing to Foreign Body-Related Infections (FBRIs) of staphylococci also designated as Device Associated Healthcare-Associated Infections (DA-HAIs).^{6,7}

S. epidermidis is involved in the pathogenesis of various infections like native and prosthetic valve endocarditis, osteomyelitis, intravenous catheters infections, Catheter Associated Blood Stream Infections (CA-BSI), Central Nervous System infections, peritonitis in peritoneal dialysis patients, Ocular infections, Catheter Associated Urinary Tract Infections (CA-UTI) and variety of cutaneous lesions.⁴

Innate and acquired immunity play an important role against *S. epidermidis* infection. Despite the presence of antibodies, it is difficult to clear *S. epidermidis*. This may be due to exopolymers that protect the bacteria from antibody recognition and lysis. Also, as it is a resident colonizer, the immune system might be less active against it.⁵

Antibiotic resistance is a growing challenge in treating the infections caused by S. epidermidis as it tends to be more multidrug resistant. Penicillin-resistant S. epidermidis isolates, responsible for fatal subacute bacterial endocarditis, were being reported as early as 1949.8 Today, as a result of huge selection pressures, it is very rare to find Penicillin-susceptible S. epidermidis isolates (10%) among hospitalized patients.^{4,7} In recent studies, the prevalence of oxacillin resistant S. epidermidis isolates has reached about 80% or more. As occurs with MRSA, MRSE isolates are more often multidrug resistant than Methicillin susceptible ones.⁷Studies have demonstrated conjugative transfer of Gentamicin resistance plasmids from CoNS to CoNS and from CoNS to S. aureus. That means CoNS, particularly S. epidermidis, may be a reservoir for antibiotic resistance genes in the hospital environment.

S. epidermidis along with *S. haemolyticus* has been found to be the commonest CoNS species exhibiting reduced susceptibility to Glycopeptides. These resistant isolates were reported long before the advent of the first *S. aureus* isolates with reduced Glycopeptide susceptibility, in 1997.⁷

In view of increasing prevalence of *S. epidermidis* infections, the present study has been undertaken to study the clinical and microbiological profile of *S. epidermidis* and its antibiogram along with detection of methicillin resistance using conventional techniques in our set up.

2. Materials and Methods

This cross sectional study was carried out over a period of one year. S. epidermidis isolated from various clinical samples either in pure or mixed culture from urine/pus samples (not more than two organisms) were included in the study. Contamination was ruled out by repeated isolation and significant colony counts. Detailed patients' history was collected. Samples were processed as per standard microbiological procedure.9,10 Identification of S. epidermidis was done based on colony morphology on Chocolate agar, Gram stain and a set of biochemical reactions; that includes, Catalase activity, inability to produce coagulase enzyme, urease activity, susceptibility to Novobiocin, negative pyrrolidonylarylamidase test (PYR test), decarboxylation of ornithine and aerobic acid production from mannose. (Image-1) The antimicrobial susceptibility test was performed by Kirby-Bauer's disc diffusion method using routine panel of antibiotics. Methicillin resistance was detected by using Cefoxitin (30µg) disc and Inducible Clindamycin resistance was detected by D-Test using Clindamycin $(2\mu g)$ and Erythromycin $(15\mu g)$. Standard reference strain of Staphylococcus aureus ATCC 25923 was included and parallel tests were carried out for quality control. Interpretation of the results was done using CLSI guidelines.¹¹

The resultant data was analyzed using descriptive statistics and presented in the form of tables.

3. Results

A total of 150 clinically significant *S. epidermidis* isolates from various clinical samples over a period of one year were considered in the present study, of which maximum of *S. epidermidis* were recovered from patients of 0 to 10 years (30.67%) followed by 21 to 30 years of age (19.33%) (Table 1). Male to female ratio is 1.54:1 (Table 1). 78% of *S. epidermidis* were isolated from hospital acquired infections and 22% from community acquired infections. Among hospital localities, 30% of total 150 isolates were recovered from intensive care units (Table 2). Maximum of *S. epidermidis* were yielded from pus samples (34.67%) followed by Blood (25.33%) and ear discharge (16.67%). Among associated risk factors, as high as 78% of patients had history of hospitalization, 73.33% had history of prior antibiotic administration, 57% of patients had foreign body in situ, 45% were ICU patients, 9.33% of the patients were diabetic and various other risk factors that encountered in lesser frequency are listed in Table 4.

Table 1: Age and gender wise distribution of S. epidermidis isolates. (n=150)

Age group	No. of isolates		Percentage			
0-1 year	28	46	18.67%	30.67%		
1-10 years	18	40	12%	50.07%		
11-20 years	10		6.6	57%		
21-30 years	29		19.33%			
31-40 years	1	16 10.679		16		67%
41-50 years	15		10%			
51-60 years	15		10%			
61-70 years	13		8.67%			
71-80 years	05 3.33%		33%			
81-90 years	01		0.67%			
Male	91		60.67%			
Female	49		39.33%			

 Table 2: Distribution of sources of S. epidermidis infections (n=150)

	No. of isolates	Percentage
Out patient	33	22%
In patient	117	78%
Wards	72	48%
ICU	45	30%
- NICU	26	17.33%
- PICU	06	04%
- SICU	05	3.33%
- IOICU	04	2.67%
- MICU	04	2.67%

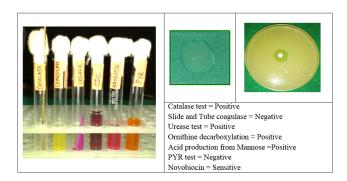


Fig. 1: Biochemical reactions of S. epidermidis

Among antibiotics maximum resistance was expressed against Penicillin (93.33%), Amoxicillin Clavulanic acid (76%) and Cotrimoxazole (71.33%) followed by Norfloxacin (64%), Ciprofloxacin (63.33%), Gentamicin (60%) and Erythromycin (55.33%) lesser resistance was expressed against Amikacin (16.67%), Tetracycline (9.33%) and Linezolid (0.67%). All the 150 isolates were 100% sensitive to Vancomycin and Teicoplanin. Inducible Clindamycin resistance is seen in 18% of the isolates and Methicillin resistance was detected among 102 isolates of *S. epidermidis* accounting for 68% of MRSE.

4. Discussion

S. epidermidis is the most frequently isolated commensal species from human epithelia. It colonizes predominantly axillae, head and nares.⁵ However it is seen as an important opportunistic pathogen. It is the most frequent cause of nosocomial infections in particular; it represents the commonest source of infection on indwelling medical devices.¹² A better understanding of the physiology of *S. epidermidis* is important to evaluate therapeutic strategies against *S. epidermidis* infections.⁵

Present study has studied 150 clinically significant *S. epidermidis* isolates with respect to the demographic profile of the patients, source of infections and associated risk factors along with their antibiotic susceptibility testing and detection of Inducible Clindamycin and methicillin resistance using conventional methodology. Results are compared with the studies conducted across the country and globe.

Maximum of *S. epidermidis* were isolated from the age group of 0 to 10 years (30.67%) followed by 21 to 30 years (19.33%). 0 to 1 year age group alone accounted for 18.67%. Which shows that pediatric age group especially infants are at higher risk of infections with *S. epidermidis*. Similar results were observed in study conducted by Lopes N et al ¹³ who isolated 24.4% of *S. epidermidis* isolates from children less 1 year of age group. Male preponderance is observed in the present study similar to the study done by Lopes N et al.¹³

S. epidermidis is isolated from all type of clinical samples of which maximum of isolates were recovered from pus samples (34.67%) followed by Blood (25.33%). Pus sample is the leading source of S. epidermidis in the studies conducted by Jayanthi RS et al (30.11%)¹⁴ and Choudary U et al (30.12%)¹⁵ and blood is second most common sample in a study conducted by Jayanthi RS et al (22.59%).¹⁴ However in a study conducted by C Roopa et al,¹⁶ 82.45% of *S. epidermidis* isolates are recovered from pus samples and contrastingly only 16.54% were recovered in the study conducted by Parashar S et al.¹⁷ Similarly blood is the commonest sample in a study conducted by Choudary U et al $(54.22\%)^{15}$ and it is less common sample in a study conducted by C Roopa et al (5.26%)¹⁶ and Asangi S et al (2%).¹⁸ Most of the blood cultures samples were received from NICU in the present study. In a study conducted by Farran CA Y et al,¹⁹ 53.9% of blood culture isolates were S. epidermidis and with greater proportion in early onset neonatal septicemia. Ahmed M M et al²⁰

Samples	Total (n=150)			Hospital acquired (n=117)		Community acquired (n=33)	
	No. of	Isolates	%	No.	%	No.	%
Pus	52						
- Pyoderma	15	10%					
- Abscess	10	6.67%					
- Orthopedic implant in situ	09	6%	34.67%	41	35.04	11	33.33
- Orthopedic implant in situ	08	5.33%	54.07%	41	55.04	11	55.55
- Osteomyelitis	02	1.33%					
- Burn wound infection	02	1.33%					
- Diabetic foot	06	4%					
Blood		38	25.33%	37	31.62	01	3.03
Ear discharge		25	16.67%	06	5.13	19	57.58
Sputum		10	6.67%	09	7.69	01	3.03
CSF		08	5.33%	08	6.84	-	-
Urine		06	4%	05	4.27	01	3.03
Vaginal swab		04	2.67%	04	3.41	-	-
Cervical swab		02	1.33%	02	1.71	-	-
Device (CVP Tip)		02	1.33%	02	1.71	-	-
Throat swab		01	0.67%	01	0.86	-	-
Ascitic fluid		01	0.67%	01	0.86	-	-
Pleural fluid		01	0.67%	01	0.86	-	-

Table 3: Sample wise distribution of S. epidermis isolates

Table 4: Antibiotic resistance pattern of S. epidermis isolates

Antibiotics	Hospital acquired (n=117)		Community acquired (n=33)		Total number of Resistant isolates (n=150)	
	No.	%	No.	%	No.	%
Penicillin	117	100	23	69.7	140	93.33%
Amoxicillin /	100	85.47	14	42.43	114	76%
Clavulanic acid						
Cotrimoxazole	97	82.91	10	30.30	107	71.33%
Norfloxacin	88	75.21	06	18.18	96	64%
Ciprofloxacin	87	74.36	08	24.24	95	63.33%
Gentamicin	82	70.09	08	24.24	90	60%
Erythromycin	65	55.56	18	54.56	83	55.33%
Clindamycin	50	42.74	23	69.70	73	48.67%
Amikacin	20	17.09	05	15.15	25	16.67%
Tetracycline	05	4.27	09	27.27	14	9.33%
Linezolid	1	0.86	00	0	1	0.67%
Teicoplanin	00	0	00	0	00	0%
Vancomycin	00	0	00	0	00	0%
Cefoxitin	95	81.2	07	21.2	102	68%

found 65.5% of Gram positive cocci causing Bacteremia and majority among them were *S. epidermidis* isolates similarly Lopes N et al¹³ recovered 60.5% of *S. epidermidis* from CA-BSI cases. Out of 52 pus samples, maximum were collected from pyoderma (15) and Abscesses (10) followed by Orthopedic implant associated wound infections (09), Surgical site infections (08) and Diabetic wound infections (06). In a study conducted by Jayanthi RS et al¹⁴30% of *S. epidermidis* were isolated from Post-Operative wound infections and in a study by Lopes N et al¹³ 5.8% of Surgical site infections were caused by *S. epidermidis* in a study conducted by Sharma L et al.²¹

Present study noticed that only 4% of *S. epidermidis* were isolated from urine samples which is in good correlation with the study conducted by Choudary U et al¹⁵ however in many other studies^{17,18,22} relatively higher number of isolates were recovered from urine samples and Lopes N et al¹³ isolated 12.8% of *S. epidermidis* from CA-UTI cases. Frequency of CSF (5.33%) sample in the present study is similar to the results seen in the studies conducted by C Roopa et al¹⁶ and Golia et al.²²

Coming to the risk factors associated with S. epidermidis infections, most of the patients in the present study

Risk factors	No. of isolates	Percentage
Prior of antibiotic administration	110	73.33
Present History of surgery	31	20.67
ICU admission	45	30%
NICU	26	17.33%
PICU	06	04%
SICU	05	3.33%
OICU	04	2.67%
MICU	04	2.67%
Foreign body insitu	57	38%
I.V. Catheter	29	19.33
Suture	14	09.33
Orthopedic Implant	09	06
Urinary Catheter	02	1.33
CVP	02	1.33
Abdominal Drain	01	0.67
Underlying condition		
Diabetes Mellitus	14	09.33
Burn	02	1.33
Pregnancy	02	1.33
COPD	03	02
Cancer	03	02
HIV Infection	01	0.67
Chronic Kidney Disease	02	1.33
Pulm. TB	01	0.67
Liver cirrhosis	01	0.67
Hospitalized patients	117	78%

Table 5: Risk factors associated with S. epidermidis infection (n=150)

had more than one underlying risk factors, commonest being hospitalization (78%) and antibiotic therapy (73.33%) followed by foreign body in situ (57%) and ICU stay (45%). Study conducted by Lopes N et al¹³ also found out that 55.8% of the patients were under antibiotics therapy and Chabi R et al²³ in their study recovered 46% of *S. epidermidis* isolates from hospital acquired infections.

Among antibiotics, maximum resistance was expressed against Penicillin (93.33%), similar results are seen in most of the studies.^{18,22,23} However Saradar SA et al^{24} and Farran CA et al 19 showed that all 100% of their isolates being resistant to Penicillin. Resistance against Amoxicillin-Clavulanic acid (76%) is comparable to the study done by Saradar SA et al²⁴ and contrastingly Golia et al²² observed only 21% resistance. 71.33% isolates were resistant to Cotrimoxazole in the present study. Correlating results are seen in the studies done by Chabi R et al²³ and Saradar SA et al,²⁴ contrasting results are shown in the studies done by Seetha KS et al.²⁵ and Farran CA et al.¹⁹ Unfortunately next highest resistance was seen against two most important alternative antibiotic options in MRSE infections i.e Fluoroquinolones (64%) and Gentamicin (60%). Similar pattern is seen in Chabi R et al²³ and Farran CA et al¹⁹ studies. However in most of the other studies.^{22,25,26} Gentamicin is shown as one of the most effective antibiotics and in our study Amikacin (16.67%)

and Tetracycline (9.3%) turns out to be the effective therapeutic options against S. epidermidis infections. Which is similar to the studies done by Ganti et al²⁶ and Seetha KS et al,25 but Chabi R et al23 showed 91.3% of resistance and contrastingly in a study done by Saradar SA et al²⁴ 100% isolates were sensitive to Tetracycline. 55.33% isolates are resistant to Erythromycin in present study which is in correlation with studies done by Asangi S et al¹⁸ and See tha KS et al.,²⁵ but not in correlation with studies done by Chabi R et al²³ and Ganti et al.²⁶ Only one out of 150 isolates is resistant to Linezolid in the present study whereas few other studies 18,22,26 show around 9% of resistance against Linezolid. Fortunately none of the 150 isolates were resistant towards Glycopeptides in the present study. Prevalence of MRSE in our study is considerably high (68%). Similar occurrence is seen in the studies done by Golia et al $(64.5\%)^{22}$ and Asangi S et al $(65\%)^{18}$ in contrast to a study conducted by C Roopa et al (21%).¹⁶ Variable Prevalence of MRSE is seen in various studies. 17,23-29

The present study has included only clinically significant *S. epidermidis* isolates. Species identification and antibiotic susceptibility tests were performed using conventional methodology that can be reproduced in any other laboratories. Detailed clinical history regarding underlying risk factors and demographic profile is analyzed to correlate the significance and pathogenicity of the isolate and the

data can be used in formulating strategies on prevention and control of the hospital acquired *S. epidermidis* infections. As the study was conducted over a period of one year which is considerably longer, hence the results can be generalized and data may be utilized in policy making on routine surveillance cultures, empirical antibiotic policies and other control measures

5. Limitations

- 1. Present study focuses mainly on species identification and antibiogram of *S. epidermidis*, further study to ascertain the pathogenic significance like biofilm productions, detection of genes contributing for the virulence and antibiotic resistance would have made sense.
- 2. Vancomycin susceptibility is tested by disc diffusion method and MIC detection was not performed.

6. Conclusions

S. epidermidis is like a double edged sword as it is always confusing for Microbiologists whether to report it or not, and will always end up in reporting with a comment as "The isolate could be a skin commensal/contaminant". Proper history, appropriate sample collection and repeated isolation confirm it's significance. Present study throws a light on importance of patient's history and significance of identification of S. epidermidis to species level in ascertaining the pathogenicity. Multidrug resistant strains of S. epidermidis higher rate of MRSE alarms that in vitro antibiotic susceptibility is must in all cases. That will guide the clinicians in treating the infected patients. There should be constant surveillance to detect emergence of Glycopeptide resistance. High degree of suspicion in addition to good infection control practices, the rational use of antimicrobial agents is one of the major steps in preventing S. epidermidis infections and antibiotic resistance.

7. Source of Funding

None.

8. Conflict of Interest

None.

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