

Original Research Article

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## Characterization and Antibiotic Susceptibility Pattern of Coagulase Negative Staphylococci with Special Reference to Methicillin Resistance

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### ABSTRACT

#### Keywords

Coagulase negative, Staphylococcus, Methicillin, Antibiotic susceptibility, Antibiotic resistance.

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Coagulase negative staphylococci (CONS) being the most common inhabitants of skin and mucous membranes are known to be the commonest opportunistic pathogens causing infections among the immune compromised hosts as well as the patients with medical devices such catheters, prosthetic heart valves and orthopaedic prosthesis. In recent years, an increase in the number of methicillin resistant coagulase negative Staphylococci (MRCONS) strains has become a serious public health problem, as the resistance for this antibiotic implies resistance to almost all the beta-lactam antibiotics. To assess the antibiotic susceptibility pattern of coagulase negative staphylococci with special reference to methicillin resistance. A total of 120 strains of CONS isolated in pure culture from various clinical samples were subjected to speciation and antibiotic susceptibility pattern with special reference to methicillin. In addition these isolates were also tested for slime production and beta-lactamase production. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method. Out of 120 CONS isolates, 65.5% were *S. epidermidis*, 15% *S. saprophyticus*, 10.83% *S. haemolyticus*, 3.33% *S. Scuiri*, 1.67% *S. hominis*, 1.67% *S. Xylosus*, 1.67% *S. Cohnii*, 0.83% *S. Simulans*. Majority of the isolates were from pus followed by blood and urine. Among the various organisms 55.83% were slime producing organisms. 37.5% isolates showed Beta-lactamase production and 40% of the species showed resistant to methicillin antibiotic. Most of the species had also showed resistance to penicillin, ampicillin and gentamicin. The increasing recognition of CONS & emergence of drug resistance demonstrates the need for characterization and determination of antibiotic susceptibility of CONS with special reference to methicillin has become warranted.

### Introduction

Coagulase negative Staphylococci (CONS) form the major part of the skin flora of man and other mammals and were considered to

be non-pathogenic in past. However infections with these have been reported since 1950 with increasing frequency.<sup>[1]</sup>

CONS had become the most common causative agents leading onto infections in immuno-compromised hosts particularly patients with cancer, burns, end stage renal disease and renal transplantation and of infections related to indwelling catheters, shunts and prosthetic device.<sup>[2]</sup> They cause infections like septicaemia, endocarditis, osteomyelitis, urinary tract infection and peritonitis.<sup>[3]</sup>

The most frequently encountered CONS species associated with human infections was *S. epidermidis*, particularly associated in patients with intravascular catheters. It is the most predominant agent of nosocomial bacteremia, endocarditis, urinary tract infection, surgical wound infection, ophthalmic infections, intravenous catheter infection and infection of various prosthetic devices. The second most frequently encountered CONS species is *S. haemolyticus*, implicated in native-valve endocarditis, septicemia, peritonitis & wounds, bone & joint infections. Similarly *S. saprophyticus* causes urinary tract infections in young healthy sexually active women. Infection with *S. lugdunensis* is seen in endocarditis with massive valve destruction.<sup>[4]</sup>

An increase in the number of methicillin resistant coagulase negative Staphylococci (MRCONS) strains has become a serious clinical and epidemiological problem as resistance to this antibiotic implies resistance to all the beta-lactum antibiotics. Accuracy and promptness in the detection of methicillin resistance is very much important in ensuring correct antibiotic treatment in the infected patients so that the control of MRCONS can be achieved in the hospital environments.<sup>[5]</sup> Slime is one of the virulence marker which is believed to make the microorganism insusceptible to certain antibiotics.<sup>[6]</sup> Detection of slime producing

and beta-lactamase producing strains help in establishment of CONS as a pathogen. As of today only very few studies had been done in southern India to assess the methicillin resistance among the CONS, so the present study has been undertaken to identify, speciate and to know the antibiotic susceptibility pattern of CONS with special reference to methicillin resistant, as they are considered to be one of the most important pathogens causing severe debilitating infections. In addition to this the isolates were also tested for slime production and beta-lactamase production

### **Materials and Methods**

The study was conducted over a period of one year in the Department of Microbiology, J.N Medical College, Belagavi. A total of 120 CONS isolated from clinical samples such as pus, urine, blood, endotracheal tube tip, cervical swab, pleural fluid, amniotic fluid and CSF were included in the study. Gram positive cocci in clusters seen in a Gram stained smear along with pus cells, repeatedly isolated from the same lesion, found relevant by critical appraisal of clinical picture were included. CONS isolated along with other bacteria (mixed culture), with no clinical correlation of symptoms were excluded.

A detailed clinical history was obtained from each patient. Isolates were identified & speciated based on Gram stain, colony morphology on Blood agar Catalase test, Oxidase test, Slide & Tube Coagulase test, Urease test, Phosphatase test, Oxidative and fermentative test, Carbohydrate utilization test, Furazolidine, Bacitracin & Novobiocin Susceptibility test done according to standard protocol.<sup>[4]</sup> Test for slime production done by tube method as described by Christensen *et al.,*, A loopful of organism from a blood agar plate

inoculated into 5ml of trypticase soya broth incubated at 35<sup>0</sup>C for 48 hrs. After the contents of tubes were aspirated and the tubes stained with safranin for 30 min. Visible safranin stained film along the wall of the tube taken as slime producer. Visible safranin stained film at the liquid-air interference taken as non slime producer.<sup>[7]</sup> Beta lactamase production was tested by chromogenic cephalosporin method using nitrocefin disk. A nitrocefin disk moistened with normal saline and the colony was rubbed over the disk with the glass rod. Pink colour development within 60 seconds is taken as positive.<sup>[4]</sup> Antimicrobial susceptibility testing was performed by the Kirby-Bauer's disk diffusion method following the clinical and Laboratory standard Institute(CLSI) guidelines.

## Results and Discussion

Table 1 shows the distribution of CONS isolates from different clinical specimens. It is inferred from the table that among the various species of CONS *S. epidermidis* (65%) found to be the most common isolate followed by *S. saprophyticus* (15%) and *S. hemolyticus* (10.8%) and the least common species were *S. hominis* (1.67%), *S. xylosum* (1.67%) and *S. cohnii* (1.67%). Most of the samples which were tested were from either the pus from the wound site or the wound swab (48.3%) followed by blood (22.5%).

The antibiotic susceptibility of CONS species was shown in table 2. Among the various antibiotics the maximum sensitivity was seen for cephalexin (71%) followed by co-trimoxazole (56.6%) and the minimum sensitivity was seen for penicillin (25%) and ampicillin (27.5%), which invariably mean that the antibiotic resistance is more for penicillin and ampicillin and about 50% of resistance is seen for erythromycin and tetracyclins.

The methicillin resistant pattern for CONS species had shown 100% resistant for *S. simulans* and *S. xylosum* and for the most common species, *S. epidermidis* the resistant level was 47.4% and the least resistance among all the species was seen for *S. saprophyticus* (5.5%) and *S. hemolyticus* (13%) (table3).

The susceptibility pattern of methicillin resistant CONS had shown resistance level of 90-100% for antibiotics like penicillin, ampicillin, erythromycin and tetracycline and methicillin sensitive CONS had shown 80-90% sensitivity to erythromycin, tetracycline and co-trimoxazole and 98% sensitivity to cephalexin (table4).

CONS have emerged as one of the important cause of nosocomial infections. During the past 10 years CONS have been recognized as major cause of septicemia in patients with various implanted medical devices.<sup>[2]</sup> Most developed countries have reported an increase in colonization & infections in hospitalized patients by CONS, which are resistance to methicillin and other antibiotics. However only few data are available on CONS infections with methicillin resistance from developing countries.<sup>[8]</sup>

In the present study most of the isolates were from pus followed by blood and urine and it is well correlated with previously published studies<sup>[9,10,11,12,13]</sup> which had clearly shown that CONS were commonly isolated from pus, urine, blood & indwelling catheters. The most common organism identified in our study was *S. epidermidis* (65.5%) followed by *S. Saprophyticus* (15%), *S. haemolyticus* (10.83%) which was almost in par with the studies conducted by Singh S *et al.*,<sup>[14]</sup> Adriana N *et al.*,<sup>[9]</sup> Pal & Ayyageri.<sup>[11]</sup> Where as Boynukara B *et al.*<sup>[13]</sup> observed that *S. hominis* the common species

(30.8%) isolated followed by *S. epidermidis* (27.7%) & *S. xylosum* (9.2%). The differences in the incidence of individual species in various publications can be attributed to the

clinical samples received from different types and section of the hospitals as well as different methodology applied by various authors for specialization.

**Table.1** Distribution of Cons Isolates from Different Clinical Specimens

Species	Pus/ Wound Swab	Blood	Cervical swab	Endo tracheal tube	Urine	Pleural fluid	Amniotic fluid	CSF	Total
<i>S.epidermidis</i>	44 (56.41%)	25 (32.05%)	04 (5.13%)	02 (2.56%)	01 (1.28%)	01 (1.28%)	01 (1.28%)	-	78 (65.0%)
<i>S.saprophyticus</i>	-	-	-	-	18 (100%)	-	-	-	18 (15.0%)
<i>S.hominis</i>	02 (100%)	-	-	-	-	-	-	-	02 (1.67%)
<i>S.simulans</i>	01 (100%)	-	-	-	-	-	-	-	01 (0.83%)
<i>S.xylosum</i>	01 (50%)	-	-	-	01 (50%)	-	-	-	02 (1.67%)
<i>S.sciuri</i>	03 (75%)	01 (25%)	-	-	-	-	-	-	04 (3.33%)
<i>S.hemolyticus</i>	06 (46.15%)	01 (7.69%)	-	03 (23.08%)	03 (23.08%)	-	-	-	13 (10.83%)
<i>S.cohnii</i>	01 (50%)	-	-	-	-	-	-	01 (50%)	02 (1.67%)

**Table.2** Antibiotic Susceptibility Pattern of Cons Species

Antibiotics	Sensitive (%)	Resistant (%)
Penicillin	30 (25%)	90 (75%)
Ampicillin	33 (27.5%)	87 (72.5%)
Gentamicin	52 (43.33%)	68 (56.67%)
Erythromycin	64 (53%)	56 (47%)
Tetracyclin	62 (51%)	58 (49%)
Co-triamoxazole	68 (56.67%)	52 (43.33%)
Cephalexin	86 (71%)	34 (29%)

**Table.3** Methicillin Resistant Pattern of Cons Species

Species	Sensitive	Resistance	Total
<i>S.epidermidis</i>	41(52.56%)	37(47.44%)	<b>78</b>
<i>S.saprophyticus</i>	17(94.44%)	01(5.56%)	<b>18</b>
<i>S.hemolyticus</i>	10(76.92%)	03(13.08%)	<b>13</b>
<i>S.sciuri</i>	01(25%)	03(75%)	<b>04</b>
<i>S.xylosum</i>	-	02(100%)	<b>02</b>
<i>S.hominis</i>	01(50%)	01(50%)	<b>02</b>
<i>S.cohnii</i>	02(100%)	-	<b>02</b>
<i>S.simulans</i>	-	01(100%)	<b>01</b>
<b>Total</b>	<b>72</b>	<b>48</b>	<b>120</b>

**Table.4** Susceptibility Pattern of Methicillin Resistance Cons (MRCONS)

Antibiotic	MRCONS		MCONS	
	Sensitive	Resistant	Sensitive	Resistant
Pencillin	-	48 (100%)	30 (41.67%)	42 (58.33%)
Ampicillin	-	48 (100%)	33 (45.83%)	39 (54.17%)
Gentamicin	2 (4.16%)	46 (95.84%)	50 (69.44%)	22 (30.56%)
Erthromycin	4 (8.33%)	44 (91.67%)	60 (83.33%)	12 (16.67%)
Tetracyclin	5 (10.42%)	43 (89.58%)	57 (79.17%)	15 (20.83%)
Co-triamoxazole	4 (8.33%)	44 (95.84%)	64 (88.89%)	8 (11.11%)
Cephalexin	16 (33.33)	32 (66.67%)	70 (97.22%)	2 (2.78%)

CONS are characterized by an ability to colonize the surfaces of biomaterial by adhering in biofilm. In the present study 55.83% were slime producing organisms, *S. epidermidis* (82.09%) was the most common species in producing slime followed by *S. haemolyticus* (8.96%) *S. Sciuri* (4.48%) which was almost similar to the results shown by few other authors.<sup>2,11,12</sup> Also Seetha KS *et al.*,<sup>[2]</sup> & Boynukara B *et al.*,<sup>[13]</sup> reported in their study that 16.07% of *S. saprophyticus* was producing slime, but interestingly in our study *S. saprophyticus* didn't produce the slime. On the other hand, it should also be considered that slime production may be affected by various factors, such as medium composition, the presence of carbohydrate, iron and CO<sub>2</sub> and oxidation.

In our study maximum resistance was observed towards penicillin (75%), followed by ampicillin (72.5%) gentamicin (56.66%), cotrimoxazole (43.33%), cephalexin (29.0%) and 40% were resistant to methicillin and these results were exactly in par with Singh S *et al.*,<sup>[14]</sup> and Seetha *et al.*,<sup>[2]</sup> whereas a study done by Singhal R *et al.*,<sup>[8]</sup> reported that CONS had maximum resistance to co-trimoxazole followed by penicillin & methicillin. Out of 120 CONS isolated in the present study, 48 strains (40%) had shown resistance to methicillin. Incidence of MRCONS is increasing day by day. High incidence of MRCONS were reported by Seetha *et al.*, (82.77%)<sup>[2]</sup>, Singhal R *et al.*, (72.3%)<sup>[8]</sup> and Jain, Agarwal and Bansal (66.0%)<sup>[16]</sup>, the incidence of MRCONS varies from 14.6%<sup>[15]</sup> to 38.0%<sup>[14]</sup>. We can

conclude that MRCONS incidences were high among the CONS isolated from the super-speciality hospitals and intensive care units of tertiary care hospitals.

Forty eight MRCONS were isolated in the present study. All the MRCONS (100%) were resistance to penicillin and ampicillin followed by gentamicin (95.83%), erythromycin and co-trimoxazole (91.67%), tetracyclin (89.58%) and least resistance to cephalexin (66.67%). Similar results were shown by Choudhari, Arora and Sharma<sup>[12]</sup> Jain, Agarwal and Bansal<sup>[15]</sup> additionally in their study they had shown that all MRCONS were sensitive to vancomycin.

Variability in the antibiotic susceptibility pattern of CONS has been observed by few author<sup>[ 8, 12, 16]</sup> which reflects the different antibiotics policies followed by different hospitals.

In conclusion, it is now well established beyond doubt that CONS can be the causative agent for many diseases. The clinical significance of CONS continues to increase as medical therapy involving invasive & interventional procedures. The widespread occurrence of methicillin resistance among these patients causes the major therapeutic problem. The Microbiologist should be alert in doing the needful investigations required for identification of such specie, which would help the patients in having a better treatment outcome.

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