



## Study of Coagulase-Negative Staphylococci Isolated from Clinical Specimens in A Tertiary Care Hospital of North East India

**\* Dr. J. Hazarika**

Assistant Professor, Department of Microbiology, LGBRIMH, Tezpur, Assam \* Corresponding Author

**Dr. Samrat Biswas**

Associate Professor, Department of Microbiology, Tezpur Medical College and Hospital, Tezpur, Assam

**ABSTRACT**

The clinical significance of CoNS will continue to increase in the field of medical science today. The present study was conducted with an aim to identify CoNS at the species level from various clinical samples and determine the antimicrobial resistance pattern of these isolates. A total of 150 samples are screened for CoNS out of which 13 are identified as CoNS were processed further. A total of 14 antibiotics are used for antibiotic susceptibility test as per Clinical and Laboratory Standards Institute guidelines. All of the 13 (100%) CoNS isolates were resistant to Penicillin G, while maximum numbers of 12 isolates were sensitive to Vancomycin. Thus taking into consideration the importance of coagulase-negative staphylococcus, a continuous surveillance and a regular update of their antibiotic susceptibility pattern are essential for maintaining good infection control program

**KEYWORDS**

Coagulase-negative staphylococcus (CoNS); antimicrobial susceptibility

**INTRODUCTION:**

Identification of staphylococci is often limited to a rapid screening test for *S. aureus*, while non-*S. aureus* isolates are simply reported as CoNS. However, because of their increasing importance, clinically significant CoNS should be identified to the species level by a reliable, simple, rapid, and, preferably, inexpensive method<sup>1</sup>. Coagulase-negative staphylococcus (CoNS) species are among the most frequently isolated bacteria in the clinical microbiology laboratory. Their important role as pathogens and their increasing incidence have been recognized and studied in recent years. Moreover, some hospital strains of coagulase-negative staphylococci demonstrate the resistance against most of the antibiotics recently. This study was conducted with an aim to identify CoNS at the species level from various clinical samples and determine the antimicrobial resistance pattern of these isolates.

**MATERIAL AND METHODS:**

**Study population:** The present study was to find out the Coagulase-negative staphylococcus (CoNS) species and to determine the antimicrobial susceptibility pattern from clinical specimens of urine, Pus, skin wound over a period of 6 months collected from the indoor and outdoor patient department of tertiary care hospital in north east of India. During this period, a total of 150 samples are screened for CoNS. Samples showing growth of staphylococcal colonies were processed further.

**Sample collection and processing**

The urine samples were collected in non-catheterized patients using the mid-stream, clean catch method into wide-mouthed, sterile, screw-capped pots, while in catheterized patients by aspirating urine with a syringe from the collecting tubes under aseptic condition. Samples of pus/other skin wounds were collected on to 2 sterile swabs which were either dry or dipped in sterile physiological saline and then inserted into sterile container tubes to be transported to the bacteriology laboratory and processed within one hour.

**Culture and Identification**

10% Sheep Blood Agar medium, MacConkey Agar medium, Cystine Lactose Electrolyte Deficient medium, Brain-heart infusion broth are used for isolation of or-

ganism. These plates were incubated aerobically at 37°C for 24 hrs and examined for growth. If growth was not seen, except for the urine, the plates were reincubated for another 24 hrs and then re-examined. Coagulase-negative staphylococci isolated from samples were identified by standard methods<sup>2,3</sup>. Media used for different biochemical tests for species identification of CoNS were prepared as per methods described by Collee et al<sup>3</sup>, and guidelines of HiMedia Ltd. The culture isolates were further identified by their morphologies and biochemical characteristics.

For urine cultures to detect bacteriuria, traditionally colony counts of  $\geq 100,000$  CFU/ml in two or more cultures of midstream urine indicate a significant bacteriuria or UTI<sup>4</sup>. Since CoNS grow relatively slowly in urine, in this study lower colony counts of 100 to 100,000 CFU/ml were considered an appropriate range for significant bacteriuria as suggested by<sup>5,6</sup>.

**Antimicrobial susceptibility testing:**

Antibiotic sensitivity testing was done by the Modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>7</sup>.

**RESULTS**

Out of 150 samples, the maximum number of 8 (61.5%) CoNS isolates was seen in the age group 20-39 years. Eight samples (61.25%) from females were culture positive, thus showing a female predominance. Table I shows the break up of the 150 samples from various clinical specimens which were processed in the laboratory. All of the 13 (100%) CoNS isolates were resistant to Penicillin G as shown in Table III.

**Table I: Breakup of the 150 samples.**

Origin of samples	No of samples screened	No of CoNS isolated	Isolation rate in %	Origin of sample in %
Urine	105	10	9.5	76.92
Pus	25	2	8	15.38
Skin wound	20	1	5	7.69
Total	150	13	22.5	100

**Table II: Species distribution from specimens**

Species isolated	Urine Sample	Pus Sample	Skin wound Sample
<i>S. saprophyticus</i>	7(70%)	0	0
<i>S. epidermidis</i>	2(20%)	1(50%)	1(100%)
<i>S. haemolyticus</i>	1(10%)	1(50%)	0
Total	10	2	1

**Table III: Sensitivity / Resistance profile of the 13 CoNS isolates**

Antibiotic	No of sensitive isolates (S)	No of resistance isolates (R)	% ( sensitive isolates )
Penicillin G(10 IU)	0	13	0
Cefoxitin(30 µgm)	5	8	38.46
Vancomycin(30 µgm)	12	1	92.3
Erythromycin(15µgm)	4	9	30.76
Clindamycin(2µgm)	8	5	61.53
Azithromycin(15µgm)	7	6	53.84
Chloramphenicol(25 µgm)	5	8	38.46
Linezolid(30µgm)	10	3	76.9
Tetracycline(30µgm)	7	6	53.84
Co-trimoxazole(23.75 µgm +1.25µgm)	8	5	61.5
Amikacin(30µgm)	4	9	30.79
Gentamicin(10µgm)	8	5	61.53
Ciprofloxacin(5µgm)	5	8	38.46
Levofloxacin(5µgm)	9	4	69.23

**DISCUSSION:**

In the present study, only 13 (8.67%) clinically relevant strains of CoNS were isolated from 150 samples of Urine, pus and skin wound specimen on the basis of standard bacteriological morphology and biochemical reactions. Gaikwad S S & Deodhar L P<sup>8</sup> reported an isolation of 3.7% CoNS from various specimens. In the present study, samples were collected from three (3) different sources. Of the 13 isolated CoNS, the highest isolation rate was seen from urine (76.92%). Mohan U et al.<sup>9</sup> reported 56% isolation rate of CoNS from urine specimens. Rajesh K.R et al.<sup>10</sup> reported 30% isolation rate from urine. The next commonest source of CoNS positive isolates was pus (15.38%). Dogra V et al.<sup>11</sup> isolated 41% from pus. In the present study, out of the total 13 isolates of CoNS, the predominant species was found to be *S. epidermidis* followed by *S. saprophyticus* and *S. haemolyticus*.

In the present study, *S. saprophyticus* was the most common species isolated (table II) from urine (70%) followed by *S. epidermidis* (20%) and *S. haemolyticus* (10%). The isolation rate of *S. haemolyticus* from pus was 50%, urine 10%. Rajesh KR et al.<sup>10</sup> reported that *S. epidermidis* was 66% and *S. saprophyticus* was 34% from various clinical cases. Dogra V et al.<sup>11</sup> reported *S. epidermidis* as the major clinical isolates (66%). B Naidu P et al.<sup>12</sup> reported that the predominant species was *S. epidermidis* (81.8%), followed by *S. saprophyticus* (11.7%), *S. haemolyticus* (5.6%) and *S. cohnii* (1.13%) from various clinical samples. Gaikwad SS and Deodhar LP<sup>8</sup> identified 55.75% as *S. epidermidis* and 44.25% as *S. saprophyticus* in various specimens from clinical infections. Nord et al.<sup>13</sup> reported *S. saprophyticus* as the most commonly associated CoNS from the urine.

All of the 13 (100%) CoNS isolates were resistant to Penicillin G, while maximum number of 12 isolates were sensitive to Vancomycin. Mohan et al.<sup>9</sup> reported 90.6% resistance towards Penicillin. Gaikwad SS and Deodhar LP<sup>8</sup> reported 50% of CoNS as resistant to Penicillin. Khanna S et al.<sup>14</sup> reported

the drug resistance of Methicillin-resistant coagulase-negative staphylococci against Penicillin as 100%. Rajesh KR et al.<sup>15</sup> reported 98% of the isolates as sensitive to Vancomycin. B Naidu P et al.<sup>12</sup> showed 85.2% sensitivity to Vancomycin whereas Raghunath R et al.<sup>16</sup>, Dogra V et al.<sup>11</sup>, and Mohan U et al.<sup>9</sup> reported 100% sensitivity to Vancomycin. It has thus been recommended that continued study of resistance mechanisms is essential to help predict and prevent the emergence of resistance to new as well as old antistaphylococcal agents<sup>17</sup>.

**CONCLUSION:**

CoNS are widespread on the human body and it becomes difficult in distinguishing the etiologic agent from contaminating normal flora in clinical specimens. The clinical significance of CoNS will continue to increase in the field of medical science today, as the invasive procedures such as replacement of damaged or missing body parts with synthetic materials and the wide spread use of catheters are widely prevalent. Most of these infections are hospital-acquired, and studies over the past several years suggest that they are often caused by strains that are transmitted among hospitalized patients. Frequent hand washing by all health care workers is of utmost importance that can reduce the incidence of infections due to coagulase-negative staphylococci both prior to and after examining patients. Meticulous surgical technique is paramount to limit intra-operative bacterial contamination. Strict attention should be paid to protocols for the insertion of intravenous and arterial catheters to minimize the risk of these infections. Thus taking into consideration the importance of coagulase-negative staphylococcus, a continuous surveillance and a regular update of their antibiotic susceptibility pattern are essential for maintaining good infection control program as the resistance of these organisms to antibiotics and the potential for change in the antibiogram makes therapy a challenge.

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