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Original Research Article

Speciation and Antibiotic Resistance Pattern of Coagulase Negative Staphylococci (CONS)- Need of Time

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ABSTRACT

Introduction: Coagulase negative staphylococci (CONS) are part of normal flora and recognized as important pathogen for immune compromised hosts, patients with medical devices and nosocomial infections.
Because of increasing clinical significance of CONS, accurate species identification of CONS and Antimicrobial susceptibility test (AST) to various classes of antimicrobials is highly desirable.
Aim and objectives: To identify species of CONS isolated from clinically significant samples and to study antibiotic susceptibility profile with reference to methicillin resistance and inducible and constitutive clindamycin resistance.
Materials and Methods: 150 CONS strains isolated from clinically significant samples were identified by different conventional methods and AST was studied by Kirby-Bauer Disk Diffusion method.
Methicillin resistance and Inducible and constitutive clindamycin resistance (D test) was detected according to CLSI guidelines.
Results: Among 150 CONS strains isolated, predominant was S. epidermidis 50.66%, followed by S. haemolyticus 24%. AST revealed resistance to penicillin in 96%, erythromycin in 70% (11.42% were inducible MLSʙ phenotype and 4.76% constitutive MLSʙ phenotype), ciprofloxacin in 67.33% and cefoxitin in 65.33 % (MRCONS).
Conclusion: The increasing importance of CONS particularly as nosocomial and opportunistic pathogen and emergence of drug resistance demonstrates need for speciation and detection of resistance pattern in CONS, which is necessary for appropriate antibiotic treatment.
Key words: Coagulase negative staphylococci (CONS), Antimicrobial susceptibility test (AST).

INTRODUCTION

Coagulase-negative Staphylococci (CONS) are known to present on external areas of the body as part of the normal skin flora, they have been considered and regarded to be non- pathogenic and their increasing incidence have been recognized and studied in recent years. Although specific virulence factors are not as clearly established as they are in Staphylococcus aureus, it seems clear that factors such as bacterial polysaccharide components are involved in attachment and/or persistence of bacteria on foreign materials. CONS are by far the most common cause of bacteremia related to indwelling devices. [1,2]

Most of these infections are hospital-acquired, [1,2] and studies over the past several years suggest that they are often caused by strains that are transmitted among hospitalized patients. Other important infections due to CONS include central

nervous system shunt infections, native or prosthetic valve endocarditis, urinary tract infections, and endophthalmitis. Intravenous treatment of systemic infections is usually required because CONS have become increasingly resistant to multiple antibiotics. [1-3]

CONS are characterized by an ability to colonize the surfaces of biomaterials by adhering in biofilm-structured communities of cells encased in a self-produced polymeric matrix, an amorphous slimy material that is loosely bound to Staphylococcal cells. Biofilm is believed to make the micro-organisms more resistant to administered antibiotics and to host defense mechanisms. [4]

Because of increasing clinical significance of CONS, accurate species identification of CONS is highly desirable, also biofilm producing strains are more resistant to antibiotics.

CONS have become a serious problem as they express methicillin resistance, which involves all β-lactam antibiotics and leads to a significant limitation in therapeutic options. Methicillin resistance is associated with the presence of the mecA gene [5] which encodes a penicillin-binding protein (PBP2a) with altered properties responsible for the observed resistance. Incidence of methicillin resistance in CONS is high, as well as, the accompanying antimicrobial resistance.

Among few therapeutic alternatives available for treatment of staphylococcal infections, clindamycin has several advantages but major barrier in its usage is development of resistance especially inducible resistance with in vitro testing and in vivo during clindamycin therapy leading to therapeutic failure. [6]

Hence this study was undertaken with the following aims and objectives.

Aim:

To identify species of CONS isolated from clinically significant samples and to study antibiotic susceptibility profile with reference to methicillin resistance and inducible and constitutive clindamycin resistance.

Objectives:

To fulfill the aim, following objectives were taken:-

1. To isolate and to identify the genus and species of CONS strains from clinically significant samples by conventional methods.
2. To study antibiotic susceptibility pattern of isolated species of CONS to various antimicrobials using Kirby-Bauer disc diffusion method.
3. To detect methicillin resistance and inducible and constitutive clindamycin resistance in the isolated species of CONS.

MATERIALS AND METHODS

Ethics Committee Approval: The study was conducted after obtaining approval from Institutional Ethics Committee.

Locus of study: Study was carried out in department of Microbiology and a rural tertiary care hospital.

Study design: Cross sectional study.

Study duration: The study was conducted from October, 2016 to April, 2018.

Sample size and source of sample: 150 CONS strains were isolated from clinically significant samples like blood, urine, indwelling catheter, pus (infected bone and joint prosthetic implants, surgical site infections etc.) and body fluids, received in department of Microbiology and processed according to conventional methods. Samples were inoculated on blood agar, MacConkey agar and incubated overnight at 37^0C.

CONS isolates from different clinical samples should not be always considered as contaminants and isolates of CONS from blood cultures should be correlated clinically and should be always interpreted with paired blood samples from two peripheral veins. [7] For other samples, to interpret CONS as pathogenic organism, repeated isolation of CONS in two consecutive samples is necessary. [8]

Various isolates were initially identified by colony morphology, gram staining, catalase and coagulase test (slide and tube method). [9] Bacitracin (0.04 u) and Furazolidone (100ug) sensitivity were done to exclude Micrococcus and Stomatococcus. [9]

Speciation of CONS was done by ornithine decarboxylase test, sugar (mannitol, trehalose, mannose, xylose) fermentation test, phosphatase production, urease activity, nitrate reduction test, pyrrolidonyl arylamidase (PYR) test, acetoin production, novobiocin and polymyxin B (50 unit) sensitivity test etc, according to standard procedure. [10]

Biofilm production was detected by tissue culture plate method, which is considered the gold standard method for biofilm detection. [11]

Antibiotic Susceptibility Test:

Antibiotic Susceptibility profile of coagulase-negative Staphylococci strains was done by Kirby Bauer Disk Diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. [12]

Lawn culture was done on Muller Hinton agar plate with broth culture of coagulase-negative Staphylococci strains (turbidity adjusted to 0.5 McFarland standards). [12]

Following antibiotics disc were put on Muller Hinton agar plate like Penicillin (10µg), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Linezolid (15µg), Tetracycline (30µg), Vancomycin (30µg), Rifampicin (5µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Norfloxacin (10 µg-only in case of urine isolates). Plates were incubated at 37º C for 16 -18 hours. Next day susceptibility profile of CONS to different antibiotics was noted according to CLSI guidelines. [12]

Methicillin resistance was detected according to CLSI guidelines by using cefoxitin (30 µg) disc [zone of inhibition ≤ 24 mm (resistant-mec A positive) and ≥ 25 mm (sensitive-mec A negative). [12]

Inducible and constitutive clindamycin resistance in erythromycin resistant (zone size ≤13mm) CONS was detected by D test according to CLSI guidelines. [11] In this test, erythromycin (15 µg disc) and clindamycin (2 µg disc) were placed at a distance of 15 mm edge to edge on a Muller Hinton agar plate already inoculated with test strain(turbidity adjusted to 0.5 McFarland standard) and incubated over night at 37º C. D test results were interpreted as per CLSI guidelines. [12]

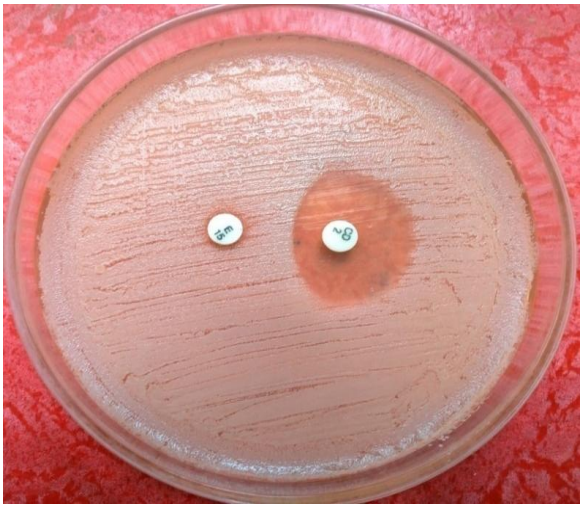

Photo1. Inducible clindamycin resistance phenotype.

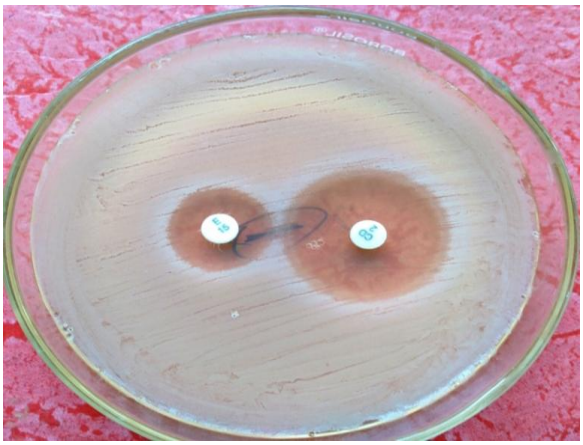

Photo2. MS phenotype.

MS Phenotype: CONS isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥ 21mm) and giving circular zone of inhibition around clindamycin was labeled as having MS phenotype. [12]

Inducible MLS ($iMLS_B$) Phenotype: CONS isolates showing resistance to erythromycin (zone size ≤ 13mm) while being sensitive to clindamycin (zone size ≥

21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having inducible clindamycin resistance phenotype. [12]

Constitutive MLS ($cMLS_B$) Phenotype: This phenotype was labelled for those CONS isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin. [12]

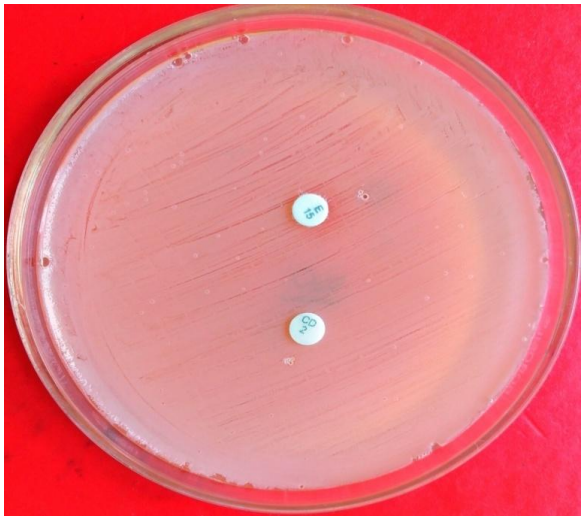

Photo3. Constitutive clindamycin resistance phenotype.

RESULTS

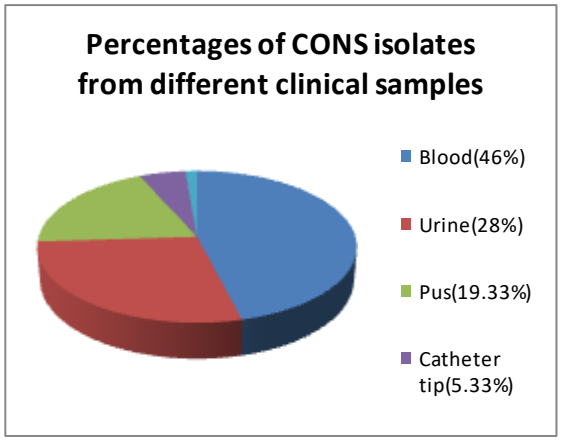

Figure.1- Sample wise distribution of CONS isolates (n=150).

Out of 150 CONS isolates, 69 (46%) isolates were from blood samples, 42 (28%) isolates were from urine samples, 29(19.33%) isolates were from pus samples, 8 (5.33%) isolates were from catheter tip samples and 2 (1.33%) isolates were from body fluids respectively (Figure-1).

Among the 150 CONS isolates, 43.33% isolates were from medicine ICU, 16% isolates were from NICU, 13.33% isolates were from paediatrics wards, 12% isolates were from surgery wards, 8 % isolates were from obstetrics and gynecology wards and 7.33% isolates were from orthopaedics wards (Figure-2).

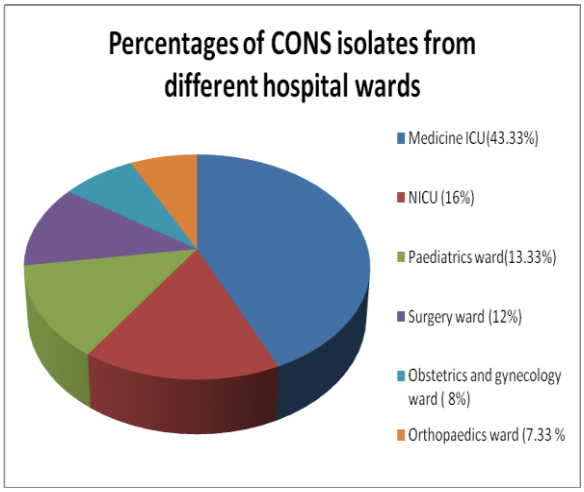

Figure.2-Ward wise distribution of CONS isolates (n=150).

Out of 150 CONS isolates, the most common isolated species was S. epidermidis (46%) followed by S.hemolyticus (25.33%) and S. saprophyticus (13.33%) from different clinical samples (Table.1).

Table.1-Species distribution of CONS from different clinical samples (n=150).

Clinical samples	S.epidermidis	S.hemolyticus	S.saprophyticus	S.schilfri	S. lugdunensis	S.hyicus
Blood	44	17	2	2	3	1
Urine	11	10	18	2	1	0
Pus	10	6	0	6	4	3
Catheter tip	04	4	0	1	0	0
Body fluids	0	1	0	0	1	0
Total	69(46%)	38(25.33%)	20(13.33%)	11(7.33%)	9(6%)	4(2.66%)

Out of 42 CONS isolated from urine samples, 18 S.saprophyticus isolates were from female patients.

Among the 150 CONS isolates, 84 (56%) were biofilm producers and 66 (44%) were non biofilm producers by Tissue culture plate method.

Antibiotic resistance pattern of CONS showed that most of the isolates were resistant to Penicillin G (96%) and all the isolates were found to be sensitive to vancomycin and linezolid. Out of 42 CONS isolated from urine samples, 20(47.61%) isolates were resistant to norfloxacin.
The resistance pattern to other antibiotics showed in Table.2.

Table.2- Antibiotic resistance pattern of CONS isolates (n=150).

Antibiotics	Total CONS strains. n=150	Biofilm producer. n=84	Non Biofilm producer. n=66
Penicillin G	146(97.33%)	84(100%)	62(93.93%)
Amikacin	51(34%)	37(44.04%)	14(21.21%)
Erythromycin	105(70%)	66(78.57%)	39(59.09%)
Clindamycin	50(33.33%)	36(42.85%)	14(21.21%)
Ciprofloxacin	101(67.33%)	70(83.33%)	31(46.96%)
Cefoxitin	98(65.33%)	64(76.19%)	34(51.51%)
Tetracycline	89(59.33%)	56(66.66%)	33(50%)
Chloramphenicol	74(49.33%)	52(61.90%)	22(33.33%)
Rifampicin	61(40.66%)	46(54.76%)	15(22.72%)
Vancomycin	0%	0%	0%
Linezolid	0%	0%	0%

Among the 150 CONS isolates, 65.33% were methicillin resistant (MRCONS) and 34.67% were methicillin sensitive (MSCONS).

Out of the 150 CONS isolates, 105 (70%) showed erythromycin resistance. Among the erythromycin-resistant isolates, 12 (11.42%) isolates were I MLS_B phenotype and showed inducible clindamycin resistance and 5(4.76%) isolates were C MLS_B phenotype (constitutive clindamycin resistance) and rests were MS phenotype.

Table.3- Resistance Phenotypes of CONS Isolates (n=150).

Erythromycin resistant (n=105)	MS Phenotype (n=45)	$iMLS_B$ (n=30)	Constitutive MLS_B (n=36)
70%	30%	20%	24%

$iMLS_B$ - Erythromycin-R, Clindamycin-S, D test- Positive
Constitutive MLS_B - Erythromycin-R, Clindamycin-R
MS Phenotype - Erythromycin-R, Clindamycin-S, D test- Negative

Table.4-Distribution of MLSB resistance phenotypes among MRCONS and MSCONS isolates.

CONS isolates(n-150)	$iMLS_B$	Constitutive MLS_B	MS Phenotype
MRCONS(n=98)	23(23.46%)	29(29.59%)	12(12.24%)
MSCONS(n=52)	7(13.46%)	7(13.46%)	33(63.46%)
Total (150)	30 (20%)	36(24%)	45(30%)

DISCUSSION

Recently, Coagulase-negative Staphylococci are emerging as opportunistic and nosocomial pathogens and CONS have become an important cause of nosocomial bloodstream infections. Now-a-days, there is a significant rise of infections caused by the drug resistant strains of CONS in hospitalized patients. [13]

In the present study, 46% CONS isolates were from blood samples, 28% isolates were from urine samples, 19.33% isolates were from pus samples and 5.33% isolates were from catheter tip samples. This is in accordance to study done by Sadhvi Parashar et al. [14] where 45.95% CONS isolates were from blood samples, 19.46% isolates were from urine samples and 15.6% isolates were from pus samples.

In the present study, 43.33% CONS isolates were from Medicine ICU. This is in accordance with study done by Maj Alina Singh et al. [7] where 48.7% CONS isolates were from Medicine ICU.

CONS species are one of the most prominent causative agents of sepsis and nosocomial infections for newborn and infants and they have been isolated from NICU and pediatrics wards. [14] In the present study, percentages of CONS isolated from NICU and pediatrics wards were 16% and 10% respectively. This is in accordance with study done by Emad Hussein et al. [15] where percentages of isolates from NICU and paediatrics wards were 11.7% and 9.3%.

In the present study, S. epidermidis was the most common species isolated 69(46%) followed by S. hemolyticus 38(25.33%), S. saprophyticus 20(13.33%), S.schilfri 11(7.33%) S. lugdunensis 9(6%) & S.hyicus 4(2.66%). This is in accordance

to study done by Vijayasri et al. [16] which showed S. epidermidis was the most common isolate (40%), followed by S. haemolyticus (26%) and S. saprophyticus (15 %).

In the present study, S. epidermidis was most commonly isolated from blood samples which is in accordance with study done by Maj Alina Singh et al. [7]

In the present study, 90% of S. saprophyticus were isolated from urine sample from female patients. This is in accordance with study done by Sheikh et al. [17] where 90.9% of S. saprophyticus isolated from urine samples of female patients.

In the present study, 84 (56%) CONS isolates were biofilm producers and 66 (44%) were non biofilm producers by Tissue culture plate method. This is in accordance with study done by Ranganathan et al. [18] and Jagatheeswari et al. [19] where 64.4% and 39.57% of CONS isolates were biofilm producers by Tissue culture plate method.

The emergence of drug resistance against CONS strains is a matter of serious concern, regular surveillance of antimicrobial susceptibility against CONS in hospital should be determined prior to treatment of these infections and its irrational use should be avoided to control the spread of infection and for better management of different infectious diseases. [14]

In present study, 96% CONS were resistant to penicillin and 70% were resistant to erythromycin. This is in accordance to study done by Asha S. Kamath et al. [20] where 95.5% isolates were resistant to penicillin and 71.6% were resistant to erythromycin.

In present study, a total of 67.33% CONS isolates were resistant to ciprofloxacin, 59.33% were resistant to tetracycline, 49.33% were resistant to chloramphenicol, 47.61% were resistant to Norfloxacin, 40.66% were resistant to Rifampicin and 34 % were found to be resistant to amikacin. This is in accordance to study done by J. Kalyani et al. [21] where 60.1% isolates were resistant to ciprofloxacin, 52.2% were resistance to Norfloxacin, 42.45 % were resistant to rifampicin and 48.6% were resistant to amikacin and Sadhvi Parashar et al. [14] where 49.19% isolates were resistant to chloramphenicol.

In the present study, all the CONS isolates were found to be sensitive to vancomycin and linezolid. This is in accordance to study done by Maj Alina Singh et al. [7]

In the present study, 65.33% CONS isolates were found to be resistant to cefoxitin, which is a surrogate marker for methicillin resistance and denotes mecA gene mediated methicillin resistance. This is in accordance to study done by Sadhvi Parashar et al. [14] where resistance to cefoxitin was 65.5%.

In the present study, there was higher antibiotic resistance in biofilm producing CONS isolates than non biofilm producers, this is in accordance with study done by Satpathy et al. [22]

In the present study, percentage of inducible clindamycin resistance ($iMLS_B$), constitutive clindamycin resistance (constitutive MLS_B) and MS phenotype in case of CONS was found to be 20%, 24% and 30% respectively. This is in accordance to study done by Neha Bansal et al. [23] where 18%, 26% and 22% of CONS isolates were inducible clindamycin resistance ($iMLS_B$), constitutive clindamycin resistance (constitutive MLS_B) and MS phenotype respectively.

In the present study, among 98 MRCONS isolates, 29.59%, 23.46% and 12.24% isolates were constitutive MLSB resistance, inducible clindamycin resistance and the MS phenotype respectively. Inducible clindamycin resistances were significantly higher in MRCONS isolates as compared to MSCONS. This is in accordance with study done by Neha Bansal et al. [23]

CONCLUSION

The clinical significance of CONS as an important cause of opportunistic and nosocomial pathogen is increasing day by day and in our study, Staphylococcus epidermidis was the most common species identified among CONS and the antibiotic resistance pattern of CONS showed resistance to multiple antibiotics. Because of this increasing clinical significance, there is an urgent need for identification up to species level by simple, inexpensive methodology and their antibiotic sensitivity for improved management of such cases and to prevent emergence of drug resistance.

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