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Detection of Antibiotic Susceptibility Pattern and Methicillin Resistance among the Clinical Isolates of Coagulase Negative Staphylococci (CONS) in a Rural Tertiary Care Hospital

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ABSTRACT

Introduction: CONS are part of the normal skin flora increasingly recognized as significant nosocomial pathogens and often causes different infections associated with implanted devices, joint prosthesis and different indwelling devices. They are very difficult to treat as they are more resistant to commonly used antimicrobial agents than others. Because of that, present study was conducted with following aims and objectives.
Aims and Objectives: To study antibiotic susceptibility profile of isolated species of CONS to various classes of antimicrobials using Kirby Bauer disc diffusion method followed by detection of methicillin resistance and inducible clindamycin resistance among the isolated species of CONS.
Materials and Methods: 170 CONS strains isolated from clinically significant samples were identified by different conventional methods and antibiotic resistance pattern was detected by Kirby-Bauer Disk Diffusion method by using different antibiotic discs. Methicillin resistance and Inducible and constitutive clindamycin resistance (D test) was detected according to CLSI guidelines.
Results: Among 170 CONS isolates, predominant isolated species were S. epidermidis (42.35%), S. haemolyticus (27.06%) and showed higher resistance to different antibiotics. 105(61.76%) isolates showed erythromycin resistance, out of which, 26 (24.76%) isolates were $iMLS_B$.113 (66.47%) isolates were MRCONS.
Conclusion: Present study showed high prevalence of MRCONS, resistant to widely used antimicrobial agents. Hence, it is necessary to have regular surveillance of MRCONS which will be useful for selecting an appropriate antibiotic.

Key words: Coagulase negative staphylococci (CONS), inducible clindamycin resistance ($iMLS_B$)

INTRODUCTION

CONS are part of the normal skin flora increasingly recognized as significant nosocomial pathogens and often causes different infections associated with implanted devices, joint prosthesis and different indwelling devices. [1] Differentiation between a clinically significant pathogenic CONS and contaminating CONS isolates is difficult and it remains a major challenge for physicians. [1-2]

Antibiotic resistance is a global problem. It is very difficult to treat those infections caused by CONS as they are more resistant to commonly used antimicrobial agents than others. [3]

Now-a-days, methicillin resistance became a serious issue among CONS strains as the number of cases of methicillin

resistance has increased massively. Because of that methicillin resistant coagulase negative Staphylococci (MRCONS) became a serious clinical problem. As the resistance to methicillin has increased massively, it implies resistance to all the beta-lactam antibiotics. [3]

To ensure correct antibiotic treatment in case of infected patients, accurate detection of methicillin resistance in hospital laboratories is very important, so that cases of MRCONS can be controlled in hospitals. [3]

Antibiotics resistance pattern has also become a serious issue with elderly people in different rural areas of India as they are irrationally taking antibiotics from local medicine shop without proper prescription or sometimes doctors in rural areas irrationally prescribing antibiotics without performing antibiotic susceptibility testing.

CONS are characterized by an ability to form adhering biofilm, whose formation is believed to make the micro-organisms more resistant to administered antibiotics and to host defense mechanisms. [4]

Because of that, present study was conducted with following aims and objectives.

Aims and Objectives:

The present study was conducted with the following aim and objectives:-

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

MATERIALS AND METHODS

Ethics Committee Approval: After obtaining approval from Institutional Ethics Committee, the study, which is a cross sectional study was conducted in department of Microbiology of Jawaharlal Nehru Medical College, Wardha, Maharashtra which is a rural tertiary care hospital.

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

After receiving in department of Microbiology, 170 CONS strains were isolated from clinically significant samples like blood, urine, indwelling catheter, pus and body fluids and processed according to conventional methods. [5-6]

The isolates were considered clinically significant when isolated in pure culture from infected sites or body fluids or if the same strain was isolated from repeat samples. [5-6]

CONS isolates were initially identified by colony morphology, gram staining, catalase and coagulase test (slide and tube method). [7] Bacitracin (0.04 u) and Furazolidone (100ug) sensitivity were done to exclude Micrococcus and Stomatococcus. [7]

Speciation of CONS was done by various conventional methods. [8]

Antibiotic Susceptibility Test:

Antibiotic Susceptibility profile of 170 CONS strains isolated from different clinically significant samples was studied by Kirby Bauer Disk Diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. [9]

The following commercially available antibiotic discs (HiMedia) were used [9] - Penicillin-G (10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Cefoxitin (30µg), Linezolid (15µg), Tetracycline (30 µg), Vancomycin (30 µg), Rifampicin (5 µg), Chloramphenicol (30µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Nitrofurantoin (300 µg). [9]

The results were interpreted using CLSI guidelines. [9]

Methicillin resistance:

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).] [9]

Inducible and constitutive clindamycin resistance in erythromycin resistant (zone size ≤13mm) CONS was detected by D test according to CLSI guidelines. [9]

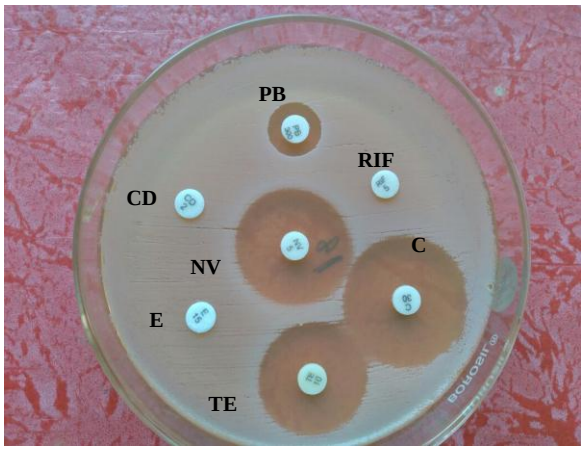

Photo-1. Antibiotic susceptibility test.
[Sensitive- PB, NB, C, TE
Resistant- RIF, E, CD]

Abbreviations: P-Penicillin–G, AK-Amikacin, VA-Vancomycin, LZ-Linezolid,
CIP-Ciprofloxacin, NIT-Nitrofurantoin, FR-Furazolidone, PB-Polymyxin-B,
RIF-Rifampicin,C-Chloramphenicol,NV-Novobiocin,TE-Tetracycline,E-Erythomycin,
CD-Clindamycin.

RESULTS

Table 1. Sample wise distribution of CONS (n=170).

Samples	No of CONS isolates (n=170)
Blood	73(42.94%)
Pus	45(26.47%)
Urine	37(21.76%)
Catheter tips	8(4.70%)
Body fluids*	7(4.12%)
Total	170

- Body fluids include [CSF (n=1), Ascitic fluid (n=3), Pleural fluid (n=3)].

Among the 170 CONS isolates, 42.94% isolates were from blood samples, 26.47% isolates from pus samples, 21.76% isolates from urine samples, 4.70% isolates from catheter tip samples and 4.12% isolates from body fluids respectively.

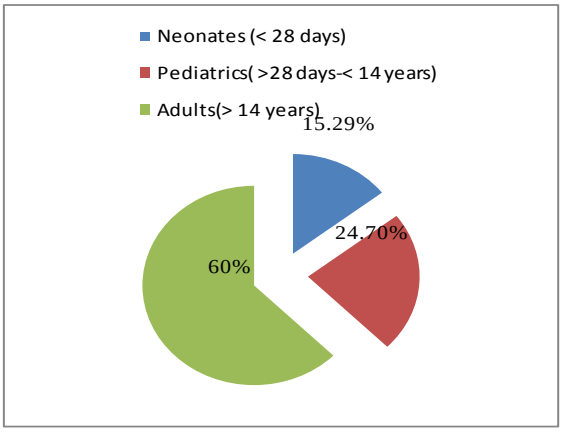

Figure 1. Age wise distribution of CONS isolates (n=170).

Among 170 CONS isolates, 26(15.29%), 42(24.70%) and 102(60%) CONS strains were isolated from neonates, pediatrics age group and adult patients respectively.

Table 2. Species distribution of CONS isolates (n=170).

Species	No of CONS isolates (n=170)
S.epidermidis	72(42.35%)
S.haemolyticus	46(27.06%)
S. schleiferi	18(10.59%)
S.lugdunensis	16(9.41%)
S.saprophyticus	10(5.88%)
S.xylosus	4(2.35%)
S.intermedius	2(1.18%)
S.warneri	1(0.59%)
S. hominis	1(0.59%)

Among 170 CONS isolates, most commonly isolated species were S. epidermidis 72(42.35%), S.haemolyticus 46(27.06%), S. schleiferi 18(10.59%) and S.lugdunensis 16(9.41%). Least commonly isolated CONS species were S.saprophyticus 10(5.88%), S.xylosus 4(2.35%), S.intermedius 2(1.18%), S.warneri 1(0.59%) and S. hominis 1 (0.59%).

Among 170 CONS isolates, 72 isolates were S.epidermidis. Out of 72 S.epidermidis isolates, all 72(100%) isolates showed resistance to penicillin-G, 51(70.83%) isolates to cefoxitin, 46(63.89%) isolates to erythromycin, 45(62.5%) isolates to tetracycline, 36(50%) isolates to amikacin, 30(41.67%) isolates to rifampicin, 37(51.39%) isolates to chloramphenicol and 37(51.39%) isolates to ciprofloxacin.

Table 3. Species wise antibiotic resistance pattern of CONS (n=170).

Species	CONS	P	CX	E	TE	AK	VA	RIF	LZ	C	CIP	NIT(n=37)
S.epidermidis	72	72 (100%)	51 (70.83%)	46 (63.89%)	45 (62.5%)	36 (50%)	0	30 (41.67%)	0	37 (51.39%)	37 (51.39%)	9(64.28%) (n=14)
S.haemolyticus	46	45 (97.83%)	31 (67.39%)	28 (60.87%)	26 (56.52%)	22 (47.83%)	0	17 (36.96%)	0	21 (45.65%)	20 (43.48%)	6(60%) (n=10)
S.schleiferi	18	18 (100%)	10 (55.55%)	11 (61.11%)	10 (55.55%)	7 (38.89%)	0	6 (33.33%)	0	8 (44.44%)	10 (55.55%)	1(50%) (n=2)
S.lugdunensis	16	15 (93.75%)	9 (56.25%)	10 (62.5%)	6 (37.5%)	7 (43.75%)	0	4 (25%)	0	6 (37.5%)	9 (56.25%)	1(33.33%) (n=3)
S.saprophyticus	10	10 (100%)	7(70%)	7(70%)	6(60%)	4(40%)	0	3(30%)	0	4(40%)	6(60%)	6(75%) (n=8)
S.xylosus	4	4(100%)	2(50%)	2(50%)	2(50%)	1(25%)	0	1(25%)	0	2(50%)	2(50%)	
S.intermedius	2	2(100%)	1(50%)	1(50%)	0	1(50%)	0	0	0	0	1(50%)	
S.warneri	1	1(100%)	1(100%)	0	0	1(100%)	0	0	0	0	1(100%)	
S. hominis	1	1(100%)	1(100%)	0	1(100%)	0	0	1(100%)	0	0	1(100%)	
Total	170	168 (98.82%)	113 (66.47%)	105 (61.76%)	96 (56.47%)	79 (46.47%)	0	62 (36.47%)	0	78 (45.88%)	87 (51.18%)	27 (62.79%)

Nitrofurantoin was only used for urine samples.

Abbreviations: P- Penicillin-G,CX-Cefoxitin,E-Erythromycin,TE-Tetracycline,AK-Amikacin,VA-Vancomycin,RIF-Rifampicin,LZ-Linezolid,C- Chloramphenicol, CIP- Ciprofloxacin, NIT- Nitrofurantoin.

Out of 46 S. haemolyticus isolates, 45(97.83%) isolates showed resistance to penicillin-G, 31(67.39%) isolates to Cefoxitin (MRCONS), 28(60.87%) isolates to erythromycin, 26(56.52%) isolates to tetracycline, 22(47.83%) isolates to amikacin, 17(36.96%) isolates to rifampicin, 21(45.65%) isolates to chloramphenicol and 20(43.48%) isolates to ciprofloxacin.

Out of 18 S. schleiferi isolates, all 18(100%) isolates showed resistance to penicillin-G, 10(55.55%) isolates to cefoxitin, 11(61.11%) isolates to erythromycin, 10(55.55%) isolates to tetracycline, 7(38.89%) isolates to amikacin, 6(33.33%) isolates to rifampicin, 8(44.44%) isolates to chloramphenicol and 10(55.55%) isolates to ciprofloxacin.

Out of 16 S. lugdunensis isolates, 15(93.75%) isolates showed resistance to penicillin-G, 9(56.25%) isolates to cefoxitin, 10(62.5%) isolates to erythromycin, 6(37.5%) isolates to tetracycline, 7(43.75%) isolates to amikacin, 4(25%) isolates to rifampicin, 6(37.5%) isolates to chloramphenicol and 9(56.25%) isolates to ciprofloxacin.

Out of 10 S. saprophyticus isolates, all 10(100%) isolates showed resistance to penicillin-G, 7(70%) isolates to cefoxitin, 7(70%) isolates to erythromycin, 6(60%) isolates to tetracycline, 4(40%) isolates to amikacin, 3(30%) isolates to rifampicin, 4(40%) isolates to chloramphenicol and 6(60%) isolates to ciprofloxacin.

Out of 4 S. xylosus isolates, all 4(100%) isolates showed resistance to penicillin-G, 2(50%) isolates to cefoxitin, 2(50%) isolates to erythromycin, 2(50%) isolates to tetracycline, 1(25%) isolates to amikacin, 1(25%) isolates to rifampicin, 2(50%) isolates to chloramphenicol and 2(50%) isolates to ciprofloxacin.

Out of 2 S. intermedius isolates, all 2(100%) isolates showed resistance to penicillin-G, 1(50%) isolates to cefoxitin, 1(50%) isolates to erythromycin, 1(50%) isolates to amikacin and 1(50%) isolates to ciprofloxacin.

Out of 200 CONS isolates, only 1 isolate was S.warneri and it was resistant to penicillin-G, cefoxitin, amikacin, ciprofloxacin and susceptible to erythromycin, tetracycline, rifampicin and chloramphenicol.

All the 170 CONS isolates showed sensitivity to vancomycin and linezolid.

Out of 37 CONS isolated from urine samples, 9(64.28%) S.epidermidis, 6(60%) S.haemolyticus, 6(75%) S.saprophyticus, 1(50%) S. schleiferi and 1(33.33%) S.lugdunensis isolates were resistant to nitrofurantoin.

So from the above table, it was observed that CONS showed variation in antibiotic resistance patterns depending upon the speciation.

Among the 170 CONS isolates, 105(61.76%) showed erythromycin resistance, out of which, 26 (24.76%) isolates showed $iMLS_B$ (inducible

clindamycin resistance), 52(49.52%) isolates showed Constitutive MLSʙ (constitutive clindamycin resistance) and 27(25.71%) isolates showed MS Phenotype.

Among the 170 CONS isolates, 113(66.47%) isolates were MRCONS and 57(33.53%) isolates were MSCONS. Out of 113 MRCONS isolates, 22(19.47%) isolates showed $iMLS_B$ phenotype, 44(38.94%) isolates showed constitutive MLSʙ phenotype and 12(10.62%) isolates were having MS Phenotype. Out of 57 MSCONS isolates, 4(7.02%) isolates showed $iMLS_B$ phenotype, 8(14.03%) isolates showed constitutive MLSʙ phenotype and 15(26.31%) isolates showed MS Phenotype.

So, it was observed that $iMLS_B$ phenotype was seen more among MRCONS isolates as compared to MSCONS isolates.

DISCUSSION

In present study, 42.94% CONS were isolated from blood samples, 26.47% isolates from pus samples, 21.76% isolates from urine samples, 4.70 % isolates from catheter tips and 4.12 % isolates were from body fluids. This observation correlates with the study done by Sadhvi Parashar et al. [10] where 45.95% CONS were isolated from blood samples, 15.6% isolates from pus samples and 19.46% isolates were from urine samples.

In present study, 60% CONS strains were isolated from adults. This study correlates with the study done by Puneet Bhatt et al. [5] where 69.4% CONS strains were isolated from adults.

In this study, predominant isolated species were S. epidermidis 72(42.35%), S.haemolyticus 46(27.06%), S. schleiferi 18(10.59%) followed by S.lugdunensis 16(9.41%) and S.saprophyticus 10(5.88%).This finding correlates with the study done by Badampudi et al. [11] where predominant isolated species were S. epidermidis (40%), S. haemolyticus (26%), S. saprophyticus (15 %) and S. schleiferi (13%). S. epidermidis was predominantly isolated species in present study and in most of the other studies. [12]

Emergence of drug resistance in CONS strains is serious cause of concern, regular surveillance of resistance pattern in CONS in hospital should be carried out prior to treatment of patient and irrational use of antibiotics should be avoided to reduce the spread of resistance and for better management of different infectious diseases. [10]

In present study, 100%, 70.83%, 63.89%, 62.50% and 51.39% of S.epidermidis showed resistance to penicillin-G, cefoxitin, erythromycin, tetracycline and ciprofloxacin respectively. This observation was seen in study done by Rahimi F et al. [13] (2016) where 98% and 86 % of S.epidermidis isolates showed resistance to penicillin-G and cefoxitin respectively and Sheikh et al. [14] where 69.23% and 57.69% S.epidermidis isolates showed resistance to erythromycin and tetracycline respectively. Another study done by Ibrahim Ali Al Tayyar et al. [15] (2015) reported 99.2%, 79.5% and 55.7% S.epidermidis isolates showed resistance to penicillin-G, erythromycin and ciprofloxacin respectively which correlates with the present study.

Another study done by Wojtyczka R D et al. [16] (2014) where 21.9%, 43.7% and 21.9% S.epidermidis isolates showed resistance to cefoxitin, erythromycin and tetracycline respectively which is significantly lower than the present study.

In present study, amongst all the S. haemolyticus isolates, 97.83%, 60.87%, 56.52%, 45.65% and 43.48% isolates showed resistance to penicillin-G, erythromycin, tetracycline, chloramphenicol and ciprofloxacin respectively. This finding was seen in study done by Sheikh et al. [14] (2012) where 95 %, 85% and 60 % S. haemolyticus isolates showed resistance to penicillin-G, erythromycin and tetracycline respectively and Wojtyczka R D et al. [16] (2014) where 54.8% S. haemolyticus isolates showed resistance to erythromycin.

Another study done by Ibrahim Ali Al Tayyar et al. [15] (2015) reported 82.7% and 76.9% S. haemolyticus isolates showed resistance to erythromycin and ciprofloxacin respectively which is significantly higher than the present study.

In present study, amongst all the S. schleiferi isolates, 100%, 55.55%, 61.11% and 55.55% isolates showed resistance to penicillin-G, cefoxitin, erythromycin and tetracycline respectively. However lower rate of resistance was seen in study done by Ragini Ananth et al. [17] (2016) where 66.67%, 33.33% and 33.33% S. schleiferi isolates showed resistance to penicillin-G, cefoxitin and erythromycin respectively.

In present study, amongst all the S.lugdunensis isolates, 56.25%, 62.50%, 37.50% and 56.25% isolates showed resistance to cefoxitin, erythromycin, chloramphenicol and ciprofloxacin respectively. This finding was seen in study done by Ragini Ananth et al. [17] (2016) where 50%, 50%, 50% and 50% S.lugdunensis isolates showed resistance to cefoxitin, erythromycin, chloramphenicol and ciprofloxacin respectively.

In present study, amongst all the S.saprophyticus isolates, 70% and 60% isolates showed resistance to erythromycin and tetracycline respectively. This finding was seen in study done by Wojtyczka R D et al. [16] (2014) where 80 % S.saprophyticus isolates showed resistance to erythromycin and Sheikh et al. [14] where 76.47% S. saprophyticus isolates showed resistance tetracycline respectively.

In present study, amongst all the S.xylosus isolates, 50% and 50% isolates showed resistance to erythromycin and tetracycline respectively. However higher rate of resistance was seen in study done by Sheikh et al. [14] (2012) where 75% and 87.5% S.xylosus isolates showed resistance to erythromycin and tetracycline respectively.

In present study, all the 170 CONS isolates showed sensitivity to vancomycin. This is in accordance to study done by Ibrahim Ali Al Tayyar et al. [15] (2015), Roopa et al. [18], Goudarzi M et al. [19] (2014) and Mane et al. [20] (2016).

In present study, all the 170 CONS isolates showed sensitivity to linezolid. This is in accordance to study done by Begum S et al. [21] (2011) and Puneet Bhatt et al. [5] (2016).

When we compared other studies with present study, it can be inferred that antibiotic resistance among species varies with geographical location and resistance is increasing in CONS species.

In the present study, among 170 CONS isolates, 105(61.76%) showed resistance to erythromycin, out of these, percentage of inducible clindamycin resistance ($iMLS_B$) was found to be 24.76%. These findings correlates with a study done by Bansal et al. [22] where 18% of CONS isolates were $iMLS_B$.

In present study, among 113 MRCONS isolates, 19.47% isolates showed $iMLS_B$. These findings correlates with a study done by Bansal et al. [22] where 25.8% of MRCONS isolates showed iMLSB. In the present study, among 57 MSCONS isolates, 7.02% isolates were $iMLS_B$. These findings correlates with a study done by Bansal et al. [22] where 13.7% of MSCONS isolates were $iMLS_B$.

Inducible clindamycin resistance was significantly higher in MRCONS isolates as compared to MSCONS. This is probably due to different antibiotic susceptibility pattern in different geographical areas. [22]

CONCLUSION

Present study showed high prevalence of MRCONS and CONS showed resistance to widely used multiple antimicrobial agents.

That's why for various life threatening CONS infections to choose an appropriate antibiotic for patients, to know the exact antibiotic resistance pattern trends and to avoid irrational use of antibiotics along with powerful antibiotics like vancomycin and linezolid, it is very

essential to have regular surveillance of MRCONS.

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