ORIGINAL RESEARCH PAPER

Volume-8 | Issue-3 | March-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SPECIES DISTRIBUTION AND ANTIMICROBIAL RESISTANCE PATTERN OF COAGULASE-NEGATIVE STAPHYLOCOCCI WITH SPECIAL REFERENCE TO INDUCIBLE CLINDAMYCIN RESISTANCE

Microbiology

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ABSTRACT

Clindamycin is commonly used for treatment of staphylococcal infections. Routine tests fail to detect inducible clindamycin resistance (ICR) due to erm genes resulting in treatment failure, so there is need to detect this by D-test.

Out of the total 360 staphylococcal isolates, it was found that 318 (88.33%) were isolates of *S. aureus* and 42 (11.67%) were CoNS isolates. *S. epidermidis* was most common species (88.10%). Among 42 CoNS isolates, 42(11.67%) isolates were MRCoNS. ICR was observed in 13 (30.95%) isolates, constitutive resistance was found in 8 (19.05%) isolates. Both patterns were higher in MRCoNS. False susceptibility tests may be obtained if staphylococci are not tested for ICR. So, D test should be used for detection of ICR.

KEYWORDS

Inducible, MRCoNS, D Test

Coagulase negative staphylococci (CoNS) are important causes of nosocomial and community acquired infections. Treatment of these infections is a growing problem because of increasing Methicillin resistance among staphylococci.[1] The increasing frequency of infections with Methicillin resistant staphylococci and changing drug susceptibility patterns have led to a renewed interest in use of Macrolide Lincosamide Streptogramin-B (MLS_B) antibiotics to treat such infections, with Clindamycin being preferred agent due to its excellent pharmacokinetic properties.[2] However, their widespread use has increased number of staphylococcal strains which are resistant to the MLS_B antibiotics.[3]

Phenotypically, such resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$).[4] In vitro staphylococcal isolates with constitutive resistance are resistant to both Erythromycin (E) and Clindamycin (CD), while isolates with inducible resistance are resistant to, but appear to be susceptible to CD.[5] Double disc diffusion (D test) is recommended by CLSI guidelines 2015 for detection of inducible Clindamycin resistance (ICR).[6]

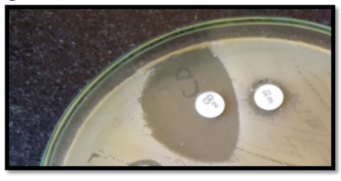
A negative result for ICR by D test confirms CD susceptibility and provides a good therapeutic option, thus necessitates detection of ICR.

MATERIAL AND METHODS

A total of 360 Staphyloccocal isolates were processed. Various specimens received at laboratory were included in study. Case history of patients was recorded. Specimens were processed by standard microbiological techniques.[7] Staphylococcal isolates which were tube coagulase negative were subjected to criteria Criteria by Ishak et al[8] for blood and Singhal et al[9] for other specimens. Species identification of CoNS was done by ornithine decarboxylase, phosphatase, nitrate reduction, urease, carbohydrate fermentation of xylose, sucrose, trehalose, lactose, fructose, maltose, mannose and novobiocin resistance test.[10]

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method and Methicillin resistance was identified by using Cefoxitin (30 µg) disc and interpreted as per CLSI guidelines.[11] 11 isolates of MRCoNS were tested for MIC to Vancomycin by E- test strips (Hi-media laboratories Pvt. Ltd. Mumbai). All staphylococcal isolates were tested for ICR by D test on Mueller Hinton agar at 35°C ± 2°C for 16-18 hours. Flattening of zone (D shape) of CD disk towards side facing E disk indicated positive D zone test.[11] (figure 1)

D Zone Test (Figure 1)



RESULTS

Out of total 360 staphylococcal isolates, it was found that 318 (88.33%) were isolates of *S. aureus* and 42 (11.67%) were CoNS isolates (Figure 2). Out of total 42 isolates of CoNS, maximum number of isolates was obtained from blood specimens (45.24%) followed by pus (38.10%) (Figure 3). With identification scheme employed in present study, species identification of all 42 isolates of CoNS was done on basis of test results shown by them. *S. epidermidis* was most common species (88.10%), followed by *S. saprophyticus* (9.52%) and *S. lugdunensis* (2.38%) (Figure 4). *S epidermidis* was most commonly isolated from blood samples (51.35%) followed by pus (40.54%) All four urinary isolates were found to be *S. saprophyticus.* In present study, only one isolate of *S. lugdunensis* was identified and it was found in pus specimen (Figure 5).

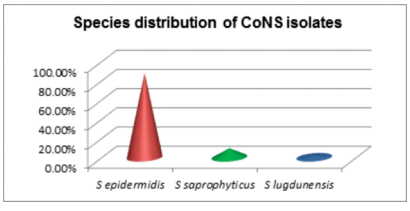
Distribution of staphylococcal isolates (Figure 2)

Total number of isolates	S. *aureus* (%)	CoNS (%)
360	318 (88.33)	42 (11.67)

Specimen wise distribution of CoNS isolates (Figure 3)

Specimen	Number of isolates (%)
Blood	19 (45.24)
Pus	16 (38.10)
Urine	4 (9.52)
Ascitic fluid	2 (4.76)
Pleural fluid	1 (2.38)
Total	42 (100)

Species distribution of CoNS isolates (Figure 4)



Distribution of CoNS species in various specimens (Figure 5)

Species	Blood (%)	Pus (%)	Urine (%)	Ascitic fluid (%)	Pleural fluid (%)	Total (%)
S. *epidermidis*	19 (51.35)	15(40.54)	0	2(5.41)	1(2.70)	37 (100)
S. *saprophyticus*	0	0	4 (100)	0	0	4 (100)
S. *lugdunensis*	0	1 (100)	0	0	0	1 (100)

Resistance for Erythromycin was 85.71% and for Clindamycin it was 54.76% (Figure 6). MRCoNS was found to be 26.19% (Figure 7). Out of 11 MRCoNS isolates that were subjected to MIC testing, all MRCoNS isolates were also found to be susceptible to Vancomycin with MICs of ≤ 4 µg/ml (Figure 8).

Antibiotic resistance pattern of staphylococci (Figure 6)

Antibiotic group	Antibiotic drug	CoNS (%)
Group A	Erythromycin	36 (85.71)
	Clindamycin	23 (54.76)
	Penicillin	42 (100)
	Cotrimoxazole	29 (69.05)
Group B	Linezolid	0
	Tetracycline	26 (61.90)
	Vancomycin*	0
Group C	Chloramphenicol	21 (50)
	Ciprofloxacin	31 (73.81)
	Gentamycin	28 (66.66)
Group U	Nitrofurantoin**	2 (50)
Group O	Amikacin	16 (38.10)
	Ofloxacin	27 (64.29)

Detection of Methicillin resistance among CoNS (Figure 7)

Organism	Methicillin resistant (%)	Methicillin sensitive (%)	Total
CoNS (n= 42)	11 (26.19)	31 (73.81)	42 (100)

Vancomycin MIC distribution in Methicillin resistant CoNS (Figure 8)

Vancomycin MIC	CoNS No. (%)
≤0.50	0
0.75	0
1	1 (9.09)
1.5	4 (36.37)
2	5 (45.45)
3	1 (9.09)
≥4	0
Total	11 (100)

Out of 42 CoNS isolates, 13 (30.95%) showed inducible phenotype, 8 (19.05%) showed constitutive phenotype, 15 (35.71%) showed MS phenotype and 6 (14.29%) isolates were susceptible to both Erythromycin and Clindamycin (Figure 9). The inducible phenotype was found to be higher than constitutive phenotype in CoNS and it was found to be statistically significant. ($p < 0.05$) (Figure 10). Out of 11 MRCoNS isolates, 45.45% showed inducible phenotype, 27.27% showed constitutive phenotype and 9.1% showed MS phenotype.

Antibiotic resistance pattern of different phenotypes of CD resistance in staphylococci was also studied. All phenotypes were sensitive to Linezolid and Vancomycin (Figure 11).

Phenotypic pattern of Clindamycin resistance among CoNS isolates (Figure 9)

Susceptibility pattern (Phenotype)	CoNS (%)
Inducible phenotype	13 (30.95)
Constitutive phenotype	8 (19.05)
MS phenotype	15 (35.71)
E and CD susceptible Phenotype	6 (14.29)
Total	42 (100)

Phenotypic pattern of Clindamycin resistance among Methicillin resistant and Methicillin sensitive CoNS (Figure 10)

Susceptibility pattern	MRCoNS (%)	MSCoNS (%)
Inducible phenotype	5 (45.45)	8 (25.81)
Constitutive phenotype	3 (27.27)	5 (16.13)
MS phenotype	1 (9.1)	14 (45.16)
E and CD susceptible phenotype	2 (18.18)	4 (12.90)
Total	11 (26.19)	31 (73.81)

Antibiotic resistance pattern of different phenotypes of clindamycin resistance in CoNS (Figure 11)

		CoNS		
Antibiotic group	Antibiotic drug	Inducible phenotype (n=13) (%)	Constitutive phenotype (n=8) (%)	MS phenotype (n=15) (%)
Group A	Penicillin	13 (100)	8 (100)	15 (100)
	Cotrimoxazole	10 (76.72)	6 (75)	12 (80)
Group B	Vancomycin*	0	0	0
	Linezolid	0	0	0
	Tetracycline	7 (53.85)	4 (50)	8 (53.33)
Group C	Chloramphenicol	7 (53.84)	5 (62.5)	10 (66.67)
	Ciprofloxacin	9 (69.23)	6 (75)	11 (73.33)
	Gentamycin	8 (61.54)	4 (50)	9 (60)
Group U	Nitrofurantoin**	1 (25)	-	1 (50)
Group O	Amikacin	6 (46.15)	5 (62.5)	8 (53.33)
	Ofloxacin	8 (61.54)	4 (50)	9 (60)

DISCUSSION

In present study, among 360 staphylococcal isolates, 318 (88.33%) were *S. aureus* and 42 (11.67%) were CoNS. The CoNS isolates were identified as clinically significant isolates on basis of Criteria by Ishak et al[8] for blood and Singhal et al[9] for other specimens. This is similar to the finding of Rao et al[12] who observed 86.11% isolates of *S. aureus* and 11.67% CoNS isolates respectively. Afridi et al[13] reported higher isolation rate of CoNS (73.5%) as compared to 26.5% of *S. aureus* isolates.

Among 42 isolates, maximum number (45.24%) of isolates were obtained from blood specimen followed by pus (38.10%) and urine (9.52%). Singhal et al[9] and Chavan et al[14] also found blood to be the commonest specimen from which CoNS were isolated. Singhal et al[9] isolated 54.2% of CoNS and Chavan et al[14] isolated 54.9% isolates of CoNS from blood specimens. However, lower isolation rate of 5.3% was reported by Roopa et al[15] from blood. All CoNS isolates were resistant to Penicillin in the present study. Mohan et al[16] reported 72.3% resistance to Penicillin. However, in a study by Chavan et al[14] maximum resistance of CoNS was seen to Penicillin (92.25%). All CoNS isolates were sensitive to Linezolid. Similar observation was done by Chavan et al[14] and Singhal et al[9] in their studies where none of isolate was found to be resistant to Linezolid.

Resistance to Erythromycin and Clindamycin was 85.71% and 54.76% respectively. Shanthi et al[17] reported 62.5% resistance to Erythromycin and 35% resistance to Clindamycin. Juyal et al[18] found 47.77% and 21.76% of resistance to Erythromycin and Clindamycin respectively. CoNS many a times are part of commensal flora and hence it might be having repeated exposure to different antibiotics and this might be reason for acquiring resistance.

In present study, resistance of CoNS to Methicillin was found to be 26.19%. This correlates with study of Juyal et al[18] and Roopa et al[15] who reported 28.35% and 33% of MRCoNS isolates respectively. However Singhal et al[9] reported higher isolation rate of 62.7% in their study. In present study, Vancomycin susceptibility was detected by E- strip method. All MRCoNS isolates were also found to be susceptible to Vancomycin with MICs of ≤ 4 µg/ml. Similarly Chavan et al[14] and Roopa et al[15] reported 100% sensitivity to Vancomycin in CoNS.

Out of 42 CoNS that were isolated in present study, 30.95% showed

inducible phenotype and 19.05% showed constitutive phenotype Similar observation was made in study by Juyal et al[18] where inducible phenotype was higher (19.40%) and lower rate of constitutive phenotype (8.96%). Certain studies demonstrated higher rate of constitutive phenotype in CoNS. Khan et al[19] reported 37.04% of isolates of inducible phenotype and 53.70% isolates of constitutive phenotype.

In present study, out of 11 MRCoNS isolates, 45.45% showed inducible phenotype and 27.27% showed constitutive phenotype. Thapa et al[20] made similar observation with higher rate of inducible phenotype i.e. 43% in MRCoNS and 50% constitutive phenotype in MRCoNS.

In present study, resistance pattern of inducible, constitutive and MS phenotypes in *S. aureus* and CoNS isolates were studied. All isolates were resistant to Penicillin and all isolates were sensitive to Linezolid. The increasing prevalence of inducible resistance as compared to that of constitutive resistance among staphylococci and indiscriminate use of antimicrobial agents has further deteriorated sensitivity pattern.

This indicates that further study of antimicrobial susceptibility of such inducible phenotypic strains may help in judicial use of drugs in serious infections.

CONCLUSION

The frequency of ICR is highly variable with regard to geographic locality, even from hospital to hospital and it also varies according to Methicillin susceptibility. Hence, local data regarding inducible clindamycin resistance is needed. Without "D test" all isolates with ICR would be erroneously classified as CD susceptible by routine testing methods. This is simple, economical and easy test and hence it must be included in routine diagnostic laboratories to prevent treatment failure.

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