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DETECTION OF INDUCIBLE AND CONSTITUTIVE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL

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ABSTRACT Clindamycin is commonly used for treatment of infections caused by *S aureus*. 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.
A total of 318 isolates of S aureus from were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. ICR was detected by D-zone test as per CLSI guidelines.
Among 318 *S.aureus* isolates, 65(20.44%) isolates were MRSA. ICR was observed in 64(20.13%) isolates, constitutive resistance was found in 43 (13.52%) isolates. Both patterns were higher in MRSA than MSSA. 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, MRSA, D Test

INTRODUCTION

Staphylococcus aureus causes variety of pyogenic infections ranging from soft tissue infections to life threatening endocarditis. The emergence of resistance to antibiotics among staphylococci is an alarming problem. The increasing frequency of infections with Methicillin resistant *S. aureus* (MRSA) 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 (CD) being preferred due to its excellent pharmacokinetic properties.[1] However, their widespread use has increased number of staphylococcal strains which are resistant to MLS_B antibiotics.[2]

Phenotypically, such resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$).[3] 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 .[4] Double disc diffusion (D test) is recommended by CLSI guidelines 2015 for detection of inducible Clindamycin resistance (ICR).[5]

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

MATERIALAND METHODS

The present study was conducted in Department of Microbiology, at tertiary care hospital from September 2015 to September 2017. A total of 318 *S. aureus* isolates were included. Various specimens received at laboratory were included in study. Case history of patients was recorded. Specimens were processed by standard microbiological techniques.[6] They were identified on basis of colony characteristics, Gram staining, catalase test, slide coagulase test, tube coagulase test, , DNase test etc.[7] 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.[5] 65 isolates of MRSA 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.[5]

FIGURE 1 Interpretation Of Phenotypes Of E And CD

Phenotype	E	CD	D test result	Character of Phenotype
E and CD susceptible	S	S		
MS Phenotype	R zone size ≤13 mm	S zone size ≥ 21 mm	Negative	Circular zone of inhibition around CD
Inducible Phenotype	R zone size ≤ 13 mm	S zone size ≥ 21 mm	Positive	D Shaped zone of inhibition around CD with flattening towards E disc
Constitutive Phenotype	R zone size ≤ 13 mm	R zone size ≤14 mm		

S – Sensitive R - Resistant

Chi square test was applied wherever applicable, p value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 318 isolates of *S. aureus* were obtained from different clinical samples. The maximum number of isolates were obtained from pus/wound swabs (55.35%) followed by blood which constituted 15.41% (Figure 2).Resistance for E was 54.72% and for CD it was 33.65%. All isolates were resistant to Penicillin (Figure 3). 65 (20.44%) isolates were MRSA (Figure 4). 65 MRSA isolates were subjected to MIC testing to Vancomycin by E - test. All isolates of MRSA were found to be susceptible to Vancomycin with MICs of ≤ 2 µg/ml. (Figure 5).

Amongst 318 isolates of *S aureus*, 20.13% showed inducible phenotype and 13.52% showed constitutive phenotype. MS phenotype was shown by 21.07% isolates and 45.28% isolates were susceptible to both E and CD. Inducible phenotype was found to be higher than constitutive phenotype and it was found to be statistically significant. ($p < 0.05$) (Figure 6). Out of 65 MRSA isolates, 24.62% showed inducible phenotype, 18.46% showed constitutive phenotype. Among 253 MSSA isolates, 18.97% showed inducible phenotype,12.25% showed constitutive phenotype and 19.76% showed MS phenotype. Inducible phenotype was higher in MRSA as compared to MSSA strains (Figure 7).

Inducible phenotype was most common in pus/wound swab (28.41%) followed by blood which showed 12.25%. Constitutive phenotype predominated in blood samples (26.53%) .MS phenotype was most common in BAL specimens (28.57%) (Figure 8). Antibiotic resistance pattern of different phenotypes of CD resistance in staphylococci was also studied. All phenotypes were sensitive to Linezolid and Vancomycin (Figure 9).

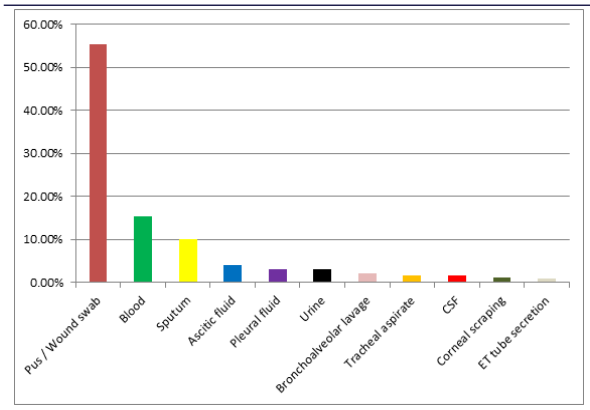

FIGURE 2 Specimen wise distribution of *S. aureus* isolates

FIGURE 3 Antibiotic resistance pattern of *S. aureus*

Antibiotic	*S aureus* (%)
Erythromycin	174 (54.72)
Clindamycin	107 (33.65)
Penicillin	318 (100)
Cotrimoxazole	214 (67.30)
Linezolid	0
Tetracycline	188 (59.12)
Vancomycin*	0
Chloramphenicol	156 (49.06)
Ciprofloxacin	224 (70.44)
Gentamycin	204 (64.15)
Nitrofurantoin**	4 (40)
Amikacin	114 (35.85)
Ofloxacin	196 (61.64)

*Vancomycin- MIC **Out of 10 urinary isolates

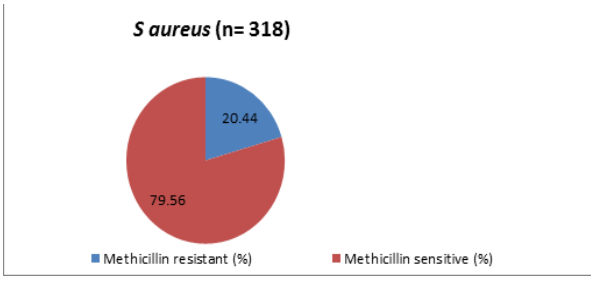

FIGURE 4 Detection Of Methicillin Resistance In *S. Aureus*

FIGURE 5 Vancomycin MIC in Methicillin resistant *S. aureus*

Vancomycin MIC	*S aureus* No. (%)
≤0.50	0
0.75	9 (13.86)
1	5 (7.69)
1.5	23 (35.38)
2	28 (43.07)
3	0
≥4	0
Total	65 (100)

FIGURE 6 Phenotypic pattern of CD Resistance among S. aureus isolates

Susceptibility pattern	*S aureus* (%)
Inducible phenotype	64 (20.13)
Constitutive phenotype	43 (13.52)
MS phenotype	67 (21.07)
E and CD susceptible Phenotype	144 (45.28)
Total	318 (100)

Applying Chi Square test, $p < 0.05$ shows statistical significance

FIGURE 7 Phenotypic pattern of CD Resistance among Methicillin resistant and Methicillin sensitive S. aureus

Susceptibility pattern	MRSA (%)	MSSA (%)
Inducible phenotype	16 (24.62)	48 (18.97)
Constitutive phenotype	12 (18.46)	31 (12.25)
MS phenotype	17 (26.15)	50 (19.76)
E and CD susceptible phenotype	20 (30.77)	124 (49.02)
Total	65 (20.44)	253 (79.56)

FIGURE 9 Antibiotic Resistance Pattern Of Different Phenotypes Of Cd Resistance

	S. aureus		
Antibiotic drug	**Inducible phenotype64) (%)**	**Constitutive phenotype (n=43) (%)**	**MS phenotype (n=67) (%)**
Penicillin	64 (100)	43 (100)	67 (100)
Cotrimoxazole	46 (71.88)	30 (69.77)	43 (64.18)
Vancomycin*	0	0	0
Linezolid	0	0	0
Tetracycline	33 (51.56)	21 (43.83)	33 (49.25)
Chloramphenicol	31 (48.43)	24 (55.81)	29 (43.28)
Ciprofloxacin	47 (73.44)	28 (65.12)	41 (61.19)
Gentamycin	37 (57.81)	25 (58.14)	36 (53.73)
Nitrofurantoin*	1* (50)	0	1* (50)
Amikacin	24 (37.5)	15 (34.89)	22 (32.84)
Ofloxacin	40 (62.5)	20 (46.52)	30 (44.78)

*urinary isolates

DISCUSSION

Most of staphylococcal isolates show multidrug resistance to commonly used antibiotics.[8] With emergence of MRSA, only a few alternatives are available to treat such infections. The MLS_B family of antibiotics is one such alternative and CD is preferred.[9]

Staphylococcal species with CD resistance can develop inducible phenotype, and gradually from such isolates, spontaneous constitutively resistant mutants arise both in vitro and in vivo during CD therapy. Hence, detection of such resistant phenotypes is of importance to minimize treatment failures.[10]

Isolation of *S. aureus* from pus and wound swabs specimens in present study was 55.35%. In study by Mokta et al[10], isolation of *S. aureus* from pus and wound swab specimen was 56%. Isolation of *S. aureus* from pus in study by Adhikari et al[11] and Bhatt MP et al[12] was 54.4% and 68% respectively. However higher isolation rate was reported by Sasirekha et al[13] (71.89%). The wide spectrum of diseases caused by *S. aureus* includes all those infections that affect skin and soft tissues, surgical site infection, infections of bones and joints. This might be reason for higher isolation of *S. aureus* from pus and wound swab samples.Tthe knowledge of current antibiotic resistance pattern of *S. aureus* strains is necessary in selection of appropriate empirical treatment.[14]In present study, E and CD resistance was 54.72% and 33.65% respectively. Similarly, Shanthi et al[15] reported 62.5% and 35% resistance to E and CD respectively. Deotale et al[16] reported 32.39% and 18.22% of E and CD resistant isolates respectively. MRSA is a major nosocomial and community pathogen causing significant morbidity and mortality. MRSA strains are important for their resistance to many other commonly used antibiotics. Vancomycin has been used to treat MRSA infections for more than three decades and there is an emergence of resistance to Vancomycin.[17] In present study, MRSA was found to be 20.44%. Similar prevalence rate of MRSA was obtained by other studies in India by Mokta et al[18] (23.42%) and Gupta et al[19] (25%). Deotale et al[16] reported 49.8% MRSA and Gade et al[20] reported 42.8% MRSA in their study. However higher isolation rate was reported in some studies. This shows a large variation in the incidence of MRSA. In present study, 43.07% of MRSA isolates showed MIC of 2 µg/ml and 35.38% showed MIC of 1.5 µg/ml. All isolates of MRSA were found to be susceptible to Vancomycin with MICs of ≤ 2 µg/ml by E - test. Similarly Bhatt MP et al[12], Gupta V[19] et al reported 100% sensitivity to Vancomycin.

In present study, *S. aureus* isolates when tested for inducible Clindamycin resistance, showed that among 318 isolates of *S. aureus*, inducible and constitutive phenotype was reported to be 20.13% and

13.52% respectively. The inducible phenotype was found to be higher than constitutive phenotype in present study. This is comparable to study by Gangurde et al[21] who reported 44 (13.53%) isolates of inducible phenotype and 12.61% of constitutive phenotype. Similar observation was made by Gade et al[20] where inducible and constitutive phenotype were 13.2% and 12.4% respectively. In a study by Patil et al[22], inducible and constitutive phenotype were found to be 11.11% and 3.55% respectively which also shows that inducible phenotype was higher than constitutive phenotype. Similar observation was made by Deotale et al[16] who also observed inducible phenotype to be higher (36%) as compared to constitutive phenotype (9%). However, there are studies in which higher constitutive resistance has been reported. Mokta et al[18] found inducible phenotype of 13.71% and constitutive phenotype of 17.14%. Similarly, Sasirekha et al[13] reported higher constitutive phenotype of 13.07% and inducible phenotype of 9.15%. Adhikari et al[11]also reported constitutive phenotype (2.25%) to be higher than inducible phenotype (11.48%). True sensitivity which is given by MS phenotype confirms CD susceptibility. MS phenotype in present study was found to be 21.07%. In another study by Deotale et al[16], 35% of MS phenotype was reported. Lower rate of MS phenotype of 8.28% was reported by Mokta et al.[18]Hence, there is a wide variation in the incidence of constitutive and inducible resistance in staphylococcal isolates. The reason for this might be geographical and environmental factors which were entirely different in different clinical set ups. The variation may depend upon sample size, age group, geographical region, population studied.

In present study, phenotypic variation of CD resistance according to Methicillin susceptibility was also studied. It was observed that inducible phenotype was higher in MRSA than MSSA but it was not found to be statistically significant.($p>0.05$)

Among 65 MRSA isolates, 24.62% showed inducible phenotype and 18.46% showed constitutive phenotype. MS phenotype was also higher in MRSA isolates (26.15%). Similar pattern has been found in various studies by Mokta et al[18], Patil et al[22] and Gangurde et al[21] who also observed inducible and constitutive resistance to be more in MRSA isolates as compared to MSSA. In the study by Mokta et al[18], 28.39% of MRSA isolates belonged to inducible phenotype and 29.62% of MRSA isolates belonged to constitutive phenotype.

In this study, a total of 176 pus/wound swab specimens were studied. Inducible phenotype was most common in pus/wound swab specimens with isolation rate of 28.41%. This is in concordance with study of Reddy et al[23]who also reported inducible phenotype most commonly in pus specimen with isolation rate of 26.53%. However, Saffar et al[24] reported lower isolation rate of 4.16% of inducible phenotype in pus and wound swabs.

Among all phenotypes, all isolates were resistant to Penicillin and all isolates were sensitive to Linezolid. Among inducible phenotypes of *S. aureus* resistance to Ciprofloxacin, Cotrimoxazole was 73.44% and 71.88% respectively.

CONCLUSION

D test is recommended for detection of ICR. 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 helpful in guiding therapy. Without "D test" all l isolates with ICR would be erroneously classified as CD susceptible by routine testing methods. As this is simple, economical and easy and hence it must be included in troutine diagnostic laboratories to prevent treatment failure.

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