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Original Research Article

Inducible and constitutive clindamycin resistance in *Staphylococcus aureus*, isolated from clinical samples

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ABSTRACT

Background: The resistance to antimicrobial agents among Staphylococci is an increasing problem. Clinical laboratories should perform D test routinely to guide the clinicians about the inducible clindamycin resistance and to prevent misuse of antibiotics.
Aims: Study aimed to isolates the Inducible and Constitutive clindamycin Resistance in Staphylococcus aureus in various clinical samples.
Materials and Methods: We analyzed the performance of disk diffusion method in 183 Staphylococci aureus strains obtained from various clinical samples of the patients collected from September 2018 to February 2020 at a tertiary care centre, Meerut.
Result: Inducible clindamycin resistance was tested by 'D test' as per CLSI guidelines. 142(77.6%) of *S. aureus* isolates were found to be methicillin resistant (MRSA) and 41 (22.4%) tested sensitive to cefoxitin i.e., methicillin sensitive *S. aureus* (MSSA).
Conclusion: Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA). The D test method showed to be simple and easy in the detection of inducible ($iMLS_B$) and constitutive clindamycin resistance ($cMLS_B$).

1. Introduction

Staphylococcus aureus is one of the most common pyogenic bacteria infecting man. The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives available to treat Staphylococcal infections. *Staphylococcus* aureus, one of the most common nosocomial and community-acquired pathogens has now emerged as an ever-increasing problem due to its increasing resistance to several antibiotics. In *Staphylococcus spp*., penicillin and methicillin resistance was first recognized in 1944 and 1961 A.D. respectively.[1] The macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due its excellent pharmacokinetic properties. Clindamycin is an alternative drug for infections due to Staphylococcus *aureus* in case of intolerance to penicillin or resistance to methicillin. Furthermore, clindamycin represents an attractive option for several reasons. First, clindamycin is available in both intravenous and oral formulations. Second, the drug has a remarkable distribution into the skin and skin structures. Third, community-acquired methicillin-resistant *S. aureus* (CA-MRSA), which has rapidly emerged in recent years as a cause of skin and soft-tissue infections, is frequently susceptible to several antibiotics, including clindamycin.[2,3]

Resistance in Gram-positive bacteria not only increases morbidity and mortality, but also the costs of management of hospitalized patients. Studies have indicated a great increase in the ratio of staphylococci resistance to MLS group and failure in the treatment with clindamycin in infections with microorganisms with inducible resistance to MLS group.[4]

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Therefore, this study was aimed to assess the prevalence and antimicrobial susceptibility pattern of methicillin and induced clindamycin-resistant *Staphylococcus aureus* from various clinical samples received in tertiary care centre, Meerut, North India.

2. Materials and Methods

2.1. Study Design and Period

A hospital-based retrospective study was conducted at Mulayam Singh Yadav Medical College & Hospital from 2018 September to 2020 February.

183 clinical isolates of *S. aureus* were subjected to D test. Out of 183 isolates, 142(77.6%) were found to be methicilin resistant *S. aureus* (MRSA) strains and 41(22.4%) methicillin sensitive *S. aureus* (MSSA) strains. Testing of methicillin resistance was done with (30μg) disc of cefoxitin as per Clinical Laboratory and Standard Institute (CLSI), 2016 guidelines. D-test was performed by placing clindamycin CLI disc (2μg) and erythromycin ERY disc (15μg) approximately 15-26 mm apart measured edge to edge on a Muller-Hinton agar plate that has been inoculated with a *Staphylococcus* isolate (0.5 McFarland standard) incubated at 35$\pm$2°C in ambient air. Flattening of the zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) = inducible clindamycin resistance [Figure 1].

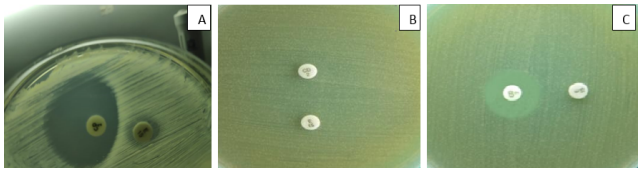

Fig. 1: (A) Positive D-test (iMSLB), **(B)** No zone (cMSLB), **(C)** MS Phenotype

D-test was performed as per Clinical Laboratory and Standard Institute (CLSI), 2016 guidelines.[5,6]

Staphylococcus aureus ATCC 25923 strains, was used to check the quality control of ERY and CLI discs. In house positive and negative controls were also used.

Interpretation of erythromycin and clindamycin zones was done according to the description given below in the [Table 1].

Table 1: Interpretation of erythromycin and clindamycin zones in *S. aureus*

	Sensitive	Intermediate	Resistant
Erythromycin	$\geq$ 23 mm	14-22 mm	$\leq$ 13 mm
Clindamycin	$\geq$ 21 mm	15-20 mm	$\leq$ 14mm

CLSI Guidelines 2017: Performance standards for Antimicrobial disc Susceptibility Tests

3. Result

In this study, 183 *Staphylococcus aureus* isolated from clinical samples in our hospital during a period of 18 months. Out of which 146(79.8%) were IPD samples and 37(20.2) samples from OPD. Predominant clinical samples being pus 112(61.2%) followed by 38(20.8%) blood, 18(9.8%) urine, 9(5%) tracheal aspirates and 6(3.2%) from other body fluid. [Figure 2]

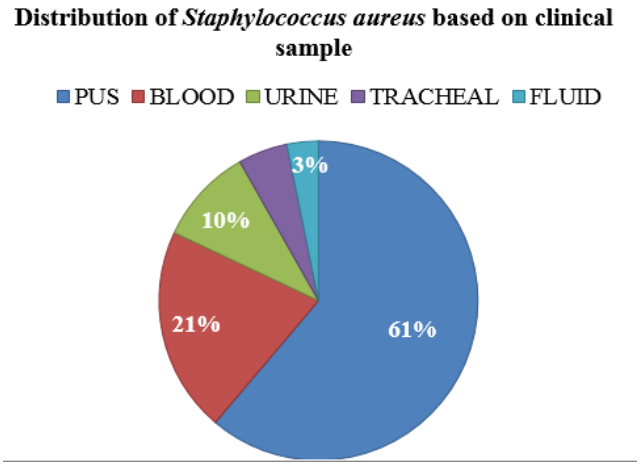

Fig. 2: Prevalence of *Staphylococcus aureus* in clinical sample

While majority of the study participants were 118(64.5%) male, and 65 (35.5%) female, the ratio was 1.8:1. Males comply poorly with hand-hygiene recommendations compared with females, and gender differences in motivation for improvement have been reported.[7] [Figure 3]

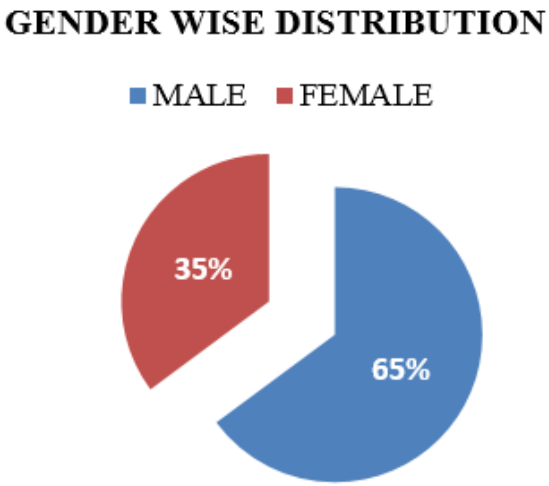

Fig. 3: Prevalence of male and female.

In our study, 183 *Staphylococcus aureus* isolates were resistant to penicillin (89.6%) and ampicillin

(88%), followed by (77.5%) co-trimoxazole, erythromycin (64.5%), Ciprofloxacin (60.6%), clindamycin (55.7%), gentamycin (25.1%) and least were resistant to vancomycin (2.1%) whereas none resistance showed in Linezolid. [Table 2]

Table 2: Antibiotic sensitivity of *Staphylococcus aureus*

S. No.	Antibiotics	Antibiotic sensitivity Resistant n (%)
1.	Penicillin	164 (89.6)
2.	Ampicillin	161 (88)
3.	Erythromycin	118 (64.5)
4.	Co-trimaxazole	142 (77.5)
5.	Clindamycin	102 (55.7)
6.	Ciprofloxacin	111 (60.6)
7.	Gentamycin	46 (25.1)
8.	Vancomycin	4 (2.1)
9.	Linezolid	-

142(77.6%) of *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA) and 41 (22.4%) tested sensitive to cefoxitin (MSSA). A total of 35% S. aureus isolates belonged to cMLSB while 20.8% iMLSB phenotype whereas 44.2% belonged to MS phenotype. [Table 3]

Table 3: Detection of constitutive and inducible-clindamycin (MLSB) phenotypes.

Organism	Inducible (iMLSB) resistance n (%)	Constitutive (cMLSB) resistance n(%)	Ms Phynotype n(%)
S. aureus	38 (20.8)	64 (35)	81(44.2)

Both constitutive and inducible resistance phenotypes were found to be significantly higher in MRSA isolates compared to MSSA. [Table 4]

Table 4: istribution among MRSA & MSSA

Susceptibility Pattern	MRSA (%)	MSSA (%)	Total (%)
Erythromycin	96(81.4%)	22(18.6%)	118(100%)
Imlsb	29(76.3%)	09(23.7%)	38(100%)
cMSLB	58(90.6%)	06(9.4%)	64(100%)
Ms Phenotype	13(16%)	68(84%)	81(100%)

4. Discussion

Our study revealed an extremely high percentage of MRSA 77.6%. In Korea, the prevalence of MRSA has been estimated to be more than 70% among all clinical isolates in early 2010s.[8] Various previous studies showed the high prevalence in their studies, Toleti et al. have reported a prevalence rate of 64.70%, and much closer rate 77.5% reported by Jarajreh et al. in their study conducted in Saudi Arabia. While 92% higher rate reported by Rameshwari et al.[9–11]

Table 5: Prevalence of Staph. aureus isolates in various clinical sample in different centres in India

S. No.	Author	Sample				
		Pus %	Blood %	Urine %	Tracheal aspirates %	Other Body fluid %
1.	Present study	61.2	20.8	9.8	5	3.2
2.	Gupta et al.	50.9	26.7	8	3	2.4
3.	Krishna et al.	63	10.5	14	-	-
4.	Kumari et al.	64	20	3.2	-	-
5.	Shrestha et al.	72.5	-	8.7	-	-
6.	Deepak et al.	43.1	1.9	13.1	-	-
7.	Mohammad et al.	-	5.1	48.5	12.5	-

In the present study, erythromycin resistance was seen in 64.5% isolates. Among the erythromycin- resistant *S. aureus*, iMLSB resistance was observed in 20.8% isolates and cMLSB in 35% and MS phenotype in 44.2%. A study carried out by Steward et al. reported maximum iMLSB phenotype 16.4% followed by cMLSB 12.5% and MS phenotype 7.8%. Similarly studies carried out by Regha et al., Deotale et al. also reported iMLSB as the predominant phenotype followed by cMLSB and then MS phenotype.[12–14]

In the present study there was significant gender difference in the study group; male was 64.5% affected where as 35.5% female and male to female ratio was 1.8:1. Similar rate reported by Patel et al. *Staphylococcus aureus* isolated were 54% form males and 46% from females.[15]

In the present study, 183 isolates of *S. aureus*, 61% were isolated from pus samples followed by 20.8% from blood, 9.8% from urine, 5% from tracheal aspirates 3.2% from other body fluid which was similar to study reported by various authors, mention in[16–21] [Table 5].

5. Conclusion

In the present study we describe D-test, it was inexpensive and easy to perform test, it can be included as a part of routine antibiotic susceptibility testing to accurately identify iMLSB and cMLSB clindamycin susceptible Ms Phenotypes. Resistance in Gram-positive bacteria not only increases morbidity and mortality, but also the costs of management of IPD or OPD patients. Studies have indicated increase rate of staphylococcus resistance in male's. In addition, D-testing can provide information about resistant

to MLS phenotype group of antibiotics and can be useful for surveillance studies related to MLS resistance in Staphylococci in clinical samples.

6. Source of Funding

None.

7. Conflict of Interest

None.

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