International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://www.theaspd.com/ijes.php

Phenotypic Detection Of Inducible Clindamycin Resistance By D-Test In Staphylococci From Various Clinical Samples

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Abstract:
Background: *Staphylococcus aureus is a major opportunistic human pathogen causing many clinical infections. Three MLSB phenotypes are known in S. aureus, a constitutive resistant phenotype (cMLSB), a clindamycin-susceptible phenotype in vitro with inducible resistance in vivo (iMLSB), and a clindamycin-susceptible and macrolide-steptogramin B-resistant phenotype (MSB).* ***Method:*** *This prospective-observational study was carried out in Department of Microbiology, NIMS medical college, Jaipur, Rajasthan from July 2023 to Sept 2024. A total of 278 Staphylococci were isolated from different types of samples. All the Staphylococcus aureus isolates that were resistant to erythromycin were tested for inducible Clindamycin resistance by double disk approximation test (D-test) as per CLSI guidelines.* ***Result:*** *A total of 278 Staphylococci isolates, MRSA (n=116), followed by MR-CONS (n=82), MSSA (n=57) and CONS (n=23).* ***Discussion:*** *In this study we found 44 (16%) inducible clindamycin resistance by D-test. Similar, findings observed in various studies also.* ***Conclusion:*** *Highest percentage of inducible resistance found in isolates from Blood sample. Clinical microbiology laboratories should report inducible Clindamycin resistance in Staphylococcus aureus and D-test can be used as a simple, auxiliary and reliable method to Delineate inducible and constitutive Clindamycin resistance in routine clinical laboratories.*

Keywords: *MLSB, D-test, ermA, msrA.*

INTRODUCTION:

Staphylococcus aureus is a major opportunistic human pathogen causing many clinical infections. The rates of infections caused by staphylococci, both community, and hospital-acquired strains, are increasing steadily.[1] Erythromycin and clindamycin are used as topical therapeutic agents for treatment of skin and soft tissue infections associated with Staphylococcal infections [2]. Three MLSB phenotypes are known in S. aureus, a constitutive resistant phenotype (cMLSB), a clindamycin-susceptible phenotype in vitro with inducible resistance in vivo (iMLSB), and a clindamycin-susceptible and macrolide-steptogramin B-resistant phenotype (MSB). Clindamycin, a macrolide–lincosamide streptogramin B (MLSB) family of antibiotics, is the drug usually advocated for treating Staphylococcal infection.[3] Resistance to MLSB arises mainly by an active efflux mechanism coded by the msrA gene or ermA gene. In inducible resistance, rRNA methylase is produced only in the presence of an inducing agent which can be any of the antibiotics in the MLSB family such as erythromycin [4,5]. Isolates with only inducible resistance are resistant to erythromycin but appear susceptible to clindamycin in vitro [6-8].

The purpose of this study is to detect inducible clindamycin resistance and antibiotic susceptibility pattern among staphylococcus aureus isolates in various clinical specimens.

MATERIALS AND METHODS:

This prospective-observational study was carried out in Department of Microbiology, NIMS medical college, Jaipur, Rajasthan from July 2023 to Sept 2024 after approval of ethical committee. The study population included patients of all age groups and sex visiting the inpatient and outpatient departments of the hospital to whom culture tests had been referred.

A total of 278 Staphylococci were isolated from different types of samples; Blood (n=161), Pus (n=64), Swabs (n=40), Respiratory samples (n=11), Fluids (n=02) etc, received in the bacteriology lab of NIMS hospital, using standard microbiological techniques. [9, 10].

Antibiotic susceptibility testing was done on Muller Hinton agar plate as per standard guidelines and procedure. All the Staphylococcus aureus isolates that were resistant to erythromycin were tested for inducible Clindamycin

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resistance by double disk approximation test (D-test) as per CLSI guidelines. In this test, turbidity of Staphylococcus aureus is compared with 0.5% McFarland's standard. The suspension was lawned over the MHA plate and the antibiotic disc was placed over the lawned plated. An erythromycin disk (15µg) and Clindamycin (2µg) were placed 15mm apart edge-to-edge on MHA plate. Methicillin resistance was identified by placing Cefoxitin (30µg) disc on MHA plate and interpreted as per CLSI guidelines. Plates were analysed after overnight of incubation at 37°C. On next day the plates were examined for the zone of inhibition around the antibiotic disc. The diameter of the inhibitory zone includes the diameter of the disc.

Result: A total of 278 Staphylococci isolates, in which MRSA were 116 (42%) followed by MR-CONS 82 (30%), MSSA 57 (20%) and CONS 23 (8%). Out of total 278 isolates, 44 (16%) showed inducible clindamycin resistance by D-test. In which MRSA were 19 (43%), MSSA 12 (28%), MR-CONS 11 (25%) and CONS 02 (4%). Inducible resistance was observed among various clinical samples like, Blood 18 (41%) followed by Pus 13 (29%), Swabs 08 (18%), Respiratory samples 05 (12%).

Table 1: Gender wise distribution of clinical Isolates		
Gender	**No of Isolates (n)**	**Percentage (%)**
Male	172	62
Female	106	38
Total	**278**	**100**

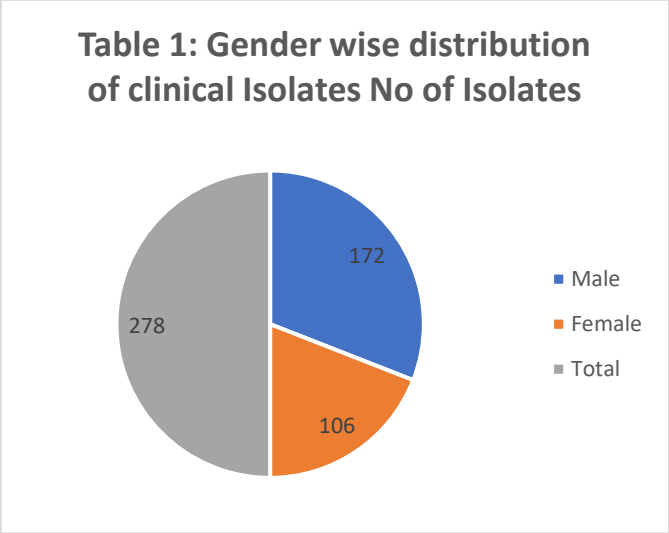

Table 2: Distribution of D-test positive according to various clinical samples				
Type of samples	**No. of samples**	**Percentage**	**D-Test Positive**	**Percentage**
Blood	161	58	18	41
Fluids	02	1	00	00
Pus	64	23	13	29
Swabs	40	14	08	18
Respiratory samples	11	4	05	12
Total	**278**	**100**	**44**	**100**

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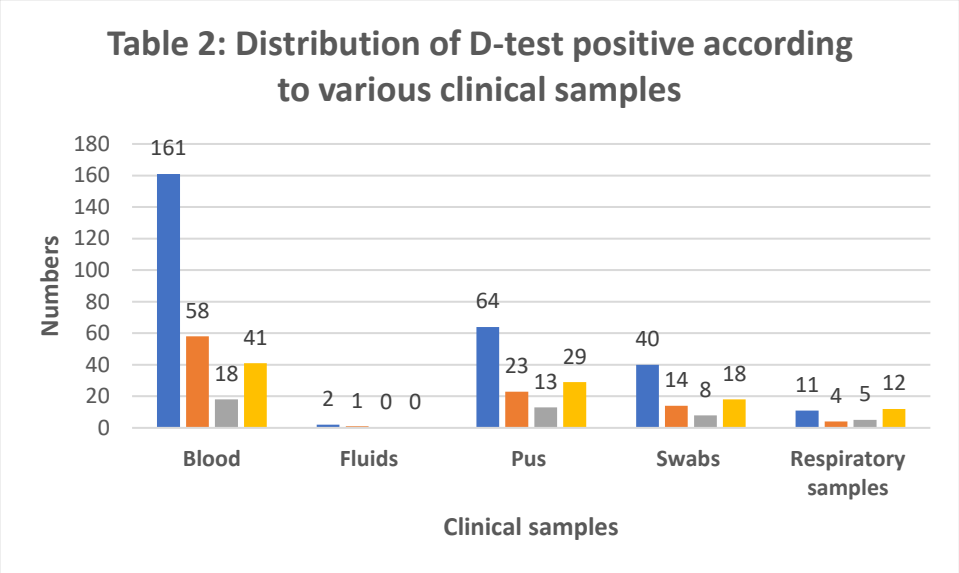
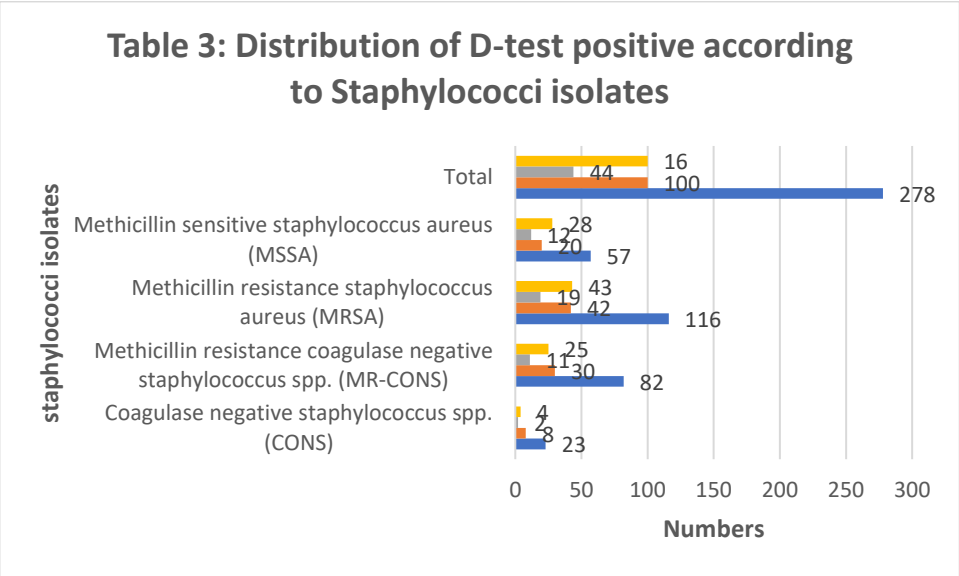


Table 3: Distribution of D-test positive according to Staphylococci isolates				
Organisms	**Number of isolates**	**Percentage**	**D-test Positive**	**Percentage**
Coagulase negative staphylococcus spp. (CONS)	23	8	02	4
Methicillin resistance coagulase negative staphylococcus spp. (MR-CONS)	82	30	11	25
Methicillin resistance staphylococcus aureus (MRSA)	116	42	19	43
Methicillin sensitive staphylococcus aureus (MSSA)	57	20	12	28
Total	**278**	**100**	**44**	**16**



DISCUSSION:

In this study we found 16% inducible clindamycin resistance by D-test. Shantala G B et al in their study observed that 57 (24.89%) had the inducible clindamycin resistance. (11)

CONCLUSION:

Highest percentage of inducible resistance found in isolates from Blood sample. Clinical microbiology laboratories should report inducible Clindamycin resistance in Staphylococcus aureus and D-test can be used as a simple, auxiliary and reliable method to Delineate inducible and constitutive Clindamycin resistance in routine clinical laboratories.

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