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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 50 *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 50 *Staphylococci* isolates, MSSA were 18 (36%) followed by CONS 12 (24%), MR-CONS 12 (24%) and MRSA 8 (16%). Out of total 50 isolates, 17 (34%) showed inducible resistant by D-test. In which MSSA were 3 (18%), CONS 5 (29%), MR-CONS 4 (24%) and MRSA 5 (29%). Inducible resistance was observed among various clinical samples like, Blood samples 10 (60%) followed by Pus 5 (30%), Wound Swab 1(5%), and High Vaginal Swab 1(5%). **Discussion:** In this study we found 34% 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 streptogr amin 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 50 Staphylococci were isolated from different types of samples; Pus (n=10), Blood (n=31), CSF (n=1), wound swab (n=4), high vaginal swab (n=2), ET (n=1), and nasal swab (n=1) 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 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 50 *Staphylococci* isolates, MSSA were 18 (36%) followed by CONS 12 (24%), MR-CONS 12 (24%) and MRSA 8 (16%). Out of total 50 isolates, 17 (34%) showed inducible resistant by D-test. In which MSSA were 3 (18%), CONS 5 (29%), MR-CONS 4 (24%) and MRSA 5 (29%). Inducible resistance was observed among various clinical samples like, Blood samples 10 (60%) followed by Pus 5 (30%), Wound Swab 1(5%), and High Vaginal Swab 1(5%).

Table 1: Gender wise distribution of clinical Isolates	
Gender	**No of Isolates**
Male	35 (70%)
Female	15 (30%)
Total	50 (100%)

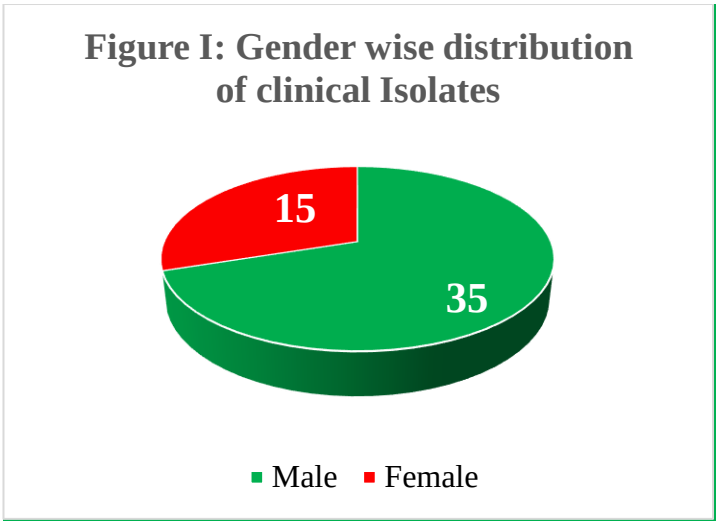
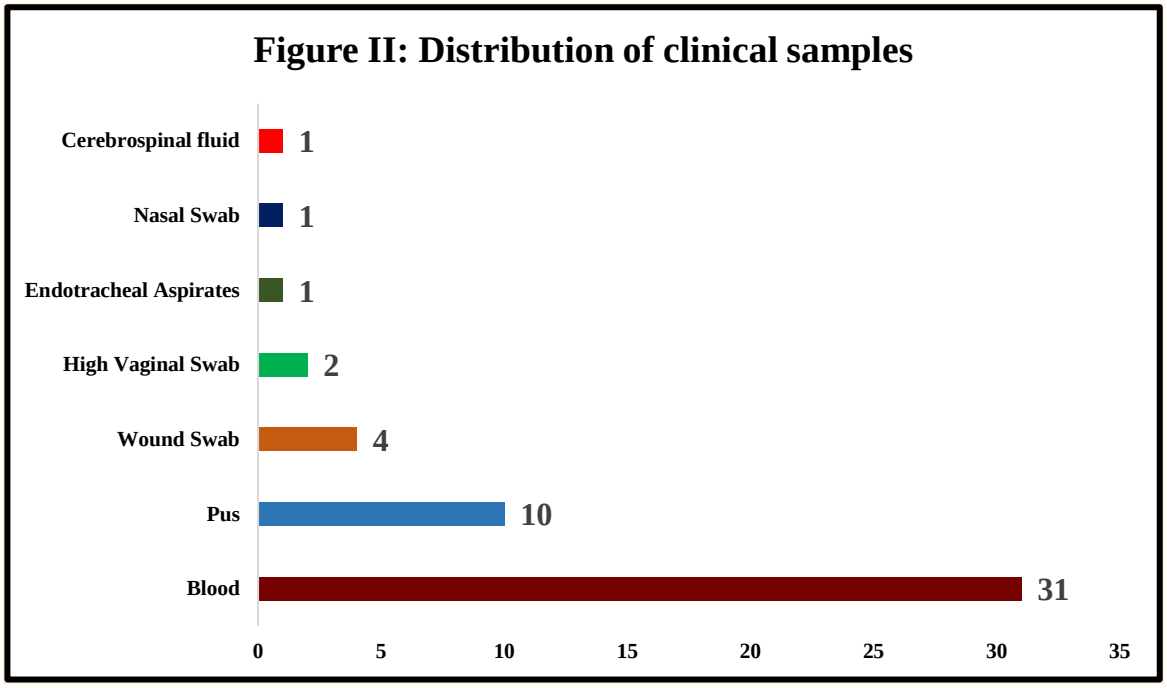
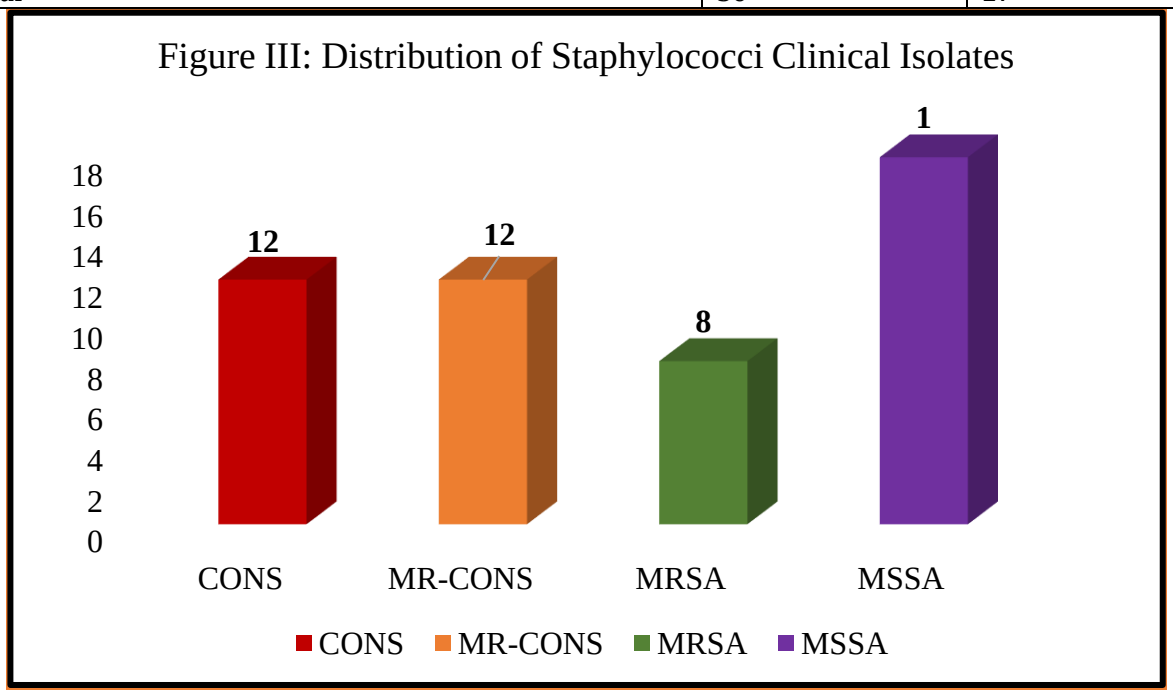


Table 2: Distribution of clinical samples according to D-test positive		
Type of samples	**No. of samples**	**D-Test Positive**
Blood	31 (62%)	10 (60%)
Pus	10 (20%)	05 (30%)
Wound Swab	04 (8%)	01 (5%)
High vaginal swab	02 (4%)	01 (5%)
Endotracheal aspirates	01 (2%)	00
Nasal swab	01 (2%)	00
Cerebrospinal fluid	01 (2%)	00
Total	**50 (100%)**	**17 (34%)**



Organisms	Number of isolates	D-test Positive
Coagulase negative staphylococcus spp. (CONS)	12 (24%)	05 (29%)
Methicillin resistance coagulase negative staphylococcus spp. (MR-CONS)	12 (24%)	04 (24%)
Methicillin resistance staphylococcus aureus (MRSA)	08 (16%)	05 (29%)
Methicillin sensitive staphylococcus aureus (MSSA)	18 (36%)	03 (18%)
Total	**50**	**17**



Discussion: In this study we found 34% 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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