

Detection of inducible clindamycin resistance in staphylococcus aureus and CONS at tertiary care hospital

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ABSTRACT

Background: The increasing prevalence of Methicillin resistant among staphylococci (MRSA) is an increasing problem. Increasing incidence of infections due to MRSA has led to emphasis on the need for safe & effective agents to treat both systemic & localized Staphylococcal infections. Clindamycin has been used to treat pneumonia & soft tissue and musculoskeletal infections due to MRSA. One important issue in Clindamycin treatment is the risk of clinical failure during therapy caused by *MLS_B* inducible resistance.

Objectives: To isolate and identify *Staphylococcus aureus* and CONS from all clinical samples & to determine the inducible Clindamycin resistance among the *Staphylococcus aureus* and CONS.

Methods: A total of 100 isolates of *Staphylococcus aureus* and CONS from various samples were isolated. Methicillin resistance was detected by using a 1 µg Oxacillin disc. The D-test was performed using the discs of Clindamycin (CL)(2µg) and Erythromycin (ER)(15µg) placed at a distance of 15mm (centre to centre) along with routine antibiotic susceptibility testing.

Results: Among *Staphylococcus aureus*, MSSA isolates were 32(47.05%) compared to MRSA isolates, 26(38.24%) and among CNS, MCONS isolates were 8(11.77%) compared to MRCONS 2(2.9%). A total of 12(17.64 %) isolates showed *iMLS_B*, of which 8(11.77%) were MRSA, 2(2.9%) were MSSA and 2(2.9%) MRCONS isolates.

Conclusion: Prevalence of inducible Clindamycin resistance among Staphylococcal isolates was significant. Hence the implementation of this D-test routinely, which is simple, reliable & inexpensive will reveals the *iMLS_B* & *cMLS_B* phenotype & prevents the therapeutic failure of Clindamycin.

Keywords: Methicillin resistant *Staphylococcus aureus* (MRSA), Inducible Clindamycin resistance, D-test, Erythromycin, *iMLS_B* phenotype.

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INTRODUCTION

Staphylococcus aureus and Coagulase negative *Staphylococci* (CONS) are recognized to be causing nosocomial and community acquired infections in every region of the world. The increasing prevalence of Methicillin resistant among staphylococci (MRSA) is an increasing problem⁽¹⁾.

Increasing incidence of a variety of infections due to *Staphylococcus aureus*, CONS & especially, the expanding role of community-associated -MRSA has led to emphasis on the need for safe & effective agents to treat both systemic & localized Staphylococcal infections.⁽²⁾

Macrolide (e.g., erythromycin), Lincosamide (e.g., Clindamycin) and Streptogramin-B (e.g., quinupristin-dalfopristin) antimicrobial agents (collectively called as MLS agents) are commonly used in the treatment of

Staphylococcal infections⁽¹⁾. Macrolide antibiotics like erythromycin are bacteriostatic agents that inhibit protein synthesis by binding reversibly to the 50S ribosomal subunits susceptible organisms⁽³⁾.

Clindamycin is a frequent choice for the Staphylococcal infections because of

- 1) Both intravenous and oral formulations (with 90% oral Bio availability).
- 2) Drug distributes well into skin and skin structures & unlike β-lactams, it is not impeded by a high bacterial burden at the infection site.
- 3) It is also less costly than same of the newer agents that might be considered for these infections.
- 4) Clindamycin may be able to inhibit production of certain toxins & other virulence factors in Staphylococci

Hence Clindamycin has been used successfully to treat pneumonia & soft tissue and musculoskeletal infections due to MRSA in adults and children.⁽²⁾ One important issue in Clindamycin treatment is the risk of clinical failure during therapy. Therapeutic failure caused by *MLS_B* inducible resistance, are being more commonly reported⁽¹⁾.

MLS family of antibiotics has 3 different mechanism of resistance

- Target site modification** on ribosome resulting in resistance to Macrolide, Lincosamide and group B Streptogramin referred as MLS_B resistance. MLS_B resistance in staphylococci is usually encoded by *erm(A)* or *erm(C)*
- Enzymatic antibiotic inactivation**
- Macrolide efflux pumps**⁽¹⁾

There are two types of Expression of MLS resistance, constitutive or inducible. Constitutively resistant strains are resistant to all MLS antibiotics & are readily detected by standard susceptibilities methods. Inducible resistance is expressed in the presence of strong inducers of methylase synthesis such as 14 membered (ER) & 15 membered (Azithromycin) macrolides, 16 membered Macrolide (Spiramycin) Lincosamide (CL) & Streptogramin B antibiotics may appear sensitive using standard & susceptibility methods because they were weak inducers of methylase synthesis. Inducible resistance can be detected by disc diffusion test. Low levels of ER are the most effective inducers of inducible MLS_B resistance.

Inducible Clindamycin resistance is not detected by standard broth microdilution, automated susceptibility testing devices, the standard disk diffusion test or E test⁽²⁾. So this study demonstrates simple, reliable and significant method (double disc diffusion test) of detecting inducible resistance to Clindamycin in isolates of *Staphylococcus aureus* and *CONS*.

AIMS AND OBJECTIVES

- To isolate and identify *Staphylococcus aureus* and *CONS* from all clinical samples.
- To determine the inducible Clindamycin resistance among the *Staphylococcus aureus* and *CONS*.

METHODOLOGY AND TECHNIQUES

A total of 100 non duplicate, consecutive isolates of *Staphylococcus aureus* and *CONS* from samples such as pus/wound swab, sputum, blood, urine, body fluids, etc were isolated. The *Staphylococcus aureus* strains and *CONS* strains were identified by using standard microbiological procedures. Antibiotic susceptibility tests were performed by the Kirby-Bauer disc diffusion method. Methicillin resistance was detected by using a

1 µg oxacillin disc.

The D-test was performed to identify the $iMLS_B$ phenotype. A lawn culture of the isolate which was adjusted to 0.5 McFarland's concentration was made on a Mueller Hinton agar plate and discs of Clindamycin (CL) (2µg) and Erythromycin (ER) (15µg) were placed at a distance of 15mm (centre to centre) along with routine antibiotic susceptibility testing. The disc diffusion test, based on the D test, showed three phenotypes.

- $iMLS_B$ Phenotype:** If ER zone is < 13mm and CL zone is > 21mm, the organism is positive for inducible resistance (D-test positive). It is noted by flattening or blunting of the CL zone adjacent to the ER disc, giving a D shape.
- MLS_B Phenotype:** If ER zone is < 13mm and CL zone is > 21mm and both have a circular zone of inhibition, the organism is negative for inducible resistance (D-test) negative. No flattening of the CL zone; Resistant to ER but susceptible to CL.
- $cMLS_B$ Phenotype:** Resistant to both ER and CL-constitutive resistance

Inclusion criteria: All isolates of staphylococci which are ER-R and CL-S are included in the study.

Exclusion criteria: Staphylococci which are ER-S are excluded.

OBSERVATION AND RESULTS

In this study, of 68 Staphylococcal isolates, 58(85.29%) were *Staphylococcus aureus* and 10(14.71%) were *CONS* isolates as shown in Fig 1.

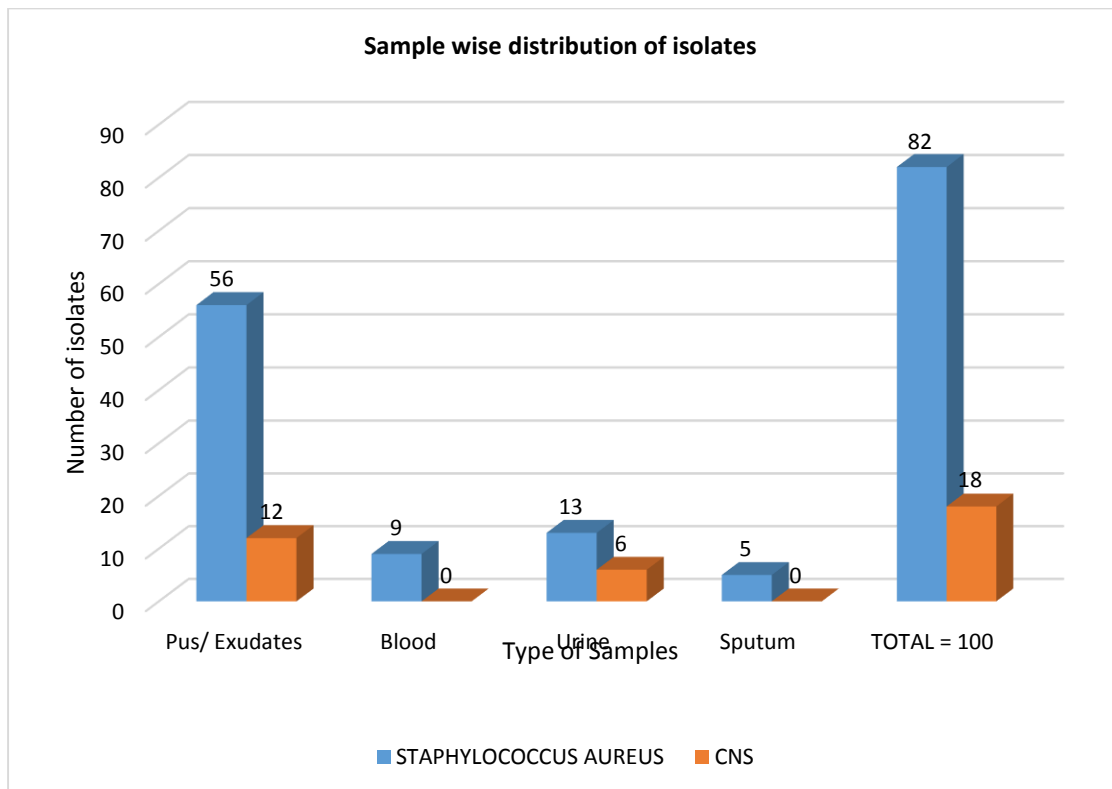
In our study, among *Staphylococcus aureus*, MSSA isolates were more i.e., 32(47.05%) compared to MRSA isolates, 26(38.24%) and among CNS, MSCNS isolates were more i.e., 8(11.77%) compared to MRCNS 2(2.9%) as shown in Fig 2. In our study there was a total of 12(17.64%) of Staphylococcal isolates showed $cMLS_B$ phenotype. Of which 6(8.8%) were of MRSA, 5(7.35%) were of MSSA and 1(1.4%) MSCNS isolates. A total of 12(17.64%) isolates showed $iMLS_B$, of which 8(11.77%) were MRSA, 2(2.9%) were MSSA and 2(2.9%) MRCNS isolates. 44(64.70%) isolates were of MS phenotype, of which 12(17.65%) were MRSA, 25(36.77%) were MSSA and 7(10.29%) were MSCNS isolates as depicted in Table 1 and Fig 3.

Table 1: Sensitivity pattern of isolates.

Phenotype	MRSA	MSSA	MRCNS	MSCNS	TOTAL
ER-R, CL-R ($cMLS_B$)	06 (8.8%)	06(7.35%)	-	03(1.47%)	15(17.64%)
ER-R, CL-S ($iMLS_B$) D test⁺	10(11.77%)	08(2.94%)	08(2.94%)	--	26(17.64%)
ER-R, CL-S (MS) D test⁻	18(17.65%)	34(36.77%)	--	07(10.29%)	59(64.70%)
Total	34(38.24%)	48(47.06%)	08(2.94%)	10(11.77%)	100

Table 2: Showing comparisons of different studies with present study

Studies	Organisms	iMLS _B	cMLS _B	MS phenotype
MR Angel et.al (2008) ⁽⁹⁾	MRSA	37 (64%)	7(12%)	14(24%)
	MSSA	6(5%)	33(25%)	88(70%)
	CNS	5(10%)	10(19%)	36(71%)
V.Deotale (2010) ⁽¹⁰⁾	MRSA	34(27%)	9(7.3%)	30(24.3%)
	MSSA	2(1.6%)	-	5(4%)
Shantala GB et.al, (2011) ⁽¹²⁾	MRSA	41(32.53%)	32(25.39%)	19(15.07%)
	MSSA	16(15.38%)	10(9.61%)	17(16.34%)
AM Ciraj et al (2009) ⁽¹⁴⁾	MRSA	16(20.51%)	4(5.1%)	-
	MSSA	10(12.82%)	-	-
	MS CNS	6(7.69%)	-	-
	MS CNS	-	4(5.1%)	-
Present study	MRSA	8(11.77%)	6(8.8%)	12(17.65%)
	MSSA	2(2.94%)	5(7.35%)	25(36.77%)
	MR CNS	2(2.94%)	-	-
	MS CNS	-	1(1.47%)	7(10.29%)

**Fig. 1: Sample wise distribution of isolates.**

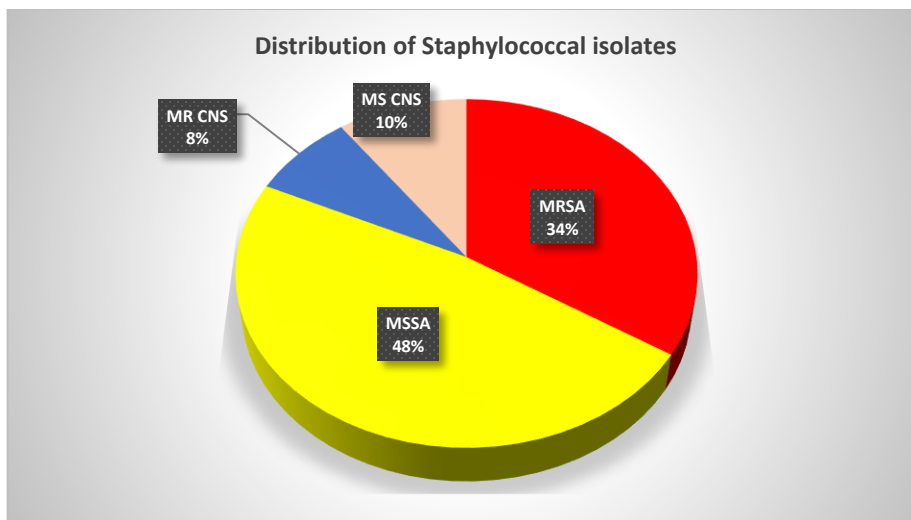


Fig. 2. Distribution of Staphylococcal isolates



Fig. 3. Shows inducible Clindamycin resistance. Note flattening or blunting of zone of inhibition around Clindamycin disc

DISCUSSION

Accurate drug susceptibility data of infecting microbe is an essential factor in making appropriate therapeutic decisions. The emergence of resistance to multiple antibiotics among Staphylococcal isolates has left very few therapeutic options for the clinicians. There are many options available for treatment of MSSA and MRSA infections, with CL being one of the good alternatives. However CL-R can develop in Staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen in vitro & in vivo during therapy⁽¹⁰⁾. Hence without checking for inducible

Clindamycin resistance it may result in therapeutic failure. Further more negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility and provides good therapeutic options.

In our study there is high percentage of MSSA [32(47.05%)] compared to MRSA [26 (38.24%)] and high percentage of MSCNS [08(11.77%)] compared to MRCNS [02(2.94%)] among all Staphylococcal isolates. Mallick et al⁽¹³⁾ had reported 189(51.6%) strains of MRSA and 177(48.3%) strains of MSSA, among Staphylococcus aureus. So in present study there is a low prevalence of MRSA compared to MSSA. In this study the inducible MLS_B (i MLS_B) and constitutive

MLS_B(cMLS_B) among the Staphylococcal isolates were 12(17.64%) strains each and MS phenotype were 44(64.70%) strains. But other ⁽¹⁰⁾ study has showed 14.5% of iMLS_B, 3.6% of cMLS_B and 14.17% of MS phenotype.

In our study iMLS_B among the MRSA isolates were 8(11.77%) strains, 2(2.94%) strains among MSSA and 2(2.94%) strains among MRCNS, and there was no iMLS_B among MSCNS in our study. AM Ciraj⁽¹⁴⁾ et al., has reported 16(20.51%) strains among MRSA are iMLS_B phenotype, 10(12.82%) strains among MSSA and 6(7.69%) among MSCNS. So there is high prevalence of iMLS_B among MRSA than MSSA. Present study has showed 6(8.8%) strains of cMLS_B among MRSA, 5(7.35%) strains among MSSA and 1(1.47%) among MS CNS. There were no cMLS_B strains among MRCNS in present study. Mr Angel ⁽⁹⁾ et al., has reported 7(12%) strains cMLS_B among MRSA, 33(25%) strains among MSSA and 10(19%) strains among CNS isolates. Similarly shantala.G.B⁽¹²⁾ et al., has reported 32(25.39%) of cMLS_B among MRSA 10(9.61%) among MSSA. In our study MS phenotype among MRSA were 17.65% among MSSA were 36.77% and among MS CNS were 10.29%. This was in concordance with Shantala.G.B⁽¹²⁾ et al., who reported (15.07%) of MS phenotype among MRSA and 17(16.34%) among MSSA as shown in Table 2. Most of the studies have indicated higher prevalence of inducible resistance compared to constitutive resistance. True incidence depends upon population studied, geographical region and methicillin susceptibility. Present study has showed higher incidence of iMLS_B as compared to cMLS_B which was similar to findings of Shantala.G.B⁽¹²⁾ et al., AM Ciraj et al⁽¹⁴⁾.

There is restricted range of antibiotics available for the treatment of MRSA infections, i.e. Vancomycin, Clindamycin should be considered for management of MRSA that are sensitive to Clindamycin. So, true sensitivity to Clindamycin can be judged only by performing 'D' test for inducible resistance among ER-R isolates. The prevalence of inducible Clindamycin resistance varies from hospital to hospital. There is no study conducted for the detection of inducible CL-R in our locality. So from our study we conclude that there is fairly high percentage of inducible CL-R among Staphylococcal isolates. Hence implementation of D test, which is simple, inexpensive, reliable method as a routine antibiotic susceptibility testing will help us to differentiate between inducible & constitutive Clindamycin resistance. So by doing this simple test routinely we can prevent the Clindamycin treatment failure, which may occur with MRSA as well as MSSA infection.

CONCLUSION

Prevalence of inducible Clindamycin resistance among Staphylococcal isolates was significant, i.e., (17.64%) isolates of 68 total isolates showed inducible

Clindamycin resistance. So the isolates with iMLS_B phenotype would have been missed, if we would have not been performed D- test, resulting in Clindamycin therapeutic failure. Hence the implementation of this D-test routinely, which is simple, reliable & inexpensive will reveals the iMLS_B& cMLS_B phenotype & prevents the therapeutic failure of Clindamycin.

Hence, clinical microbiology laboratories should consider performing routine D testing and reporting for inducible Clindamycin resistance in Staphylococcal isolates to ensure that clinicians can rely on Clindamycin susceptibility test results. It is also recommended to avoid switch therapy from Erythromycin to Clindamycin in a patient resistant to erythromycin.

List of Figures with Captions:

1. Sample wise distribution of isolates.
2. Distribution of Staphylococcal isolates.
3. Shows inducible Clindamycin resistance. Note flattening or blunting of zone of inhibition around Clindamycin disc.

List of Abbreviations:

1. MRSA- Methicillin resistant Staphylococcus aureus.
2. MSSA- Methicillin sensitive Staphylococcus aureus.
3. MRCONS- Methicillin resistant Coagulase negative staphylococci.
4. MSCONS- Methicillin resistant Coagulase negative staphylococci.
5. MLS_B- Macrolide, Lincosamide, group B streptogramins.
6. ER-Erythromycin.
7. CL- Clindamycin.
8. iMLS_B- Inducible Clindamycin resistance.
9. cMLS_B-Constitutive Clindamycin resistance.

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Conflict of Interest: NIL.

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