



Inducible clindamycin resistance in *Staphylococcus aureus*: Reason for treatment failure

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Abstract:

Emergence of methicillin resistance in *Staphylococcus aureus* (*S.aureus*) has left us with very few therapeutic alternatives available to treat staphylococcal infection. The widespread use of macrolide-lincosamide-streptogramin B (MLS_B) antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics. This study was done to investigate the infections by hospital and community acquired “erythromycin- induced clindamycin resistant” strains or D-test positives of clinical isolates of *Staphylococcus aureus* (*S.aureus*) in a hospital. Three hundred isolates of *S.aureus* were subjected to routine antibiotic susceptibility testing including Cefoxitin (30µg) by modified disc diffusion method. Inducible resistance to clindamycin in *S.aureus* was tested by D-test as per CLSI guidelines. Among 300 *S.aureus* isolates, 114 (38%) were methicillin resistant *Staphylococcus aureus* (MRSA) and 186 (62%) methicillin susceptible *Staphylococcus aureus* (MSSA). Forty one (13.67%) isolates showed induced clindamycin resistance, 49(16.33%) showed constitutive resistance and 94 (31.33%) showed the MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA compared to MSSA (22.81%, 23.68% and 8.1%, 11.8% respectively). D-test should be included as a part of routine antibiotic susceptibility testing to detect induced clindamycin resistance to prevent treatment failure.

Key words: Erythromycin, Clindamycin, D-test, MRSA, MSSA

Introduction:

Emergence of methicillin resistance in *Staphylococcus aureus* (*S.aureus*) has left us with very few therapeutic alternatives available to treat staphylococcal infection. The Macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.¹

However, widespread use of (MLS_B) antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics.² The most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype). Strains with inducible resistance to clindamycin are difficult to

detect in routine laboratory as they appear erythromycin resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other. In such cases, *in vivo* therapy with clindamycin may select constitutive *erm* mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* as well as *in vitro* and strain does not typically become clindamycin resistant during therapy.³

The study aimed to find out the percentage of *S.aureus* having inducible clindamycin resistance (iMLS_B) in our hospital using D-test.^{1,4} Also, we tried to ascertain the relationship between methicillin- resistant *Staphylococcus aureus* (MRSA) and inducible clindamycin resistance.

Materials & Methods:

The prospective study was conducted for a period of one year from July 2013 to June 2014. We analyzed 300 non-duplicate consecutive isolates of *S.aureus* isolated from various clinical specimens like pus, wound swab, aspirates, blood, and sterile fluids. Age and sex of the patients were recorded. The isolates were first identified as *S.aureus* by standard conventional techniques.⁵ They were then subjected to susceptibility testing by Kirby Bauer's disc diffusion on Mueller Hinton agar plates using amikacin (30µg), ciprofloxacin (5µg), clindamycin (2µg), fusidic acid (10µg), cotrimoxazole (1µg), erythromycin (15µg), linezolid (30µg), tetracycline (10µg) and vancomycin (30µg). MRSA was detected using Oxacillin (1µg, Hi Media, Mumbai, India) further confirmed by using Cefoxitin disc (30µg, Hi Media, Mumbai, India) which is an accurate surrogate marker for *mecA* gene detection. The diameter of zone of inhibition was recorded as per CLSI guidelines.⁴

Those isolates which were erythromycin resistant were further subjected to D-test as per CLSI guidelines.⁴ Briefly, erythromycin (15µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2µg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37°C, flattening of zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance. (**Figure I**) Quality control (QC) of erythromycin and clindamycin discs was performed with *S.aureus* ATCC 25923 according to standard disc diffusion QC procedure. Additional QC was performed with separate in-house selected *S.aureus* strains that demonstrated positive and negative D-test. Ethical clearance for the study was obtained from the institution.

Three different phenotypes were appreciated after testing and then interpreted. This interpretation was done for erythromycin-resistant *S.aureus* strains. All the erythromycin sensitive isolates were excluded.

1. **MS phenotype:** Resistant to erythromycin (zone \leq 13mm)
Susceptible to clindamycin (zone \geq 21mm)
D-test negative
2. **Inducible MLS_B phenotype:**
Resistant to erythromycin (zone \leq 13mm)
Susceptible to clindamycin (zone \geq 21mm)
D-test positive (**Figure I**)
3. **Constitutive MLS_B phenotype:**
Resistant to erythromycin (zone \leq 13mm)
Resistant to clindamycin (zone \leq 21mm)

Results were tabulated and analysed statistically (software used was Epi-Info 5. Results were expressed in proportions. Statistical test used was Chi-Square test) as shown in **Table I**.

Results:

The age range of the study group was 19-60 years. Males were 177 and females 123. Out of 300 *S.aureus* isolates, 114 (38%) were MRSA and 186 (62%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Inducible resistance to clindamycin (erythromycin resistant, clindamycin sensitive and D-test positive, (iMLS_B) was observed in 26 (22.81%) of MRSA and 15 (8.1%) of MSSA isolates. The incidence of inducible MLS_B (iMLS_B) and constitutive MLS_B (cMLS_B) phenotypes was significantly higher in MRSA than in MSSA (Chi-square test, P less than 0.01). MLS_B resistant and

Table I: Distribution of isolates

Susceptibility pattern (phenotype)	MRSA No (%)	MSSA No (%)	Total No (%)	P value
ER-S, CL-S	36 (31.58)	80 (43.0)	116(38.67)	
ER-R, CL-R (cMLS _B)	27 (23.68)	22 (11.8)	49(16.33)	Chi square - 7.27 P value - 0.0070 Very significant
ER-R, CL-S, D test negative (MS)	25 (21.93)	69 (37.1)	94(31.33)	
ER-R, CL-S, D test positive (iMLS _B)	26 (22.81)	15 (8.1)	41(13.67)	Chi square -13.02 P value - 0.0003 Very significant
Total	114 (38)	186 (62)	300 (100)	

ER-S: Erythromycin susceptible; CL-S Clindamycin susceptible; ER-S: Erythromycin resistant; CL-S Clindamycin resistant; cMLS_B- Constitutive clindamycin resistant; MS- MS phenotype, iMLS_B- Inducible clindamycin resistant

sensitive phenotypes among *S.aureus* are shown in **Table I**.

Discussion:

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms. *S.aureus* is one of the important pathogens causing nosocomial and community-acquired infections. It facilitates disease by its propensity to develop multidrug resistance which complicates treatment, well exemplified by MRSA, leaving few therapeutic options.⁶ Clindamycin is a good substitute to treat both MRSA and MSSA infections. Its advantages are its low cost, availability of oral and parenteral forms, lack of need for renal adjustment, good tissue penetration, fewer side effects, ability to directly inhibit toxin production and the fact that it is not impeded by high bacterial burden.⁷ Furthermore, it is a useful option in the treatment of penicillin allergy patients.¹

However, resistance to clindamycin can develop in staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro as well as in vivo during clindamycin therapy.⁸ Reporting *S.aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand, negative for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.⁹

In our study, we found high percentage of *S.aureus* isolates [184 (61.33%)] resistant to erythromycin. Amongst them, 41 (22.3%) tested positive for inducible clindamycin resistance by D-test, which is compared with other studies in **Table II**. These findings suggest that if D-test had not been performed, nearly one-third of the erythromycin resistant isolates would have been misinterpreted as clindamycin sensitive resulting in therapeutic failure.

We also observed that inducible MRSA (22.81%) as compared to MSSA (8.1%) isolates which is in correlation with previous studies.^{8, 10, 11, 12} while Deotale V et al¹³ reported 27.6% in MRSA and 1.6% in MSSA. Some studies have shown a very high frequency of inducible resistance to MRSA.¹⁴ On the contrary, few studies have showed higher percentage of inducible resistance in MSSA as compared to MRSA as shown in **Table II**.^{15, 16}

Accurate susceptibility data are important for appropriate therapy decisions. The pattern of macrolide resistance in *S.aureus* varies in different regions. Depending upon this prescription rate will not be uniform in different regions. There is no substantial data regarding clindamycin prescription from India. It is kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending upon antibiotic susceptibility results. Further, by using clindamycin, vancomycin can be avoided.¹⁷ However,

clindamycin resistant was higher among A expression of inducible resistance to clindamycin could limit the effectiveness of this drug.¹⁸

The true susceptibility to clindamycin can only be judged after performing D-test on the erythromycin resistant isolate. The prevalence of inducible clindamycin resistance may vary from hospital to hospital. From our study, we can conclude that there is fairly high percentage of inducible clindamycin resistance amongst the staphylococcal isolates which shows erythromycin resistance.

Inducible clindamycin resistance cannot be detected by standard broth micro-dilution testing, automated susceptibility testing devices and standard disc diffusion test or E-test.¹⁹ The D-test identifies inducible clindamycin resistance, is simple, inexpensive, easy-to-perform, reproducible, and can be included as a part of routine antibiotic susceptibility testing.^{14, 20}

Table II: Various studies showing prevalence of iMLS_B in *S.aureus* isolates

Authors	MRSA (%)	MSSA (%)
Sathish JV et al. 2014 (present study)	22.81	8.1
Sexena S., et al. ²¹ 2014	28.9	12.6
Dhanalakshmi TA et al. ²² 2013	13.1	6
Samant SA et al. ¹⁵ 2013	36	50
Kumar S., et al. ¹⁰ 2012	22.6	11.8
Prabhu K et al. ¹¹ 2011	20	6.2
Pal N et al. ²³ 2010	43.6	6.93
Shenoy MS et al. ²⁴ 2010	15.65	-
Deotale V et al. ¹³ 2010	27.6	1.6
Gupta V et al. ¹⁷ 2009	20	17.33
Shrestha B et al. ²⁵ 2009	39.7	0
Vandana KE et al. ⁶ 2009	48.7	9.5
Ciraj AM et al. ²⁶ 2009	38.4	12.9
Ajantha GS et al. ¹⁴ 2008	74	45
Angel MR et al. ¹⁶ 2008	12	25
Rahabar M et al. ¹² 2007	22.6	4
Yilmaz G et al. ⁸ 2007	24.4	14.8
Gadepalli R et al. ² 2006	30	10

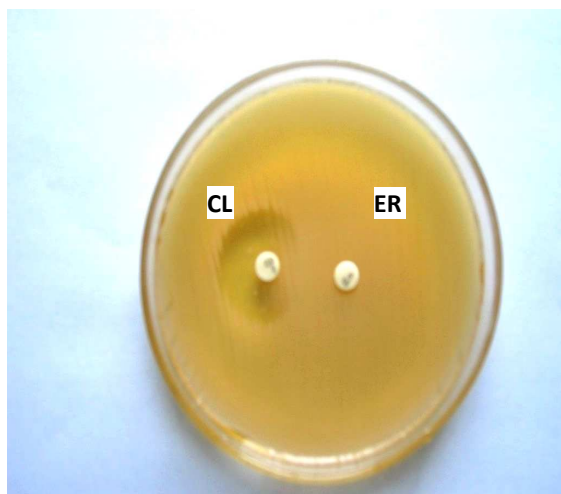


Figure I: D-test positive isolate showing flattening of clindamycin (CL) zone adjacent to erythromycin (ER) disc

In conclusion, increased rate of inducible clindamycin resistance (iMLS_B) among *staphylococcal* isolates indicates the importance of identification of such strains by D-test to avoid treatment failure when clindamycin is used. Thanks to the early detection of iMLS_B, such a measure will enable the clinician to save time. Consequently, treatment using CL can be omitted in patient with infections caused by inducibly resistant strains, and therapeutic failures may thus be avoided.

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