

Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolates in a Community Setting

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Abstract: *Background: The resistance to antimicrobial agents among Staphylococci is an increasing problem. This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat Staphylococcus aureus (S. aureus) infections. The resistance to macrolide can be mediated by msr A gene coding for efflux mechanism or via erm gene encoding for enzymes that confer inducible or constitutive resistance to MLS_B antibiotics. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to erm genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D test on a routine basis. Objective: This study was carried out to determine the prevalence of MLS_Bi resistance in both S. aureus isolates, including Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA). Results: Out of the 88 Staphylococcus aureus isolates, 18 (20%) were MRSA and 70 (80%) were MSSA. 41 (47%) isolates were erythromycin resistant. These erythromycin resistant isolates, when subjected to 'D' test, 17 isolates showed MS phenotype, 13 showed inducible MLS_B phenotype and 11 isolates showed constitutive MLS_B phenotype. Out of 18 MRSA isolates 08 (44%) showed Inducible MLS_B phenotype and 02 (11%) showed Constitutive MLS_B phenotype, while in 70 methicillin sensitive Staphylococcal isolates 05 (7%) showed Inducible MLS_B phenotype and 09 (13%) showed Constitutive MLS_B phenotype. The percentage of inducible resistance was higher amongst MRSA isolates as compared to MSSA isolates. Conclusions: Clindamycin is kept as a reserve drug and is usually advocated in severe MRSA infections depending upon the antimicrobial susceptibility results. This study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in Staphylococci for the optimum treatment of patients.*

Keywords: Constitutive clindamycin resistance, D test, Inducible clindamycin resistance, MRSA, Staphylococcus aureus

1. Introduction

Staphylococcus aureus is an important bacterial pathogen causing infection in both hospital and community settings. Macrolide, lincosamide and streptogramin B (MLS_B) antibiotics are commonly used in treatment of staphylococcal infections (Nikam et al, 2017; Lall a & Sahni, 2014). Extensive use of MLS_B antibiotics has led to rise in resistance to these antibiotics especially clindamycin, amongst staphylococcal strains (Ratna & Basudha, 2017; Lim et al, 2002; Lina et al, 1999; Drinkovic et al, 2001). Macrolides such as erythromycin, roxithromycin, clarithromycin and lincosamides such as clindamycin and lincomycin belong to different classes of antimicrobials. Although they are structurally different, their mode of action is similar. These acts through the same mechanism by inhibit bacterial protein synthesis by binding to 23S rRNA (Nikam et al, 2017; Lall a & Sahni, 2014; Steward et al, 2005). Clindamycin has been used for treating both methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) infections. Clindamycin has been considered as the favored agent due to its superb pharmacokinetic properties including good penetration and distribution in to the skin and other soft tissues and acceptable oral absorption with no dosage adjustment in renal disorders (Nikam et al, 2017; Lall a & Sahni, 2014; Patel et al, 2006; Delialioglu et al, 2005; Leclercq et al, 2002). Expression of inducible resistance to clindamycin could limit the effectiveness of this drug due to prolonged usage (Belbase et al, 2017; Ratna & Basudha, 2017; Lall a & Sahni, 2014; Delialioglu et al, 2005).

Macrolide resistances may be constitutive or inducible in the presence of a macrolide inducer (More et al, 2017; Lall a & Sahni, 2014; Leclercq et al, 2002). This mechanism can be constitutive, where methylase is always produced, or can be inducible, where methylase is produced only in presence of a macrolide inducer. Among MLS_B drugs only macrolides are good inducers of the enzyme erythromycin ribosome methylase (erm). Once induced, the gene product confers cross-resistance to other members of the group including lincosamides and streptogramin B (Nikam et al, 2017; Lall a & Sahni, 2014; Fiebelkorn et al, 2003). *S. aureus* isolates with constitutive resistance show resistance to erythromycin and clindamycin on in vitro testing, whereas isolates with inducible resistance show resistance to erythromycin but appear sensitive to clindamycin on disc diffusion testing. A double disc diffusion test (D test) for detecting inducible resistance to clindamycin in erythromycin-resistant isolates can be performed by placing a 15 µg erythromycin disc in proximity to a 2 µg clindamycin disc in adjacent positions (More et al, 2017; Lall a & Sahni, 2014; Kumar et al, 2010; Sasirekha et al, 2014). This test helps to distinguish staphylococci that have inducible resistance from those with constitutive resistance (Ratna & Basudha, 2017; Lyall et al, 2013; Prabhu et al, 2011). For erythromycin-resistant isolates, D test can help to determine whether clindamycin could be used as a therapeutic option (reported as susceptible when the D test is negative or reported as resistant when the D test is positive) (Nikam et al, 2017; Hiva et al, 2016). Data describing MLS_Bi prevalence or clinical predictors of the presence of macrolide-

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lincosamide-streptogramin B resistance (MLS_{Bi}) among community acquired methicillin resistant *S. aureus* (CA-MRSA) and hospital acquired methicillin resistant *S. aureus* (HA-MRSA) isolates are limited (More et al, 2017; Lall a & Sahni, 2014; Hiva et al, 2016). In this study, we aimed to determine the prevalence of MLS_{Bi} resistance in both *S. aureus* isolates, including MRSA and MSSA.

2. Materials and Methods

This study included 88 non duplicate isolates of *S. aureus* from households in a selected semi urban community in Ratmalana, Sri Lanka (Abeygoonawardena et al, 2015). Isolated microorganisms were identified by using conventional methods (colony morphology, Gram stain, catalase test, slide and tube coagulase test and DNase test). Methicillin resistance was detected using 30 µg cefoxitin disc on a swab inoculated Mueller Hinton agar plate supplemented with 2% NaCl and incubating at 35 °C for 24 h. Antimicrobial susceptibilities were studied by Kirby Bauer disc diffusion method as per guidelines from Clinical and Laboratory Standards Institute (CLSI). Interpretation of the diameters of zones of inhibition was as depicted in Table 1.

To detect inducible clindamycin resistance, 15 µg erythromycin and 2 µg clindamycin discs were placed on Mueller Hinton plate that had been inoculated with a staphylococcal isolate. The discs were placed at a distance of 15-20 mm edge to edge from each other. Plates were incubated overnight at 37 °C. *S. aureus* ATCC 25923 was used as control for these tests.

A positive D test was taken as flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (D shaped zone of inhibition) and was defined as inducible MLS_{Bi} resistance (Fig. 1). Strains that were resistant to both erythromycin and clindamycin were defined as exhibiting constitutive MLSB resistance, and those that were resistant to erythromycin and sensitive to clindamycin were the MS phenotype (Lall a & Sahni, 2014). The D test phenotype categories were recorded as noted in Table 2.

3. Statistical Analysis

Univariate analysis was carried out. Chi-square test was used for categorical variables and student's 't' test was carried out for quantitative variables.

Table 1: Interpretation of erythromycin and clindamycin zone sizes in *S. aureus*.^a

	Sensitive	Intermediate	Resistance
Erythromycin	≥23 mm	14-22 mm	≤ 13 mm
Clindamycin	≥21 mm	15-20 mm	≤ 14 mm

^aCLSI guidelines 2016

4. Results

During the study period, 88 *S. aureus* isolates were collected prospectively among 317 participants. Among these 18 (20%) were MRSA and 70 (80%) were MSSA. The difference in proportion was found to be statistically

significant between the MRSA and MSSA (p <0.001). The presence of MLS_{Bi} was confirmed by the D test. A blunted edge with otherwise clear zone of inhibition around clindamycin disc was seen in D test positive strains. The overall prevalence of MLS_{Bi} among all Staphylococcus isolates was 15% (Table 3). In MRSA 44% exhibited the MLS_{Bi}, 11% exhibited the constitutive phenotype while 11% strains exhibited the MS phenotype.

Amongst MSSA, 7% exhibited the MLS_{Bi}, 13% the constitutive resistance phenotype while 21% exhibited MS phenotype. When the results were compared statistically for presence of inducible resistance in MRSA and MSSA, there was a highly significant difference amongst MRSA showing much higher proportion of MLS_{Bi} than MSSA (44%, 7% respectively).

Table 2: D test phenotype categories and their characteristics

D test phenotype	Resistance phenotype	CLI result	ERY result	Double disc test description
D+	Inducible MLSB	S	R	Blunted, D shaped clear zone around CLI disc proximal to ERY disc
D-	MS	S	R	clear zone around CLI disc
R	Constitutive MLSB	R	R	Growth up to CLI and ERY discs
S	No resistance	S	S	Clear zones around discs

S = Sensitive, R = Resistant, CLI = Clindamycin, ERY = Erythromycin.

Table 3: MLSB resistance phenotype of *S. aureus*

	Total isolates	Constitutive MLSB resistance (ERY -R, CLIN-R)	Inducible MLSB resistance (ERY -R, CLIN-S, D+)	MS phenotype (ERY -R, CLIN-S, D-)
<i>S. aureus</i>	88	-	-	-
MSSA	70(80%)	09 (13%)	05(07%)	15(21%)
MRSA	18(20%)	02(11%)	08(44%)	02(11%)

ERY- Erythromycin; CLIN- Clindamycin

5. Discussion

In recent times, Clindamycin has become an excellent drug for some of the staphylococci infections, particularly skin and soft tissue infections because of good penetration into skin, soft tissues and it can be use as alternative for penicillin allergy patients (Nikam et al, 2017). Clindamycin has superior oral bioavailability and is useful for outpatient therapy or as oral agent. If necessary, can be followed after intravenous therapy. Clindamycin able to inhibit production of some toxins and virulence factors by staphylococci and is cost effective as well. Changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin (Ratna & Basudha,

2017). Empirical outpatient treatment options for staphylococcal infections have become more limited as concerns about the prevalence of MRSA have increased. However therapeutic failures caused by MLSBi resistant strains are now being reported commonly. Resistance to macrolide, lincosamide and streptogramin B (MLSB) antibiotics results from acquisition of *erm* gene. Expression of MLSB resistance can be constitutive or inducible.

Staphylococcal strains with MLSBi are resistant to inducer macrolides (eg. erythromycin) but susceptible to non inducer macrolides such as spiramycin and lincosamides (clindamycin) and streptogramin B (quinupristin). Inducible clindamycin resistance may not be detected if erythromycin and clindamycin discs are placed in nonadjacent positions (Belbase et al, 2017 & Sanjay et al, 2017).

We found a high prevalence of 44% of MLSBi amongst MRSA isolates. A study conducted in Delhi, India observed a prevalence of MLSBi as 37.5 % (Lall a & Sahni, 2014) and another study conducted in Maharashtra, India observed a prevalence of MLSBi as 30% (Archana et al, 2017). Another study done in tertiary care hospital in Maharashtra, India prevalence was observed as 25.7% (Nikam et al, 2017). Similar study done in Nepal with clinical isolates found the percentage as 24.5% (Ratna & Basudha, 2017). Different studies have found varying prevalence rates of inducible clindamycin resistance. When compared to other studies, our results are higher than those. The possible explanation given for this difference was that pooling of the data from multiple sites can obscure trends those may exist within individual communities or countries according to the differences in patient populations and due to clindamycin usage patterns.



Figure 01^a



Figure 02^b

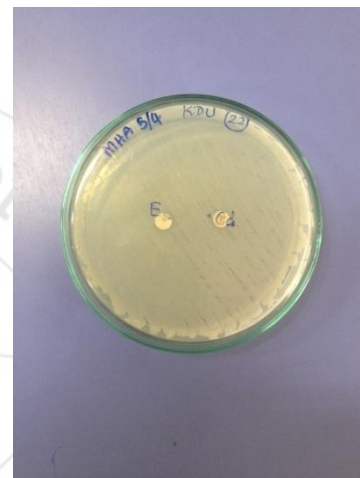


Figure 03^c

^aInducible MLSBi resistance

^bMS phenotype

^cConstitutive MLSB resistance

Staphylococci exhibiting inducible resistance to MLS antibiotics are now common in clinical practice. Only a few reports describing patients who received clindamycin for *S. aureus* infections with MLSBi are available, and some of these patients developed constitutive resistance during therapy (Christine et al, 2005). One should be cautious about using clindamycin in patients with major infections, especially where treatment is likely to be prolonged or infection difficult to eradicate as constitutive mutants can be selected during the course of clindamycin therapy in patients with MLSBi (Bernard & Veronika, 1969). Conversely labeling all erythromycin-resistant staphylococci as clindamycin resistant would prevent the use of clindamycin in infections caused by truly clindamycin susceptible isolates (Roland, 2002). Clindamycin may be useful for non-MLSBi infections esp. less severe *S. aureus* infections (Roland, 2002). This study reflects the prevalence of MLSBi at a community; however prevalence may differ from community to community based on geographical and socio-economical status. Microbiological laboratories should adopt testing for MLSBi among *S. aureus* isolates and report isolates exhibiting MLSBi as clindamycin resistant.

6. Conclusion

The current study, we can conclude that there is a high percentage of inducible clindamycin resistance amongst the staphylococcal isolates. If D-test would not have been performed, many inducible clindamycin resistant *S. aureus* could have been easily misidentified as clindamycin susceptible leading to therapeutic failure. Thus, simple and reliable D-test can be incorporated into routine Kirby–Bauer disk diffusion method in clinical microbiology laboratory. This will enable us in guiding the clinicians regarding judicious use of Clindamycin in skin and soft tissue infections as Clindamycin is not a suitable drug for D test positive isolates; while it can definitely prove to be a drug of choice in case of D test negative isolates. Routine and constitutively performing the D test in the diagnostic bench adds the early detection of its phenotypic resistance pattern that ultimately guide the clinician to avoid the treatment failure.

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