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Detection of Inducible Clindamycin Resistance with Erythromycin in Clinical Isolates and Its Prevalence among Methicillin Resistant *Staphylococcus aureus*

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A B S T R A C T

Clindamycin is a substitute choice for mild to moderate Staphylococcal infections especially in penicillin-allergic patients. *Staphylococcus aureus* is one of the most common pathogen with ability to cause wide range of infections in humans. Clindamycin has been used to treat serious infections for more than 30 years in children. It remains effective for many infections caused by community-acquired and hospital acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA/ HA- MRSA). The clinical manifestations of a positive D-test begin with a perception of cross resistance among three antibiotic families that share a common binding site— Macrolides (e.g., Erythromycin) Lincosamide (e.g.; Clindamycin), and group B Streptogrammins. A positive D-test indicates the presence of MLSBi genotype, though, sub inhibitory concentration of Erythromycin is a common inducer of Inducible Clindamycin resistance (ICR). When Erythromycin diffuses on a plate streaked with *Staphylococcus aureus*, it induces, resistance to Clindamycin and results in flattening of the Clindamycin zone of inhibition just next to the Erythromycin disk, making a D shape, so this method is called D- test. Susceptibility testing was performed according to CLSI (Clinical and laboratory standard Institute) on isolates of *Staphylococcus aureus* by using the method of disk diffusion. Almost one third of erythromycin resistant strains have expressed inducible clindamycin resistance. MRSA isolates has higher inducible clindamycin resistance than MSSA. Henceforth D test becomes a vital part in routine laboratory procedures for the detection of inducible resistance than leading to therapeutic failure.

Introduction

Staphylococcus aureus (*S. aureus*) causes varied range of infections in humans counting nosocomial and community-acquired infections in every part of the world. The increase incidence of MRSA is an emergent problem (Yilmaz *et al.*, 2007). Even though healthy children have a small risk for serious infections, the children might be common carriers of this organism. Numerous clinical infections may develop as well as spread from nasal carrier individuals.

Very few alternating antibiotics options were left behind for the treatment of methicillin resistance Staphylococcal infections. One of such effective alternative is macrolide-

lincosamide – streptogramin B (MLS_B) family of antibiotics. Of these, macrolide group (Erythromycin) and lincosamide class (Clindamycin), that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacteria as their mechanism of action against the organisms.

With excellent pharmacokinetic properties, Clindamycin has taken over as the most ideal antibiotic. (Delialioglu *et al.,* 2005) However, extensive use of MLS_B antibiotics has led the strains to acquire resistance to these antibiotics (Deotale *et al.,* 2010; Ajantha *et al.,* 2008).

Staphylococcus aureus offers resistance to these two distinct classes of antimicrobial agents through methylation of their ribosomal target site. This Target site modification mechanism of resistance is mediated by *erm* genes. The resistance can be expressed either constitutively (constitutive MLS B phenotype) or inducibly (inducible MLS B phenotype) (Steward *et al.,* 2005).

Strains with inducible clindamycin resistance, when not placed adjacent to each other become difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive. In such cases, clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. Another mechanism of resistance, efflux of antibiotic which is mediated through *msrA* genes, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* and *in vitro* and the strain does not typically become clindamycin resistant during therapy

This study is conducted to find the prevalence of Erythromycin resistant *Staphylococcus aureus* strains and its antibiotic susceptibility pattern with clindamycin by doing a feasible D test and also to elicit the relationship between MRSA and Erythromycin resistance.

Materials and Methods

The study was conducted from April 2017 to July 2017. A total of 180 *S. aureus* were isolated from various clinical specimens like pus, wound swab, aspirates, blood, and sterile fluids and tested. The isolates were first identified as *S. aureus* by conventional standard biochemical techniques (Milles and Amyes, 1999) and then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), and cefoxitin (30 µg) as per CLSI guidelines (Clinical and Laboratory Standards Institute, 2017). An inhibition zone of 19 mm or less around cefoxitin disc indicates MRSA.

'D test' to test Inducible resistance to clindamycin was done as per CLSI guidelines (Clinical and Laboratory Standards Institute, 2017). On previously inoculated on Mueller-Hinton agar plate with 1 McFarland standard bacterial suspensions, Erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg).

Following overnight incubation at 37°C, three different types of erythromycin resistance pattern was observed. They are as follows

MS phenotype - Isolates showing resistance to erythromycin (zone size ≤13 mm) while susceptible to clindamycin (zone size ≥21 mm) with regular, circular shape zone of inhibition around clindamycin belongs to this phenotype.

Inducible MLS_B ($iMLS_B$) phenotype - Isolates exhibiting resistance to erythromycin (zone size ≤13 mm) while being susceptible to clindamycin (zone size ≥21 mm) and with flattening towards erythromycin disc giving D-shaped zone of inhibition around clindamycin belongs to this phenotype.

Constitutive MLS_B phenotype - this phenotype was labeled for those *Staphylococcal* isolates, which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around clindamycin.

Results and Discussion

Total number of strains included in the study is 180. Out of this 180 isolates erythromycin resistance and clindamycin resistance were observed in 69 (38.3%) and 28 (15.5%) respectively. Among erythromycin resistant isolates, clindamycin was resistant in 9 (13%) isolates which accounts for the Constitutive MLS_B Phenotype. Total clindamycin susceptibility was observed in 152 isolates. Among clindamycin susceptible strains, erythromycin resistance was seen in 60 isolates.

D test positive – 22 (31.8%)
(Inducible MLS_B Phenotype)

D test negative – 38 (55%)
(MS Phenotype)

Wide range of infections are caused by *Staphylococcus aureus* in humans. The occurrence of invasive infections has been rising with emergence of Hospital Acquired (HA) and community acquired (CA) Methicillin Resistant *Staphylococcus aureus*. (MRSA). Clindamycin claims to be the one of the effective alternative antimicrobial agent for treating Methicillin resistant staphylococci infections mainly in soft tissue infections. Though the claim to be, it has to be investigated prior for its hidden inducible resistance along with erythromycin to prevent clinical failure of the treatment. On the alternative, negative inducible clindamycin resistance settles clindamycin susceptibility and provides a very good therapeutic option as it has a good oral bioavailability (Deotale *et al.,* 2010). In routine antimicrobial susceptibility test for all *S. aureus* D-test becomes a domineering part (Gupta *et al.,* 2009) because by using standard susceptibility test methods the $iMLS_B$ resistance mechanism is not predictable also its prevalence differs according to geographic location.

In our study, the erythromycin resistant were found in 69 (38.3%) isolates. Similar studies by (Prabhu Kavitha, 2017) and (Deotale *et al.,* 2010) reported erythromycin resistance to be 28.42% and 32.4% respectively, which actually quotes on the higher range.

Assessment of erythromycin resistance among MRSA and MSSA

S. No.	Erythromycin Pattern	MRSA(n=56)	MSSA (n=124)	TOTAL
1.	Constitutive MLS_B	6(10.7%)	3(2.4%)	9
2.	$iMLS_B$(D+)	16(28.5%)	6(4.8%)	22
3.	MS phenotype	27(48.2%)	11(8.8%)	38

Among erythromycin resistance isolates, around 32% were tested positive for inducible clindamycin resistance with a positive D test. D test negative were seen in 47 strains of which constitutive resistance and MS phenotype accounted for 38 (55%) and 9 (13%) respectively. Almost one third of erythromycin resistant strains have projected inducible clindamycin resistance and if this D test is not performed routinely, it would eventually lead to therapeutic failure. Similar findings were obtained in other studies too by (Ciraj *et al.,* 2009).

The assessment of Erythromycin susceptibility pattern among MRSA and

MSSA also showed that MRSA isolates has upper range of all three types of erythromycin resistance. Inducible MLS_B and MS phenotype in MRSA were 28.5% and 48.2%, respectively which are higher when compared to MSSA (4.8% and 8.8%). Similar study by (Yilmaz *et al.,* 2007) found inducible MLS_B resistance of 24.4% in MRSA and 14.8% in MSSA; (Rahabar and Hajia, 2007) reported 22.6% in MRSA and 4% in MSSA. On the conflicting side, Schreckenberger *et al.,* (2004) and Levin *et al.,* (2005) showed higher percentage of inducible resistance in MSSA as compared to MRSA.

In our study Constitutive MLS_B resistance was seen in 10.7% of MRSA isolates and 2.4% in MSSA, which is similar to other studies by Deotale *et al.,* (2010) (7.3% of MRSA isolates). Also contrary to this result, the only study that was reported by Angel *et al.,* from India (Angel *et al.,* 2008) which did not find it in any of the strains

Antibiotic options for the treatment of Methicillin resistance Staphylococcal infections are very limited. Of which Vancomycin would be reserved for life threatening infections. Hence, clindamycin should be considered for the controlling of serious soft tissue infections with MRSA that are truly sensitive to clindamycin. Though, the effectiveness of the drug is restricted by the expression of inducible clindamycin resistance.

So, clinical microbiology laboratories should perform the simple, supporting and reliable D test to report and to differentiate between inducible and constitutive clindamycin resistance routinely in all Staphylococcal isolates that are isolated from all samples received in the laboratories. Likewise D test enable us in guiding the clinicians regarding judicious use of clindamycin in skin and soft tissue infections.

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