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Original Article

Inducible Clindamycin Resistance (ICR) in *Staphylococcus Aureus* Among Various Clinical Samples

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Abstract

Introduction: Staphylococcus aureus is increasingly recognized as a cause of hospital associated (HA) and community associated (CA) infections. The Macrolide-lincosamide-StreptograminB (MLS_B), family of antibiotics serves as one such alternative, clindamycin being the preferred agent due to its excellent pharmacokinetic properties. However widespread use of clindamycin led to increase in resistance due to target site modification mediated by erm genes which can be expressed either constitutively or inducibely so use of D-test in a routine laboratory enables us to guide clinicians in judicious use of clindamycin. *Aims and Objective:* To study prevalence of inducible and constitutive clindamycin resistance among *Staphylococcus aureus* and to compare in between MRSA and MSSA isolates. *Material and Methods:* A total of 107 *Staphylococcus aureus* isolates were subjected to routine antibiotic susceptibility testing including cefoxitin (30mcg) by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by using D test, as per CLSI guidelines on erythromycin resistant isolates. *Results:* A total of 67 isolates were resistant to erythromycin. Among 67 isolates, 17(25.37%) showed inducible Clindamycin resistance, 27(40.2%) showed MS phenotype and Constitutive resistance was seen in 23(34.3%) isolates. Constitutive and inducible clindamycin resistance was found to be higher in MRSA as compared to MSSA. *Conclusion:* For efficient use of clindamycin, D-test should be used as a mandatory method in routine disc diffusion testing to detect Inducible clindamycin resistance.

Keywords: Constitutive; Inducible Clindamycin Resistance; MRSA; MSSA.

Introduction

Staphylococcus aureus is increasingly recognized as cause of hospital associated (HA) and community associated (CA) infections. Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives such as vancomycin and linezolid to treat such methicillin resistant Staphylococcus aureus (MRSA) infections [1]. But recently emergence of vancomycin resistance was reported in few studies. So there is strong need to use alternative antimicrobial agents to treat such infections keeping vancomycin as reservoir drug [2].

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 [3]. Macrolides such as erythromycin, roxithromycin,

clarithromycin and lincosamides (clindamycin and lincomycin) are most commonly used in treatment of Staphylococcal infections. However, widespread use led to increase in resistance to these antibiotics especially clindamycin. The most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively or inducibely [2]. Treatment of an infection using clindamycin, caused by a strain carrying inducible *erm* gene, can lead to clinical failure [4]. Detection of inducible clindamycin resistance, a disc approximation test can be performed by placing a 2mcg clindamycin disc, 15-26mm away from the edge of a 15mcg erythromycin disc on Mueller Hinton agar plate at 37^0C for 16-18 hrs [5].

So, the present study was conducted to know the prevalence of constitutive and inducible resistance pattern among methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistance Staphylococcus aureus (MRSA).

Aims and Objective

To study prevalence of inducible and constitutive clindamycin resistance among *Staphylococcus aureus* and to compare in between MRSA and MSSA isolates.

Materials and Methods

The present study was conducted in a tertiary care hospital from April to June 2015. A total of 107 isolates of *Staphylococcus aureus* isolated from various clinical specimens like pus, wound swab, aspirates, blood and body fluids. These isolates were identified as *Staphylococcus aureus* by using conventional methods [6]. Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method using various antimicrobial agents like Penicillin [10units], Cefoxitin[30mcg], Gentamycin [10mcg], Ciprofloxacin [5mcg], Erythromycin [15mcg] , Clindamycin [2mcg], Amoxicillin/ Clavulanicacid [20/10mcg], levofloxacin [5mcg], Netilmycin [30mcg], Linezolid [30mcg], Teicoplanin [30mcg] as per CLSI guidelines [7]. For detection of methicillin resistance, cefoxitin [30mcg] disc was placed and plates were incubated at 37^0C for 24 hrs. Isolates with zone diameters < 21mm were labelled as methicillin resistant. For detection of inducible clindamycin resistance, a disc approximation test was performed by placing a 2mcg clindamycin disc, 15-26 mm away from the edge of a 15mcg erythromycin disc on Mueller Hinton agar plate at 37^0C for 16-18 hrs [5].

Following overnight incubation at 37^0C, three different phenotypes were appreciated and interpreted as follow.

MS Phenotype

Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤ 13mm), while sensitive to clindamycin (zone size ≥ 21mm) and giving circular zone of inhibition around clindamycin (D test negative).

Inducible MLS_B phenotype

Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13mm) while being sensitive to clindamycin (zone size ≥ 21mm) and giving D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype (D test negative).

Constitutive MLS_B Phenotype

This phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤ 13mm) and Clindamycin (zone size ≤ 14mm) with circular shape of zone of inhibition if any around clindamycin.

Results and Observations

Among 107 isolates of *Staphylococcus aureus*, 67(62.61%) showed resistance to erythromycin. These isolates were subjected to D test which showed various phenotypes.

Among 67 isolates of *Staphylococcus aureus* resistant to erythromycin, 56(83.58%) were MRSA and 11(16.42%) were MSSA. Inducible and constitutive clindamycin resistance was 17(25.3%) and 23(34.33%) respectively (Table 1). Overall inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA isolates but it was found to be statistically insignificant. (Feisher's exact test)

All the strains were sensitive to vancomycin, linezolid and teicoplanin and resistant to penicillin. D-test positive isolates showed more resistance to antibiotic like Gentamycin,ciprofloxacin and Netilmycin as compared to D Test negative isolates (Table 2).

Table 1: MLS_B Resistant phenotype of *Staphylococcus aureus*

	MRSA	MSSA	Total
Constitutive MLS_B Resistance	21 (91.30 %)	2 (8.70%)	23 (100%)
Inducible MLS_B Resistance	16 (94.11%)	1 (5.89%)	17 (100%)
MS Phenotype	19 (70.37%)	8 (29.63%)	27 (100%)
Total	56 (83.58 %)	11 (16.42 %)	67 (100%)

MLS_B –macrolid-lincosamide-streptogramin B

Table 2: Percentage of antimicrobial resistance in D test positive & negative isolates

Antibiotics	D test –ve (n=27)	D test +ve (n=17)
Penicillin	27(100%)	17(100%)
Gentamycin	16(59.26%)	16(94.11%)
Ciprofloxacin	14(51.85%)	14(82.35%)
Amoxycillin/Clavulanic acid	19(70.37%)	16(94.11%)
Levofloxacin	6(22.22%)	7(41.18%)
Netilmycin	0 (0)	3(17.65%)

Discussion

In the era of increasing multidrug resistance it is necessary to determine the antimicrobial susceptibility of a clinical isolate so that appropriate treatment can be given to infected patients. Few therapeutic options are available for treatment of MRSA. Clindamycin is rapidly absorbed after oral ingestion and widely distributed in body fluids and blood (including bones), also used as an alternative for patients allergic to penicillin [3]. However some strains carrying *erm* gene give rise to inducible phenotype of Staphylococcal isolates and such isolates give rise to spontaneous constitutively resistant mutants in vivo during Clindamycin therapy leading to clinical failure [4]. So use of D-Test in a routine laboratory enables us in guiding clinicians for judicious use of clindamycin.

Among 107 *Staphylococcus aureus* isolates studied, 62.61% were erythromycin resistant, which is similar to lyall et al (51.5% [2]) and higher compared to other studies (28.4% [8],32.4% [9]), ICR was observed in 25.37% of isolates, which was higher compared to studies conducted by Ciraj AM et al [4] (13%) and prabhu K et al [8] (10%).while other studies reported higher prevalence as compared to our study (45% [9], 50% [10], 49% [11]) .

In our study, ICR (23.88%) was much higher in MRSA than in MSSA (1.49%) similarly study conducted by Mohamed Rahabar et al reported 22.6% in MRSA and 4% in MSSA [12], while the percentage was almost equal among MRSA and MSSA (33.2% and 34.6% respectively) in a study conducted by Lyall et al [2].

Similar to our study Lyall et al [2] reported that resistance to different antibiotics was more among D-Test positive isolates as compared to D-Test negative isolates.

Conclusion

To conclude, reporting of staphylococcal isolates as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate therapy while negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provide a very good treatment option.

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