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Inducible Clindamycin Resistance in Staphylococcus aureus isolated from pus samples in an Orthopaedic tertiary care centre.

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Introduction: Clindamycin is a commonly used antibiotic to treat skin and soft tissue infections caused by Staphylococcus aureus particularly Methicillin-Resistant Staphylococcus aureus (MRSA) infection. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D - test on a routine basis. **Materials and Methods:** 165 isolates of Staphylococcus aureus were subjected to routine antibiotic susceptibility testing including Oxacillin (1µg) and Cefoxitin (30µg) by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by D test as per CLSI guidelines on erythromycin resistant isolates. **Results:** 24 (14.5%) isolates showed inducible clindamycin resistance, 8 (4.84%) showed constitutive resistance while the remaining 59 (35.75%) showed MS phenotype. Inducible clindamycin resistance and MS phenotype were found higher in MRSA (21.42%, 40.47%) as compared to MSSA (7.40%, 30.86%). **Conclusion:** This study showed that the D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

Keywords: Inducible, Constitutive, MS phenotype, MRSA, MSSA

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Introduction

Staphylococcus aureus (S.aureus) is recognised as one of the important organism causing hospital-acquired and community-acquired skin and soft tissue infections in most parts of the world. The increasing prevalence of methicillin resistance among Staphylococci is an important concern [1]. The emergence of resistance to methicillin in Staphylococcus aureus has left with very few therapeutic options. The macrolide – lincosamide – streptogramin B (MLSB) family of antibiotic serves as one such alternative, with clindamycin being the preferred drug due to its excellent pharmacokinetic properties [2].

Clindamycin is considered a useful alternative in Penicillin allergic patients for the treatment of skin and soft tissue infections caused by S.aureus. It gets accumulated in abscesses and no renal dosage adjustment is required. It has excellent tissue penetration except into the central nervous system, where it does not cross the blood-brain barrier, even in the presence of inflamed meninges [3]. Good oral absorption makes it an attractive option for outpatient prescription or as a follow-up drug after intravenous therapy [4].

This helps in the early shift to outpatient management of susceptible infection without the need for continued intravenous access [5]. Widespread use of MLSB antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLSB antibiotics [2,6]. The most common mechanism for such resistance is target site modification mediated by erm genes which can be expressed either constitutively (constitutive MLSB phenotype) or inducibly (inducible MLSB phenotype).

Strains with inducible resistance to clindamycin are difficult to detect in the 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 the case of another mechanism of resistance mediated through MSR A genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive with both in vivo and in vitro and the strain does not typically become clindamycin resistant during therapy [7].

This study helps to demonstrate a very simple and useful method of detecting inducible resistance to clindamycin in erythromycin resistant Staphylococcal isolates i.e." D test" as described by Fiebelkorn *et al*.[8,9]. Also, we tried to establish the relationship between methicillin-resistant Staphylococcus aureus (MRSA) and inducible clindamycin resistance.

Materials and Methods

The study was conducted from January 2018 to January 2019. A total of 165 isolates of Staphylococcus isolated from pus, wound swab, aspirates were tested. The isolates were first identified as Staphylococcus aureus by standard biochemical techniques [10] and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Muller Hinton agar (MHA) plates using Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Oxacillin (1µg), Ciprofloxacin (5µg), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30µg) as per CLSI guidelines [9]. An inhibition zone of 10 mm or less around the Oxacillin disc and 19 mm or less around the Cefoxitin disc indicates MRSA.

Inducible Clindamycin resistance was tested by D test as per CLSI guidelines [9]. Briefly Erythromycin (15mg) disc was placed at a distance of 15 mm (edge to edge) from Clindamycin (2mg) disc on a Muller Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspension.

Following overnight incubation at 37ºC flattening of the zone (D shaped) around Clindamycin in the area between the two discs, indicated inducible clindamycin resistance[9]. [Fig 1]

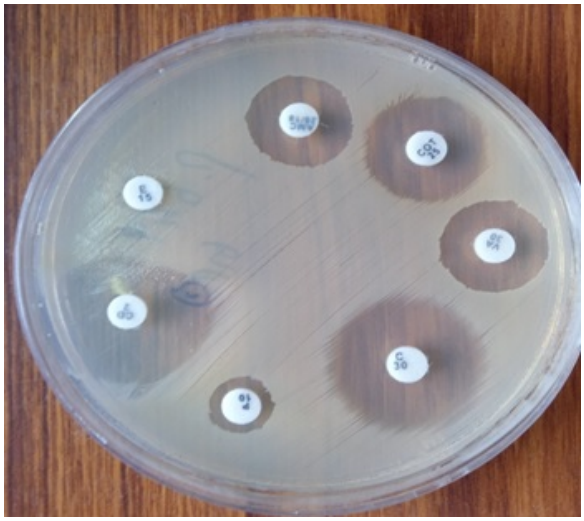

Fig 1. Showing D shaped zone around clindamycin when clindamycin and erythromycin are placed at a distance of 15mm indicating D test positive.

Three phenotypes were identified :

01. **MS phenotype:** Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤ 13mm) while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin was labelled as having MS phenotype.
02. **Inducible MLSB (iMLSB) phenotype:** Staphylococcal isolates sharing 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 was labelled as having iMLSB phenotype.
03. **Constitutive MLSB (cMLSB) phenotype:** This phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤ 13mm) and clindamycin (zone size ≤14 mm) with a circular shape of the zone of inhibition around the discs.

Results

Results were tabulated and analyzed statistically. One hundred and sixty-five Staphylococcus aureus isolates were tested for susceptibility to erythromycin and other antibiotics by routine disc diffusion testing.

Table-1: Susceptibility to Erythromycin and Clindamycin among Staphylococcus aureus isolates

S. no.	Susceptibility pattern (phenotype)	Number of isolates	Percentage
1	ERY – S CL – S	74	44.84% (74/165)
2	ERY – R CL – R (cMLSB)	08	4.84% (8/165)
3	ERY – R CL – S (D test positive iMLSB)	24	14.54% (24/165)
4	ERY – R CL – S (D test negative MS)	59	35.75% (59/165)
TOTAL		165	100%

ERY – Erythromycin, CL – Clindamycin, S – Sensitive, R – Resistant, cMLSB – constitutive MLSB phenotype, iMLSB – inducible MLSB phenotype, MS – MS phenotype.

Table–2: Association of Clindamycin resistance with Methicillin resistance.

Clindamycin resistance	Methicillin resistance (Cefoxitin)		
	MRSA n=84	MSSA n=81	Total 165
ERY – S, CL – S	27 (32.14%)	47 (58.02%)	74
ERY – R, CL – R (cMLSB)	05 (5.95%)	03 (3.70%)	08
ERY - R, CL – S (D test positive, iMLSB)	18 (21.42%)	06 (7.40%)	24
ERY – R, CL – S (D test negative, MS)	34 (40.47%)	25 (30.86%)	59

ERY – Erythromycin, CL – Clindamycin, S – Sensitive, R – Resistant, cMLSB – constitutive MLSB phenotype, iMLSB – inducible MLSB phenotype, MS – MS phenotype, MRSA – Methicillin-resistant Staphylococcus aureus, MSSA – Methicillin sensitive Staphylococcus aureus.

Discussion

Because of the increase in the resistance and emergence of multidrug-resistant organisms, accurate antimicrobial susceptibility data of an isolate is crucial for appropriate therapy decisions. Empirical outpatient treatment options for Staphylococcal infections have become more limited as concern about the prevalence of MRSA has increased [11,12]. Among the options available for MRSA and MSSA infections, clindamycin has evolved much interest, as it is a very good alternative because of its excellent pharmacokinetic properties [2,13].

However, resistance to clindamycin is highly variable, and the incidence of constitutive and inducible MLSB resistant phenotype varies by geographic regions and even between hospitals [8]. So 'D test' becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of Staphylococcus aureus [14].

In this study of 165 Staphylococcus aureus studied over 12 months [1 year]. Erythromycin resistance MS phenotype was seen is 59 (35.75%) of the isolates. Among the erythromycin resistant Staphylococcus aureus iMLSB resistance was observed in 24 (14.54%) isolates, constitutive MLSB resistance was observed in 8 (4.84%) of the isolates. These observations suggest that if the D test had not been performed, nearly 1/3rd of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

It was also observed that percentages of inducible resistance and MS phenotype were higher amongst MRSA (21.42% and 40.47%) as compared to MSSA (7.40% and 30.86%). This was in concordance with a few of the studies reported earlier. Deotale v et al. found inducible resistance of 27.6% in MRSA and 1.6% in MSSA and MS phenotype were high amongst MRSA (24.3%) as compared to MSSA (4%) [2]. Yilmaz et al [1] found inducible resistance of 24.4% in MRSA and 14.8% in MSSA, Gadepalli et al. [6] showed it to be 30% in MRSA and 10% in MSSA, While Mohamed Rahabar et al. [15] reported 22.6% in MRSA and 4% in MSSA.

Constitutive resistance in our study was seen in 5 (5.95%) of MRSA isolates and 3 (3.70%) in MSSA isolates. This was in concordance with Kavitha Prabhu et al. where the percentages of constitutive resistance among MRSA and MSSA were (16.66%) and 6.15% respectively [16]. Also this was in concordance with a few of the studies reported earlier [1]. Some studies have shown a very high frequency of inducible resistance among MRSA [11]. On the contrary few studies have shown a higher percentage of inducible resistance in MSSA as compared to MRSA [17,18].

Because of a restricted range of antibiotics for the treatment of MRSA infection and the known limitations of vancomycin, Clindamycin should be considered for the management of serious skin and soft tissue infection with MRSA that are sensitive to clindamycin [19]. The true sensitivity to clindamycin can only be judged after performing a D test on erythromycin resistant isolates, as the prevalence of inducible clindamycin resistance (Macrolide resistance) in Staphylococcus aureus varies in different regions and from hospital to hospital.

Conclusion

From our study we can conclude that there is a fairly high percentage of inducible clindamycin resistance amongst the Staphylococcal isolates which shows erythromycin resistance.

What does the study add to the existing knowledge?

So clinical Microbiology laboratories should report inducible clindamycin resistance in Staphylococcus aureus, and the D test can be used as a simple and reliable method to delineate inducible and constitutive clindamycin resistance in routine clinical laboratories.

These will also enable guiding the clinicians regarding judicious use of Clindamycin in the skin and soft tissue infections.

Author's contribution

Pratibha S: Manuscript preparation, **Praveen Kumar:** Concept, study design

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