ORIGINAL ARTICLE

Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolates Recovered in Specimen from Tertiary Care Hospital

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ABSTRACT

Objective: To find out the percentage of *Staphylococcus aureus* having inducible Clindamycin resistance in our hospital using D-Test and to know the relationship between Methicillin-resistant *Staphylococcus aureus* (MRSA) and inducible Clindamycin resistance.

Materials and methods: This was a descriptive cross-sectional study conducted on 93 *Staph aureus* isolates in a tertiary care hospital of Lahore during period of April 2012 to June 2012. Susceptibility to Penicillin (10µg), Cefoxitin (30µg), Erythromycin (15µg), Clindamycin (2µg), Linezolid (30µg), Ciprofloxacin (15µg), Gentamycin (10µg) Trimethoprim/Sulfmethoxazole (1.25/23.75µg) discs by Kirby Bauer disc diffusion method was determined as per NCCLS guideline. A disc containing Erythromycin (15 µg) was placed 15mm from centre to centre of a Clindamycin (2 µg) disc. Inducible resistance to Clindamycin is manifested by flattening or blunting of the Clindamycin zone of inhibition adjacent to the Erythromycin disc, giving a D-shape to the zone of inhibited growth. D-shaped Clindamycin susceptibility patterns where considered as D-test positive.

Results: Total 93 *Staph aureus* was isolated from different clinical samples, 42 (45%) were Methicillin-resistant *Staphylococcus aureus* (MRSA) and 51 (55%) were Methicillin-sensitive *Staphylococcus aureus* (MSSA). Maximum isolates of *Staph aureus* were recovered from pus. Out of 42 MRSA isolates, 5 (12%) were D-Test positive and out of 51 MSSA only 1 (2%) isolate was D-Test positive. Maximum resistance was observed with Penicillin (96%) followed by Trimethoprim/ sulfamethoxazole (91%). All isolates were sensitive to Linezolid (100%), sensitivity to Clindamycin and Erythromycin was 73% and 51% respectively. Out of total 93 *Staph aureus* isolates 43(46%) were sensitive to both Erythromycin and Clindamycin, 20(22%) isolates were resistant to both antibiotics and 6 (6%) isolates were resistant to Erythromycin and sensitive to Clindamycin (D-Test positive) 24 (26%) isolates were resistant to Erythromycin and sensitive to Clindamycin (D-Test negative). Out of 30 isolates which were resistant to Erythromycin and sensitive to Clindamycin, 6 isolates (20%) were D-Test positive and 24 isolates (80%) were D-Test negative.

Conclusion: All laboratories should routinely evaluate *Staph aureus* isolates that initially test as resistant to erythromycin and susceptible to clindamycin for inducible Clindamycin resistance using the "D- test." When inducible Clindamycin resistance is present, the isolate is presumed to be resistant, and use of an alternative agent should be considered.

Keywords: D-Test, Inducible Clindamycin resistance, Methicillin-resistant *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is an important agent in hospital and community-associated infections, causing high morbidity and mortality. The resistance to antimicrobial agents among Staphylococci is an increasing problem. Introduction of the new antimicrobial classes for this pathogen has been usually followed by the emergence of resistant strains through multiple mechanisms[1]. Clindamycin is a lincosamide antibiotic used in staphylococcal infections. It is being widely used by clinicians because it is available for parenteral and oral use, distributes well in tissues and is highly bacteriostatic against *Staphylococcus aureus*[2]. Macrolide resistance is the most widespread and clinically important mechanism of resistance encountered with Gram-positive organisms Clindamycin resistance may be constitutive or inducible[3]. 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 inducible (inducible MLS_B 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

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clinical therapeutic failure. The phenotypes are not differentiated by using standard susceptibility test methods, but can be distinguished by erythromycin-Clindamycin disc approximation test (D-test) and demonstration of resistance genes by molecular methods[4,5].

PATIENTS AND METHODS

This was a descriptive cross sectional study conducted in the Microbiology laboratory of PGMI Lahore during the period from 1st April 2012 to 30th June 2012. A total of 93 isolates of *Staphylococcus aureus* were recovered from different clinical specimens during this study period. S. aureus was identified using conventional methodology (colony morphology, gram staining, catalase, coagulase test and DNase test). Oxacillin resistance was detected by taking Cefoxitin as a surrogate marker. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method using Penicillin (10µg), Cefoxitin (30µg), Erythromycin (15µg), Clindamycin (2µg), Linezolid (30µg), Ciprofloxacin (15µg), Gentamycin (10µg) and Trimethoprim/Sulfmethoxazole (1.25/23.75 µg) discs and interpreted according to Clinical Laboratory Standard Institute (CLSI) break points. Erythromycin resistant isolates were examined for inducible Clindamycin resistance by using double disc approximation test (D-Test). A disc containing Erythromycin (15µg) was placed 15mm from centre to centre of a Clindamycin (2µg) disc. Inducible resistance to Clindamycin is manifested by flattening or blunting of the Clindamycin zone of inhibition adjacent to the erythromycin disc, giving a D-shape to the zone of inhibited growth. Interpretation of the diameters of zones of inhibition for D-Test was: Isolates showing circular zones of inhibition with diameter of <13mm for ER and >21mm for CL were interpreted as negative for inducible resistance (D-test negative). Isolates with same inhibitory diameters as above but a D-shaped zone around the CL, were interpreted as positive for inducible resistance (D-test positive)[6].

RESULTS

Total 93 isolates of *Staph aureus* were included. Out of 93 *Staph aureus*, 42 were MRSA and 51 were MSSA as shown in Figure 1.Out of 42 MRSA isolates, 5 were D-Test positive and out of 51 MSSA only one isolate was D-Test positive as shown in Figure 2. Maximum isolates of *Staph aureus* were recovered from pus. Erythromycin and Clindamycin disc were used for detection of inducible Clindamycin resistance by double disc diffusion method. Among pus isolates 46 were D-test negative and 2 isolates were D-Test positive (Table 1). *Staph aureus* recovered from blood specimen 20 were D-Test negative and 4 were D-Test positive. From other specimens like urine, CSF, sputum, fluids and HVS, *Staph aureus* isolated with D-Test negative were 3, 1, 1, 2 and 3 respectively. Sensitivity pattern of *Staph aureus* to different antibiotics is shown in Table 2. Penicillin (4.3%), Cefoxitin (54.8%), erythromycin (50.3%), Clindamycin (73.2%), Linezolid (100%), Ciprofloxacin (53.7%), Gentamycin (60.2%), Trimethoprim/Sulfmethoxazole (8.6%) were sensitive. Out of total 93 *Staph aureus* isolates 43(46.2%) were sensitive to both Erythromycin and Clindamycin, 20 (21.5%) isolates were resistant to both antibiotics and 6 (6.5%) isolates were resistant to Erythromycin and sensitive to Clindamycin (D-Test positive) 24 (25.8%) isolates were resistant to Erythromycin and sensitive to Clindamycin (D-Test negative) [Table 3]. Out of 30 isolates which were resistant to Erythromycin and sensitive to Clindamycin, 6 isolates (20%) were D-Test positive and 24 isolates (80%) were D-Test negative as shown in Figure 3. Inducible resistance to Clidamycin is manifested in Figure 4 by flattening of the Clindamycin zone of inhibition adjacent to the Erythromycin disc.

Fig. 1: Number of MRSA and MSSA isolated from clinical specimens (n=93)

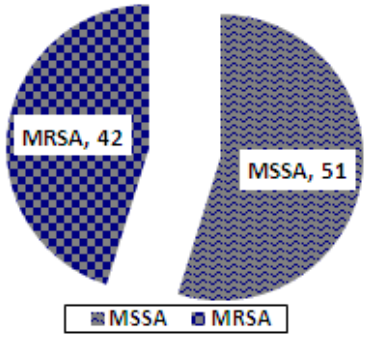


Fig. 2: Positive D-Test in MRSA & MSSA isolates (n=93)

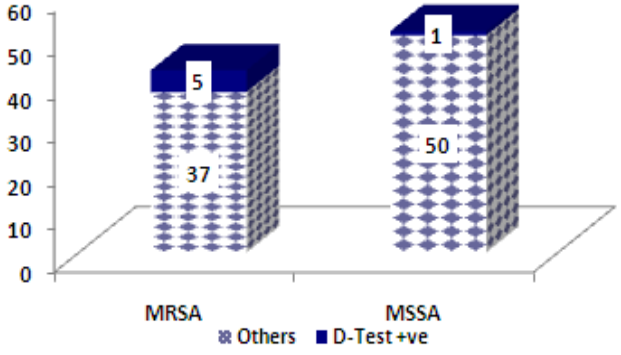


Table 1: Number of *Staph aureus* isolated from clinical specimens (n=93)

Specimen	*S. aureus* with D-Test negative	*S. aureus* with D-Test positive
Pus	46	2
Blood	22	4
Urine	3	-
CSF	2	-
Sputum	1	-
Fluid	2	-
HVS	3	-
Total	87	6

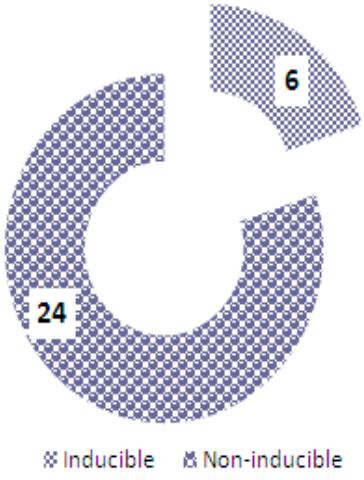
Table 2: Sensitivity pattern of *Staph aureus* to different Antibiotics (n=93)

Antibiotics	Sensitive	Resistant
Penicillin	4(4.3%)	89(95.7%)
Cefoxitin	51(54.8%)	42(45.2%)
Erythromycin	47(50.3%)	46(49.5%)
Clindamycin	68(73.2%)	25(26.9%)
Linezolid	93(100%)	-
Ciprofloxacine	50(53.7%)	43(46.2%)
Gentamycin	56(60.2%)	37(39.8%)
Trimethoprim/ sulfamethoxazole	8(8.6%)	85(91.4%)

Table 3: Susceptibility to Erythromycin and Clindamycin among all *S. aureus* isolates (n = 93)

Susceptibility pattern	No.	%
Erythromycin-sensitive Clindamycin-sensitive	43	46.2
Erythromycin-resistant Clindamycin- resistant	20	21.5
Erythromycin- resistant Clindamycin-sensitive (D-Test positive)	6	6.5
Erythromycin-resistant Clindamycin- sensitive (D-Test negative)	24	25.8

Fig. 3: Frequency distribution of inducible and non-inducible Clindamycin resistance (n=30)



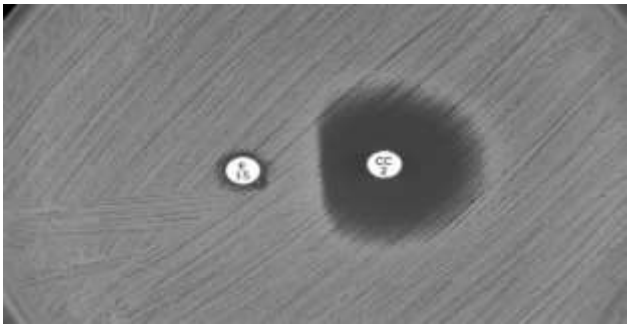


Fig. 4: Positive D-Test result or double disc diffusion assay

DISCUSSION

Increasing frequency of methicillin resistant *Staph aureus* infections and changing patterns of antimicrobial resistance in every region of the world have led to renewed interest in the use of alternative agents in this infection. Traditionally, Clindamycin has been used to combat both MRSA and MSSA because of its cost effectiveness and well tolerated orally. However major concern with regard to the use of this drug is the possible presence of constitutive or inducible resistance. In the present study out of 93 *Staph aureus* isolated from different clinical specimens, 42(45%) were MRSA and 51(55%) were MSSA as shown in Fig. 1. The increasing incidence of a variety of infections due to *Staph aureus* especially the expanding role of methicillin-resistant *Staph aureus* (MRSA) has led to emphasis on the need for safe and effective agents to treat both systemic and localized staphylococcal infections. Although Clindamycin is an appropriate antimicrobial agent for treatment of MRSA infections, however, many of erythromycin-resistant MRSA isolates have inducible Clindamycin resistance that may lead to treatment failures. In our study inducible Clindamycin resistance was 5(12%) out of 42 MRSA isolates and inducible Clindamycin was only one (2%) in MSSA isolates as shown in Fig. 2, these results shows that percentages of inducible resistance were higher amongst MRSA as compared to MSSA. Our study is comparable to study conducted by Seif et al[7] in Mashad, which shows 42% isolates were MRSA and 58% MSSA and inducible Clindamycin was 20% in MRSA and 7% in MSSA. Another study conducted by Fomda et al[8] in Srinagar shows 49% isolates were MRSA and 51% isolates were MSSA and inducible Clindamycin was 15% in MRSA and 11% in MSSA. Table 2 shows that maximum resistant was observed with Penicillin (96%) followed by Trimethoprim/ sulfamethoxazole (91%). All isolates were sensitive to Linezolid (100%), sensitivity to Clindamycin and Erythromycin was 73% and 51% respectively. The susceptibility pattern vary from region to region and different in different hospital setups depending upon

the prescription pattern. As shown in Fig. 3 total 30 isolates which were resistant to Erythromycin and sensitive to Clindamycin, among these isolates 6(20%) were inducible Clindamycin resistance (D-Test positive) and 24(80%) were non-inducible Clindamycin resistance (D-Test negative). Study conducted by Pal et al[9] and Deotale et al[10] in India revealed inducible Clindamycin resistance 23% and 15% respectively. These studies are comparable with study conducted by Yelmaz et al[11] in Turkey which shows 22% inducible Clindamycin resistance.

CONCLUSION

Clindamycin should keep as a reserve drug for severe MRSA infections depending upon the antimicrobial susceptibility results. Use of D test in a routine laboratory will enable us in guiding the clinicians regarding the correct use of Clindamycin in skin and soft tissue infections; when inducible Clindamycin resistance is present, the isolate is presumed to be resistant and use of alternative agent should be considered. While it can definitely prove to be a drug of choice in case of D test negative isolates. Decision about Clindamycin use for Staphylococci with the Erythromycin resistance/ Clindamycin sensitive phenotype should be made according to the local prevalence data.

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