

Research Article

Detection of inducible clindamycin resistance and susceptibilities to other antimicrobial agents in clinical isolates of *Staphylococcus aureus*

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ABSTRACT

Background: The resistance to antimicrobial agents among staphylococci is an increasing problem. Clindamycin is commonly used for the treatment of skin and soft tissue infections produced by *Staphylococcus aureus* and its widespread use has led to its resistance by different mechanisms & hence it is important to detect this. *In vitro*, routine tests 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: To find out the percentage of inducible clindamycin resistance in our hospital using D-test and their susceptibilities to other antimicrobial agents to guide therapy.

Methods: One hundred and two *S. aureus* isolates from various clinical samples were evaluated and methicillin resistance was determined using cefoxitin (30 mcg) disc and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines. Antibiotic susceptibility to other antimicrobial agents was done by Kirby Bauer's disc diffusion method.

Results: Nineteen (18%) isolates showed inducible clindamycin resistance, 12 (11%) showed constitutive resistance and 22 (21%) showed MS phenotype. All the three resistance patterns were higher in Methicillin Resistant *Staphylococcus aureus* (MRSA) as compared to Methicillin Sensitive *Staphylococcus aureus* (MSSA).

Conclusion: Our study showed, that D-test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance for optimum treatment of patients.

Keywords: D-test, Inducible clindamycin resistance, MRSA

INTRODUCTION

Staphylococcus aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections in every region of the world. The increasing prevalence of methicillin resistance among *Staphylococci* is an increasing problem.¹ This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.² However,

widespread use of MLS_B antibiotics has led to an increase in the number of staphylococcal strains acquiring resistance to MLS_B antibiotics.³

Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible.⁴ 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 inducibly (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 clinical therapeutic failure. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and clindamycin -sensitive both *in vivo* and *in vitro* (MS phenotype) and the strains do not typically become clindamycin resistant during therapy.²

The aim of this study was to determine the rate of inducible clindamycin resistance in both methicillin-resistant and susceptible strains of Staphylococci in our hospital and their susceptibility to other antibiotics.

METHODS

During the period of October 2012 to January 2014, 102 *S. aureus* isolates from various clinical samples like pus or wound swab, urine, sputum, aspirates, blood and body fluids from patients attending NRI General Hospital, Guntur were first identified by standard biochemical techniques⁵ and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method as per CLSI guidelines⁶ using antibiotics such as erythromycin (15 mcg), clindamycin (2 mcg), cefoxitin (30 mcg), ciprofloxacin (5 mcg), Trimethoprim-Sulphamethoxazole (TMP-SMX) (1.25/23.75 mcg), linezolid (30 mcg), vancomycin (30 mcg), gentamicin (10 mcg), cefepime (30 mcg), amikacin (30 mcg), sparfloxacin (5 mcg), teicoplanin (30 mcg) & ceftazidime (30 mcg).

Methicillin resistance was determined using cefoxitin (30 mcg) disc and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines (2011).⁶

The D-test was performed by placing the erythromycin (E- 15 mcg) and Clindamycin (CD- 2 mcg) discs side by side with edge to edge distance of 15 mm on Mueller-Hinton agar plate. Plates were analyzed after 18hours of incubation at 35°C. Flattening of zone around clindamycin in the area adjacent to the erythromycin (producing D shape) was looked for, which was designated D-test positive, indicating inducible clindamycin resistance.⁷

RESULTS

Different phenotypes were noticed. Induction phenotypes are the ones with D zone. These were further divided into D with a clear D- shaped zone around clindamycin disc (Figure 1) and D+ with colonies within the D-shaped zone (Figure 2).

Non-induction phenotypes were of four types: MS phenotype (erythromycin resistant and clindamycin sensitive without any D zone) (Figure 3); HD phenotype (hazy D zone), with two zones of growth around

clindamycin disc, one zone is a light, hazy growth up to clindamycin disc and the second zone is heavy growth & showing "D" (Figure 4); R phenotype, which is resistant to both clindamycin and erythromycin (Figure 5) and S phenotype, which is sensitive to both clindamycin and erythromycin.



Figure 1: Inducible MLS_B isolate - D phenotype, clindamycin therapy failure.



Figure 2: Inducible MLS_B isolate - D⁺ phenotype, clindamycin therapy failure.



Figure 3: MS phenotype - successful treatment with clindamycin.



Figure 4: HD phenotype - two zones of growth around clindamycin disc.



Figure 5: Constitutive MLS_B resistance.

Among 102 *S. aureus* isolates, MRSA were 66 (64.70%) and MSSA were 36 (35.29%). Fortynine (48%) showed sensitivity to both E and CD. Twelve (11%) were resistant to both E & CD indicating constitutive resistance (Table 1).

Forty one (40.19%) were susceptible to clindamycin and resistant to erythromycin. Out of these twenty-two were MS phenotype, two showed hazy D zone (HD phenotype), seven were D^+ phenotype and 10 were D phenotype (Table 2). The two isolates showing HD phenotype were MRSA, among the D^+ phenotypes five were MRSA and two were MSSA and among the D phenotypes eight were MRSA and two were MSSA.

Susceptibility of *iMLS_B* phenotypes isolated were Ciprofloxacin (33.33%), TMP-SMX (21.05%), linezolid (94.73%), vancomycin (94.73%), gentamicin (52.63%), cefepime (31.57%), amikacin (78.94), sparfloxacin (15.78%), teicoplanin (94.73%) and ceftazidime (5.26%).

Table 1: Distribution of various phenotypes.

(Phenotype) Susceptibility pattern	MRSA ^{††} (%)	MSSA ^{‡‡} (%)	Total (%)
ERY [*] -S [‡] , CL [†] -S	22 (33.33%)	27 (75%)	49 (48%)
ERY-R [§] , CL-R (cMLS _B)	11 (16.66%)	1 (2.77%)	12 (11%)
ERY-R, CL-S, D test positive (iMLS _B) ^{¶¶}	15 (22.72%)	4 (11.11%)	19 (18%)
ERY-R, CL-S, D test negative (MS) ^{**}	18 (27.27%)	4 (11.11%)	22(21%)
Total	66 (64.70%)	36 (35.29%)	102

ERY^{*} - Erythromycin; CL[†] - Clindamycin; S[‡] - Sensitive, R[§] - Resistant; cMLS_B^{||} - Constitutive resistance to clindamycin; iMLS_B^{¶¶} - Inducible resistance to clindamycin; MS^{**} - MS phenotype; MRSA^{††} - Methicillin-resistant *Staphylococcus aureus*; MSSA^{‡‡} - Methicillin sensitive *Staphylococcus aureus*

Table 2: Various phenotypes in erythromycin resistant and clindamycin sensitive isolates.

Phenotype	MRSA	MSSA ^{¶¶}	Total
MS [†] phenotype	18	4	22
HD [‡] phenotype	2	-	2
D^+ [§] phenotype	5	2	7
D [*] phenotype	8	2	10

D^{*} - D zone positive; MS[†] - Resistant to erythromycin and sensitive to clindamycin without D zone; HD[‡] - Hazy D zone with two zones of growth around clindamycin disc; D^+ [§] - Small colonies growing towards clindamycin disc inside D zone; MRSA^{||} - Methicillin-resistant *Staphylococcus aureus*; MSSA^{¶¶} - Methicillin-sensitive *Staphylococcus aureus*

DISCUSSION

In recent times, clindamycin has become an excellent drug for some *Staphylococcal* infections, particularly skin and soft tissue infections and as an alternative in penicillin- allergic patients.⁸ Also, clindamycin has good oral bio availability making it a good option for outpatient therapy and changeover after intravenous antibiotics.⁹ However, clindamycin resistance can develop in *Staphylococcal* isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both during *in vitro* testing and *in vivo* during clindamycin therapy.¹ Reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.² Since the *iMLS_B* resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an

imperative part of routine antimicrobial susceptibility test for all clinical isolates of *S. aureus*.¹⁰

In the present study, it was found that inducible clindamycin resistance is more in MRSA (22.72%) compared to MSSA (11.11%). This is in concordance with few studies reported in India. Deo tale et al.¹¹ found 27.6% i MLS_B in MRSA and 1.6% in MSSA. Gupta et al.⁴ showed it to be 20% in MRSA and 17.33% in MSSA. Constitutive resistance in our study was found to be 16.66% in MRSA and 2.77% in MSSA. Other studies done in India showed 16.66% in MRSA & 6.15% in MSSA by Prabhu et al.¹² and shantala et al.¹³ showed 25.39% in MRSA and 9.61% in MSSA which is similar to our study showing that cMLS_B is more in MRSA than MSSA. We found MS phenotype also more in MRSA (27.27%) compared to MSSA (11.11%), like that showed by Deo tale et al.,¹¹ 24.3% in MRSA & 4% in MSSA. These observations suggest that had D-test not been performed, nearly one-third of the erythromycin-resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

Highest susceptibility rates for i MLS_B isolates were seen for linezolid, vancomycin and teicoplanin (94.73%). 60.60% of our isolates of MRSA were sensitive to clindamycin, against which it would be safe & appropriate to use clindamycin or other macrolides. It correlates with previous studies who have reported 57% of susceptibility towards clindamycin among MRSA strains.¹⁴ However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug. So, clinical microbiology laboratories should report inducible clindamycin resistance in *S. aureus* and D-test can be used as a simple, auxiliary and reliable method to delineate inducible and constitutive clindamycin resistance in routine clinical laboratories.

CONCLUSION

Our observations suggest that, D-test should be mandatory for all microbiological laboratories before reporting clindamycin susceptibility 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. Therefore, regular surveillance of antimicrobial susceptibility pattern of MRSA, determination of phenotypic pattern of inducible clindamycin resistance and formulation of a definite antibiotic policy may be helpful in reducing the burden of MRSA infections & failures in clindamycin treatment in the hospital.

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