Original article

Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from wound infection in a Tertiary care hospital of North India

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ABSTRACT

Introduction: Wound infection is an important cause of morbidity and mortality of patients. *Staphylococcus aureus* (*S. aureus*) is the commonest Gram positive organism isolated from wounds. Both methicillin resistant and sensitive isolates of *S. aureus* are effectively treated by clindamycin. However, inducible clindamycin resistance may develop during therapy leading to treatment failure.

Objective: The present study was done to determine the prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from wound infection.

Materials & Methods: A hospital based cross-sectional study was done from November 2015 to October 2016. A total of 232 *S. aureus* isolates derived from wound infection were evaluated for antimicrobial susceptibility testing by Kirby Bauer disk diffusion method. Methicillin resistance was detected using cefoxitin (30 µg) disk and inducible clindamycin resistance was determined in all erythromycin resistant isolates by using D-zone test.

Results: Out of 232 *S. aureus* isolates, 85 (36.6%) were methicillin resistant (MRSA) and 147 (63.4%) were methicillin sensitive (MSSA). 128 (55.2%) were erythromycin resistant on which D-zone test was done. The prevalence of inducible clindamycin resistance was 11.2%, with percentage of isolates with constitutive resistance and MS phenotype (true clindamycin susceptible) to be 19.4% and 24.6% respectively. All the isolates showing inducible clindamycin resistance were 100% sensitive to linezolid and vancomycin.

Conclusion: Due to high prevalence of erythromycin resistance amongst *S. aureus* isolates, we suggest that D-zone test should be routinely done in all laboratories for appropriate prescription of clindamycin and thereby preventing emergence of inducible resistant strains and treatment failure.

Keywords: *Staphylococcus aureus*, inducible clindamycin resistance, wound infection

INTRODUCTION

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue.[1,2] Wounds can be accidental, pathological or post-operative. An infection of this breach in continuity constitutes wound infection. Wound infection is thus the presence of pus in a lesion as well as the general or local features of sepsis such as pyrexia, pain and induration. Infection is

believed to occur when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system.[3] Wound infection is important in the morbidity and mortality of patients irrespective of the cause of the wound. It is also important because it can delay healing and cause wound breakdown.[4] This is also associated with longer hospital stay and increased cost of healthcare. Wound infections are also significant in that they are the most common nosocomial infection.[5,6]

Staphylococcus aureus (*S. aureus*) is the most common Gram positive organism isolated from wound and sepsis.[7,8] Resistance to antibacterial agents in this organism has become an ever-increasing problem. The emergence and spread of methicillin resistance in *S. aureus* (MRSA) has further limited the therapeutic options.[9,10] This has led to renewed interest in the usage of alternative drugs such as Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat such infections, with clindamycin (a lincosamide) being the preferred agent due to its excellent pharmacokinetic properties and good penetration into various tissues even bones. It accumulates in abscesses, and no dosage adjustments are required in the presence of renal disease.[11,12] It is also used in the treatment of staphylococcal skin and soft tissue infections in patients allergic to penicillin.[13]

However, widespread use of MLS_B antibiotics has led to an increase in the number of staphylococcal strains acquiring resistance to these antibiotics due to production of enzyme methylases and efflux proteins.[14,15] The efflux pump encoded by *msr*A gene leads to resistance to the macrolides and the type B streptogramins, but spares lincosamides (clindamycin). These isolates are known as the MS phenotypes. The enzyme r-RNA methylase encoded by *erm* gene cause methylation of 23S rRNA of 50S subunit of the ribosome, thereby reducing the binding of MLS_B agents to the ribosome, hence, leading to resistance which is known as MLS_B resistance phenotype.[8,13] This resistance can be either constitutive ($cMLS_B$) or inducible ($iMLS_B$). As both MS phenotype and $iMLS_B$ phenotype (in the absence of inducer) show *in vitro* resistance to erythromycin and susceptibility to clindamycin, they are indistinguishable by using standard susceptibility test methods, including the Vitek system.[16,17]

Clinically, bacterial strains exhibiting $iMLS_B$ phenotype have a high rate of spontaneous mutation to constitutive resistance and use of non-inducer antibiotics such as clindamycin can lead to selection of constitutive mutants during treatment, ultimately leading to therapeutic failure.[18-21] As strains with MS phenotype show susceptibility to clindamycin, *in vitro* as well as *in vivo* (true clindamycin susceptible) and these isolates do not become resistant to it during therapy, clindamycin can safely be given to treat infections caused by organisms of this phenotype without any risk of clinical failure.[13] Therefore, it is important that clinical microbiologists should be able to differentiate these two mechanisms of resistance and thus help in guiding the clindamycin therapy effectively.[22]

Although *S. aureus* isolates with $iMLS_B$ phenotype cannot be identified by routine tests but it can be easily detected in the presence of an inducing agent. Erythromycin is an effective inducer of $iMLS_B$ phenotype, and this forms the basis of the erythromycin-clindamycin disk approximation test (D-zone test) which is recommended by Clinical and Laboratory Standards Institute (CLSI) for phenotypic detection of inducible clindamycin resistant isolates.[23] Since prevalence of inducible clindamycin resistance amongst staphylococci varies according to geographical location, therefore, the present study was undertaken to find

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out the prevalence of inducible clindamycin resistant *S. aureus* isolated from pus and wound infection in our geographic area by using simple phenotypic D-zone test.

MATERIALS AND METHODS

The hospital based cross-sectional study was done over a period of 1 year from November 2015 to October 2016, in the Department of Microbiology, Hind Institute of Medical Sciences, Mau, Ataria, Sitapur. The study was approved by Institutional Ethics Committee. A total of 232 consecutive, non duplicate strains of *S. aureus* isolated from post-operative wound infection and pus arising due to any other cause, collected from patients attending outpatients department and those admitted in wards (inpatients) were included in the study. Gram positive cocci other than *S. aureus*, Gram negative bacilli and yeast isolates were excluded from the study. The isolates were identified as *S. aureus* by conventional methodology (Gram staining, colony characteristics, catalase test, slide and tube coagulase test, mannitol fermentation test).[24] Antibiotic susceptibility testing was performed on Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) by Kirby Bauer disk diffusion method as per CLSI guidelines using antibiotic disks (HiMedia Laboratories, Mumbai, India) such as penicillin (10 units), gentamicin (10µg), tetracycline (30µg), linezolid (30µg),trimethoprim-sulfamethoxazole (1.25/23.75µg), cefoxitin (30µg), erythromycin (15µg), clindamycin (2µg) and ciprofloxacin (5µg). *Staphylococcus aureus* ATCC 25923 was used as standard quality control strain.[23]

Methicillin resistance amongst *S. aureus* was determined using cefoxitin (30µg) disk on Mueller-Hinton agar as per CLSI guidelines, and results were read after 18 hours of incubation at 35°C. The *S. aureus* isolates which showed zone size ≥ 22mm were considered methicillin sensitive (MSSA) and those with zone size ≤ 21mm were considered as methicillin-resistant *S. aureus* (MRSA).[23] Susceptibility of MRSA strains to vancomycin was tested by agar dilution method as per CLSI guidelines by inoculating 0.5 McFarland bacterial suspensions on Mueller-Hinton agar (MHA) plates by using sterile swabs. The plates were analyzed after 24 hours of incubation at 35°C. Minimal inhibitory concentration (MIC) of vancomycin of ≤ 2µg/mL for *S. aureus* was considered as susceptible to vancomycin.[23]

D-zone test (disk approximation test):

The isolates which were resistant to erythromycin were further tested by D-zone test which was performed as per CLSI guidelines by inoculating 0.5 McFarland bacterial suspensions on the Mueller-Hinton agar plates with the help of sterile swabs and placing the erythromycin (E-15µg) and clindamycin (CD-2µg) disks side by side with edge to edge distance of 15mm. Plates were analyzed after 18 hours of incubation at 35°C.[23]

Three different phenotypes of erythromycin resistant isolates were interpreted as follows:

1. The constitutive MLS_B phenotype ($cMLS_B$): The *S. aureus* isolates resistant to both E (zone size ≤ 13mm) and CD (zone size ≤ 14mm), with circular shape of zone of inhibition if any around clindamycin.
2. The MS phenotype: The *S. aureus* isolates which showed resistance to E (zone size ≤ 13mm) and a complete circular zone of inhibition around CD (zone size ≥ 21mm), indicated negative D-zone test.
3. The inducible MLS_B phenotype ($iMLS_B$): The *S. aureus* isolates which showed resistance to E (zone size ≤ 13mm) and susceptibility to CD (zone size ≥ 21mm) with flattening of zone of inhibition around clindamycin in the area adjacent to the erythromycin (D shaped zone), indicated positive D-zone test.

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Statistical Analysis

The collected data was statistically analyzed using SPSS Data Editor Software, Chicago, version 20. The statistical association between inducible clindamycin resistance phenotype and methicillin resistant *S. aureus* isolates were evaluated using Chi-square test and $p < 0.05$ was considered as statistically significant.

RESULTS

Among 232 *S. aureus* isolates included in our study, 184 (79.3%) were isolated from pus samples and 48 (20.7%) were isolated from post-operative wound infection, as shown in Table 1. Out of 232 *S. aureus* isolates, 85 (36.6%) were methicillin-resistant (MRSA) and 147 (63.4%) were methicillin-sensitive *S. aureus* (MSSA). Although, majority of the MRSA isolates were derived from pus samples (68.2%), however, the *S. aureus* isolates derived from post-operative wound infection were mostly MRSA (56.3%, 27/48). This finding was found to be statistically significant ($p = 0.002$). Out of 232 *S. aureus* isolates, majority were resistant to erythromycin (55.2%, 128/232) as shown in Figure 1.

A high percentage of erythromycin resistant *S. aureus* isolates (55.2%, 128/232) was detected of which 82.4% (70/85) were MRSA and 39.5% (58/147) were MSSA. All the erythromycin resistant isolates were subjected to D-zone test and the resulting distribution of *S. aureus* isolates was shown in Table 2. A total of 26 *S. aureus* isolates showed inducible clindamycin resistance by giving a positive D-zone test, hence, its prevalence was found to be 11.2% (26/232), with percentage distribution of $cMLS_B$ phenotype and MS phenotypes in all *S. aureus* isolates as 19.4% and 24.6% respectively. The susceptible phenotype (E-S and CD-S) predominated in MSSA (60.5%) as compared to MRSA (17.6%). Both the constitutive resistant ($cMLS_B$) and the inducible resistant ($iMLS_B$) phenotypes predominated in MRSA (41.2% and 22.4% respectively) as compared to MSSA (6.8% and 4.8% respectively), wheras, MS phenotype predominated in MSSA (27.9%) as compared to MRSA (18.8%). This finding was found to be highly significant ($p < 0.001$) statistically.

Table 3 shows the distribution of *S. aureus* isolates on the basis of the source of collected pus sample (inpatients or outpatients) and their susceptibility pattern to erythromycin and clindamycin disks. Out of 232 *S. aureus* isolates, 145 (62.5%) were derived from samples of outpatients and 87 (37.5%) were derived from samples of inpatients. The frequency of inducible clindamycin resistant isolates ($iMLS_B$ phenotypes) and MS phenotypes predominated in samples of outpatients (57.7% and 63.2% respectively) as compared to inpatients (42.3% and 36.8% respectively). This reveals that inducible clindamycin resistance is both community (outpatients) as well as hospital (inpatients) acquired in our geographic location. However, constitutive resistant isolates ($cMLS_B$ phenotypes) predominated in samples of inpatients (55.6%) as compared to samples of outpatients (44.4%). This finding was found to be statistically significant ($p = 0.020$).

The antimicrobial susceptibility test result of all the 26 *S. aureus* isolates with $iMLS_B$ phenotype revealed that they were 100% sensitive to vancomycin and linezolid, with moderate sensitivity (69.2%) to ciprofloxacin, and least sensitivity (15.4%) to tetracycline as shown in Table 4.

Table 1: Distribution of *Staphylococcus aureus* isolates on the basis of sample and susceptibility to cefoxitin (30 µg) disk.

Sample	*Staphylococcus aureus*			Chi-Square (χ^2) & *p value
	Resistant to cefoxitin (MRSA) N = 85 (36.6%)	**Susceptible to cefoxitin (MSSA) N = 147 (63.4%)**	**Total isolates N = 232 (100%)**	
Pus due to any other cause, N (%)	58 (68.2%)	126 (85.7%)	184 (79.3%)	$\chi^2 = 10.028$ df = 1 p = 0.002
Post-operative Wound infection, N (%)	27 (31.8)%	21 (14.3%)	48 (20.7%)	

N = Number of isolates. **MRSA** = Methicillin resistant *Staphylococcus aureus*; **MSSA** = Methicillin sensitive *Staphylococcus aureus.* *p value < 0.05 was considered as statistically significant.

Table 2: Distribution of *Staphylococcus aureus* isolates on the basis of their susceptibility to erythromycin and clindamycin disks placed adjacent to each other.

Antibiotic susceptibility pattern	*Staphylococcus aureus*			Chi-Square (χ^2) & *p value
	MRSA N = 85 (36.6%)	**MSSA N = 147 (63.4%)**	**Total isolates N = 232 (100%)**	
E-S, CD-S (Susceptible phenotype)	15 (17.6%)	89 (60.5%)	104 (44.8%)	$\chi^2 = 71.590$ df = 3 p < 0.001
E-R, CD-R ($cMLS_B$ phenotype)	35 (41.2)%	10 (6.8%)	45 (19.4%)	
E-R, CD-S (MS phenotype)	16 (18.8%)	41 (27.9%)	57 (24.6%)	
E-R, CD-S ($iMLS_B$ phenotype)	19 (22.4%)	7 (4.8%)	26 (11.2%)	

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N = Number of isolates. **MRSA** = Methicillin resistant *Staphylococcus aureus*; **MSSA** = Methicillin sensitive *Staphylococcus aureus*; **E** = Erythromycin (15 µg) disk; **CD =** Clindamycin (2 µg) disk; **S =** Sensitive; **R** = Resistant; **cMLS$_B$ phenotype =** isolates with constitutive resistance to clindamycin; **MS phenotype** = isolates with susceptibility to clindamycin (circular zone of inhibition) and negative D-zone test; **iMLS$_B$ phenotype** = isolates with inducible resistance to clindamycin and positive D-zone test. *p value < 0.05 was considered as statistically significant.

Table 3: Distribution of *Staphylococcus aureus* isolates on the basis of their antibiotic susceptibility pattern and the source of collected pus sample.

Susceptibility pattern	**Source of *Staphylococcus aureus* isolates**			**Chi-Square (χ^2) & *p value**
	Outpatient N = 145 (62.5%)	**Inpatient N = 87 (37.5%)**	**Total N = 232 (100%)**	
E-S, CD-S (Susceptible phenotype)	74 (71.2%)	30 (28.8%)	104 (100%)	$\chi^2 = 9.849$ df = 3 p = 0.020
E-R, CD-R (cMLS$_B$ phenotype)	20 (44.4)%	25 (55.6%)	45 (100%)	
E-R, CD-S (MS phenotype)	36 (63.2%)	21 (36.8%)	57 (100%)	
E-R, CD-S (iMLS$_B$ phenotype)	15 (57.7%)	11 (42.3%)	26 (100%)	

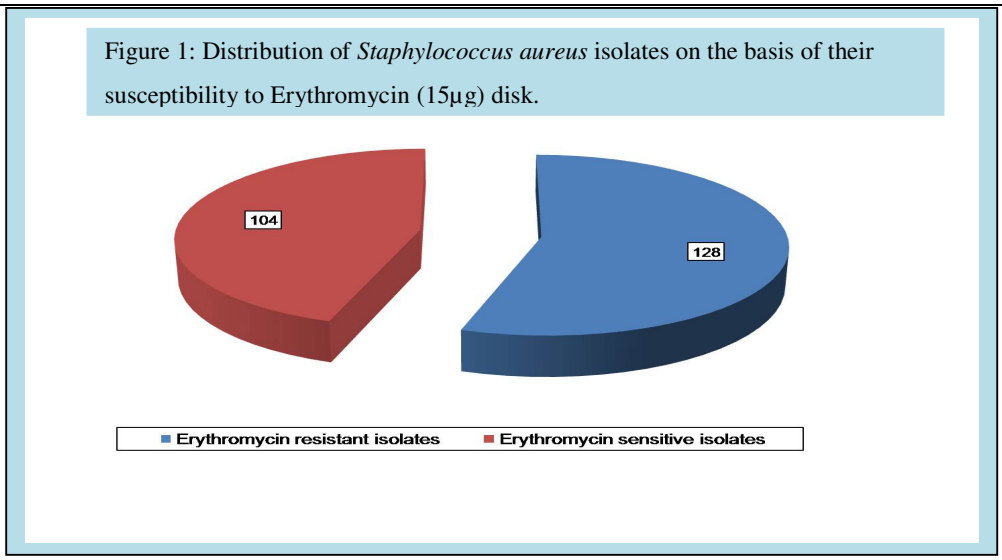
N = Number of isolates. **E** = Erythromycin (15 µg) disk; **CD =** Clindamycin (2 µg) disk; **S =** Sensitive; **R** = Resistant; **cMLS$_B$ phenotype =** isolates with constitutive resistance to clindamycin; **MS phenotype** = isolates with susceptibility to clindamycin (circular zone of inhibition) and negative D-zone test; **iMLS$_B$ phenotype** = isolates with inducible resistance to clindamycin and positive D-zone test. *p value < 0.05 was considered as statistically significant.

Table 4: Antibiotic susceptibility pattern of inducible clindamycin resistant *Staphylococcus aureus* isolates (iMLS$_B$ phenotypes) derived from pus and wound infection.

Antibiotic tested	iMLS$_B$ phenotypes N = 26 (100%)	
	Resistant N (%)	Sensitive N (%)
Penicillin	20 (76.9)	6 (23.1)
Gentamicin	12 (46.2)	14 (53.8)
Tetracycline	22 (84.6)	04 (15.4)
Linezolid	0 (0)	26 (100)
Vancomycin	0 (0)	26 (100)
Trimethoprim-sulfamethoxazole	17 (65.4)	9 (34.6)
Cefoxitin	19 (73.1)	07 (26.9)
Ciprofloxacin	8 (30.8)	18 (69.2)

N = Number of isolates.

Figure 1: Distribution of *Staphylococcus aureus* isolates on the basis of their susceptibility to Erythromycin (15µg) disk.



DISCUSSION

In the present scenario of increase in antibiotic resistance and emergence of multidrug resistant *S. aureus*, it is often crucial to determine antimicrobial susceptibility of all clinical isolates for optimal therapy of infected patients.[9] Due to limited range of antibiotics available for the treatment of methicillin-resistant staphylococcal infections and the known limitations of vancomycin, clindamycin should be considered for

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the management of serious soft tissue infections with MRSA that are sensitive to clindamycin.[25] However, clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen during clindamycin therapy.[26] Reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy and hence therapeutic failure. On the other hand negative result for inducible clindamycin resistance (MS phenotype) confirms true clindamycin susceptibility and as these isolates do not become resistant to it during therapy, clindamycin can act as a good therapeutic option in such cases.[13] The true sensitivity to clindamycin can only be judged after performing D-zone test on the erythromycin resistant *S. aureus* isolates.[9]

In our study we have included 232 *S. aureus* isolates derived from pus (79.3%) and post-operative wound infection (20.7%) from both outpatients and inpatients of orthopaedic department of our institution. The prevalence of methicillin resistance amongst all *S. aureus* isolates was found to be 36.6%. The prevalence of MRSA in other studies was found to vary from 12.9% to 49.8% with the geographical area under study.[8,9]

In the present study, the susceptible phenotypes (susceptible to both erythromycin and clindamycin) were found to predominate in MSSA (60.5%) as compared to MRSA (17.6%). A high percentage of erythromycin resistant *S. aureus* isolates (55.2%, 128/232) were detected of which 82.4% (70/85) were MRSA and 39.5% (58/147) were MSSA. All these were tested for D-zone test. Amongst them maximum isolates (44.5%, 57/128) were of MS phenotype (true sensitivity to clindamycin and D-zone test negative), followed by constitutive and inducible resistance phenotype. This suggests that majority of the erythromycin resistant *S. aureus* isolates can still be treated successfully with clindamycin. In our study the percentage of inducible clindamycin resistance ($iMLS_B$ phenotype, which gave positive D-zone test) amongst erythromycin resistant isolates was 20.3% (26/128). This is in agreement to studies from Chandigarh and Bangalore which reported inducible resistance to be 26.1% and 22.2% respectively among erythromycin resistant isolates.[21,27] While in two different studies from Karnataka, the $iMLS_B$ phenotype was seen to be quite high in 63% and 55.26% isolates respectively among the erythromycin resistant strains of *S. aureus*. [22,28]

In the present study it was found that the percentage of both constitutive resistance and inducible clindamycin resistance was higher amongst MRSA (41.2% and 22.4% respectively) as compared to MSSA (6.8% and 4.8% respectively), whereas, MS phenotype was found to predominate among MSSA (27.9%) as compared to MRSA (18.8%). This was in concordance with a study from Kolkata which showed higher inducible resistance and constitutive resistance in MRSA compared to MSSA (22.6%, 35.5%, and 11.8%, 11.8%, respectively), whereas, MS phenotype predominated in MSSA compared to MRSA (17.6% and 16.1% respectively).[29] A study from Chandigarh also showed higher inducible and constitutive resistance in MRSA isolates (20% and 46% respectively) as compared to MSSA isolates (17.3% and 10% respectively), whereas, MS phenotype was predominant among MSSA (37.3%) as compared to MRSA (16%).[21] Another study from Karnataka also showed higher constitutive and inducible resistance amongst MRSA (15.4% and 38.5% respectively) as compared to MSSA (0% and 12.9% respectively). However, they did not report any MS phenotype.[14] A study from Maharashtra also showed higher percentage of

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inducible resistance amongst MRSA as compared to MSSA (27.6% and 1.6% respectively). However, they also reported higher MS phenotype amongst MRSA (24.3%) than in MSSA (4%), and constitutive resistance of 7.3% in MRSA and none amongst MSSA.[9] On the other hand, few studies have shown higher percentage of inducible resistance in MSSA as compared to MRSA.[16,30]

In our study higher incidence of inducible clindamycin resistance was detected among isolates derived from outpatients (community acquired) as compared to inpatients or hospital acquired (57.7% and 42.3% respectively). This finding was similar to another study which also reported higher incidence of inducible clindamycin resistance from community (66.67%) than from hospital (33.33%).[21] This may be due to the fact that clindamycin being an oral drug has been increasingly prescribed by the physicians in outdoor clinical settings, thus leading to increased incidence of community-acquired inducible clindamycin resistance.

In our study we also looked forward for treatment options for inducible clindamycin resistant *S. aureus* isolates by detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with $iMLS_B$ phenotype were 100% susceptible to linezolid and vancomycin, followed by moderate susceptibility to ciprofloxacin (69.2%). This finding is in concordance to other studies that also found that all the $iMLS_B$ isolates were uniformly susceptible to linezolid and vancomycin.[21,27,31]

CONCLUSION

We conclude that clindamycin is an effective oral drug for both methicillin resistant as well as methicillin sensitive *S. aureus*, and is commonly used to treat staphylococcal skin and soft tissue infections. However, it is important for laboratories to be aware of the local prevalence of inducible clindamycin resistant isolates. A therapeutic decision is not possible without the relevant antibiotic susceptibility data. This is where the D-zone test becomes significant, as in the absence of D-zone test many erythromycin resistant *S. aureus* isolates would have been misidentified as clindamycin sensitive, but these isolates develop resistance to it during therapy resulting in clinical failure. On the other hand avoiding clindamycin therapy in every erythromycin resistant *S. aureus* isolates would be inappropriate. Therefore, as recommended by Clinical and Laboratory Standards Institute, D-zone test should be routinely performed in all laboratories and thus enabling the laboratory physicians to guide the clinicians regarding judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not a suitable drug for D-zone test positive isolates ($iMLS_B$ phenotypes), while it can definitely prove to be a drug of choice in case of D-zone test negative isolates (MS phenotypes).

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