

Inducible Clindamycin Resistance in Methicillin Resistant *Staphylococcus aureus*

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Abstract The resistance to antimicrobial agents among staphylococci is an increasing problem. This has led to renewed interest in the usage of macrolide-lincosamide-streptogramin B (MLS_B) antibiotics to treat *Staphylococcus aureus* infections. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance thus necessitating the need to detect such resistance by a simple D-test on routine basis. The objective of this study was to investigate *S. aureus* collected isolates for MLS_B phenotypes, in particular inducible clindamycin resistance (MLS_{Bi}). **Methods:** Four hundred and forty six *S. aureus* isolates from samples were evaluated and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines (2012). **Results:** Among 224 isolates of staphylococci studied, 101 (21.4%) were methicillin-resistant *S. aureus* (MRSA) and 123 (26.1%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Of the 224 staphylococcal isolates 62 (27.7%) were resistant to erythromycin of which 10 (4.46%) showed inducible clindamycin resistance and belonged to the MLS_{Bi} phenotype. Among the 10 MLS_{Bi} phenotype 7 (6.93%) were MRSA and 3 (2.44%) were MSSA. **Conclusion:** D-test should be included as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in staphylococci for the optimum treatment of patients.

Keywords: *staphylococcus, inducible clindamycin - resistance, D-test*

Cite This Article: Saleh.H. Baiu, and Nadia. E. Al-Abdli, "Inducible Clindamycin Resistance in Methicillin Resistant *Staphylococcus aureus*." *American Journal of Infectious Diseases and Microbiology*, vol. 4, no. 1 (2016): 25-27. doi: 10.12691/ajidm-4-1-5.

1. Introduction

Staphylococcus aureus is responsible for both nosocomial and community-acquired infections worldwide. [1,2] In humans *S. aureus* is able to cause a wide variety of different diseases, owing to its frequent association with multidrug resistance, *S. aureus* cause hard-to-treat infections because these are resistant to most of the antibiotics such as beta-lactams, aminoglycosides and macrolides. antibiotics are structurally unrelated; however, they are related microbiologically because of their similar mode of action. They inhibit bacterial protein synthesis by binding to 23s rRNA, which is a part of large ribosomal subunit. [3,4] For years, macrolides have been used as an alternative to penicillin and cephalosporins in the treatment of infections caused by gram positive bacteria, but the worldwide development of macrolide resistance has now limited the use of these antibiotics. Macrolide resistance is by diverse mechanisms. The resistance to macrolide can be mediated by *msr(A)* gene coding for efflux mechanism or via *erm* gene encoding for enzymes that confer inducible or constitutive resistance to MLS_B antibiotics. In constitutive resistance, r-RNA methylase is always produced (cMLS_B); where as in inducible, methylase is produced only in the presence of an inducing agent (iMLS_B). [5] Erythromycin is an effective inducer

whereas clindamycin is a weak inducer. In vitro, *S. aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin whereas those with inducible resistance are resistant to erythromycin and appear sensitive to clindamycin (iMLS_B). [3,6] Failure to identify inducible CL resistance leads to incorrect laboratory reports and treatment problems. [7,8]. The treatment of patients harboring iMLS_B staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure. [9,10] Thus, the aim of the present study was to detect the inducible CL resistance in staphylococci by the D-test.

2. Material and Methods

2.1. Collection of Samples and Isolation of Bacteria

This study was performed from April to August 2013 in ten hospitals of Benghazi, Libya. (Psychiatric hospital and Al-Erada sanatorium, Benghazi Medical Center, 7th of October hospital, Benghazi Childrens hospital, Al-Joumhouria hospital, Cardiac Center, Nephrology Center, Al-Jala Hospital, Urology and ENT Centers and Eye hospital). This study was done on a total of 472 HCWs. The samples were collected from the right and left anterior nares by using swabs.

2.2. Cultivation and Identification

Specimens were collected from the anterior nares with sterile dry cotton swabs (SPA Cultiplast, Melano-Italy), dipped in normal saline (0.9%). All swabs were inoculated on blood agar (BA-HiMedia, India) and subsequently on Mannitol salt agar plates (MSA-HiMedia, India) and were incubated at 37°C for 24-48 hours. Well isolated colonies were initially Gram-stained and then biochemical tests such as catalase, DNase and coagulase tests [11].

2.3. Methicillin-Resistance Test

Methicillin resistance was tested using Mueller- Hinton agar with Cefoxitin disc (30 µg) by Kirby-Bauer disc diffusion method [11]. Zone diameters were measured and recorded after a 24h incubation at 37°C. A zone size of >22 mm was considered sensitive and < 21 was considered resistant [12].

2.4. Antibiotic Susceptibility Testing

All the isolates were tested for their susceptibility to penicillin (10 µg), gentamicin (10 µg), Rifampicin (5 µg), cotrimoxazole (25 µg), erythromycin (15 µg), ciprofloxacin (5 µg), amoxicillin and clavulanate (20 µg), vancomycin (30 µg), Fusidic acid (10µg) and oxacillin (1 µg) by Kirby Bauer disc diffusion method using criteria of CLSI (2012), and was performed on Mueller-Hinton agar. Single isolated colonies were selected and inoculated in Mueller-Hinton broth and placed in incubator for 24 hours at 37°C. When its turbidity is comparable to 0.5 McFarland turbidity standards, the plates were inoculated with each broth culture and left to dry before the application of antibiotic discs. The plates were inverted and incubated at 35-37°C for 18-24 hours.

2.5. Detection of Inducible Clindamycin Resistance Phenotypic Inducible Resistance to Clindamycin by D- test.

Isolates were plated on a Muller Hinton agar plate at a McFarland concentration of 0.5 to eventually cover the agar surface. Clindamycin and Erythromycin disks, containing µg and 15µg each respectively were placed in the center of the plate separated by a distance of 15 cm between the edges. Plates were incubated at 37° C for 24 hr. Inducible resistance to Clindamycin was defined as blunting of the clear circular area of no growth around the Clindamycin disk on the side adjacent to the Erythromycin disk and was designated D - test positive. Absence of a blunted zone of inhibition was designated D - test negative. Three different phenotypes were interpreted as follows [13,14].

1. MS phenotype: isolates showing circular zone of inhibition around Clindamycin (Zone size > 21mm) and resistance to Erythromycin (Zone size <13 mm) was labeled as MS phenotype.

2. Inducible MLSB phenotype: Staphylococcal isolates showing resistance to Erythromycin (zone size <13 mm) and sensitive to Clindamycin (Zone size >21mm) giving D - shaped zone of inhibition around Clindamycin disc were labeled as Inducible MLSB phenotype.

3. Constitutive MLSB phenotype: Staphylococcal isolates showed resistance to both Erythromycin (Zone

size <13 mm) and Clindamycin (Zone size < 14mm) with circular shape of zone of inhibition if any around Clindamycin.

3. Results

Among Four hundred and seventy two *S.aureus* isolated from HCWs in Benghazi hospitals were 101 (21.4%) MRSA and 123 (26.1%) MSSA. Panel of antibiotics were tested by routine disc diffusion method including erythromycin and clindamycin discs quite apart from each other. Out of Two hundred and twenty four tested, 62 (27.7%) were resistant to erythromycin and (7.5%) were resistant to clindamycin, D- test was performed for these isolates and observed that 16 (7.14%) isolates were resistant to clindamycin indicating the percentage of constitutive MLSBc phenotype. (Figure 1). It is further observed that high percentage of both inducible MLSB and constitutive MLSB among MRSA isolates (6.93%, 8.91%) when compared to (2.44%, 5.69%) MSSA. MS phenotypes were identified more among MSSA (3.25%) when compared to MRSA (1.9%) (Table 1).

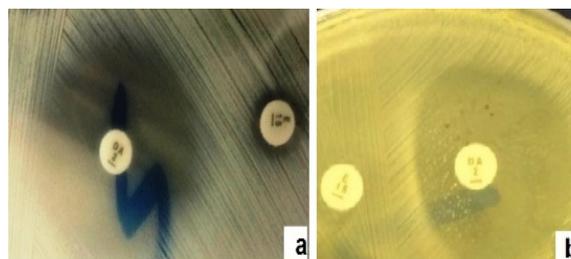


Figure 1. This figure shows Phenotypic inducible resistance to clindamycin by D- test. a) MS phenotype. b) MLSB phenotype

Table 1. D-test result among the erythromycin resistant *S. aureus*

(Phenotype)	MSSA (%)	MRSA (%)	Total
MLSBC	7 (5.69%)	9 (8.91%)	16 (7.14%)
MLSBI	3 (2.44%)	7 (6.93%)	10 (4.46%)
MS	4 (3.25%)	2(1.9%)	6 (2.68%)

4. Discussion

The prime step before initiating the antimicrobial therapy of infected individuals is performing the antimicrobial susceptibility testing for clinical isolates to avoid indiscriminate usage of antibiotics. Commonest antibiotic being preferred while treatment of these staphylococcal infections in case of failure to beta-lactam antibiotics is clindamycin. [15] Reporting *S. aureus* strains as susceptible to clindamycin without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy. Considering the high prevalence of clindamycin resistance among the clinical isolates, we feel that the laboratories should routinely test *S. aureus* strains for inducible MLSB. As the D-test is simple, inexpensive and easy to perform, it can be included as a part of routine antibiotic susceptibility testing. The benefit of routine D-testing is that we can clearly identify those strains that remain susceptible to clindamycin despite macrolide resistance. Among them

inducible clindamycin resistance (D-test positive). In our study we found high prevalence of erythromycin resistance isolates. Among these isolates tested positive for inducible clindamycin resistance by D-test. It was found that inducible clindamycin resistance is more in MRSA compared to MSSA. This was in concordance with a few of the studies reported before [16,17,18]. We found MS phenotype is more in MSSA than MRSA. This is similar to the studies done by [9]. The other studies done in Tripoli, Libya showed cMLSB is more in MRSA than MSSA which is similar to our study. In our study, we have described simple, reliable methods to detect inducible resistance to clindamycin in erythromycin resistant isolates of *S. aureus*.

5. Conclusion

This study highlights the crucial role of antibiotic susceptibility testing. As clindamycin is one of the most commonly used antibiotics for MRSA isolates, this study shows high prevalence of inducible clindamycin resistance among MRSA isolates. The increasing clindamycin resistance in the form of iMLSB and cMLSB limits the therapeutic options for MRSA to the antibiotics like linezolid and vancomycin. The inducible clindamycin resistance can be easily missed by routine *in vitro* susceptibility tests, when the erythromycin and the clindamycin discs are placed in non adjacent positions. In view of the therapeutic implications, the D test is a simple, reliable and inexpensive test to perform along with routine susceptibility testing which delineates the inducible (iMLSB) and the constitutive (cMLSB) resistance. Use of D-test in a routine laboratory will enable us in guiding the clinicians regarding the judicious use of clindamycin. Therefore, it should be used as a routine test in all microbiology laboratories.

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