Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus*

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**Background:** *Staphylococcus aureus* is one of the most important agents causing nosocomial infections. Inducible clindamycin resistance is an important concern, because common laboratory tests could not detect it.

**Materials and Methods:** A total of 209 clinical *S. aureus* isolates were collected and identified by conventional phenotypic tests. Antibiotic susceptibility pattern was detected by disc diffusion method. D-test was done using clindamycin (2 μg) and erythromycin (15 μg) discs according to the protocols of Clinical and Laboratory Standards Institute (CLSI). To detect methicillin resistant *Staphylococcus aureus* (MRSA), oxacillin disc was used and the results were confirmed by detection of *mecA* gene.

**Results:** Of all 209 clinical *S. aureus* isolates, 207 (99%) were resistant to amoxicillin. All isolates were susceptible to vancomycin and linezolid. The rate of clindamycin inducible resistance was 4% (n = 8). This phenotype was not observed in MRSA strains. There was no significant difference between methicillin resistant and susceptible strains. Resistance to clindamycin and erythromycin was higher in MRSA strains. D⁷ phenotype was detected in 1 (1%) of all isolates. Methicillin resistance was detected in 66 (32%) isolates by oxacillin disc and *mecA* gene was detected by PCR.

**Conclusions:** In our study, inducible clindamycin resistance rate was 4%; so it is necessary to conduct D-test regularly by disc diffusion for this bacterium. Resistance to erythromycin, clindamycin, ciprofloxacin and gentamicin was significantly higher in MRSA isolates than methicillin susceptible *Staphylococcus aureus* (MSSA), although the methicillin resistance prevalence was low.

**Keywords:** *Staphylococcus aureus*; Methicillin-Resistant *Staphylococcus aureus*; D-test; Inducible Clindamycin Resistance

1. Background

*Staphylococcus aureus* is one of the most important nosocomial pathogens colonizing on surface and epithelial tissue (1-3). Methicillin resistant *Staphylococcus aureus* (MRSA) produces penicillin binding protein 2a (PBP-2a), the enzyme causing resistance against semi-synthetic beta lactam antibiotics), which is encoded by the chromosomal gene, *mecA*. MRSA isolates are found on the body surface of about 2% of people and cause more systemic and potentially lethal infections in comparison to the methicillin susceptible (MSSA) clinical isolates. The MRSA prevalence is about 45-55%, according to the previous reports from Iran (4-7), although results may be lower based on the studied area or other related reasons (8, 9). MRSA strains may infect people in hospital (HA-MRSA) or the community (CA-MRSA). HA-MRSA isolates have a wide antibiotic resistance (10, 11). Clindamycin (a lincosamide) and erythromycin (a macrolide) have efficient antibacterial activities against *S. aureus* strains. Clindamycin is the preferred agent due to its excellent pharmacokinetic properties and good penetration into various tissues (12). Resistance to these antimicrobial agents varies among the countries (7, 12-25), which is related to the differences in the use of antibiotics and regional factors. Clindamycin is an efficient antibiotic for MRSA infections that is used as an alternative of vancomycin (14). Inducible resistance to clindamycin is important because the common laboratory tests could not recognize it, and there is a difference between laboratory results and clinical treatment, unless doing D-test. Macrolide resistance is a great concern, and clinically important mechanism in *S. aureus* and other gram positive bacteria. Clindamycin resistance can be constitutive or inducible. Clindamycin and erythromycin inhibit bacterial growth by preventing the protein synthesis of 50s ribosomal subunit. Moreover, it is used in children pneumonia (due to MRSA) and can inhibit the production of Staphylococcal virulence factors.
Resistance to these antibiotics occurs by methylation of ribosomal target, causing by erm gene encoded proteins. Another mechanism is through the enzymatic degradation of lincosamide caused by proteins encoded by 

3. Materials and Methods

Clinical isolates collected from September 2011 to January 2012 (6-month-period); 209 S. aureus clinical isolates were collected from patients (57% male and 43% female) admitted to Loghman Hospital. Samples were taken from tra- chea, blood, skin lesions, tissue cultures and other origins. The identification tests such as mannitol fermentation on MSA mediume, DNase and tube coagulase and colony morphology were done. For antibiotic susceptibility test, antibiot- ic discs including vancomycin (30 µg), linezolid (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), amoxicillin (10 µg), oxacillin (1 µg), cotrimoxazole (25 µg), gentamicin (10 µg) and ciprofloxacin (5 µg) were used (provided from MAST, UK). After 18-24 hours the results were observed and recorded. S. aureus ATCC25923 was used as control.

Clindamycin inducible resistance (D-test) was carried out using locating clindamycin and erythromycin discs (10, 16, 19). Antibiogram test was concluded after preparing a 0.5 Mc Farland turbidity of each isolate in sterile saline serum and culturing on Mueller Hinton Agar using the Clinical and Laboratory Standards Institute (CLSI) recommendations. In D-test, induced resistance was observed in isolates resistant to erythromycin (inhibition zone of 14 mm ≥) and susceptible to clindamycin (inhibition zone of 21 mm ≤) by a D shaped zone, when using the both antibiotics (IMSLB, Figure 1). In the laboratory, the phenomenon can be detected by D-test on Mueller Hinton Agar (MHA) and placing these two antibiotics close to each other (in a 14-22 mm distance) (17).

The following phenotypes were observed during D-test process:
R: resistant to both (erythromycin and clindamycin), S: sensitive to both mentioned antibiotics, D: induced resistant to clindamycin, D positive (D+): growth of a few colonies in the D-shaped sensitivity zone.

3.1. Primer Assay

Primer sequences were as follow: F: GTG AAG ATA TAC CAA GTG ATT and R: ATG CGC TATAGATTGAAA GGA. PCR reaction mixture included: 9.5 µL dd water, 2 µL dNTPs (1 mM, 1 to 10 diluted), 1.5 µL MgCl₂ (50 mM, 1 to 10 diluted), 1 µL of each primer, 3 µL 10X buffer, 2 µL Taq polymerase (500 U, 1 to 10 diluted) and 5 µL DNA template. Thermo cycler conditions comprised a 94˚C for 5 minutes followed by 55˚C for 30 cycles, 72˚C (30 seconds) for 30 cycles and final 72˚C for 4 minutes and then hold at 4˚C for 5 minutes (18). The products were visualized under UV Trans illuminator gel, then 5 µL of mecA product mixed with 2 µL of both staining dyes (red gel and loading buffer (Sina gen, Iran) were used and then run in each well.

4. Results

4.1. Antibiotic Susceptibility Test

The samples including trachea 74% (n = 154), blood 14.3% (n = 30), skin lesions 13% (n = 27), tissue culture 1.4% (n = 3) and some other specimens, had shown resistance to the following antibiotics: clindamycin 27% (n = 57), erythromycin 31% (n = 64), cotrimoxazole 11% (n = 23), tetracycline 43% (n = 90), ciprofloxacin 31% (n = 65), gentamicin 19% (n = 40), amoxicillin 99% (n = 207) and oxacillin 32% (n = 66). All of the isolates were susceptible to vancomycin and linezolid. Cotrimoxazole and gentamicin had antimicrobial effects on the clinical isolates, after vancomycin and linezolid. Antimicrobial resistance in MRSA isolates were as fol- low: tetracycline 36% (n = 24), erythromycin 35% (n = 23), clindamycin 39% (n = 26), ciprofloxacin 36% (n = 26), amoxicillin 90% (n = 74), cotrimoxazole 31% (n = 21), gentamicin 46% (n = 31) and in MSSA were: erythromycin 12% (n = 25), clindamycin 6.66% (n = 14), tetracycline 31.1% (n = 65), ciprofloxacin 11.1% (n = 23), amoxicillin 87% (n = 182), cotrimoxazole 31% (n = 66) and gentamicin 4.4% (n = 10). There was significant difference in antibiotic resistance to erythromycin, clindamycin, ciprofloxacin and gentamicin between MRSA and MSSA isolates. Ex- cluding the vancomycin and linezolid antibiotics, gentamicin (4.4%) and clindamycin (6.66%) were the most effective antibiotics inhibiting MSSA isolates, while cotrimoxazole (31%) and erythromycin (35%) have the highest effect on MRSA isolates.

4.2. D-test

Susceptibilities to antimicrobial agents detected by D-test during disc diffusion were as follow: (a) 50% (n = 104) were susceptible to both clindamycin and erythromycin (S), (b) 15% (n = 31) were resistant to both mentioned agents (R), (c) 4% (n = 8) were inducible clindamycin resistant (D) (Figure 2 A) and all were MSSA, (d) 1% (n = 3) were inducible positive (D+) (Figure 2 B).

4.3. PCR Assay

The results to detect mecA gene (MRSA isolates) confirmed the oxacillin disc diffusion. In this study 32% (n = 66) of isolates were detected as MRSA by confirmation of mecA gene using PCR assay (Figure 3).
5. Discussion

Inducible resistance to Macrolide-Lincosamide and Streptogramin (iMLSb) is a very important concern as these antibiotics are among the few restricted effective agents. The resistance to macrolides can be mediated by msrA gene encoding efflux proteins or via erm gene encoding enzymes that confer inducible or constitutive resistance to MLSB antibiotics. Clindamycin is an excellent effective drug particularly for the treatment of Staphylococcal skin and soft tissue infections and as an alternative in penicillin-allergic patients, which has yet good oral bioavailability. Metillin-resistant isolates have a greater widespread antibiotic resistance. Inducible resistance could not be detected, unless doing D-test with both clindamycin and erythromycin disks. Therefore, D-test is an imperative part of routine antimicrobial susceptibility test for all clinical isolates of S. aureus. Strains with this character are resistant to macrolides (erythromycin) and susceptible to clindamycin. In this study inducible resistance was seen in eight isolates (4%) and D positive in three isolates (1%), which were methicillin susceptible. D and D+ inducible resistance have the same outcome in patient, however the causing genes differ. There are various reports of inducible resistance, in Vivian study, among 81 erythromycin resistant isolates, 10 were susceptible to clindamycin and 6.2% of them had inducible resistance. In a report, 10% of the isolates were inducible resistant and the incidence of R and S phenotypes were 9% and 8%, respectively; and MRSA strains had higher antibiotic resistance (14). Deotale et al. detected that 14% of the isolates had inducible resistance, which was more prevalent among MSSA isolates (20). Other reports of inducible resistance to clindamycin are as follow: Sedighi found that 5% of CA-MRSA and 6.3% of HA-MRSA isolates had inducible clindamycin resistant (D-test) (22), and Rahbar et al. stated that inducible resistance was 9.7% (23), while reported phenotypes in studies from other countries are variable, especially from India (17, 24-27). In this study all of erythromycin-resistant and clindamycin-susceptible isolates showed inducible resistance. None of methicillin-resistant clinical isolates showed inducible resistance to clindamycin, but had higher resistance to both erythromycin and clindamycin; some of the reports indicated that MRSA isolates were more capable to resist against clindamycin and other antibiotics (28-31) and these results are in alignment with our study. Fortunately, inducible resistance was low in comparison to the other countries, but the laboratories should survey the probability of inducible resistance besides antibiotic susceptibility testing, because the resistance to these antibiotics increases by more consumption during the time. In this study, 20 (9.56%) of MRSA isolates were resistant to all of the antibiotics, except vancomycin and linezolid, while gentamicin (19%) and co-trimoxazole (11%) were more effective compared to the other antibiotics. This study showed inducible resistance and other phenotypes in Loghman Hospital of Tehran, Iran. In this study, inducible resistance to clindamycin was low; it should be detected by D-test because of the important effects of clindamycin.

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Authors’ Contributions

Abdolmajid Ghasemian performed the laboratory work. Najar Peerayeh inspected and guided the study. Dr. Bakhshi was the supervisor of this study. Mohsen Mirzaei was co-worker and helped in data analyzing.

Financial Disclosure

The authors declared that they had no conflict of interest and will not.

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