

The prevalence of *icaADBC* Genes among Clindamycin Inducible Resistant *Staphylococcus aureus* Isolates

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Background: Clindamycin inducible resistant *Staphylococcus aureus* (*S.aureus*) isolates can cause failure in treatment with this antibiotic. Biofilm production via polysaccharide intercellular adhesion (PIA) contributes in the colonization of *S. aureus*, resulting in the initiation of different diseases. The aim of this study was to detect *icaADBC* genes among isolates of *S.aureus* with inducible resistance to clindamycin.

Materials and Methods: A total of 209 clinical *S.aureus* isolates were collected and identified by conventional phenotypic tests. Isolates with inducible resistance to clindamycin were detected by double disk diffusion test (D-Test) using clindamycin (2 µg) and erythromycin (15 µg). Oxacillin was used to detect Methicillin resistant *Staphylococcus aureus* (MRSA) isolates. Polymerase Chain Reaction (PCR) was performed to detect the *icaADBC* genes.

Results: The rate of clindamycin inducible resistance was 4% (n=8). All the isolates were susceptible to methicillin. Four isolates (50%) contained the whole *icaADBC* genes. The prevalence of *icaA*, *icaB*, *icaC* and *icaD* genes were 5 (62.5%), 4 (50%), 6 (75%) and 5 (62.5%), respectively.

Conclusion: The results indicate that the prevalence of *icaADBC* genes among clindamycin inducible resistant strains was low, and also these strains were susceptible to methicillin.

Keywords: *Staphylococcus aureus*, inducible resistance, D-test, MRSA, *icaADBC* operon

1. Background

Staphylococcus aureus isolates including nosocomial and community associated pathogens colonize on the surface and epithelium of the body (1-3). Clindamycin (as a lincosamide) and erythromycin (as a macrolide) have remained among the few efficient antibacterial drugs against *S. aureus* strains. Clindamycin has excellent pharmacokinetic properties and can penetrate into various tissues (4). Resistance to these antibiotics has gradually become widespread among the countries (5), due to the differences in the consumption of antibiotics and various regional factors. Clindamycin is a proper antibiotic for the treatment of staphylococcal infections as an alternative after vancomycin (6). Resistance to these antibiotics occurs via methylation of ribosomal drugs target. This alteration is called macrolide-lincosamide-streptogramin (MLS_B) resistance (7).

The *icaADBC* genes play an important role in the biofilm formation among both isolates of *S. aureus* and *S. epidermidis*. In this operon, *ica* genes, *icaA* encodes the major enzyme essential for PIA synthesis. Likewise, this enzyme might require *icaD* gene product (called IcaD) for efficient activity (8). Co-expression of *icaA* with *icaD* provokes induction of higher enzymatic activity (9). The other genes within *ica* operon are *icaB* (polysaccharide deacetylase), *icaC* (transporter of PIA) and *icaR* (the inhibitor gene). In a study by Akiyama (2003), all *S. aureus* strains studied in the skin lesions of impetigo, atopic dermatitis and pemphigus had capability to produce glycocalyx and formed microcolonies (10). Most strains of *S. aureus* contained all four genes of *ica* operon, however several reports have detected some of these genes (11).

2. Objectives

The aim of this study was to detect the *icaADBC* genes among *S. aureus* with inducible resistance to clindamycin.

3. Materials and methods

3.1. Bacterial isolates

A total of 209 *S. aureus* isolates were collected from various clinical origins of infection in hospitalized patients, including blood, trachea, wound and sputum from July 2012 to January 2013. Biochemical tests were conducted for the identification of the isolates, including mannitol fermentation on Mannitol Salt Agar (MSA) medium, coagulase (including slide and tube) and DNase tests, and colony morphology on blood agar medium.

3.2. Clindamycin inducible resistance

The Double disk/D test was performed on Mueller Hinton Agar medium (similar to disk diffusion test) using clindamycin (2µg) and erythromycin (15µg) antibiotics according to Clinical and Laboratory Standards Institute (CLSI) guidelines (version of 2012).

3.3. Detection of MRSA strains

Oxacillin (1µg) was used in phenotypic test for the detection of MRSA strains with the antibiotic susceptibility test. Moreover, PCR assay was performed to detect *mecA* gene with specific primers, Table 1.

3.4. Extraction of genomic DNA

One or two colony of each bacterial isolate was suspended in 200 µl of TE buffer, and then the enzyme lysostaphin was added (a total of 200 µl of TE buffer and 20 µl of 2 µgml⁻¹ lysostaphin). Genomic DNA was isolated as described in the method described by Gey and colleagues (13).

3.5. Polymerase Chain Reaction (PCR)

Simplex PCR was conducted to determine *mecA* gene in MRSA and the *icaADBC* genes with specific primers, Table 1.

PCR for *mecA* gene was performed with the mixture of 9.5 µl distilled water (DW), 1 µl primer (100pm), 1.5 µl MgCl₂ (50 mM), 3 µl 10x buffer, 2 µl dNTPs (10 mM), 2 µl Taq polymerase (500 U) and 5 µl DNA template. PCR conditions were as 94 °C (5 min), followed by 30 cycles of 94 °C (30 s), 55 °C (30 s), 72 °C (30 s) and then 72 °C for 5 min. PCR for *icaADBC* genes was performed with mixture of 9.5 µl DW, 1 µl primer, 1.5 µl MgCl₂, 3 µl 10x buffer, 2.5 µl dNTPs, 2µl Taq polymerase and 5 µl DNA template. PCR conditions were partially different for these genes. For example for *icaADBC* genes, 30 cycles were set. For these genes, 94 °C for 5min was conducted and was followed by 94 °C for 1min, except for *icaD* (30 s). Then the annealing temperature for *icaA* (55 °C for 1min), *icaB* (52 °C for 30 s), *icaC* (55 °C for 30 s) and *icaD* (55 °C for 30 s) was set up. The extension step of 72 °C included: *icaA* (1min), *icaB* (1.5min), *icaC* (30 s) and *icaD* (1 min).

Table1. Sequence of the *icaA*, *icaB*, *icaC* and *icaD* primers used in this study.

Primer	Sequence (5' to 3')	Product size (bp)	Reference
<i>mecA</i>	F: GTG AAG ATA TAC CAA GTG ATT R: ATG CGC TATAGATTGAAA GGA	147	12
<i>icaA</i>	F: ACACCTGTCTGGCGCAGTCAA R: TCTGGAACCAACATCCAACA	188	15
<i>icaB</i>	F: AGAATCGTGAAGTATAGAAAATT R: TCTAATCTTTTTCATGGAATCCGT	900	15
<i>icaC</i>	F:ATGGGACGGATTCCATGAAAAAGA R: TAATAAGCATTAAATGTCAATT	1100	15
<i>icaD</i>	F: ATGGTCAAGCCAGACAGAG R: AGTATTTTCAATGTTAAAGCAA	198	15

3.6. Statistical Analysis

Pearson Chi-Square was used for data analysis using SPSS software version 19. The P-value less than 0.05 were considered as significant.

4. Results

4.1. The phenotypic tests

All the isolates with inducible resistance to clindamycin (equal to 8) were susceptible to methicillin (MSSA) (Figure1). Moreover, all the isolates were susceptible to vancomycin and linezolid. Six isolates (75%) were resistant to amoxicillin. Resistance to tetracycline, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole were observed in 4 (50%), 3 (37.5%), 3 (37.5%) and 2 (25%) isolates, respectively.

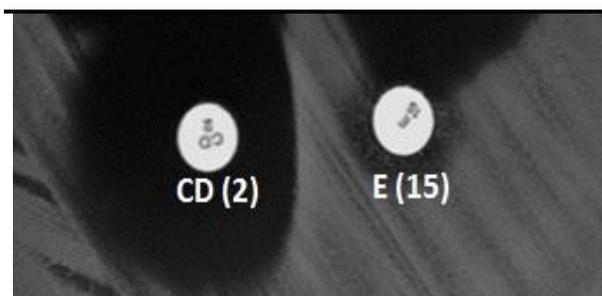


Figure1. Clindamycin inducible resistance; the distortion of the susceptibility zone indicates this phenomenon. CD: clindamycin, E: erythromycin

4.2. The presence of *mecA* and *icaADBC* genes

The *mecA* gene was not detected in clindamycin inducible resistant isolates. Four (50%) isolates contained all the *icaADBC* genes. The frequency of *icaA*, *icaB*, *icaC* and *icaD* genes were: 62.5% (n=5), 50% (n=4), 75% (n=6) and 62.5% (n=5), respectively. Among the total of 209 isolates, there was no significant difference between MSSA and MRSA strains regarding the presence of *icaADBC* genes (P-value = 0.14). Two blood isolates with inducible resistance harbored all *icaADBC* genes (Fig. 2).

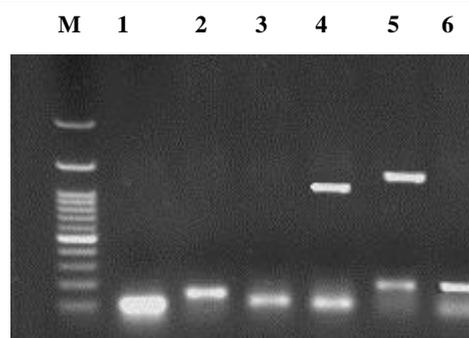


Figure2. The *icaADBC* genes. M: DNA size marker; Lanes 1 and 2 positive control for *mecA* and *icaA* genes, respectively; lanes 3 and 4 indicate *icaA* gene (188bp); lanes 5 and 6 indicate *icaD* gene with 198bp size; lanes 4 and 5 also show *icaB* (900bp) and *icaC* (1100bp), respectively.

5. Discussion

In our study, eight (4%) isolates had inducible resistance to clindamycin. In our previous survey, we depicted that several previous surveys had reported a variety of results. Morbidity and mortality rates due to a variety of *S. aureus* infections have been reported continuously from several areas (14). In our previous study, we determined that the majority of the isolates belonged to accessory gene regulator (*agr*) group I (15), but in inducible resistant isolates *agrII* was more frequent than *agrI*. Besides this, we observed no relationship between virulence genes and *agr* groups. The *agr* locus in *S. aureus* isolates, works as a global regulator of virulence genes, including secreted virulence components and surface proteins. We also observed that half of the inducible resistant isolates harbored the whole four *icaADBC*, showing an inevitable role of this operon in biofilm production. To our knowledge, there is no previous study regarding the presence of these genes in clindamycin inducible resistant strains of *S.aureus*. However, several surveys have displayed that *icaAD* genes are present in a majority of isolates of *S.aureus*; especially when producing biofilm phenotypically (16, 17). Furthermore, most of the previous studies have conducted surveys on the *icaAD* genes that encode PIA. For instance, Nasra and colleagues (2012) reported that the *icaAD* genes were present in 32% of blood and catheter isolates (18). In the study by Szweda and colleagues (2012), 36 of 46 *Staphylococcal* isolates harbored *icaA* and *icaD* genes; however, Grinholc and colleagues did not detect *icaD*, however, all strains contained *icaA* (19). In the study by Kara Terki and colleagues (2013), the *icaAD* genes have been detected in 17(38.5%) of the 44 *Staphylococcal* isolates from urinary tract (20). Moreover, in the present study, two blood isolates with inducible resistance contained all the *icaADBC* genes, suggesting that more studies are required for the relationship between clinical infections and presence of these genes. It is suggested that

several factors, such as epidemiological aspects, the strains and origins should be included in studies on the frequency of *icaADBC* genes.

6. Conclusion

In this study, half of the isolates with inducible resistance to clindamycin harbored all the *icaADBC* genes, suggesting that the presence of these genes is important for biofilm production.

Conflict of Interests

The authors declare they have no conflict of interests.

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Authors' Contributions

Abdolmajid Ghsemian performed the laboratory work. Dr. Shahin Najar Peerayeh guided the process, Dr. Bita Bakhshi advised the study, Mohsen Mirzaee helped the laboratory work.

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