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Frequency of Class I, II Integrons, and Aminoglycoside-Resistance Genes in Clinical Isolates of *Staphylococcus aureus* in northern Iran

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Abstract

Staphylococcus aureus is known as a hospital pathogenic bacterium that can cause a wide range of infections. Aminoglycosides are one of the drugs of choice in the treatment of septicemia caused by this bacterium. The aim of this study is to investigate the level of resistance to aminoglycosides, determine the frequency of aminoglycosides modifying enzyme genes and the frequency of class I and II integrons among clinical isolates of aminoglycosides resistant S. *aureus*. In this study, the resistance of 200 isolates of S. *aureus* to aminoglycosides including gentamycin, kanamycin, amikacin and streptomycin were investigated by Kirby-Boyer disc diffusion method. The frequency of *aac(6')-Ie-aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia* genes and and class 1 and 2 integrons in test isolates were determined by PCR. Out of 200 isolates, 134 isolates (67%) were resistant to at least one aminoglycoside. Of this number, the frequency of *aac(6')-Ie-aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia* genes were 35.07%, 29.1% and 20%, respectively. Class I and II Integrons were detected in respectively 66% and 19% of isolates. All of isolates carrying class I integron were aminoglycoside resistant and positive for aminoglycoside modifying genes. The results showed high resistance to aminoglycosides and high frequency of aminoglycoside modifying genes in clinical S. *aureus* isolates carrying calss I and II integrons.

Key words: S. *aureus*, aminoglycosides, integron, *aac(6')-Ie-aph(2'')* , *aph(3')-IIIa* , *ant(4')-Ia*

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Introduction

Staphylococcus aureus is the major cause of nosocomial infections in both developed and developing countries. This bacterium can cause various types of infection, including soft tissue infections, pneumonia, endocarditis, sepsis, pneumonia, catheter-related infections (Mahdiyoun et al., 2016, Hu 2015) .S. *aureus* has a broad spectrum of virulence agents that allow it to be resistant to a large group of antibiotics including aminoglycosides, and provoking the emergence of multidrug-resistant (MDR) isolates. Also because of its ability to adapt to host defense stress S. *aureus* is considered as a successful pathogen (Yoo et al., 2013, Walker et al., 2016).

Aminoglycosides are a group of antibacterial agents that are used to treat many bacterial infections, particularly those caused by staphylococci (Gade, 2014). Aminoglycosides exert antibacterial effects by targeting the bacterial ribosome and disrupt protein synthesis (Jana et al, 2006). The three mechanisms of resistance to aminoglycosides include: change in the ribosomal position of the drug, reduced permeability of the drug and the enzymatic deactivation of the drug which is the most common resistance mechanism of aminoglycoside antibiotics. These enzymes are classified into three main categories based on their modifying activity: aminoglycoside acetyl transferases (AACs), aminoglycoside phosphotransferases (APHs), and aminoglycoside nucleotidyltransferases (ANTs). These three enzymes are encoded by *aac (6')-Ie/aph (2'')*, *aph (3)-IIIa*, and *ant (4)-Ia* genes, respectively (Alli O et al., 2015, Soleimani et al 2010, Nikaido et al 2001, Zuo et al 2014).

On the other hand, studies showed that several different mechanisms including mobile genetic elements consisting of plasmids, transposons and integrons play an important role in acquiring and spreading antibiotic resistance genes (Partridge et al, 2018). Integrons are one of the mobile genetic factors that are able to carry and spread antibiotic resistance genes among these bacteria, and their horizontal transfer among bacteria is one of the most important ways of spreading resistance genes and creating resistant strains. (Hall et al, 2007). The present study was aimed to investigate the level of resistance to aminoglycosides, determine the frequency of aminoglycosides modifying enzyme genes and the frequency of class I and II integrons among clinical isolates of S. *aureus* resistant to aminoglycosides.

Materials and Methods

Sampling and isolation of bacteria

In this cross- sectional study, clinical samples were collected from patients' blood, urine, and skin lesions in Gilan province, northern Iran during 2021. In total, 200 non-duplicate S. *aureus* isolates were included in this study and duplicate samples of patients were excluded. To isolate test bacteria, samples culture was performed on mannitol salt agar and blood agar (Merck, Germany). Coagulase, catalase and DNase production was investigated, and subsequently S. *aureus* isolates were identified by a pair of 23SrRNA specific primers as described previously (Salasia et al 2011, Straub,1999). S. *aureus* ATCC43300 strain has been used as a positive control.

Antimicrobial susceptibility testing

Aminoglycoside resistant isolates detected in phenotypic assay were screened for aminoglycoside resistance using Kirby-Bauer disc diffusion method according to the CLSI (2023) guideline. The disk of antibiotics (High Media-India), including gentamicin (30µg), kanamycin (30µg), streptomycin (10µg), and amikacin (30µg), were used to determine the antibiotic sensitivity of S.*aureus* isolates.

Identifying aminoglycoside resistance genes

Three aminoglycoside resistance encoding genes including *aac(6')-Ie/aph(2'')*, *ant(4')-Ia*, *aph(3')-IIIa* were amplified with PCR method. For this purpose, the polymerase chain reaction (PCR) was carried out using gene-specific primers (Table 1) in a total volume of 25 µl; 0.5 µl dNTPS (10 µM), 5 µl enzyme buffer (10×), 3 µl reverse and forward primers (10 pM), 2 µl template DNA (2 µg), 0.5 µl Pfu enzyme (2.5 units) (Bioneer, South Korea), and 14 µL distilled water. The thermocycler (Analytic Jena,GmbH Co.) thermal treatment consisted of initial denaturation step of 94 °C for 4 min, 30 cycle of 94 °C for 50 s, 60 °C for 45 s, 72 °C 60 s, and a final

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Table 1. Nucleotid sequences of primers used for PCR

Gene	Primer Sequence (5'-3')	Amplicon size (bp)	Annealing Tem.	Ref.
aac(6')-Ie/aph(2")	F:CCAAGAGCAATAAGGGCATACC R:CACACTATCATAACCACTACCG	222	45	(Mahdiyoun et al 2016)
ant(4')-Ia	F:AATCGGTAGAAGCCCAA R:GCACCTGCCATTGCTA	135	47	(Mahdiyoun et al 2016)
aph(3')-IIIa	F:CTGATCGAAAAATACCGCTGC R:TCATACTCTTCCGAGCAAAGG	269	51	(Mahdiyoun et al 2016)
intI1	F: CCT CCC GCA CGA TGA TC R: TCC ACG CAT CGT CAG GC	280	55	(Moura et al., 2007)
intI2	F: TTA TTG CTG GGA TTA GGC R: ACG GCT ACC CTC TGT TAT C	233	50	(Moura et al., 2007)

extension of 72 °C for 10 min. The PCR product was then electrophoresed on 1.5% agarose gel, which was examined via UV transilluminator.

Detection of class I and II integrons

All test isolates were screened for class I and II integrons in PCR reaction as described previously (Moura et al., 2007).

Results

Aminoglycoside resistance in test isolates

Out of 200 isolates, 134 isolates (67%) were resistant to at least one aminoglycoside. Among them highest and lowest resistance were detected against streptomycin (89.15%) and gentamicin (44.15%) and resistance against amikacin and Kanamycin were detected in 59%, and 50% respectively.

Frequency of aminoglycoside resistance genes

The frequency of *aac(6')-Ie-aph(2")*, *aph(3')-IIIa* and *ant(4')-Ia* genes in phenotypic detected aminoglycoside resistant S. *aureus* isolates were 35.07%, 29.1% and 20%, respectively.

Investigating the presence of intI and int II genes

Class I and II Integrons was detected in respectively 66% and 19% isolates. All of isolates carrying class I integrone were aminoglycoside resistant and positive for aminoglycoside modifying genes.

Discussion

In the present study, the prevalence of aminoglycoside resistance in S. *aureus* isolates was evaluated. Tested isolates showed the high level of resistance to aminoglycosides. Increase in frequency of antibiotic resistance gene such as gene encoding for resistance to aminoglycosides (aacA-D and aph), is a challenge for treatment of infections caused by S. *aureus* (Mahdiyoun et al., 2016). Several mechanisms including the presence of genetic mobile factors encoding drug resistance genes, such as plasmids and transposons, help in the spread of antibiotic resistance among these bacteria. In addition, the role of integrons in the spread of antibiotic resistance has been proven in many bacteria, including *Staphylococcus aureus* (Yang, 2004). In the present study, the presence of intI and int II genes was detected in 66% and 19% of the isolates, respectively. All of isolates carrying class I integrone were aminoglycoside resistant and positive for aminoglycoside modifying genes.

During a descriptive study conducted by Gudarzi et al., on 80 S. *aureus* strains isolated from patients hospitalized in ICU departments in five hospitals in Tehran, class 1 and 2 were detected in 56.3% and 18.7%, respectively (Goudarzi M, 2016). In another study conducted by Yahaghi et al., on 200 strains of S. *aureus* in 2013, only 1% of the strains (two isolates) contained class I integron (Yahaghi, 2014).

In the present study, more than 67% of S.*aureus* isolates showed aminoglycoside resistant

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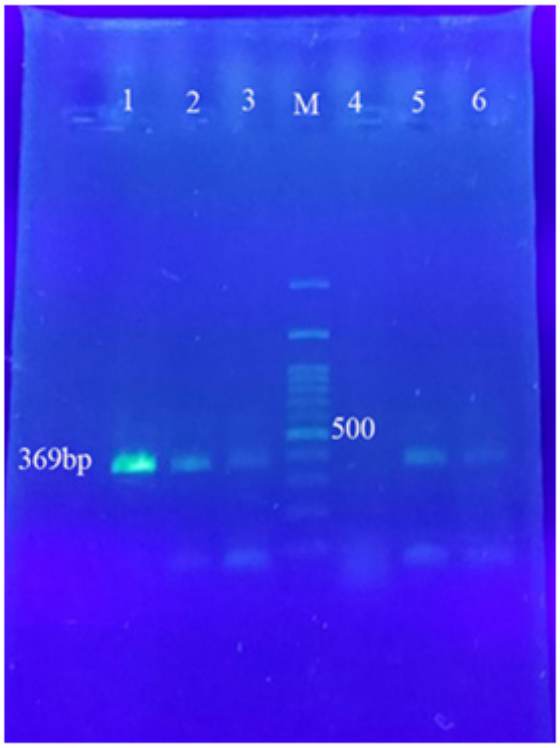

Figure 1. Agarose gel electrophoresis of *aac(6)-le-aph (2)* genes. Lanes 1-7: 369bp PCR amplicons of aac(6)-le-aph(2), Lane M:100bp DNA marker

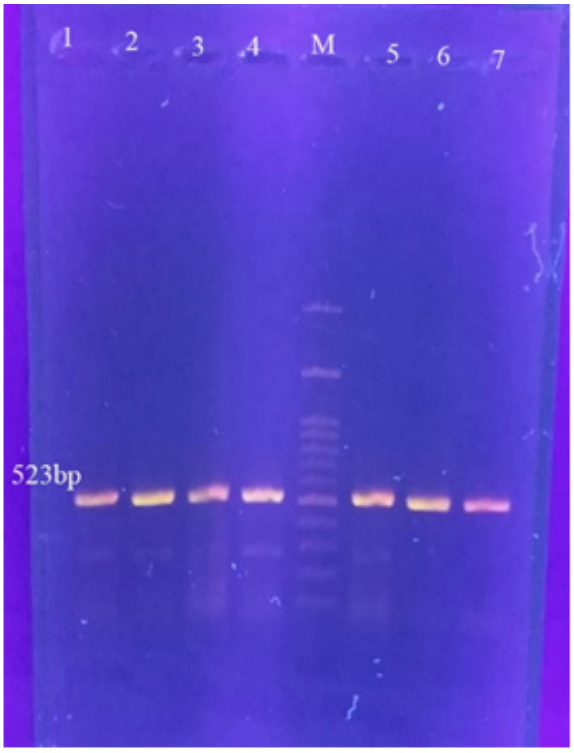

Figure 2. Agarose gel electrophoresis of of *aph(3)-llla* gene PCR amplicons. Lanes 1-7: 523bp PCR amplicons of *aph(3)-llla* , Lane M:100bp DNA marker

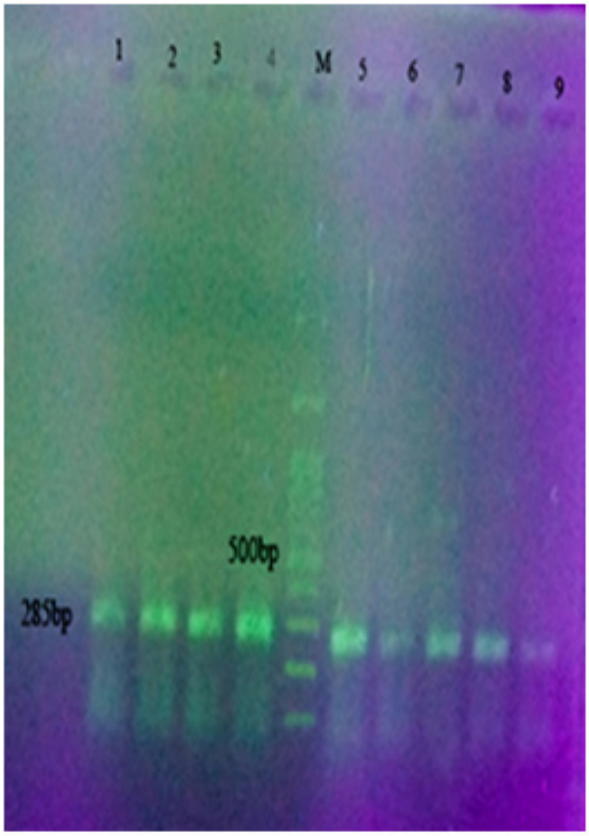

Figure 3. Agarose gel electrophoresis of *IntI* gene PCR amplicons. Lanes 1-9: 285bp PCR amplicons of *IntI*. Lane M:100bp DNA marker.

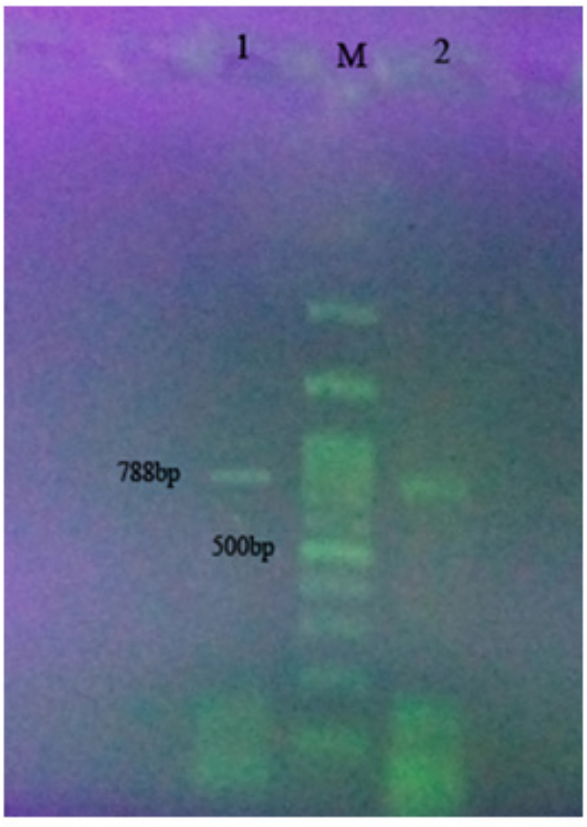

Figure 4. Agarose gel electrophoresis of *IntII* gene PCR amplicons. Lanes 1-22: 788bp PCR amplicons of *IntII*. Lane M:100bp DNA marker.

phenotype and the genes *aac(6')-Ie/aph(2")*, *aph(3')-IIIa* and a*nt(4')-Ia* encoding for aminoglycoside modifying enzyme were detected in 35.07%, 29.1% and 20%, isolates respectively. In accordance with present finding Mahdiyoun et al., showed that the aac (6')/aph (2') gene is the most prevalent gene (77%) encoding AME enzymes among clinical MRSA isolates in Sari and Tehran. These researchers also reported high frequency of *aph (3')-IIIa* (68.4%) and *ant (4')-Ia* (70.1%) genes among MRSA isolates from hospitalized patients (Mahdiyoun et al., 2016). Also, A Systematic Review and Meta-analysis, introduced *aac(6')-Ie/aph(2")*, as the most common AMEs gene in Gram positive cocci including enterococci and MRSA in Iran (Arabestani et al., 2015).

During the last two decades, the results of various studies in other countries showed that the *aac(6')/aph(2")* gene is the most abundant gene encoding aminoglycoside-modifying enzymes in MRSA clinical isolates (Vanhoof et al., 1994, Choi et al., 2003, Sareyyupoglu et al., 2006, Fatholahzadeh et al., 2009). In the present study, the prevalence of *ant (4')-Ia* gene was 20%. According to our studies, the frequency of the *aac(6')-Ie/aph(2")* gene in the investigated strains was 35.07%, while the findings from Turkey and Korea show that in clinical strains, the *aac(6')/aph(2")-Ia* gene is the most abundant gene (66% and 65%) among genes encoding aminoglycoside-modifying enzymes (AME) (Sareyyupoglu et al., 2006, Nihonyanagi et al., 2012). Also, high frequency of aminoglycoside resistance genes including *ant (6')-Ia* (79.59%) and *aph(3')-III* (73.47%) has been reported in a study conducted by Zhang Z et al., (2023).

Conclusion

The results showed high resistance to aminoglycosides and high frequency of aminoglycoside modifying genes in clinical S. *aureus* isolates carrying calss I integron. Rapid identification of these strains and treatment of related disease are required to prevent the spread of these bacteria.

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