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Identification of class I integrons gene in staphylococcus strains isolated from clinical samples

Parasto Veise[1], Rashid Ramazanzadeh[2], Zahra Dailami Khiababi[1], Bahare Derakhshi[3, *], Nour Amirmozafari[4]

[1]Microbiology department, Islamic Azad University of Zanjan
[2]Cellular & Molecular Research Center and Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj- Iran
[3]Student Research Committee, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj- Iran
[4]Microbiology department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Email address:
b.derakhshi@yahoo.com (B. Derakhshi)

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Abstract: *Introduction and Objectives:* Antimicrobial resistance is a major contemporary public health threat. Strategies to contain antimicrobial resistance have been comprehensively set forth, however in developing countries where the need for effective antimicrobials is greatest implementation has proved problematic. Staphylococcus is an important Nasocomial infectious agent which is notorious for rapidly gaining antimicrobial resistance genes. Integrons are a series of mobile genetic elements that are able to express gene cassettes encoding various antibiotic resistances. This study aimed to identify integron class I gene cassettes in clinical Staphylococcus isolates recovered from patients in Sanandaj, Iran hospitals. *Materials and Methods:* A total of 200 Staphylococci spp. was recovered from nose and throat swabs of patients (ICU and infection wards) in Toohid and Beasat hospitals in Sanandaj, Iran. Following bacterial DNA extraction, Class I Integron gene was detected by PCR. *Results:* Out of the 200 Staphylococci spp. , 81 (40.5%) isolates were carriers of class I integron . The integron expressing isolates included 35 cases (23.5%) of *Staphylococcus epidermidis*, 37 cases (40.1%) of *Staphylococcus aureus*, and 9 cases (36%) of *Staphylococcus saprophyticus. Conclusion:* Results indicated that frequency of class I integron gene is quite high among clinical Staphylococcus isolates in Sanandaj area. For control of antibiotic resistance spread, screening of clinical samples for these genes and elucidation of their genetic diversity is crucial.

Keywords: Staphylococcus Aureus, Coagulase Negative Staphylococcus, Integrons Class I Gene

1. Introduction

Antimicrobial resistance is a major contemporary public health threat. Strategies to contain antimicrobial resistance have been comprehensively set forth, however in developing countries where the need for effective antimicrobials is greatest implementation has proved problematic. Treatment of serious life-threatening multi-drug-resistant organisms poses a serious problem due to the limited therapeutic options.. Staphylococci are Gram positive bacteria, non-spore former, immobile, facultative anaerobe, and pigmentogenic with production of golden, yellow and white pigments [(1,2)] . These bacteria are responsible for most Nosocomial Infections and about 80% of purulent diseases [(3)]. Resistance to antimicrobial agents is very widespread among these isolates and most of them can spread by mobile genetic agents.

Integrons are a series of movable genetic elements that are able to express gene cassettes encoding antibiotic resistance. They are composed of two distinct regions which include one or more resistance genes[(4-6)]. Integrons expressing numerous gene cassettes can cause multiple drug resistance [(7-13)] . Based on the homology of the integrase proteins, they are classified into 10 different classes. Five of these classes are among the causes of antibiotic resistance in bacteria [(6,14).] The class I integron seems to be the most prevalent integron class in a majority of clinical important Gram-positive bacteria. Class I integrons are located in bacterial chromosome and are primarily associated with the Tn3 transposon family (Tn21 or Tn1696) [(14).] The purpose of this study was to detect

integron class I in Staphylococci spp. isolated from patients in Sanandaj hospitals.

2. Materials and Methods

Two hundred sterile swab samples were collected from the throat and nose of patients admitted to the intensive care unit and Infection wards in Beasat and Toohid hospitals. The samples were cultured immediately in mannitol salt agar. *Staphylococcus aureus* and coagulase-negative Staphylococci were identified with conventional biochemical tests which included colony shape and color, Gram stain, catalase production, slide and tube coagulase test, mannitol fermentation, DNase production and novobiocin sensitivity. For genomic DNA extraction of the Staphylococcal strains, a commercial DNA extraction kit was used (DNA Cinna Pure kit, Cinagene Co. Tehran, Iran).

The PCR reaction for class I integrons was conducted as follows: First, class I integrons gene specific primers were prepared.

Table 1 : *patients biography and samples*

sample source		Age group	Sex		Samples						Units		
Throat	Nose	14-87	Female	Male	S.T* Saprophyuticu		S.T* Epidermidis		S.T* aureus		ICU	Infection	CCU
55	145		110	90	Throat 6	nose 19	Throat 26	Nose 59	Throat 22	nose 68	39	60	101

Table 2: *primer sequences size in tracing of class I integron gene*

Primer	Sequence
intI1-U	ACGAGCGCAAGGTTTCGGT-3'-5'
intI1-D	GAAAGGTCTGGTCATACATG-3'-5'

PCR was performed in a final volume of 25 microliters, using DFS Master Mix kit (Cinagene co.). This kit includes Taq DNA polymerase, $MgCl_2$, dNTPs, $(NH4)_2SO4$, Tris-HCl, and Tween 20 . The PCR program was as follows; 94 ˚C Primary denaturation for 5 min, 94 ˚C Denaturation for 1 min, 54-58 ˚C Annealing for 1 min, 72 ˚C Extension for 45 sec (30 cycle) and 72 ˚C Final extension for 10 min.

Electrophoresis of PCR products was performed in agarose gel followed by staining with ethidium bromide. Data analysis and statistical comparisons was performed with Microsoft Excel software and SPSS 11.5 and 2χ test.

Table3 *: materials amount used in PCR reaction for intI1 gene*

Reagent	Concentration
Master Mix	12.5
Taq polymerase	0.2
intI1-U	1
intI1-D	1
Distilled water	8.3
DNA template	2
Total	25

Table 4: *PCR tempreture conditions for intI1 gene*

Primary denaturation	94 ˚c	5 min	1 cycle
Denaturation	94 ˚c	1 min	
Annealing (Touch down)	58-54 ˚c	1 min	8+30 cycles
Extension	72 ˚c	45 s	
Final Extension	72 ˚c	10 min	1 cycle

3. Discussion and Conclusion

Out of 85 strains of *Staphylococcus epidermidis*, 35 (41.2%) strains; 90 strains of *Staphylococcus aureus,* 37 (41.1%) strains; and 25 strains of *Staphylococcus saprophyticus*, 9 (36%) were shown to be in possession of the class I integron gene. In the total 200 samples, 81 (40.5%) of them were integron-positive and 119 (59.5%) were free of the gene.

Table 5***:*** *Frequency distribution of class I integron gene Staphylococcus strains isolated from infection, intensive care and coronary care units in Sanandaj hospitals*

Organism	*Integron* PCR(+)	PCR(-)	Total
Staphylococcus aureus	37(%40/1)	53(%59/9)	90(%100)
Staphylococcus epidermidis	35(%23/9)	50(%76/1)	85(%100)
Staphylococcus saprophyticus	9(%36)	16(%64)	25(%100)
Total	81(%40/5)	119(%59/5)	200

Table 6 *Distribution of class I integron genes in Staphylococcus strains isolated from clinical samples in Coagulase-positive and negative groups*

Organisms	integron PCR(+)	PCR(-)	Total
Staphylococcus aureus	37(%45/7)	53(%54/3)	90(%45)
coagulase negative Staphylococcus	44(%45/7)	66(%54/3)	110(%55)
Total	81(%40/5)	119(%59/5)	200

In a study by Xu and colleagues who investigated the expression of class I integron as a determinant factor for antibiotic resistance on nosocomial MRSA strains during 2001-2006, 76 of the 179 (5/42%) isolates were carriers' of the genes [7]. A separate study conducted in a hospital in Guangzhou, China during 2007, investigators looked at *Staphylococcus aureus* (n = 30) for the presence of class I

integrons and found 16 (53%)of them to contain the gene [(11).] In another study by Xu *et al*, six strains of MRSA of nosocomial origin were tested by Southern hybridization and all have a copy of the class I integrons with gene cassettes located on aadA2 locus on the Chromosomes [(14)].

In Liu 'study, out of the 1,019 cases, 743 (72.9%) opportunistic pathogens were isolated. The top five commons organism identified were Coagulase-negative staphylococcus, Staphylococcus aureus, Haemophilus influenzae , Streptococcus pneumoniae and Klebsiella pneumoniae . The isolated rates of S. aureus and H. influenzae were decreased with aging.[(15)]

In the study done in nine European countries, 43.0% (70/163) of isolates were shown to be integron-positive. these isolates were statistically more likely to be resistant to aminoglycoside, quinolone and beta8-lactam compounds, including third-generation cephalosporins and monobactams, but There was no association between the presence of integrons and susceptibility to cefepime, amikacin and the carbapenems, to which at least 97% of isolates were fully susceptible. [(16)] Results of the above studies are in accordance to those obtained in this report.

The results of this study show high prevalence of antibiotic resistance in the Sanandaj hospitals, due to the indiscriminate use of antibiotics.

Although the resistance gained by the bacterial populations may be due to mutates in their genes due to continuous use of antibiotic, but this more likely that the new propagation mechanism of class I integron gene may have played the main role in the transfer of resistance gens in the bacterial strains[(17-19)]

Based on the results of other studies, antibiotic resistance was caused by gene cassette of the antibiotic resistance genes in class I integron gene and this resistance is considered as a limitation for empirical treatment of infections caused by Staphylococcus [(20, 23).]

In this study, it was shown that the environment and equipment used in the infection and intensive care units could colonize Staphylococcus bacteria as an agent of nosocomial infections. As this colonization is determined as a source of infection for patients, class I integron gene, derived from Staphylococcus aureus strains isolated from these units could be the main contributors to antibiotic resistance.

As the role of class I integron gene in the development of antibiotic resistance has been established, the use of appropriate antibiotics could prevent the resistance find in bacteria

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