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Molecular Study of *Staphylococcus Epidermidis* Strains Isolated from Clinical Specimens from Different parts of Rouhani Hospital (Babol, Iran)

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Abstract

Introduction: *Staphylococcus epidermidis* is known as the most predominant member of coagulase negative staphylococci which can cause nosocomial infections especially in ICU and NICU wards.

Aim: The aim of this study was to investigate the molecular characterization of *S. epidermidis* strains obtained from the Rouhani Hospital in Babol, Iran.

Materials and Methods: In this descriptive study, a total of 60 *S. epidermidis* strains were collected. Thereafter, the antimicrobial susceptibility testing, the prevalence of *mecA* and *icaD* gene was evaluated. Finally the molecular pattern of isolates was determined by using RAPD-PCR technique.

Results: A total of 50 clinical strains and 10 environmental isolates were obtained from hospitalized patients from different specimens such as bloodstream, urine, catheter, body fluids and etc. by disc diffusion method the high rates of resistance were belonged to oxacillin (70.5%) and ciprofloxacin (63.9%). The prevalence of *mecA* and *icaD* genes was reported 85% and 41.6% respectively. 24 RAPD-Type was identified by using RAPD-PCR method which indicates the high genotypic diversity in the *S. epidermidis* isolates.

Conclusion: The high risk of transmission of infection between the wards and also the Rouhani Hospital staff should be taken seriously.

Keywords: *Staphylococcus Epidermidis*; Molecular Typing; ICU; Nosocomial Infections

Introduction

Staphylococci are known as a gram-positive, non-motile and non-spore forming cocci which are divided into two mains coagulase-negative and coagulase-positive staphylococci [1]. *Staphylococcus epidermidis* with the highest prevalence rate is recognized as the most significant member of the family of coagulase-negative staphylococci and plays an important role in nosocomial infections [2]. *S. epidermidis* is considered as the main contributor in medical equipment infections. In recent decades, due to the clinical importance and emergence of methicillin-resistant *S. epidermidis* strains, this organism has become a challenge in the treatment of patients [3]. Several virulence factors and resistance genes were identified in which the *mecA* and *ica* gene are the important one [4]. Many strains of *S. epidermidis* produce an N acetylglucosamine (PNAG) homopolymer, called PIA which is associated in biofilm formation [5].

PIA is the main element of the extracellular matrix that is produced by the *ica* gene. The *ica* gene products include *icaA*, *icaD*, *icaB* and *icaC* [2]. Identification of these virulence factors is essential in control and prevention of outbreak infections. One of the most effective techniques in this issue is using molecular typing methods. Due to *S. epidermidis* related infections various typing methods were described such as analysis of plasmid and Restriction enzymes, DNA hybridization, RAPD-PCR, SCCmec typing, PFGE

and MLST [2]. As mentioned methods Random Amplification of Polymorphic DNA (RAPD) is a type of modified PCR method in which short-term random sequences are able to attach in different locations of the genome and can produce a range of amplified PCR products [6,7]. The aim of this study was to investigate the molecular characterization of *S. epidermidis* strains obtained from the Rouhani Hospital in Babol, Iran.

Materials and Methods

Isolation and Identification

A total of 50 *S. epidermidis* strains were collected from bloodstream, urine, catheter, shunt, body fluids and wound infections from different wards including open heart surgery, orthopedics, neurology, infectious disease, ICU and NICU of Rouhani Hospital. In addition a total of 10 *S. epidermidis* strains were obtained from hospital staff for RAPD-PCR test. The isolates were referred to the laboratory of the Babol University of Medical Sciences for confirmation by microbiological test such as gram staining, catalase, coagulase, mannitol fermentation on mannitol salt agar media and sensitivity to polymyxin B and finally stored at -20 °C.

Antimicrobial Susceptibility Testing

Antibiogram test was performed by disk diffusion method according to CLSI 2016 standards. In this test, Muller Hinton Agar (Merck - Germany) and antibiotic disks included Cefoxitin (30 µg), Oxacillin (1 µg), Gentamicin (10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Ciprofloxacin (5 µg), Fusidic acid (10 µg), Levofloxacin (30 µg), Trimethoprim/Sulfamethoxazole (1.25/23.73 µg), Vancomycin (30 µg), and Linezolid (30 µg) (ROSCO - Denmark) was used.

PCR Amplification for mecA and icaD Genes

In order to perform PCR, the MasterMix was prepared in a final volume of 25µl, including: 12.5µl Super MasterMix 2X, 1µl of each primer (Forward & Reverse), 3µl DNA template and 7.5µl Sterile Deionized Water. PCR cycles was perform with initial denaturation temperature of 94°C for 5 minutes, then 30 cycles with a denaturation temperature of 94°C for 30 seconds, annealing temperature of 55°C for 30 seconds, and an extension temperature of 72°C for 40 seconds and a final extension cycle was performed at a temperature of 72°C for 5 minutes. The specific primers are shown in (Table 1).

Table 1: Specific primers for amplification of mecA and icaD genes.

Gene	Nucleotide sequences	Size (bp)	Reference
mecA	F: 5'-AGTTCTGCAGTACCGGATTTGC-3'	533	[7]
	R: 5'-AAAATCGATGGTAAAGGTTGGC-3'		
icaD	F: 5'-GAACCGCTTGCCATGTGTTG-3'	483	[8]
	R: 5'-GCTTGACCATGTTGCGTAACC3'		

RAPD Analysis

The DNA genomic was extracted by commercial kit (Yekta-Tajhiz, Iran) and RAPD-PCR assay was carried out according to procedure described previously by Burucoa [8,9]. PCR was performed in a final volume of 25µl containing 12.5µl Super MasterMix 2X, 1µl Primer (5'-CCG CAG CCA A-3'), 3µl DNA Template and 8.5µl Sterile Deionized Water [10]. The PCR program was done as 95 °C for 1 min as an initial hot start followed by 94 ºC for 1 min, 30ºC for 1 min, and 72ºC for 2 min for 4 cycles, followed by 36 cycles of 94ºC for 1 min, 36ºC for 1 min, and 72ºC for 2 min and a final extension step of 72ºC for 5 min. Finally, RAPD-PCR profiles were analyzed by using GelQuest software with 80% similarity cut-off point with clustered using the Dice's coefficient, and a dendrogram generated based on the Unweighted Pair Group Method with Arithmetic mean (UPGMA).

Results

Table 2: *S. epidermidis* strains in details including isolation ward, RAPD-Type, *mecA* and *icaD* genes and resistance pattern.

Strain	Ward	RAPD Type	*mecA* Gene	*ica* Gene	Resistance pattern
7	ICU	R-1	Positive	Negative	OXA-FU-CLI-FOX
8	ICU	R-1	Positive	Negative	E-GM-FOX-OXA-CLI-CIP-SXT-LEV
9	NICU	R-1	Positive	Negative	OXA-GM-CLI-E-CIP-FU-FOX
4	CCU	R-1	Positive	Positive	LEV-CLI-CIP-OXA-GM-E-FOX
11	Pulmonary	R-1	Positive	Positive	E-FOX-OXA-CIP-GM-SXT-LEV-CLI
15	NICU	R-2	Positive	Positive	OXA-FOX
16	CCU	R-2	Positive	Positive	CLI-FOX
17	ENT	R-2	Positive	Negative	OXA-FOX-CIP-GM-E-CLI-SXT
36	NICU	R-3	Positive	Positive	CIP-GM-E-FOX-OXA-LEV-CLI-FU
12	ICU	R-4	Positive	Positive	OXA-GM-CIP-FOX-E-FU-CLI-LEV
13	ICU	R-4	Positive	Negative	OXA-GM-CIP-FOX-E-FU-CLI-LEV
14	CCU	R-4	Positive	Positive	CIP-GM-E-FOX-OXA-SXT-CLI-FU
28	ICU	R-4	Positive	Positive	CIP-FOX-OXA-GM-E-LEV-SXT-CLI

24	ICU	R-5	Positive	Negative	OXA-GM-FOX-CLI-SXT-LEV-CIP-E
2	Infectious Diseases	R-6	Positive	Positive	FOX-CLI
3	ICU	R-6	Positive	Positive	OXA-LEV-FOX-CIP
23	Infectious Diseases	R-6	Positive	Negative	OXA-LEV-FOX-CIP-E
22	Infectious Diseases	R-7	Positive	Negative	OXA-FOX-GM-CIP-LEV-SXT-E
25	Infectious Diseases	R-7	Positive	Negative	CLI-LEV-SXT-CIP-GM-E-FOX-OXA
29	Infectious Diseases	R-7	Negative	Positive	SXT-LEV-E
30	ICU	R-7	Positive	Positive	OXA-SXT-E-CIP-FOX-LEV
33	Infectious Diseases	R-8	Positive	Negative	OXA-CIP-LEV-FOX
35	Cardiovascular	R-8	Positive	Positive	OXA-CLI-CIP-E-FU-FOX-LEV
27	Surgery	R-8	Positive	Negative	E-OXA-CIP-LEV-FOX-SXT
32	Emergency	R-9	Negative	Positive	E-CLI
60	Environmental	R-9	Negative	Negative	E-GM-CLI
34	ICU	R-10	Positive	Negative	OXA-GM-FOX-CLI-SXT-LEV-CIP-E
45	ICU	R-10	Negative	Positive	OXA
37	NICU	R-11	Positive	Positive	FOX-E-CIP-OXA-LEV
39	ICU	R-12	Positive	Positive	CIP-OXA-LEV-FOX
40	ICU	R-12	Positive	Positive	FOX-LEV-SXT-CIP-GM-E-OXA-FU
48	Environmental	R-12	Negative	Negative	CLI-LEV-SXT-CIP-GM-E-OXA
52	Environmental	R-12	Positive	Negative	CLI-E-OXA-FOX
18	Infectious Diseases	R-13	Positive	Negative	FOX-CIP-GM-OXA-E
19	Gastroenterology	R-14	Positive	Positive	OXA-CIP-GM-E-FOX-CLI-LEV
42	ICU	R-14	Positive	Negative	CLI-SXT-CIP-GM-E-OXA-FOX-LEV
43	Thorax	R-15	Positive	Positive	SXT-LEV-CLI-E-OXA-FOX-GM-CIP-FU
44	Pulmonary	R-15	Negative	Negative	OXA-CIP-FU
20	Thorax	R-15	Positive	Positive	GM-OXA-E-FOX-CIP-CLI-SXT-CIP-LEV
21	Emergency	R-16	Positive	Positive	OXA-GM-CIP-E-FOX-CLI-SXT-LEV-FU
58	Environmental	R-16	Positive	Negative	OXA-GM-CLI-SXT-LEV-CIP-E-FOX
46	Pulmonary	R-17	Positive	Positive	LEV-SXT-CLI-OXA-E-FOX
59	Environmental	R-17	Positive	Negative	OXA-SXT-FOX
47	Infectious Diseases	R-18	Positive	Negative	LEV-SXT-CLI-OXA-E-FOX
54	Environmental	R-18	Negative	Negative	CLI-E-GM-CIP
49	Environmental	R-18	Negative	Negative	CLI-E
55	Environmental	R-18	Positive	Negative	GM -CIP-E-LEV-FOX
5	Infectious Diseases	R-19	Positive	Negative	OXA-FOX-E-LEV-CIP-CLI-SXT-GM-FU
6	Cardiovascular	R-19	Positive	Negative	OXA-FOX-GM-CIP-LEV-SXT
50	Environmental	R-20	Positive	Negative	OXA-FOX-CIP
56	Environmental	R-20	Positive	Negative	GM-CLI-SXT-LEV-CIP-E
38	Surgery	R-20	Positive	Positive	OXA-CIP-FOX
1	ENT	R-21	Negative	Negative	E
10	Thorax	R-21	Positive	Positive	CLI-LEV-CIP-GM-OXA-FOX
41	NICU	R-21	Positive	Negative	OXA-CLI-FOX
51	Environmental	R-22	Positive	Negative	GM-E-FOX-OXA-CLI
53	Environmental	R-23	Positive	Negative	CLI-E-FOX
57	Environmental	R-23	Positive	Negative	GM-E-FOX-OXA-CLI-LEV-FU
26	Surgery	R-24	Positive	Negative	FOX-GM-CLI-SXT-LEV-CIP-E
31	Emergency	R-24	Positive	Negative	CIP-GM-E-FOX-OXA-CLI-LEV-FU

A total of 60 *S. epidermidis* strains were collected from different wards of Rouhani Hospital. Due to our results the infectious diseases and ICU wards had the highest rate of specimens in comparison to other wards. 53% of isolates were belonged to female gender. By disc diffusion method no resistance was reported to vancomycin and linezolid, while the rate of resistance to oxacillin, gentamicin, cefoxitin, clindamycin, ciprofloxacin and erythromycin were 70.5%, 45.9%, 55.7%, 55.7%, 63.9% and 59% respectively. On the other hand, resistance to trimethoprim/sulfamethoxazole (39.4%), fusidic acid (25.4%) and levofloxacin (41%) was noticeable (Figure 1). The prevalence of *mecA* and *icaD* genes was 85% and 41.6% respectively. There were no significant correlation between detected genes and gender (P-value > 0.05). In addition, twenty-four different RAPD-Types were analysis by 80% similarity cut-off point which indicates the high genotypic diversity in our isolates (Figure 2). Table 2 is shown the complete details of all studied isolates.

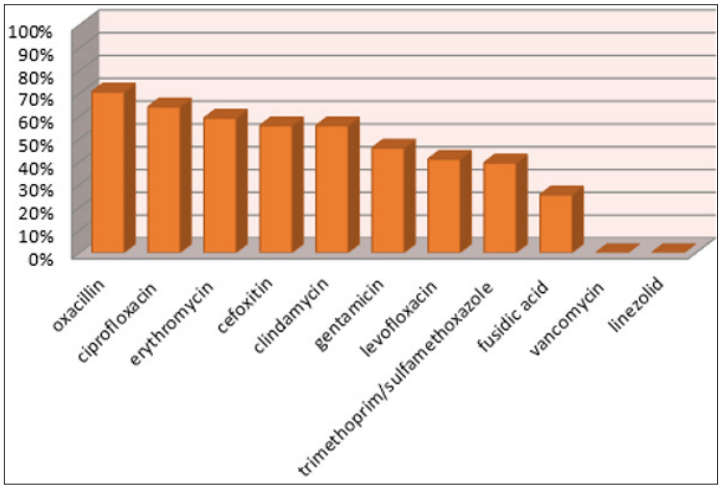


Figure 1: Antibiotic Resistance pattern of *S. epidermidis* strains isolated from Rouhani Hospital.

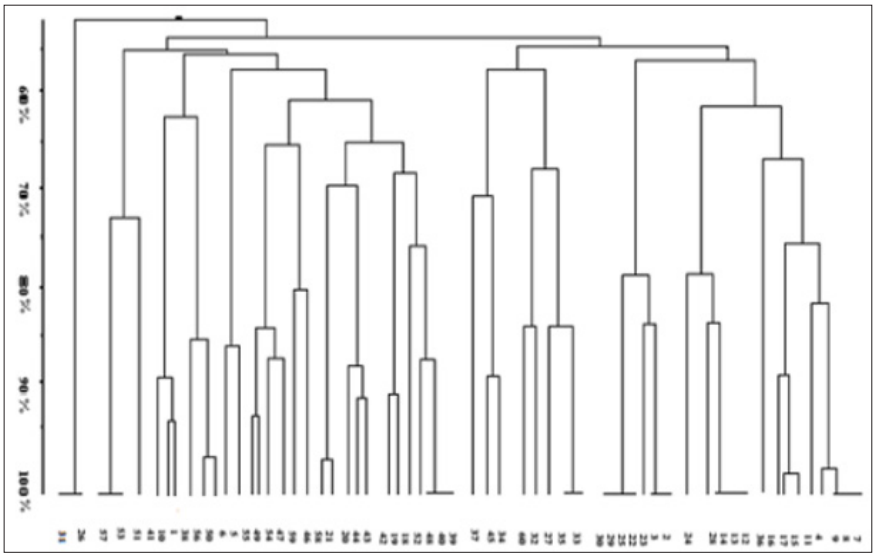


Figure 2: Dendrogram Cluster Analysis of RAPD-PCR Data for 60 *S. epidermidis* Isolates.

Discussion

According to the results, the presence of *mecA* and *icaD* genes among S. epidermidis strains obtained from Rouhani Hospital in Babol was 85% and 41.6%, respectively. The frequency of these genes among similar studies is reported differently. In a study conducted by Najar-Peerayeh et al. The presence of the *mecA* gene was 92.2% [11]. In the study of Pourmand et al., the frequency of *mecA* gene was reported 95%, which is higher than of all conducted studies in Iran. Also, resistance to clindamycin and erythromycin in this study was 77% and 79%, respectively which is higher than our study [12]. Moreover, in Pishva et al. research in Al-Zahra Hospital in Isfahan, 75.3% of *S. epidermidis* strains harbored *mecA* gene [13]. In a study by Soroush et al., on 80 *S. epidermidis* strains which was collected from children, 41% had *icaD* gene. In this study 90% of isolates were found multi-drug resistance, in which the rate of resistance to co-trimoxazole was reported 91.2%. On the other hand, no resistance was observed to linezolid and vancomycin [14].

Moreover, the RAPD-PCR results revealed that RAPD-Type 1 with 5 member followed by RAPD-Type 4, 7, 12 and 18 with four member were the main clusters in current study in comparison

to RAPD-Type 3, 5, 11, 13 and 22. These results indicated that the variety of *S. epidermidis* strains in different wards of the Rouhani Hospital in Babol was high, consequently, due to the wide range of pathogenic factors, *S. epidermidis* related infections is expected in different wards of the hospital. According to (Table 2), the RAPD-PCR method showed that a number of RAPD-Types had the same genotypic profiles and also the same antimicrobial resistance patterns such as RAPD-Type 1, 4, 7, etc. while, the isolation wards were different. There is a significant genetic relationship between the member of some clusters for instance RAPD-Type 4. However, a number of RAPD-Types (9, 12, 16, 17, 18 and 20) illustrated that there was coloration between clinical and environmental isolates. Due to our results, the bacterial infection between various wards and also staff hands and patients is a major concern. Although much progress has been made in molecular typing and more precise methods have been developed, such as PFGE and MLST, but RAPD-PCR has maintained its importance as a reliable, cost-effective and user-friendly method. We used RAPD-PCR in this study to find the phylogenetic relationship between *S. epidermidis* obtained from the Rouhani Hospital in Babol.

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