Short Communication

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Antibiotic Susceptibility Profile and Erythromycin Resistance Genes in the Staphylococcus Epidermidis Strains Isolated by Multiplex-PCR

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ABSTRACT

BACKGROUND AND OBJECTIVE: Staphylococcus epidermidis is one of the most common coagulase negative Staphylococcal strains and has an important role in the infection related to medical device in human and is a crucial public in the antibiotic therapy. The aim of this study was to identify genes erythromycin resistant Staphylococcus epidermidis is isolated from children with Multiplex-PCR method.

METHODS: In the cross-sectional study, a total of 60 Staphylococcus epidermidis were collected from the Amir-Kabir Hospital in Arak. Antibiotic susceptibility test was performed on the Muller Hinton agar according to the clinical and laboratory standard institute (CLSI). Then all strains were evaluated for ermA and ermC genes by multiplex-PCR method.

FINDINGS: In the present study, the highest and lowest samples were related to urinary catheter (31 strains, 51.6%) and wound samples (11 isolates, 18.3%). All isolates were susceptible to vancomycin. The prevalence of ermA and ermC genes were 4 (6.6%) and 45 (75%), respectively. the results showed that the highest and lowest strains carried these genes were ermC and ermA, respectively.

CONCLUSION: Control of transmission of the microorganisms are important infection control and classification methods phenotypic and genotypic diagnosis of clonality of isolates and better control they will be very beneficial.

KEY WORDS: *Staphylococcus epidermidis, erm genes, Multiplex PCR.*

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Introduction

Negative coagulase staphylococci are among the most abundant microorganisms isolated from clinical specimens in medical laboratories (1). Due to the presence of these organisms as normal skin flora and human mucous membranes, their separation from patient specimens was considered as a culture contamination, while in the past two decades, these bacteria have become more important as random pathogens, especially in the hospital environment. Today, more than 30% of cases of hospital bacteremia are caused by CoNS (2). These bacteria usually cause infections in infants and people with immune deficiency, and the infections caused by these organisms are mainly related to external devices inside the body such as intravenous catheters, cerebrospinal shunt, artificial heart valves And artificial joints (3). Erythromycin, an antibiotic, is a macrolide group indicator that consists of a lactone ring attached to two sugars. Erythromycin is one of the inhibitors of protein production in bacteria and is considered as a selective drug for the treatment of various infections such as Staphylococcus epidermidis, Haemophilus influenzae, Mycoplasma pneumoniae, Corynebacterium diphtheria and etc (4).

Resistance to this antibiotic can be constructive or inducible, other macrolides of dissonamine cladinosis are derived from erythromycin and include Clarithromycin, Roxithromycin, Azithromycin, Dithiromicin, there are two important mechanisms in resistance to macrolides, there are two important mechanisms in resistance to macrolides, the most important of which is the change in the target position caused by erm coded genes and resistance to macrolides and lincosamides, which results in streptogramin B (MLSB) and an efflux pump system located in the membrane which encoded by the mef (A/E), msr genes, the erm genes are responsible for coding methyltransferases (5, 6).

These enzymes induce the demethylation of an adenine group (2059A/2058A) from the second V at the site of the peptidyl transferase of 23S ribosomal subunit, which ultimately reduces the binding affinity and induces resistance to macrolides, lincosamides, and streptogramin B (MLSB). The erm genes have been identified in many transposons (7). Ionic pumps consist of twelve sections that pass through the width of the cytoplasmic membrane and interfere with the generation of energy and proton driving forces. The mefA ion pump has several substrates, such as erythromycin and its derivatives such as azithromycin (8). Recent studies on staphylococci have shown that the resistance of staphylococci to erythromycin (ery) is higher than 70-80%. Most of the erythromycin-resistant isolated staphylococci isolates that have been studied have ermA, ermC and msr genes, and the ermC gene is the most abundant (higher than 70%) and msr has the least gene combination with erm genes (9). The frequency of ermA and ermC genes is also dependent on the geographic region (10). Today, it is thought that treating patients with Staphylococcus with induction resistance may ق‌esulting in structural resistance in addition to treatment failure (11). Therefore, the aim of this study was to identify erythromycin-resistant genes in Staphylococcus epidermidis using Multiplex-PCR and to determine the pattern of drug resistance in these strains.

Methods

In this cross-sectional study, 60 non-repeat samples including blood, urinary tract, ulcer and sputum were collected from Amir Kabir's educational hospital in Arak for 7 months (from the beginning of April to the end of November, 2016). After transferring the specimens onto blood agar media (Merck, Germany), the strains were identified using standard biochemical and microbiological methods such as gram staining, catalase, coagulase, and mannitol fermentation using Manitol Salt Agar (MSA), DNase test, sensitivity to basitracin and resistance to novobiocin. An antibiotic susceptibility test was performed using the Kirby-Baer diffusion method according to the CLSI instruction on the Muller Hinton Agar (12).

Antibiotic resistance was performed for all isolates of Staphylococcus epidermidis using Cefoxitin 30 µg, gentamicin 30µg, vancomycin 30µg, penicillin 10IU, clindamycin 2µg, ciprofloxacin 5µg, oxacillin 1µg and erythromycin 15µg. For this purpose, the disks were placed by sterilized forceps with appropriate distance from each other on the Muller Hinton Agar medium and then incubated for 24 hours at 37 °C. Then the diameter of the inhibition zone of bacterial growth was measured and compared with the standard table (12). Primers were ordered to the Pishgam Company to perform PCR. Genomic DNA was extracted from strains cultured in BHI medium using CinnaPure-DNA kit (Gram positive). To confirm the purity of the DNA extracted nanodrop spectrophotometer (THERMO, USA) was used and the ratio of the absorbance ratio of

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260 A/280 A in micrograms of DNA per ml was calculated. Also, to evaluate the integrity of the extracted DNA from the samples, electrophoresis was performed on 1% agarose gel. The oligonucleotide sequence of primers in table 1 was used (13).

Table1. Primers used in this study

PCR size(bp)	Primer sequences (5'→3')	Gene
421	F=5'-GTTCAAGAACAATCAATACA GAG-3' R=5'-GGATCAGGAAAAGGACATTTTAC-3'	*ermA*
572	F=5'-GCTAATATTGTTTAAATCGTCAATTCC-3' R=5'-GGATCAGGAAAAGGACATTTTAC-3'	*ermC*

Following the BLAST primers selected on the NCBI site, the Multiplex-PCR reaction to a final volume of 25 µl containing 5.5 µl PCR master mix 5X (Sinoclon, Iran) containing Taq DNA polymerase (0.05 U/µl), MgCl2 (3 mM) and (0.04 mM) dNTPs, 1 µl of each primer at a concentration of 0.8 µm, 1 µL of a template DNA (10 ng) and 10.5 µl of sterilized distilled water using a thermocycler gradient (Epandorf, Germany) for 34 cycles was followed by the selection of the related program as follows: First step, secondary denaturation, 94 degrees Celsius 50 seconds Second Step initialization 56°C for 30 seconds, step three initial expansion 72 °C for 2 minutes and final expansion 72 degree was considered for 10 minutes. In the end, M-PCR products were electrophoresed in 1% agarose gel containing ethidium bromide (0.5 µg/ml). The second step was to connect the primer, 56 °C for 30 seconds; the third step was the initial expansion, 72°C for 2 minutes and a final expansion, 72 °C for 10 minutes. In the end, M-PCR products were electrophoresed in 1% Agarose gel containing ethidium bromide (0.5 µg/ml).

Results

From the number of samples received, 60 samples of Staphylococcus epidermidis were identified. The highest number of urinary cord samples (31 strains, 51.7%), the least number of ulcer samples (11 isolates, 18.3%) and 18 samples (30%) is also for blood sample. All 60 studied strains were sensitive to vancomycin. The highest resistance was related to penicillin (table2). The MPCR test to determine whether exsist or not ermC genes and ermA (encode resistance to erythromycin) on each of the 60 strains isolated from clinical cases: the frequency of ermC and ermA genes were 4 strains (6.6%) and 45 isolates (75%). These results showed that the highest and lowest number of carrier strains transmitted to ermC and ermA resistance, respectively (Fig 1).

Table2. Pattern of antibiotic susceptibility testing in isolates under study

Antibiogram results	Resistant(R) N(%)	Semi-sensitive(I)	Sensitive(S) N(%)
Antibiotic disk		N(%)	
Penicillin	60(100)	0(0)	0(0)
Gentamicin	38(63.3)	5(8.3)	17(28.3)
Erythromycin	51(85)	0(0)	9(15)
Ciprofloxacin	46(76.6)	1(1.6)	13(21.6)
Clindamycin	41(68.3)	1(1.7)	18(30)
Oxaziline	23(38.3)	2(3.3)	35(58.4)
Cefoxitin	33(51.6)	1(1.7)	28(46.7)
Vancomycin	0(0)	0(0)	100(100)

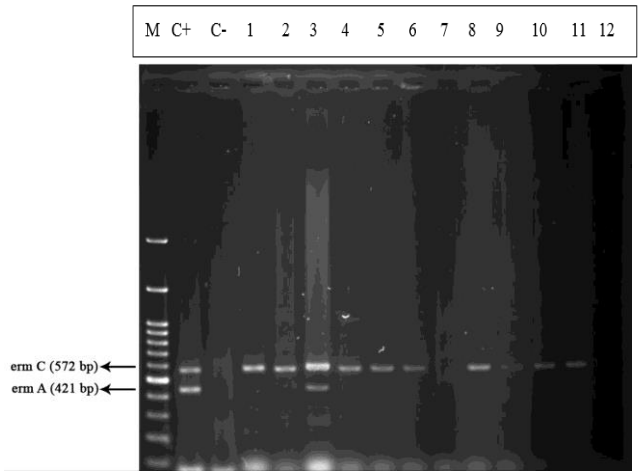

Figure1. This image shows the result of multiple PCR testing in strains 13-24 selected isolates. From right to left, M; DNA marker 100 bp plus (prepared from Formantase Co.), well number 1, positive control (Staphylococcus epidermidis ATCC 12228), wells 1-12 strains obtained from clinical specimens.

Discussion

All strains of Staphylococcus epidermidis were resistant to penicillin antibiotics. Also, the highest levels of resistance for erythromycin, ciprofloxacin, clindamycin and gentamicin were 85%, 76.6%, 68.3% and 63.3% respectively which was consistent with Rahimi et al. (14). All strains were susceptible to Vancomycin, which was consistent with the study by Rahimi et al. (14) and Tavakoli et al. (15). Rahimi et al. reported that the highest resistance of Staphylococcus epidermidis to antibiotics was related

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to penicillin and erythromycin (14). 23 oxacillin-resistant strains were considered as Methicillin-resistant Staphylococcus epidermidis (MRSE), while 33 isolates were considered as MRSE in the screening test for Cefoxitin disks. The Oxacillin disk diffusion test (ODD) showed a lower MRSE phenotype than the Cefoxitin disc. These findings are consistent with the results of Anand et al. (16).

Broekema et al. (17) showed that the Oxacillin disk diffusion test using cefoxitin disk is far superior to other phenotypic tests, such as ODD, Oxacillin Screen agar Testing (OST), and now the approved method to identify methicillin-resistant strains in many reference groups, such as the CLSI. By using the diffusion in the disk criteria and based on the CLSI instruction, the diameter of the inhibition zone for resistant and susceptible cefoxitin strains was 21 ≥ and 22≥ mm, respectively, and its specificity and sensitivity were 100% in all strains, while OST was not suitable. The frequency of ermC and ermA genes were about 4 strains (6.6%) and 45 isolates (75%), respectively. Abdollahi et al. (18) reported the prevalence of ermA and ermC genes in 48 isolates of Staphylococcus coagulase negative resistant to erythromycin about 4.5% and 2.1% respectively. Tavakoli et al. (15) found in their study that of the 150 studied subjects, about 90% had resistance to methysilone, all resistant to clindamycin and erythromycin, and 52.6% (n=79) and 41.3% (69 = n), respectively, had ermA and ermC genes. The researchers found that the ermA gene was the most important factor in resistance to erythromycin. Anand et al in their study reported the prevalence of erm gene for ermA and ermC in Staphylococcus coagulase negative isolates about 41% and 5% respectively. Syrogiannopoulos et al. (19) reviewed and identified erythromycin resistant factors through PCR. Erythromycin resistant agents include erm, efflux pumps and deactivating enzymes. By studying Tavakoli et al. (15), it can be concluded that the frequency of ermC gene in Arak region is high and is the most important factor in resistance to erythromycin. In this study, more than half of the staphylococci were resistant to erythromycin and clindamycin. The prevalence of resistance to a wide range of antibiotics among Staphylococcus epidermidis strains indicates the dispersal and diffusion of these strains in hospitals. Determining the exact pattern of antibiotic resistance can be effective in preventing and treating infections. The results of this study indicate that resistance to erythromycin in Staphylococcus epidermidis is mainly due to the ermC gene.

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