Determination of antibiotic resistance in clinical isolates of coagulase negative Staphylococci from hospitalized patients in selected hospitals of Tehran

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ABSTRACT

To investigate the prevalence of methicillin and aminoglycoside resistance and gene encoding staphylococcal cassette chromosome mec (SCC*mec*) and aminoglycoside modifying enzymes in clinical isolates of coagulase negative staphylococci(CoNS) from hospitalized patients. One hundred and three isolates of coagulase negative staphylococci(CoNS) were recovered from various clinical samples from August 2013 to November 2014. All the specimens were identified by conventional microbiological methods. These tests were contained colony morphology, gram stain, catalase, slide and tube coagulase. To determine the sensibility of CoNS to antimicrobial components including Cefoxitin(30µg), Tobramycin(10µg), Kanamycin(30µg), Amikacin(30µg) and Gentamicin(10µg), disk diffusion method was performed by Kirby Bauer antibiotic testing. In order to show the presence of methicillin and aminoglycoside resistant CoNS genes, PCR were demonstrated. In our study the rate of resistance to Cefoxitin, Kanamycin, Gentamicin, Tobramycin and Amikacin were 74(71.8%), 54(52,4%), 51(49.5%), 45(43.7%) and 16(15.5%), respectively. Some strains of CoNS have been detected with intermediate resistance to Kanamycin 4(3.9%), Tobramycin and Amikacin 2(1.9%). In our study, the distribution of *mec*A gene among clinical isolates of CoNS was 89(86.4%). The prevalence of aminoglycoside resistance genes like *ant*(4 ')-Ia, *aac*(6 ')/*aph*(2 '') and *aph*(3 ')-IIIa were 89(86.4%), 87(84.5%) and 68(66%), respectively. The rate of coexistence of *aac*(6')-Ie-*aph*(2'') with *aph*(3')-IIIa and *aac*(6')-Ie-*aph*(2'') with *ant*(4')-Ia was 65(63%) and 77(74%), respectively. Resistance to aminoglycosides, develops quickly in coagulase-negative staphylococci from clinical areas where these antimicrobial agents are widely used. Therefore, higher investments should be directed towards identifying coagulase-negative staphylococcus species in healthcare institutions and in the community. Overall, Knowing the epidemiology and antibiotic resistance of CoNS is essential to implement the prevention strategies and reducing antibiotic consumptions.

Keywords: Coagulase negative staphylococcus, Aminoglycoside resistance.

INTRODUCTION

Coagulase negative staphylococcus as a microbial flora which is associated with nosocomial infection due to its connection with medical devices[1]. CoNS can become involved in infection such as heart valves, pacemaker lines and boxes, cerebrospinal shunts, and prosthetic joints. People at high risk for developing infections were considered to be low birth weight infants, and individuals who are immunocompromised by diseases such as cancer and the chemotherapy and dialysis patients[1-3].

High-level resistance to methicillin is caused by the *mec*A gene, which encodes an alternative penicillin-binding protein, PBP 2a (PBP2'), located at the staphylococcal cassette chromosome *mec* (SCC*mec*). Although eighth types of SCC*mec*(I-VIII) have been recognized between staphylococci, types IV and V are currently found in *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*[4, 5]. It has been supposed that SCC*mec* can be transferred between staphylococci like coagulase negative staphylococcus(MRCoNS)[6].

Many anti-staphylococcal therapies inhibit protein biosynthesis like aminoglycosides which play an important role in perish of bacteria[7, 8]. The main mechanism of aminoglycoside resistance is drug

inactivation by cellular aminoglycoside-modifying enzymes. Modifying enzymes have been encoded with several distinct genes including aminoglycoside-6'-N-acetyltransferase/2''-O-phosphoryltransferase [AAC(6')/APH(2'')], aminoglycoside-4'-O-nucleotidyltransferaseI [ANT(4')-I] or aminoglycoside-3'-O-phosphoryltransferase III [APH(3')-III] [9, 10]. Aminoglycosides modified at amino groups by AAC enzymes or at hydroxyl groups by ANT or APH enzymes, lose their ribosome-binding ability and inhibit protein synthesis[11]. The bifunctional enzyme, AAC(6')/APH(2''), is encoded on composite transposon Tn4001, mediates resistance to gentamicin and concomitant resistance to tobramycin and kanamycin in coagulase negative staphylococcus[11]. ANT(4')-I , is often carried on small plasmids, and then integrated into larger conjugative plasmids, such as pSK41, and subsequently into the mec region of the chromosome of some S. aureus isolates, mediates resistance to neomycin, kanamycin, tobramycin and amikacin in staphylococci[11]. According to APH(3')-III enzyme can mediate resistance to neomycin and kanamycin, it may be located on both the chromosome and plasmids[12]. In an attempt to determine the prevalence of methicillin and aminoglycoside resistance and gene encoding staphylococcal cassette chromosome mec (SCC*mec*) and aminoglycoside modifying enzymes in clinical isolates of coagulase negative staphylococci(CoNS) from hospitalized patients.

MATERIALS AND METHODS

Bacterial strains

From August 2013 to November 2014, 103 coagulase negative staphylococcus that were isolated from males 58(56.3%) and female 45(43.7%) were recovered from clinical samples including blood, wound, pus, urine, Central nervous system, catheter, sputum, bronchoalveolar, prosthetic joints and fluid body from hospitalized patients in Taleghani, Mofid, khatam, Motahari, Sasan and Pars hospitals, Tehran, Iran. The isolates were identified by their phenotypic characteristic like colony morphology, gram stain, catalase, slide and tube coagulase.

Identification and susceptibility testing

All isolates were tested, using a disc diffusion method, for resistance to Gentamicin(10µg), Tobramycin(10µg), Kanamycin(30µg), Amikacin(30µg) and Cefoxitin(30µg) (MAST, Merseyside. U.K) according to the guidelines provided by the CLSI[13].

DNA Extraction and Amplification

Total DNA template was extracted by Kit(Qiagen N.V.). The primers used for PCR amplification are presented in table one. The PCR reactions were carried out in Intellectica thermo cycler by using the PCR Master Kit (Cinna Clone Inc., Iran) according to the manufacturer guideline. PCR condition was as follows: Initial denaturation at 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 1 minute, annealing for *mec*A gene 1 minute at 57°C,*aac*(6')/*aph*(2'') 45 second at 56°C, *ant*(4')-I 30 second at 48°C and *aph*(3')-III 45 second at 57.5°C , extension at 72°C for 30 second.

The final extension step was continued for another *mec*A gene 8 minutes at 72°C and the rest of aminoglycoside gene 1 minutes at 72°C. DNA fragments were analyzed by electrophoresis in a 1% agarose gel at 95 V for 45 minute in 1X TBE containing ethidium bromide.

Statistical Analysis

Statistical analysis was performed using SPSS software for Windows, version 17.0 (SPSS Inc., Chicago, IL) and Chi-squared test.

Table1. PCR primers used to detect aminoglycoside resistance genes in this study

Primer (5' → 3')	Amplicon size(bp)	gene
GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	*mec*A
AATCGGTAGAAGCCCAA GCACCTGCCATTGCTA	135	ant(4´)-Ia
CCAAGAGCAATAAGGGCATACC CACACTATCATAACCACT	222	aac(6´)-Ie/aph(2˝)
CTTGATCGAAAAATACCGCTGC TCATACTCTTCCGAGCAAA	269	aph(3´)-IIIa

RESULTS

A total of 103 strains of coagulase negative staphylococcus(blood=27, wound=37, pus=1, urine=15, CNS=2, catheter=4, sputum=9, bronchoalveolar=2, prosthetic joints=1 and fluid body=5) were recovered from six hospitals of Tehran, Iran. The rates of resistance to different antibiotic are shown in Table 2.

Table 2. Antimicrobial susceptibilities of 103 coagulase-negative staphylococci isolates to 6 antibiotic in

Hospital	Antibiotics No.(%)				
	Tobramycin	Kanamycin	Amikacin	Gentamicin	Cefoxitin
K	17(65.4)	20(76.9)	20(76.9)	20(76.9)	25(96.2)
M	7(30.4)	7(30.4)	1(4.3)	8(34.8)	12(52.2)
P	8(33.3)	9(37.5)	4(16.7)	8(33.3)	18(75)
MO	6(46.2)	8(61.5)	2(15.4)	7(53.8)	9(69.2)
S	1(11.1)	3(33.3)	0	2(22.2)	3(33.3)
T	6(75)	7(87.5)	3(37.5)	6(75)	7(87.5)
Total	45(43.5)	54(54.5)	30(25.1)	51(49.3)	74(68.9)

K= Khatam, M= Motahari, P= Pars, MO= Mofid, S=Sasan and T= Taleghani

Some strains of CoNS have been detected with intermediate resistance to Kanamycin 4(3.9%), Tobramycin and Amikacin 2(1.9%).

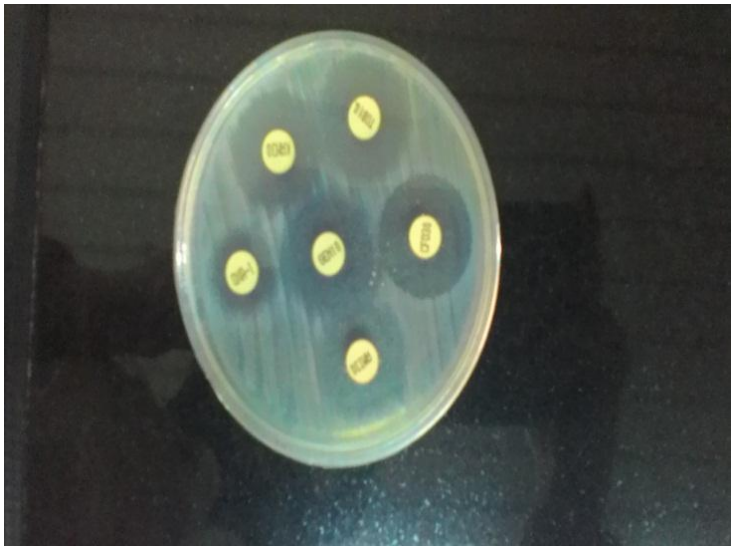

Figure 1. Antimicrobial susceptibility testing CoNS isolates

According to table 1, the rate of resistance to kanamycin, Gentamicin, Tobramycin and Amikacin were 54.5% , 49,3%, 43.5% and 25.1%, respectively. Also, regarding to table 2, the proportion of MRCNS which showed resistance to the aminoglycosides in this study, was higher than that of the MSCNS isolates. There was no significant difference between aminoglycoside and their sites of isolation. since more than half of the isolates carrying *mec* A gene, the relationship between methicillin and aminoglycoside resistance was noticeable (table 2). The most prevalence of aminoglycoside resistance genes amongst isolates of coagulase negative staphylococcus was *ant*(4')-I, occurring in 87.6% of MRCNS. However, The rate of *aac*(6')/*aph*(2'') was pretty great. The least common was *aph*(3')-III, found in 66.3% of MRCNS. The rate of coexistence of *aac*(6')-Ie-*aph*(2'') with *aph*(3')-IIIa and *aac*(6')-Ie-*aph*(2'') with *ant*(4')-Ia was 65(63%) and 77(74%), respectively.

Table 3. The distribution of aminoglycoside resistance in isolates of coagulase-negative staphylococci (CNS) in relation to methicillin resistance and sites of infection

sites of isolation	Tobramycin	Kanamycin	Amikacin	Gentamicin
MSCNS				
wound	0	1(6.3)	0	1(6.3)
Blood	2(40)	4(80)	1(20)	3(60)
urine	1(25)	1(25)	0	1(25)
sputum	2(100)	2(100)	1(50)	1(50)
Fluid body	1(50)	1(50)	1(50)	1(50)
catheter	0	1(100)	0	1(100)
Total	6(35.8)	10(60.2)	3(20)	8(48.5)
MRCNS				
wound	13(36.1)	13(36.1)	3(8.3)	13(36.1)
Blood	15(68.2)	16(72.7)	8(36.4)	15(68.2)
urine	4(26.7)	5(33.3)	2(13.3)	6(40)
sputum	5(71.4)	7(100)	2(28.6)	6(85.7)
Fluid body	0	1(25)	0	0
catheter	2(66.7)	1(33.3)	0	1(33.3)
BAL	2(100)	2(100)	2(100)	2(100)
CNS	1(50)	1(50)	1(50)	1(50)
prosthetic joints	1(100)	1(100)	1(100)	1(100)
pus	1(100)	1(100)	1(100)	1(100)
Total	44(68.7)	48(65)	20(54.5)	46(68.1)

Table 4. The distribution of aminoglycoside resistance genes in isolates of coagulase negative staphylococci (CNS) in relation to methicillin resistance

Resistant gene	MSSA (n=14)	MRSA (n=89)
aac(6')/aph(2'')	13(92.9)	74(83.1)
aph(3')-III	9(64.3)	59(66.3)
ant(4')-I	11(78.9)	78(87.6)

Abbreviations: MSCNS, methicillin susceptible CNS; MRCNS, methicillin-resistant CNS

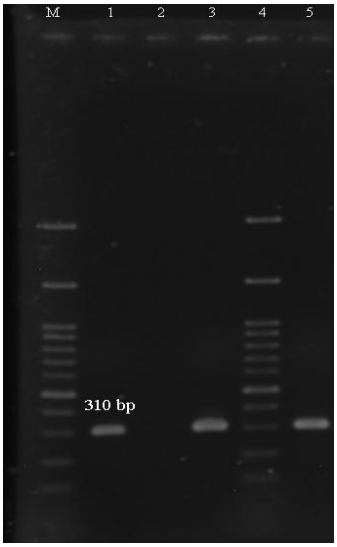

Figure 2. Detection of genes encoding *mec* A gene in coagulase negative staphylococci by PCR. M, 100 pb DNA ladder (Fermentas); lane 1, positive control; lane 2, negative control; lane 3 and 5 patient samples

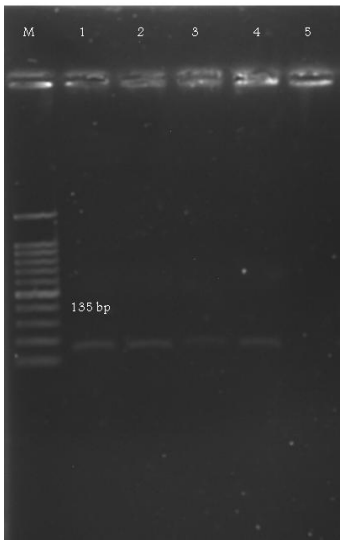

Figure 3. Detection of genes encoding *ant(4')*-I gene in coagulase negative staphylococci by PCR. M, 100 pb DNA ladder (Fermentas); lane 1, positive control; lane 5, negative control; lane 2-4 patient samples

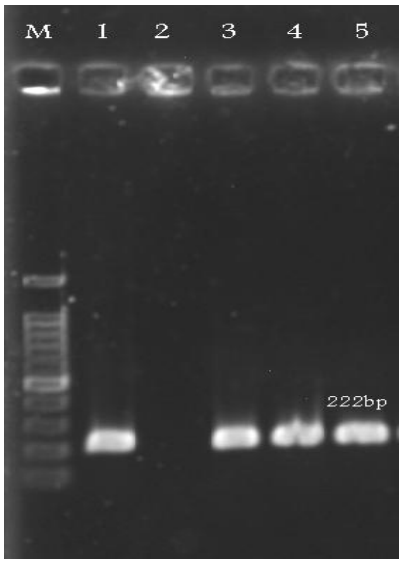

Figure 3. Detection of genes encoding *aac(6')-aph(2'')*gene in coagulase negative staphylococci by PCR. M, 100 pb DNA ladder (Fermentas); lane 1, positive control; lane 2, negative control; lane 3 and 5 patient samples

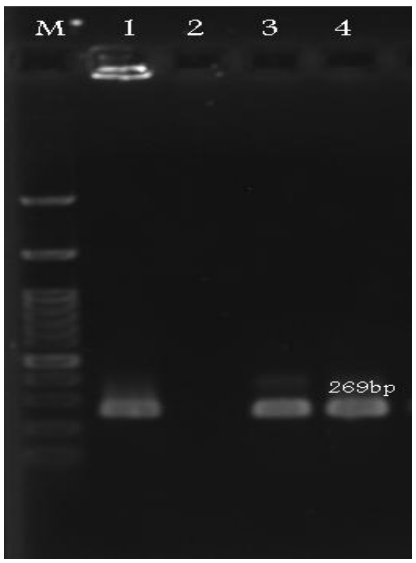

Figure 4. Detection of genes encoding *aph(3')*-III in coagulase negative staphylococci by PCR. M, 100 pb DNA ladder (Fermentas); lane 1, positive control; lane 2, negative control; lane 3 and 4 patient samples

DISCUSSION

Excessive consume of different antibiotic is caused to emerge multi-drug resistance in developing countries[14]. Aminoglycosides have been an important group of antibiotics in treatment of serious bacterial infections, especially gram negative bacteria, but current reports indicated the emergence of resistance to aminoglycosides in Coagulase negative staphylococcus isolates in different parts of the world[15]. The goal of this study was to investigate the prevalence of methicillin and aminoglycoside resistance and gene encoding staphylococcal cassette chromosome mec (SCC*mec*) and aminoglycoside modifying enzymes in clinical isolates of coagulase negative staphylococci(CoNS) from hospitalized patients.

The methicillin resistance rate of 86%(89/103) among our CoNS isolates was approximately 13% higher than those reported among Colombia, Brazil and Egyptian medical centers[16-18]. In the current study, despite many studies which had shown the rate of *aac*(6')/*aph*(2'') gene was more common than other ones[19-21], In this study the most prevalence of aminoglycoside resistance genes between our isolates was ant(4')-I (87.6%) that this result was similar to reports from Kuwait and Japan[22, 23].

The second most prevalent AMEs gene was *aac*(6')-Ie-*aph*(2''), this gene enables to inactivate gentamicin, kanamycin, tobramycin, neomycin, and amikacin[24]. The third one was *aph*(3)-IIIa. Although this gene, which causes to inactivate kanamycin and amikacin[24], was the lowest in our article, its amount was higher than the study had been done in different studies[20, 25].

We detected the probable coexistence of all three enzymes in vast majority of our isolates, as did two researchers in their study[23]. According to the existence of two *mec*A and AMEs genes, some studies have shown, there is a correlation between aminoglycoside and methicillin resistance that leading to spread of multi drug resistant (MDR) isolates [19, 23, 25].

High level of resistance among CNS isolates and spread of MDR isolates is threat for hospitalized patients and limits the use of antimicrobial agents for therapy [26].

In conclusion, continuous surveillance should be directed towards identifying coagulase-negative staphylococcus species in healthcare institutions and in the community and also, prescribing antibiotics including aminoglycoside should be revised.

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