Characterization of coagulase-negative staphylococci isolated from hospitalized patients in Tehran, Iran

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ABSTRACT

Coagulase-negative staphylococci (CNS) are a main cause of nosocomial infection. The main purpose of this study was to determination of frequency of CNS isolates in in hospitalized patients and their susceptibility pattern to antimicrobial agents. During 11 month study, 65 CNS clinical isolates were recovered from hospitalized patients in different wards of hospital. In vitro susceptibility of isolates to 12 antimicrobial agents Penicillin; Ampicilin; Cephalothin; Cefoxitin; linezolid; Nitofurantoin; Erythromycin; Norfloxacin; Gentamicin; Vancomycin; Chloramphenicol and Oxacillin was performed by Kirby-Bauer’s Disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) criteria. Out of 1875 samples of hospitalized patients 65(3.47%) patients were infected with CNS. Twenty one (32.3 %) were isolated from the urine samples, 17(26.1%) from sputum, 15(23.1%) from pus samples, 8(12.3 %) from ear swabs, 3(4.7%) from fluid and 1(1.5%) from blood sample. All of CNS isolates were sensitive to nitrofurantoin. The rates of resistance to the majority of antibiotics tested varied between 4.5% and 100 %. The rate of resistance to beta lactam antibiotics, Chloramphenicol, erythromycin, gentamycin was high (more than 70%). The most of isolates remained susceptible to linezolid, and nitrofurantoin. All of isolates were susceptible to vancomycin. Multi-drug resistant CNS with reduced susceptibility to linezolid and nitrofurantoin are emerging pathogens of clinical concern. Monitoring of antibiotic resistance with attention to multi-resistant profile and aware to practitioners in the field is necessary.

Keywords: Coagulase-negative staphylococci; Antimicrobial susceptibility; Nosocomial infection.

INTRODUCTION

Nosocomial infections are important public health problems in developing countries as well as in developed countries. Nosocomial or hospital-acquired infections are usually defined as infections that are identified at least 48-72 hours following admission to hospital and health care facility [1]. The most frequent types of nosocomial infections are bloodstream infection (BSI), urinary tract infection (UTI), pneumonia and surgical-wound infection [2].

Coagulase-negative staphylococci (CNS) are a group of micro-organisms that known as normal biota of human skin and mucous membranes. CNS are consisting of 39 Species and 16 Species of them are known to cause infection in human. Since the 1970s, CNS is recognized as important etiologic agents of a wide variety of human nosocomial infections. They account for 9% of nosocomial infections [4].

The two most frequently encountered CNS species in clinical samples are Staphlococcus epidermidis and Staphlococcus saprophyticus. Overall, S. epidermidis is the predominant agent in nosocomial infection, bacteremia, intravascular catheter-related infections, endocarditis, central nervous system shunt infections, urinary tract infections, ophthalmologic infections, dialysis-related infections and surgical wounds while S. saprophyticus is more associated with urinary tract
infections in young females. Therefore, CNS isolates have received more attention recently as a cause for above mentioned infections [4,5]. The distinguishing between true pathogen and contaminating isolates is very difficult and remains a main problem. Accurate species level identification of CNS isolates is expensive and time consuming. According to past researches, identification to species level is not necessary for good patient management and treatment [6]. Nowadays the role of CNS as potentially agent in nosocomial blood stream infections and UTI has been recognized. CNS isolates account for approximately 30% of all nosocomial blood stream infections. Among CNS isolates Staphylococcus epidermidis is most frequently associated with blood stream infection [7]. The use of indwelling medical devices such as central and peripheral venous catheters, artificial heart valves, valvular prostheses, pace-makers and orthopaedic prostheses in patients is the one of main predisposing agents S.epidermidis bacteremia[8]. UTI is one of the most frequent types of nosocomial infections and probably affects about one-half of all people during their lifetimes. Many of clinicians are commonly encountered with UTIs in developing countries. UTI refer to the existence of microbial pathogens in the urinary tract and defined as the growth of a single pathogen of >10^5 colony-forming units per milliliter from properly collected mid-stream urine specimens [9,10]. UTIs are often caused by different bacteria. Bacterial agents are responsible for a spectrum of UTI that can be ranged from mild irritative voiding to bacteremia, sepsis and death. UTIs can often be asymptomatic or asymptomatic [11]. The major causatives of UTIs are Escherichia coli and other Enterobacteriaceae. Although relative frequency of the pathogens varies depending upon age, sex, catheterization and hospitalization but in complicated urinary tract infections and hospitalized patients, Gram-negative rods (Pseudomonas spp) and gram positive cocci (coagulase negative Staphylococci, Staphylococcus aureus, Streptococcus group B, Enterococci) are comparatively more common [12].

Recently, in all over the world, resistance to antibiotics among CNS isolates has been reported. The infections associated with CNS requiring antimicrobial therapy. Inappropriate use of antimicrobial agents in order to treatment of patients recently has led to the spread of antimicrobial resistance among CNS isolates. On the other hand, widespread resistance among CNS isolates is major problem for the empirical treatment of nosocomial infections [11,12].

During the past decade, CNS isolates exhibited a remarkable ability to rapidly develop antibiotic resistance. Area-specific monitoring studies in order to detection of antimicrobial resistance patterns, effective treatment and decrease mortality rates is necessary [12].

Considering lack of information about antimicrobial resistance profiles of CNS clinical isolates and increasing infections, the object of this study was to investigate the frequency of CNS isolates in hospitalized patients and their susceptibility pattern to commonly used antimicrobial agents.

**MATERIALS AND METHODS**

**Bacterial isolates**

The present descriptive study was performed on cases who were hospitalized in different wards of hospital. A total of 65 clinically significant CNS isolates collected from different clinical samples for a period of 11 months from during Jun 2012 to May 2013. CNSs were isolated from sputum, blood, pus, urine and body fluid samples. Isolates were diagnosed as true pathogen when isolated in pure culture from infected sites.

UTI refer to the existence of microbial pathogens in the urinary tract and defined as the growth of a single pathogen of >10^5 colony-forming units per milliliter (CFU/ml) from properly collected mid-stream urine specimens [13]. All the cases had history of nosocomial infection and clinical examination was conducted by physician to exclude community-acquired infections.

The plates were incubated in aerobic conditions at 37°C for 24–48 hours. Negative cultures were maintained in incubator up to 2 days. Identification of specimens was performed by Gram staining, catalase, manitol fermentation and coagulase tests and other conventional biochemical tests. Coagulase test was done both slide and tube methods [14, 15]. Samples confirmed as a CNS isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C and were subjected to further investigation.
Antimicrobial susceptibility testing

To evaluate antimicrobial susceptibility of isolates Kirby-Bauer’s Disk diffusion method was done according Clinical Laboratory and Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) criteria [16]. The following antimicrobial agents were used in this study: Penicillin; Ampicillin; Cephalothin; Cefoxitin; linezolid; Nitrofurantoin; Erythromycin; Norfloxacin; Gentamicin; Vancomycin; Chloramphenicol and Oxacillin. Antibiotic disks used in this research were supplied by MAST Laboratories Ltd (Bootle, Merseyside, UK). Briefly, the bacterial suspension obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). Diameter of inhibition zones was measured after incubation at 35°C for 18-24 hours, and data were reported as susceptible, intermediate, and resistant. Staphylococcus aureus ATCC 25923 was used as reference strains for susceptibility testing. Data were analyzed using SPSS version 13 software.

RESULTS

In this study, a total of 1875 samples of hospitalized patients were screened during a period of 11 months. 65(3.47%) patients were infected with CNS of which there were 36 (55.4%) females and 29 (44.6%) males. The age range of the patients was from 3 to 72 years. Among 65 isolates of CNS, 21 (32.3%) were isolated from the urine samples, 17(26.1%) from sputum, 15(23.1%) from pus samples, 8(12.3%) from ear swabs, 3(4.7%) from fluid and 1(1.5%) from blood sample. All isolates of CNS were negative for free and tube coagulase.

Occurrence of infection with CNS was highest in the age group 21-45 year (60%) and the lowest in the age less than or equal to 20 years (3.1%). In males, majority of the CNS 18(27.6) found in the age group 21-45 years and in females, 21(32.4) in found in age group 25-32 years. Frequency of CNS in different age groups is shown in Table 1. The patients were distributed in 6 hospital department. Twenty-four isolates (36.9%) of CNS was obtained from internal medicine, 14 isolates (21.6%) from infectious ward, 10 isolates (15.4%) from surgical ward, 8 isolates (12.3%) from gastroenterology, 6 isolates (9.2%) from Intensive Care Unit and 3 isolates (4.6%) from Pediatric ward.

No significant difference was found between isolated bacteria and age of the patients. The profile of isolated bacterial showed wide different level of resistance for tested antibiotics (Data are shown in Table 2). Antibiotic susceptibility testing of the isolates showed maximum resistance to penicillin (95.4%) and ampicillin (89.2%) followed by gentamycin (86.2%), cefoxitin (84.7%), cefalotin (83%), erythromycin (76.9%), chloramphenicol (73.8%), norfloxacin (69.2%), oxacillin (58.5%), linezolid (23.1%) and nitrofurantoin (3.1%). All of isolates were sensitive to vancomycin. Multidrug-resistant (MDR) was defined as resistance to at least three or more antibiotics (17,18). Of 65 isolates tested 51(47.8%) were MDR. Frequencies of MDR to four, five, six or more antibiotics were 19(37.2%), 15(29.4%), 12(23.5%) and 5(9.8%). MDR strains to three or more tested antibiotics were isolates from hospitalized patients in internal medicine, surgery, infectious, ICU and gastroenterology wards respectively. The predominant resistance profile among our isolates were included resistance to 4 antibiotics (penicillin, Ampicillin, Gentamicin ad cefalotin), 5 antibiotics (penicillin, Ampicillin, Gentamicin, cefoxitin and chloramphenicol) and 6 antibiotics (penicillin, Ampicillin, Gentamicin, cefoxitin, chloramphenicol and erythromycin) which were common among 14(27.4%), 11 (21.6%) and 7(13.7%) isolates.

Table 1. Frequency of CNS in different age groups

<table>
<thead>
<tr>
<th>Gender</th>
<th>&lt;20 N(%)</th>
<th>21-45 N(%)</th>
<th>46-60 N(%)</th>
<th>&gt;60 N(%)</th>
<th>Total N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1(1.55)</td>
<td>18(27.6)</td>
<td>6(9.3)</td>
<td>4(6.1)</td>
<td>29(44.6)</td>
</tr>
<tr>
<td>Female</td>
<td>1(1.55)</td>
<td>21(32.4)</td>
<td>8(12.3)</td>
<td>6(9.2)</td>
<td>36(55.4)</td>
</tr>
<tr>
<td>Total</td>
<td>2(3.1)</td>
<td>39(60)</td>
<td>14(21.6)</td>
<td>10(15.3)</td>
<td>65(100)</td>
</tr>
</tbody>
</table>
DISCUSSION

Nosocomial infection is a global problem that affects both developed and developing countries. Recent studies have revealed the importance of CNS as one of the causes of nosocomial or healthcare related infections. Many of nosocomial infections are associated with microorganisms that are resistant to antibiotics and can easily spread by hospital environment and personnel. Monitoring of antimicrobial susceptibility can aid to clinicians for prescript appropriate antibiotics and prevent the development of drug resistance [15]. Effective treatment of patients with UTI and blood stream infections associated with CNS commonly relays on the identification of the type of organisms and the selection of an effective antibiotic agent to the organism in question. The pattern of antimicrobial resistance of CNS producing infection varies in different regions and especially different wards [16].

In this study, the frequency of CNS isolated from hospitalized patient in different wards was 65(3.47%) of which there were 36 (55.4%) females and 29 (44.6%) males. Among different clinical samples, CNS isolates were mainly isolated from 21 (32.3 %) urine samples in female.

This result is consistent with the results of recent studies in India [17,18]. In a study done by Vaez et al in 2012, CNS was mostly isolated from blood cultures that are in contrary with our study (Iran5). In another study done by Banerjee et al, 72 of 150 strains of CNS (60%) were isolated from blood samples, 36(24%) from pus samples, 15(10%) from urinary catheter tip and 12(8%) from the urine samples [19].

High frequency of UTI associated with CNS in our study, may be attributed to contamination during sampling, anatomy and microflora in genitourinary system, host factors, hygiene practices and healthcare practices. The sex distribution of patients in our study mainly was female (55.4%) between 21 to 45 years. This result is similar to those reported from many other researchers [16].

In this study, we investigated the frequency and antimicrobial susceptibility patterns of CNS isolated from hospitalized patients in Tehran, Iran.

This study revealed that all of CNS isolates were fully sensitive to vancomycin. Our finding about vancomycin is in accordance with studies done in India 2012, Spain 2002, England 2004, Asia-Pacific region 2007, USA 2007 and Turkey 2007[20-24]

In our study, the highest resistance rate of the CNS was against penicillin (95.4%) followed by ampicillin (89.2%) followed by gentamycin (86.2%), cefoxitin(84.7%),cefalotin(83%),erythromycin(76 .9%), chloramphenicol (73.8%), norfloxacin (69.2%) and oxacillin(58.5%). This study correlates with a study by Asangi et al, where the antibiotic susceptibility testing showed maximum resistance to penicillin and ampicillin with 85%-95%. In study Sader et al. 85%-95% of CNS were resistance to ampicillin and penicillin. High resistances to these antibiotics have been reported by several researchers [17]. Resistance to

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Susceptible(%)</th>
<th>Intermediate(%)</th>
<th>Resistance(%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>2(3.1)</td>
<td>1(1.5)</td>
<td>62(95.4)</td>
<td>65(100)</td>
</tr>
<tr>
<td>ampicillin</td>
<td>5(7.7)</td>
<td>2(3.1)</td>
<td>58(89.2)</td>
<td>65(100)</td>
</tr>
<tr>
<td>gentamycin</td>
<td>7(10.7)</td>
<td>2(3.1)</td>
<td>56(86.2)</td>
<td>65(100)</td>
</tr>
<tr>
<td>cefoxitin</td>
<td>7(10.7)</td>
<td>3(4.6)</td>
<td>55(84.7)</td>
<td>65(100)</td>
</tr>
<tr>
<td>cefalotin</td>
<td>10(15.5)</td>
<td>1(1.5)</td>
<td>54(83)</td>
<td>65(100)</td>
</tr>
<tr>
<td>erythromycin</td>
<td>12(18.5)</td>
<td>3(4.6)</td>
<td>50(76.9)</td>
<td>65(100)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>10(15.5)</td>
<td>7(10.7)</td>
<td>48(73.8)</td>
<td>65(100)</td>
</tr>
<tr>
<td>norfloxacin</td>
<td>16(24.6)</td>
<td>4(6.2)</td>
<td>45(69.2)</td>
<td>65(100)</td>
</tr>
<tr>
<td>oxacillin</td>
<td>22(33.8)</td>
<td>5(7.7)</td>
<td>38(58.5)</td>
<td>65(100)</td>
</tr>
<tr>
<td>linezolid</td>
<td>48(73.8)</td>
<td>2(3.1)</td>
<td>15(23.1)</td>
<td>65(100)</td>
</tr>
<tr>
<td>nitofurantoin</td>
<td>63(96.9)</td>
<td>0(0)</td>
<td>2(3.1)</td>
<td>65(100)</td>
</tr>
<tr>
<td>vancomycin</td>
<td>65(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>65(100)</td>
</tr>
</tbody>
</table>

Table 2. Frequency and antimicrobial susceptibility pattern of 65 CNS isolates to 12 antimicrobial agents
Resistance to ampicillin, penicillin, cefoxitin and cefalotin among our isolates may be related to improper usage of this antibiotic for treatment of other infections, increase use of other beta lactam antibiotics in hospital and acquisition of resistant during hospitalization.

Resistances to erythromycin have been reported differently by several researchers. In Spain, resistance to erythromycin increased progressively in CNS from 41% in 1986 to 63% in 2002 [20]. Asangi et al. showed high level of resistance to erythromycin among CNS isolates [17]. In two surveillance studies, performed in 2006, in US and Europe CNS isolates had increased resistance rate to erythromycin [25]. The possible reasons of high resistant rate to erythromycin may be related to use of erythromycin in treatment of disease caused by CNS and common infections, increase exposure of this isolates to new macrolide, efflux of the drug and ribosomal methylation. Cross resistance between clindamycin and macrolides is well described by several investigators.

The resistance rate to linzolid was 23.1% in our study, which was higher than Spain, US and Turkey, Englands, Australia and Irland [20-26]. The resistance rate to gentamicin in among CNS isolates was higher than other studies [27,28]. The data from our investigation exhibited that ampicillin, penicillin, cefoxitin, cefalotin, gentamicin, norfloxacin and erythromycin had not good activity against CNS isolates.

Surprisingly, in our study the resistance to linezolid in comparison with other studies done by several researcher was relatively high [4, 24,29, 30] which the reason for resistance to these antibiotics could be mediated by their widespread use in the hospital and the community.

A high incidence of MDR strains was found in internal medicine and infectious wards. It should not be ignored that MDR strains of CNS can serve as a reservoir of resistance genes and can spread to the other microorganisms. Therefore, in order to prevent further spread of multi-drug resistant CNS, the use of antibiotics should be monitored and implementation of infection control. In the other hand, continued use of antibiotic for treatment of infections associated with CNS isolates should be supported by monitoring of antimicrobial susceptibility to prevent the spread of resistant isolates and also eliminate the use of antibiotics for a prolonged period.

Although resistant to linzolid and nitrofurantoin has seen among our isolates but it seems that them can be effective drugs for treatment of infections associated with CNS isolates. According to our findings, ampicillin, penicillin, cefoxitin, cefalotin, gentamicin, norfloxacin and erythromycin are not effective drugs for treatment of infections associated with CNS. Progressive increase in resistant to these antibiotics and multiple resistances in present study, may be related to increased usage of these antibiotics for treatment and also ability of strains in acquisition of resistance genes to other organisms of different species. Our investigation also exhibited that use of linzolid in order to decrease spread of resistance gene among CNS isolates must be revised.

In conclusion, high level of resistance among CNS isolates limits the use of antimicrobial agents for therapy and also the spread of MDR isolates is threat for hospitalized patients. Continuous Surveillance for multidrug-resistant strains is necessary to prevent the further spread of resistant isolates.

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