Prevalence of Oxacillin-Resistant Coagulase-Negative Staphylococci Strains Isolated from Nosocomial Bloodstream Infection

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Accurate detection of oxacillin resistant coagulate negative Staphylococci (CoNS) is very important for the management of infected patients and for selecting the appropriate infection control measures. Accordingly, different methods for phenotypic characterization of mecA-mediated oxacillin resistance were compared with genotypic reference testing. After identification, the isolates were tested for antimicrobial sensitivity using disc diffusion and automation system methods. The presence of mecA gene was identified by the polymerase chain reaction technique. The mecA gene was detected in 54 (90%) strains, whereas analysis of the sensitivity profiles revealed a high rate of resistance to multiple classes of antimicrobial agents. Analyses of the clinical significance of CoNS isolates represent important factors for the accurate choice of antibiotic therapy.

Key words: Staphylococcus aureus, Nosocomial, Vancomycin, Oxacillin, Cefoxitin.

Nosocomial bloodstream infections (NBSI) are the most common problem in hospitals and represent a serious complication in critically ill patients as newborns and Immunocompromised patients1,2. NBSI represents about 15% of all nosocomial infections and affects approximately 1% of all hospitalized patients. It is associated with high mortality and prolonged hospitalization1,5.

During the 60s and 70s, Gram-negative were more frequently isolated from patients with NBSI. Since then, due to the increased use of antimicrobial agents and increased number of hospitalized immunocompromised patients Gram positive cocci, especially coagulate negative staphylococci (CoNS), have emerged as a major etiological agent of these infections5,8. Also, CoNS staphylococci constitute one of the most common contaminants in blood cultures. For this reason, it is necessary to use different clinical and laboratory criteria to distinguish bacteremia contamination clinically significant5,7,8.

According to Centers for Disease Control and Prevention (CDC), the CoNS are responsible for 20.2% of NBSI. In Egypt, this percentage is between 12% and 20%, and 70% to 90% of isolates are resistant to oxacilina10. This resistance is related to the presence of chromosomal mecA gene, which codes for the production of penicillin binding proteins (PBP) altered, so called PBP2a or PBP2’ with low affinity for β-lactamase antibiotics10.

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In recent years, the substantial increase in the rates of oxacillin resistance in CoNS, has created much concern about accurate laboratory diagnostics, since they are essential guide to therapy and to promote the rational use of glycopeptides antibiotics. As a result, different phenotypic methodologies have been employed to characterize the oxacillin resistance in CoNS. However, the emergence of strains with heterogeneous resistance complicates the interpretation of disk diffusion testing in routine testing.

Since 2004, the Clinical and Laboratory Standards Institute (CLSI) recommended the use of cefoxitin disk (30µg) in conjunction with oxacillin (1µg) for detecting oxacillin resistance mediated by the \textit{mecA} gene. In 2009, the CLSI canceled the oxacillin disk in anticipation of CoNS resistance against \beta-lactamases agents. The detection of the \textit{mecA} gene by molecular methods is considered gold standard for qualitative evaluation of oxacillin resistance. However, these methods are rather prevalent routine diagnostic.

The aim of this study was to evaluate bacteremia and sensitivity profiles of CoNS isolated in blood cultures from patients admitted to the Tanta University Hospital (TUH), Delta, Egypt during the period from December 2010 to January 2012. Furthermore, we compared phenotypic methods with genotypic testing reference in the detection of oxacillin resistance mediated by the \textit{mecA} gene.

**MATERIALS AND METHODS**

**Isolation and identification**

During the period from December 2010 to January 2012, the patients were selected consecutively, 60 samples of isolated CoNS blood cultures performed on the automated system Bactec9240® (Becton Dickinson, Sparks, MD) in patients treated at TUH. The identification of these strains was performed using the system automated MicroScan® (Siemens). No identical isolates from a single patient were included. As a result, the strains were stored in simple broth with 15% glycerol at -20 °C. This study was approved by the Ethics Committee of TUH.

**Inclusion criteria**

Inclusion Strains Criteria were selected from monomicrobial blood cultures which exhibited oxacillin resistance as the least one of the phenotypic tests performed previously (automation and/or disk diffusion).

**Clinical significance of the isolates**

The analysis was done based on the medical records of patients. Variables were evaluated clinically; results were basically tested in laboratory, antimicrobial therapy was active, length of stay and risk factors were related to NBSI (immunosuppression and use of invasive medical devices).

**Sensitivity tests**

The isolates were tested for susceptibility to oxacillin, gentamicin, levofloxacin, erythromycin, clindamycin, ciprofloxacin, rifampicin, cefoxitin and sulfamethoxazole / trimethoprim using disk diffusion method on Mueller Hinton agar (Difco Laboratories, Detroit, Mich.) according to CLSI 2009. Despite prediction of oxacillin resistance was no longer recommended since 2009, oxacillin was included in this study in order to compare its performance with cefoxitin disk. The new antimicrobial susceptibility tigecycline was also evaluated in the disk diffusion technique. However, there is no interpretation criteria defined by CLSI and therefore values were considered to be approved by the U. S. Food and Drug Administration (FDA).

Sensitivity tests using automation MicroScan® (Siemens) were also performed. In this analysis, no cefoxitin and tigecycline were evaluated, but were included in the antimicrobial vancomycin. Micro dilution for vancomycin as document M7 - A6, NCCLS / CLSI 2003 was also performed. The strain of \textit{S. aureus} ATCC43330 was used as control.

**Detection of the \textit{mecA} gene**

**DNA extraction**

Bacterial DNA was extracted according to the method previously described by Ida et al. 2001. Briefly, colonies obtained from overnight \textit{S. aureus} cultures from sheep blood agar were harvested and suspended in 100 ml of lysis solution (20 mM Tris HCl, 140 mM NaCl, 5 mM EDTA [pH 8.0]). Three units of lysostaphine were added and the suspension was incubated at 37°C for 3 hours. 200 ml of distilled water was added and incubated at 95°C for five minutes. Phenolchloroform extraction and ethanol precipitation steps were then performed for DNA...
extraction which was stored at -20 °C until analysis.

**PCR amplification**

Polymerase chain reaction was performed to detect the mecA gene using the methodology previously described by Kearns et al., 1999\(^6\). We used the following pair of primers: F-1 (5'CGG TAA CAT TGA TCG CAA CGT TCA3') and F-2 (5'CTT TGG GAT GCC AAC TAA CAT TCT3') (Ludwig Biotec®). All reactions were carried out with two microliters of DNA and 48µl of the mix, which contained 15µmol of primers, 200µM of deoxyribonucleotides (Ludwig Biotec®) 1x Taq buffer (Ludwig Biotech®) and 5U Taq DNA polymerase (Ludwig Biotech®).

**Evaluation of the pattern of DNA amplicons**

After amplification, 17 µl of PCR sample was loaded on a 1 % (w/v) agarose gel (Bioline, London, UK) containing 0.5 gr/ml ethidium bromide and run in a horizontal gel electrophoresis unit (Mini-Sub DNA cell, BioRad). The running buffer was TAE [40 mM Tris, 20 mM acetic acid, 1 mM ethylene diamine tetra-acetic acid (EDTA), pH 8.0]. Electrophoresis was carried out at 100 V for 2 h on an Amersham- Pharmacia Biotech (Uppsala, Sweden) power supplier unit ECPS3000/150. The stained bands were visualized with UV light (309 nm) using a trans-illuminator and gels were recorded as digital TIFF images using a gel documentation system (UVI-Tech). The positive result for the presence of the mecA gene was demonstrated by the amplification of the fragment of 214 pair base. This was confirmed by the positive control and marker molecular weight.

**RESULTS**

During the period from December 2010 to January 2012, approximately 4,379 blood cultures were requested to the microbiology laboratory analysis of TUH. Of this total, 625 (14 %) were positive and from these, 124 (19.8 %) resulted in isolated CoNS. To perform this study, we selected 60 strains of CoNS oxacillin resistant (60 out of 124/48,4%).

Among the 60 selected strains, the most prevalent species was S. epidermidis (40 out of 60/67%). The others were identified as S. haemolyticus (12 out of 60/20%), CoNS (7 out of 60/11%) and S. hominis subs novobiosepticus (1 out of 60/2%).

The hospital sectors in which there was a prevalence of strains selected were the intensive care units (ICUs) adult (15 out of 60/25%) and neonatal (12 out of 60/20%), followed by the Center Bone marrow transplantation (CBMT) (5 out of 60/8%) and central treatment of children with cancer (CTCC) (5 out of 60/8%), totaling 61% of the isolates.

The evaluation of clinical significance could be done for 38 strains (38 out of 60/63,3%). Already for 22 strains, there were difficulties in achieving this analysis due to insufficient clinical data. Thus, it was found that most of the isolates were responsible for true bacteremia (30 out of 38/78,9%) and only eight were shown to be contaminants (8 out of 38/21,1%).

According to antimicrobials sensitivity

### Table 1. Sensitivity to antimicrobial agents of 60 strains of CoNS isolated from blood cultures performed in TUH, from December 2010 to January 2012, using the disk diffusion method

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>3</td>
<td>5,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24</td>
<td>40,0</td>
<td>4</td>
<td>7,0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>23</td>
<td>38,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>9</td>
<td>15,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>17</td>
<td>28,0</td>
<td>6</td>
<td>10,0</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>28</td>
<td>47,0</td>
<td>5</td>
<td>8,0</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>6</td>
<td>10,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>43</td>
<td>72,0</td>
<td>1</td>
<td>2,0</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>20</td>
<td>33,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>60</td>
<td>100,0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
that performed by the disk diffusion method (Table 1), CoNS strains showed significant resistance to many classes of antimicrobial agents. The highest sensistivities were obtained against rifampicin antibiotic (43 out of 60 / 72%) and tigecycline (60 out of 60 / 100%). In addition, seven antimicrobial sensitivity prevalent profiles were identified. Those who did not fit among prevalent profiles were quite distinct from each others. For this reason they were grouped as other groups (Table 2).

The automated method MicroScan® (Siemens) has also showed a high rate of resistance
among selected CoNS strains (Table 3).

Regarding to the vancomycin antibiotic, all strains showed equal sensitivity, showing MIC \( \leq 2\mu g/ml \) from the automation and broth microdilution methods.

In Table 4, the results of the tests for characterization of phenotypic resistance to methicillin are listed as well as genotypic test results (detection of the mecA gene). In the disk diffusion (54 out of 60/90%) were resistant to the CoNS and oxacillin (57 out of 60/95%) were resistant to cefoxitin. The mecA gene was detected at (54 out of 60/90%) of the samples tested (Figure 1).

Through statistical analysis of the results, there obtained 92% sensitivity for oxacillin and 96% for cefoxitin when compared to genotypic testing reference.

**DISKUSSION**

In this study, the CoNS strains were prevalent to formulate *S. epidermidis* (40 out of 60 / 67%) and *S. haemolyticus* (12 out of 60 / 20%). These two species have been reported in national and international studies as the most frequent among isolates of CoNS nosocomial infections 10,11,17,18.

From an epidemiological viewpoint, *S. epidermidis* has developed interesting strategies to conquer the environment of hospital and become a notorious pathogen. We highlight its ability to colonize the inert surface of medical devices offensively, forming biofilms that are difficult to treat, as well as the Entrainment of different mobile genetic elements which are oxacilina that are responsible for resistance 19.

The *S. haemolyticus* also plays a considerable role in opportunistic infections related to medical installed devices. However, for this species, the molecular basis of biofilm formation has not yet been fully elucidated. The high levels of antimicrobial resistance, including heteroresistant glycopeptides are important features to treat infections caused by *S. haemolyticus* 20.

In this study, seven isolates of CoNS (7 out of 60 / 20%) were not satisfactorily identified to the species level by the system automated. Despite the advantages offered by this system, such as logistics workflow and speed in providing results, it may have limitations in identifying and determining susceptibility of some pathogens 21.

Due to the emergence of nosocomial infections associated with CoNS, the identification of strains to the species level becomes an important factor in order to define its meaning in clinical and epidemiological studies 21.

The percentage of clinical significance of our isolates was higher than that described in the literature10. However, one must consider that inclusion criteria may have influenced this result, that their employment has delayed the inclusion of a greater number of strains, possibly contaminants.

CoNS strains selected for this study were derived, mainly of patients admitted to critical care units (ICUs adult and neonatal) and hematological units. Thus, the data obtained in this study are in agreement with published reports in which the prevalence of CoNS was observed in blood cultures from patients in CTIs10, newborns, immunocompromises 4,22 and marrow transplant bone5. In these hospital sectors, most NBSI is related to offensive procedures, use of catheters and immunosuppression23. In addition, an important feature of these strains is the significant rate of oxacillin resistance, which hinders treatment, leading to the use of multiple antimicrobial and selective pressure23.

The isolates showed an index of considerable resistance to antimicrobials (Tables 1, 2 and 3). Some variations between results from the conventional method (Table 1) and automated (Table 3) were detected. The greater sensitivity to clindamycin, sulfaemethoxazole / trimethoprim and levofloxacin, evidenced in automation can lead to higher mistakes since there is a possibility of false existing strains sensitivity. With the antibiotics of gentamicin and oxacillin, the results of disk diffusion may show the larger errors.

As shown in Table 2 (disk diffusion), the strains with profiles I and V were characterized by resistance to most antimicrobials tested, showing sensitivity only to tigecycline. The profile V is different from profile I due to present intermediate resistance to levofloxacin. Already strains with pattern II were susceptible to tigecycline and rifampin, resembling the strains with the profile VI, in which there were intermediate resistance of...
gentamicin. Profiles III and IV showed higher sensitivity of those previously mentioned, the strains are sensitive to tigecycline, rifampin, clindamycin, ciprofloxacin and levofloxacin. Additionally, we found that strains with these seven prevalent sensitivity profiles were derived, mainly of patients admitted to ICUs. This is an important feature of the strains of hospital origin, regarding that in critical units, there is greater multidrug resistance.

From the genotypic method, it was found that 54 (54 out of 60 / 90%) strains carried the mecA gene and that six (6 out of 60 / 10%) were absent. Already in disk diffusion, the mecA gene detected sensitivity of 96% and 92%, using the disks of cefoxitin (30µg) and oxacillin (1µg), respectively (Table 4). This finding is compatible with the other described studies in which the disk cefoxitin also showed a higher correlation with the gold standard. Analyzing the contents of oxacillin resistance obtained automation and cefoxitin disk, it could be seen over estimation occurred compared to that found in the gold standard. The Oxacillin resistance without the presence of mecA gene may be due to hyper production of penicillinase or modification in proteins binding penicillin (PBP 1, 2 and 4).

With regard to sensitivity to glycopeptides, all isolates were susceptible to vancomycin. However reports of strains of CoNS and S. aureus showing reduced susceptibility to vancomycin in several countries, including Egypt, have generated therapeutic dilemmas in clinical practice.

In recent years, new therapeutic options have emerged as alternatives of vancomycin. Daptomycin and quinupristin / dalfopristin were recently approved by the FDA for the treatment of bacteremia caused by multiresistant gram-positive cocci. Tigecycline, oritavancina, linezolid and ceftobiprole, also make part of this new therapeutic agents, but have not yet been approved for the treatment of infections of the bloodstream.

In this context, it is noted that the analysis of the clinical significance of CoNS isolated from blood cultures, as well as accurate detection resistance to oxacillin, represent key factors to avoid a higher mortality rate among patients with bacteremia. Vancomycin remains the treatment of choice for serious infections associated with this CoNS oxacillin resistant tertiary hospital. However, due to the emergence of strains of vancomycin-resistant enterococcus (VRE) and Staphylococcus spp with reduced susceptibility to vancomycin to reduce the use of this antimicrobial agent has been recommended. Therefore, it is suggested that new therapeutic options are used to protect this glycopeptide.

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