Clonal relatedness of methicillin-resistant coagulase-negative staphylococci in the haemodialysis unit of a single university centre in Greece

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Abstract

Background. Coagulase-negative staphylococci (CoNS) are frequently encountered pathogens in hospital environment. Dialysis patients, often carrying central venous catheters, are prone to CoNS infections. Methicillin-resistant (MR) staphylococci in hospitals are resistant to multiple antibiotics and may cause an overall increase in the incidence of staphylococcal infections rather than simply replacing the more susceptible strains. The aim of this study was to evaluate the antimicrobial resistance and the clonal relatedness of all clinically significant CoNS isolates recovered from haemodialysis patient infections treated in a tertiary care centre, the University Hospital of Larissa, in central Greece. In addition, the CoNS isolates from carriers among health-care workers of the local haemodialysis unit were tested.

Methods. All staphylococci recovered from chronic haemodialysis patients who developed CoNS infections according to Herwaldt criteria in the University Hospital of Larissa, from October 2002 to October 2005, were included. In addition, isolates from the palms and the nasal mucosa of the nursing and medical personnel in the haemodialysis unit were also collected. Isolates were identified and tested for antimicrobial resistance by conventional microbiological methods. The clonal relationship of both patients’ and carriers’ isolates was tested by pulsed-field gel electrophoresis (PFGE) analysis.

Results. Forty-two CoNS isolates were recovered from clinical culture specimens of patients hospitalized for various reasons. In 37 out of 42 CoNS isolates, methicillin resistance was determined. The majority of the MR Staphylococcus epidermidis isolates from patients belonged to one main clone (27 out of 32), arbitrarily named clone z. Clone z was also found to colonize 40% of the haemodialysis unit personnel.

Conclusions. The high prevalence of clone z emphasizes the great capacity of CoNS to colonize patients with central venous catheters such as haemodialysis patients and personnel. This emphasizes the need for the establishment of control and prevention measures.

Keywords: central venous catheters; coagulase-negative staphylococci; dialysis care providers; Greece; haemodialysis

Introduction

Coagulase-negative staphylococci (CoNS) are important and frequently encountered pathogens in hospital environment, and they account for ~10% of all nosocomial infections [1,2]. Predominant CoNS species associated with clinically relevant infections are Staphylococcus epidermidis followed by Staphylococcus haemolyticus and Staphylococcus hominis. In addition, the introduction of methicillin in clinical practice during the 1960s resulted in acquisition of resistance to methicillin [3]. Nosocomial methicillin-resistant (MR) CoNS strains are frequently resistant to multiple antibiotics and may cause an overall increase in the incidence of staphylococcal infections in hospitals rather than simply replacing the more susceptible strains [4].

Infections with CoNS are typically associated with implanted foreign bodies and central venous catheters. Colonization of the foreign body is a necessary first pathogenic step, in which slime production and biofilm formation on polymeric surfaces are very important factors. The production of biofilm was correlated with the presence of the polysaccharide intercellular adhesin (PIA) encoded by genes of the ica operon (icaA, icaB, icaC, icaD) [5].

In haemodialysis patients with end-stage renal disease (ESRD), bacterial infections are still a major cause of morbidity and mortality [6,7]. In particular, mortality caused by sepsis is several times higher in haemodialysis patients compared to the general population [6], and the incidence
of bacteraemia is highest (ranging from 3.9 to 16.7 episodes per 100 patient-months) among patients dialyzing through central venous catheters [8,9]. In catheter-related bacteraemia episodes, CoNS are the most frequent pathogens involved [1]. In a haemodialysis unit cross-transmission of these pathogens might occur either directly between patients in contact with each other or indirectly through the contaminated hands of health-care workers or environmental surfaces [10]. In addition, the haemodialysis unit provides an ideal setting for the cross-transmission of CoNS because regular haemodialysis is required three times per week for several hours in a closed setting and because health-care workers provide concurrent care to multiple patients [11].

The aim of this study was to evaluate antimicrobial drug resistance and clonal relatedness of all clinically relevant CoNS isolates in haemodialysis patients treated for CoNS infection from October 2002 to October 2005 in a tertiary care centre, the University Hospital of Larissa, in central Greece. In order to further elucidate possible cross-transmission, the same characteristics of CoNS isolates from carriers among health-care workers of the unit were also evaluated.

### Methods

#### Bacterial strains

Clinical CoNS isolates obtained from chronic haemodialysis patients, admitted from October 2002 to October 2005 in the University Hospital of Larissa, were investigated. Larissa is a semi-rural area in central Greece with 279 305 inhabitants and 220 patients with ESRD on haemodialysis. During hospitalization, haemodialysis was applied in the renal unit of the University Hospital of Larissa, in central Greece. In order to further elucidate possible cross-transmission, the same characteristics of CoNS isolates from carriers among health-care workers of the unit were also evaluated.

#### Identification and susceptibility testing

Identification of staphylococci to species level was performed by Gram stain, catalase, coagulase and by the API® ID 32 Staph System (bioMérieux, Marcy l’Etoile, France). Susceptibility to various antimicrobial agents (penicillin, oxacillin, trimethoprim-sulphamethoxazole, ofloxacin, clindamycin, erythromycin, gentamicin, tobramycin, rifampin, tetracycline, fusidic acid, vancomycin, teicoplanin and linezolid) was performed by the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines [13,14]. Confirmation of methicillin resistance was done by the detection of penicillin-binding protein 2a (PBP2a) production using a latex agglutination test (SlideX MRSA Detection; bioMérieux). Determination of minimal inhibitor concentration (MIC) to oxacillin was performed by E-test (AB Biodisk, Solna, Sweden) according to the instructions of the manufacturer [15,16].

#### Pulsed-field gel electrophoresis

Molecular typing of CoNS was performed by pulsed-field gel electrophoresis (PFGE) of Smal DNA digests and clones were defined after comparison between isolates from patients, health-care personnel and predominant clones, previously isolated in our hospital [17]. Isolates were designated as genetically identical according to the criteria established by Tenover et al., i.e. if their restriction pattern had the same number of bands and the corresponding bands had the same apparent size [18].

#### Detection of biofilm production and the icaABCD genes

Production of biofilm was tested by a qualitative method involving glass test tubes described by Christensen et al. [19]. The four genes of the ica operon (icaABCD) were detected by the polymerase chain reaction using the following primers: icaA forward 5'-gacctgagcaagtcgagt-3', icaA reverse 5'-cccatacgttgttagctgcc-3'; icaB forward 5'-atggatgttaacgaaacaacgca-3', icaB reverse 5'-cattaacttgctcgttgacag-3'; icaC forward 5'-ataactcagctgtatgtt-3', icaC reverse 5'-atataataactctcttacaa-3' and icaD forward 5'-ggttttttaatgaaatttgctc-3', icaD reverse 5'-agtgtaacagccagacag-3' [20].

### Results

In total, 42 CoNS isolates were recovered from clinical specimens (22 blood cultures, 20 central venous catheter) obtained from chronic haemodialysis patients hospitalized for various reasons in the University Hospital of Larissa during the study. Isolation of CoNS was evenly distributed over the study period with no evidence of outbreaks. Mean age of patients was 63.5 years and 26 out 42 (62%) were men. The distribution of the isolates to the species level was as follows: 36 S. epidermidis, 4 S. hominis and 2 S. haemolyticus (Table 1). Among them, methicillin resistance was identified in 37 isolates (37 out of 42 isolates, 88.1%). All these 37 isolates (32 S. epidermidis,

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### Table 1. Species distribution of coagulase-negative staphylococci (CoNS) isolated from a variety of clinical sources

<table>
<thead>
<tr>
<th>CoNS species</th>
<th>Total</th>
<th>Blood cultures n (%)</th>
<th>Catheter cultures n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>36 (85.7)</td>
<td>19 (86.4)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>S. hominis</td>
<td>4 (9.52)</td>
<td>2 (9.1)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>2 (4.76)</td>
<td>1 (4.5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>42 (100)</td>
<td>22 (100)</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>
Table 2. Resistance phenotypes of all methicillin-resistant (MR), coagulase-negative staphylococcus (CoNS) isolates ($n = 37$) to the following seven antibiotics

<table>
<thead>
<tr>
<th>MR CoNS</th>
<th>Total</th>
<th>β-Lact</th>
<th>ERY</th>
<th>OFLX</th>
<th>FUS</th>
<th>TMP/SMX</th>
<th>GEM</th>
<th>TET</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>32</td>
<td>31</td>
<td>28</td>
<td>18</td>
<td>25</td>
<td>19</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. hominis</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>All</td>
<td>37</td>
<td>36</td>
<td>33</td>
<td>21</td>
<td>30</td>
<td>22</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

β-Lact, β-lactam; ERY, erythromycin; OFLX, ofloxacin; FUS, fusidic acid; TMP/SMX, trimethoprim-sulphamethoxazole; GEM, gentamicin; TET, tetracycline.

3 $S. hominis$ and 2 $S. haemolyticus$ had MIC $\geq 0.5$ mg/l to oxacillin and produced PBP2a, while the remaining 5 isolates ($4$ $S. epidermidis$ and 1 $S. hominis$) had MIC <0.5 mg/l to oxacillin and were PBP2a negative.

Apart from β-lactams, MR CoNS were also resistant to other antibiotics: erythromycin 33 (89.1%), ofloxacin 21 (56.7%), fusidic acid 30 (81%), trimethoprim-sulphamethoxazole 22 (59.4%), gentamicin 17 (45.9%) and tetracycline 12 (32.4%). The resistant phenotypes of each species are presented in Table 2. None of the isolates showed reduced susceptibility to vancomycin, teicoplanin and linezolid.

PFGE analysis showed that the majority of MR $S. epidermidis$ (27 out 32 isolates) fell into one pulsotype, arbitrarily named clone z. The remaining five $S. epidermidis$ isolates, as well as the $S. haemolyticus$ and the $S. hominis$ isolates, fell into different clonal unrelated pulsotypes (Figure 1). The ica operon, comprising the icaABCD genes, and biofilm production were detected in all MR staphylococcal isolates that belonged to clone z.

CoNS isolates from 5 out of 13 health-care personnel were identified as $S. epidermidis$; these isolates were MR, belonged to clone z, produced biofilm and carried the entire ica operon (icaABCD).

**Discussion**

This is the first survey from Greece about the species distribution, antimicrobial drug resistance and clonal relatedness of CoNS in haemodialysis patients. It is well known that ESRD is accompanied by disturbances of the immune system and subsequent susceptibility to infections. Infections are responsible for $\sim 16\%$ of deaths in haemodialysis patients, the second leading cause of death in this population [7]. Mortality caused by sepsis is 100–300 times higher in haemodialysis patients compared to the general population and it remains 50 times higher even after adjustments for age, race, sex, diabetes mellitus and possible record errors [6]. In ESRD, acquired immunity disturbances are caused by uraemia per se, the haemodialysis procedure, chronic renal failure complications and therapeutic interventions for their treatment. Most infections of haemodialysis patients are caused by Gram-positive bacteria [8,11].

In our study, $S. epidermidis$, $S. hominis$ and $S. haemolyticus$ predominate among CoNS species isolated from hospitalized haemodialysis patients infections. The species distribution in our collection is similar to published studies in non-haemodialysis patients [16,21]. However, the prevalence of methicillin resistance among CoNS isolates in haemodialysis patients proved to be higher than that previously shown for CoNS isolates in other wards of our hospital (88.1 versus 59.5%) [16].
Clonal analysis by PFGE revealed a high prevalence of the clone z among \textit{S. epidermidis} isolates that predominated among isolates associated with sepsis and catheter infections in hospitalized haemodialysis patients. This clonal type was identified for the first time in our hospital and was not related to previously detected CoNS clones in other wards [16].

Furthermore, the clonal relatedness of isolates strongly suggests that CoNS infections were probably contracted from a common source in the haemodialysis unit and that this clone was transferred by patient-to-patient transmission leading to infections. In agreement to this notion, a considerable number of haemodialysis health-care workers, tested in this study, were colonized by clone z. Unfortunately, cultures from nares and hands of the personnel were obtained at the end of the study period and therefore it cannot be stated with absolute certainty that colonization might be the source or the result of cross-transmission.

Empirical treatment of catheter-related infections in our unit included vancomycin or β-lactam in combination with aminoglycoside. The frequent administration of β-lactams and aminoglycosides as first-line antimicrobial drugs for potential bacterial infection in haemodialysis patients, as well as the ability of biofilm production by CoNS strains, might be the main factors that principally facilitate the dissemination of clone z in our haemodialysis unit.

Central venous catheters as a haemodialysis access are incriminated in the development of severe and often life-threatening infections. Nevertheless, their use is often inevitable. In these cases, patients’ and health-care providers’ strict adherence to sterility measures during catheter placement and everyday handling is the cornerstone for preventing catheter-related infections. In addition, use of indwelling central catheters should be restricted to dialysis purposes and their use for infusions or blood sampling should be abandoned. Today, CoNS infections have not been eliminated from haemodialysis units probably due to certain properties some strains possess, including antibiotic resistance, biofilm production and the ability to colonize multiple hosts [22]. Therefore, in order to prevent and reduce MR CoNS infections, additional effective control measures should be established since the decrease in catheter-related infections has been shown to improve survival in haemodialysis patients [23]. The benefit from the use of local antibiotic agents like mupirocin or polysporin at the catheter’s exit site or of antibiotic-locking solutions and antibiotic-impregnated catheters should be further investigated in accordance with previous reports of a high proportion of identically typed organisms in skin and catheter cultures [5].

The Centers for Disease Control and Prevention Guidelines for the prevention of intravascular catheter-related infections do not routinely recommend topical application of an antibiotic ointment [24]. However, considering the present results and taking into account that nasal carriage, at least for \textit{S. aureus}, is associated with an increased relative risk (1.8–4.7) for haemodialysis catheter-related infections [25], eradication of staphylococci nasal carriage from the dialysis personnel and/or patients should be re-evaluated. Finally, application of molecular methods like PFGE to determine epidemiologic relatedness of isolates could offer valuable information towards recognizing possible ways of dissemination, evaluating preventive measures and controlling infections more efficiently.

It is well known that biofilm production forms a barrier around bacteria, protecting them from antibiotics and phagocytes and making treatment of infections very difficult. In medicine, biofilm has been hypothesized to be involved in more than 60% of all infections [26]. In this study, the predominant clone, recovered from clinically significant isolates, as well as among colonizing isolates, was found to carry the \textit{ica} operon and produce biofilm. Biofilm-producing CoNS lead to the development of severe infections (sepsis, etc.), especially in the presence of catheter dysfunction, since flow stagnation offers an ideal environment for bacteria proliferation [27]. Measures adopted in order to inhibit biofilm formation (silver or antibiotic coating) do not always ensure \textit{in vivo} anti-infective efficacy [28]. Therefore, universal application of the above-mentioned preventive measures should be the main approach towards preventing catheter-related infections.

In conclusion, the findings of our study strongly suggest the need for the establishment of control program measures in order to prevent and reduce MR CoNS infections in haemodialysis patients. In this context, molecular epidemiology and the surveillance by PFGE might be very useful to understand the transmission patterns and to control nosocomial infections induced by multi-resistant strains.

\textit{Conflict of interest statement}. None declared.

\textbf{References}

22. Lok CE. Avoiding trouble down the line: the management and prevention of hemodialysis catheter-related infections. *Adv Chronic Kidney Dis* 2006; 13: 225–244

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