Comparison of Coagulase-Negative Staphylococci Isolated from Blood Cultures as a True Bacteremia Agent and Contaminant in Terms of Slime Production and Methicillin Resistance

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Abstract

Objective: The aim of this study is to determine the species distribution, slime activity, and methicillin resistance of coagulase-negative staphylococci (CoNS) isolated from blood cultures as either contaminants or true bacteremia agents.

Materials and Methods: In this study, 13,268 blood culture samples sent to our laboratory from various clinics during a two-year period were examined in terms of the presence of CoNS to clarify whether the isolates are true bacteremia agents, as defined by Centers for Disease Control and Prevention (CDC) criteria. The slime activities of true bacteremia agents (58 CoNS strains) and contaminants (50 randomly selected CoNS strains) were investigated by the Christensen method. The methicillin susceptibilities of the strains were determined by the disk diffusion method.

Results: Although the frequency of slime production was 39.7% among the true bacteremia CoNS agents, it was 18% in CoNS that were judged to be contaminants (p<0.05). S. epidermidis was the most frequently isolated species for both the true bacteremia agent group (58 CoNS strains) and contaminants (50 randomly selected CoNS strains) were investigated by the Christensen method. The methicillin susceptibilities of the strains were determined by the disk diffusion method.

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Conclusion: The presence of slime activity in CoNS isolated from blood culture samples is supportive evidence that they are most likely the agents of true bacteremia cases.

Key Words: True bacteremia, blood culture, coagulase-negative staphylococcus, methicillin resistance, slime production

Özet

Amaç: Bu çalışmada kontaminant ve gerçek bakteremi etkeni olarak saptanan koagülaz negatif stafilokokların (KNS), tür düzeyinde tanımlanması, slime aktiviteleri, metisilin direnci oranlarının saptanması amaçlanmıştır.


Bulgular: Slime üreteceği oranan gerçek bakteremi etkeni olarak saptanan KNS'lerde %39,7 iken kontaminan KNS'lerde ise bu oran %18 olarak saptanmıştır (p<0,05). İzole edilen KNS'lerden S. epidermidis gerçek bakteremi etkenleri arasında %56,9, kontaminant olarak değerlendirilen grupda ise %74 oranında en sık izole edilen türüdür. Ayrıca zamanda bu bakteri her iki grupta da en sık slime üreten türüdür. Gerçek bakteremi etkisi olarak izole edilen KNS'lerin %82,6'sı slime üretiyor iken, kontaminan olarak izole edilen KNS'lerde bu oran %77,8'di.

Sonuç: Kan kültür örneğinde saptanan KNS'lerde slime varlığı gerçek bakteremi destekleyici bir bulgu olduğunu görülmektedir. Anahtar Kelimeler: Gerçek bakteremi, kan kültür, koagülaz negatif stafilokok, metisilin direnci, slime üretimi
Introduction

Coagulase-negative staphylococci (CoNS) typically reside on healthy human skin and mucous membranes, rarely cause disease, and are most frequently encountered by clinicians as contaminants of microbiological cultures [1]. In the past, CoNS were often discarded as contaminants. However, this situation has changed over the last decade, and CoNS are now being recognized as important causes of nosocomial infections [2].

The increasing importance of CoNS may also be attributable in part to the growing appreciation of this group of organisms as opportunistic pathogens and to the increase in the use of medical devices, such as intravascular catheters and prosthetic devices, in seriously ill and immunocompromised patients (such as intensive care patients, premature newborns, and cancer and transplant patients) [2]. CoNS are characterized by their ability to adhere to and grow on solid surfaces and, subsequently, to produce a polysaccharide slime; this extracellular slime may protect the bacteria against immunological host defense mechanisms and antimicrobial therapy [3].

The predominant Staphylococcus species of humans, S. epidermidis, is widely distributed over the body surface. At the same time, S. epidermidis is by far the most frequently recovered organism in bacteremia cases, accounting for 50% to over 80% of isolates. Other commonly implicated species include S. haemolyticus, S. lugdunensis, S. schleiferi, S. warneri, S. hominis, S. simulans, and S. saccharolyticus [4].

In this study, the aim was to determine the species distribution, slime activity, and methicillin resistance of CoNS strains isolated from blood cultures as either contaminants or true bacteremia agents.

Materials and Methods

In this study, 13,268 blood culture samples sent to our laboratory from various clinics of Ataturk University Research Hospital during a two-year period were examined in terms of CoNS presence. For this aim, culture bottles were incubated using the Bactec 9240 system (Becton Dickinson). Subcultures of the positive bottles were performed on 5% blood agar, and the plates were incubated at 35°C for 24-48 h. CoNS were defined based on colony morphology, Gram staining, positive catalase reaction, and the absence of tube coagulase activity.

To clarify whether the CoNS isolated from these blood cultures are true bacteremia agents, laboratory-confirmed bloodstream infection criteria defined by the Centers for Disease Control and Prevention (CDC) were used [5]. Cultures that did not meet with the criteria defined by the CDC for true bacteremia cases were evaluated as contaminants. If repeated cultures were sent from the same patient and CoNS were isolated as true bacteremia agents each time, only one of the isolates was utilized as part of the study. Among the many CoNS strains evaluated as contaminants, only 50 that belonged to different patients were included in the study by random selection.

The strains were then identified to the species level using the API ID 32 Staph kit (bioMérieux, France). The slime activity of each strain was investigated by the Christensen method [6]. S. epidermidis ATCC 35983 (tube-adherence test positive) and S. hominis ATCC 35982 (tube-adherence test negative) were used as control organisms.

The methicillin susceptibilities of the CoNS strains were determined by the disk diffusion method on Mueller-Hinton agar (bioMérieux, France) according to the Clinical and Laboratory Standards Institute (CLSI). Disks containing 30 µg sefoxitin (Oxoid) (a zone diameter ≥25 mm is sensitive, ≤24 mm is resistant) were used for this test [7]. A standard methicillin sensitive S. aureus strain (S. aureus ATCC 43300) and methicillin resistance S. aureus strain (S. aureus ATCC 25923) were used as quality control organisms. The statistical p-value was calculated by Fisher’s exact test. p<0.05 was considered significant.

Results

In this study, 13,268 blood culture samples were examined, and CoNS were isolated from 947 (7.1%). Of the strains, 818 (68.4%) were evaluated as contaminants. The number of strains defined as true bacteremia agents was 129; these were isolated from the cultures of 58 patients (2 samples from each of 46 patients, 3 from each of 11 patients, and 4 from 1 patient). The CoNS from true bacteremia cases consisted of six different species, whereas the contaminant group contained seven different species.

The species distribution of these isolates (58 true bacteremia agents and 50 randomly selected species) and their slime-producing activities are shown in Tables 1 and 2. It was found that although the frequency of slime production was 39.7% among the true bacteremia agent CoNS, the frequency was only 18% in CoNS that were considered to be contaminants (p<0.05).

S. epidermidis was the most frequently isolated species in both the true bacteremia agent group (56.9%) and contaminant group (74%). Additionally, S. epidermidis was the species that produced slime most frequently in both groups. The slime positivity rate of S. epidermidis was 51.5% in the true bacteremia agent group (17 of the 33 strains) and 21.6% in the contaminant group (8 of the 37 strains) (p<0.05).
Methicillin resistance in the slime-producing CoNS was determined to be 82.6% in the true bacteremia agent group and 77.8% in the contaminant group. With regard to the non-slime-producing CoNS, the methicillin resistance rates were detected as 48.6% and 51.2% in the true septicemia agent group and contaminant group, respectively. The distribution of the true bacteremia agent and contaminant CoNS according to their slime activity and methicillin resistance is shown in Table 3.

Slime positivity was 39.7% in the CoNS strains recovered from true bacteremia cases, 19 (82.6%) of which were methicillin resistant and 4 (17.4%) methicillin sensitive. Methicillin resistance was found to be significantly higher in the slime-positive strains (82.6%) than in the slime-negative strains (48.6%) (p<0.05) (Table 3).

The methicillin resistance rates of the slime-positive CoNS strains belonging to both the true bacteremia agent and contaminant groups were similar, as were the rates for the slime-negative strains (Table 3).

**Table 1. Slime activity rates of CoNS species isolated from true bacteremia cases**

<table>
<thead>
<tr>
<th>CoNS</th>
<th>Slime positive n (%)</th>
<th>Slime negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis (n=33)</td>
<td>17 (51.5)</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>S. haemolyticus (n=8)</td>
<td>2 (25.0)</td>
<td>6 (75.0)</td>
</tr>
<tr>
<td>S. hominis (n=9)</td>
<td>3 (33.3)</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>S. xylosus (n=3)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S. warneri (n=2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. simulans (n=3)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total (n=58)</td>
<td>23 (39.7)</td>
<td>35 (60.3)</td>
</tr>
</tbody>
</table>

**Table 2. Slime activity frequencies of CoNS species isolated as contaminants**

<table>
<thead>
<tr>
<th>CoNS</th>
<th>Slime positive n (%)</th>
<th>Slime negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis (n=37)</td>
<td>8 (21.6)</td>
<td>29 (78.4)</td>
</tr>
<tr>
<td>S. haemolyticus (n=5)</td>
<td>0</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>S. hominis (n=3)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. xylosus (n=2)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. simulans (n=1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. schleiferi (n=1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. cohnii (n=1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total (n=50)</td>
<td>9 (18.0)</td>
<td>41 (82.0)</td>
</tr>
</tbody>
</table>

**Discussion**

CoNS are among the most important microorganisms in nosocomial bloodstream infections. These bacteria can be pathogenic, especially in immunocompromised patients with hematological and other malignancies and in patients with foreign bodies, such as invasive catheters [8]. CoNS are resident flora of the human body and are not harmful to ordinary individuals. However, they cause serious infections in compromised hosts, especially patients with prosthetic valves, prosthetic joints, cerebrospinal fluid shunts, or intravascular catheters. Recent progress in medicine has resulted in an increase number of compromised hosts. Because of this, CoNS has become one of the most common causes of nosocomial infection [2]. Epidemiological studies of CoNS infection are difficult because this organism is a normal commensal on the skin; thus, differentiating infection from colonization and contamination may be difficult [2].

In a previous study of blood culture from 4,234 patients, CoNS was isolated from 109 patients (2.6%), and 94% of all CoNS isolates were contaminants [9]. In the study of Kirchhoff and Sheagren [10], 85% of all CoNS isolated during the study period were judged to be contaminants. In the present study, 13,268 blood culture samples were examined within a two-year period, and 947 (7.1%) CoNS were isolated. Of these, 86.4% of the CoNS isolates (818/947) were considered to be contaminants, whereas 129 were evaluated as true bacteremia agents; these 129 strains were isolated from 58 different patients. Our research demonstrated that the majority of CoNS isolated from blood culture samples were not true bacteremia agents.

CoNS have few virulence factors, with biofilm production, delta toxin, and presence of the mecA gene being the best described. Among these factors, the production of exopolysaccharide or slime has been indicated as an epidemiological marker of infection by several authors [5, 11, 12]. Both CoNS evaluated as either true bacteremia agents or contaminants can produce slime. However, the frequency of slime production in CoNS evaluated as true bacteremia agents is known to be higher than the frequency in contaminant CoNS.

In a study conducted by Garcia et al. [13], slime positivity was found in 20 of 35 CoNS isolates (57.1%) evaluated as true bacteremia agents but in only 7 of 27 (25.9%) isolates evaluated as contaminants. In the present study, the slime positivity rates were 39.7% and 18.0% in the CoNS isolates evaluated as true bacteremia agents (23 out of 58) and as contaminants (9 out of 50), respectively.

*S. epidermidis* has been reported as the most frequently isolated species among the CoNS evaluated as sepsis agents, with a frequency of 43.5%, followed by *S. haemolyticus* (11.5%), *S. hominis* (9.5%), *S. lugdunensis* (9.0%), *S. capitis*...
and S. xylosus (5.0%) [14]. S. epidermidis was also the most frequently isolated species in our study, with a frequency of 56.9% as a true bacteremia agent among the CoNS. The other species were as follows: S. hominis (15.5%), S. haemolyticus (13.8%), and S. xylosus (5.2%).

Of CoNS species, slime production is most frequently found for S. epidermidis. Koksal et al. [14] found that the slime positivity rates of CoNS isolates in terms of species were as follows: S. epidermidis (71%), S. haemolyticus (35%), and S. hominis (26%). In our study, the frequencies of slime production were 51.5%, 25%, and 33.3% for the same species, respectively. Considering the results of both studies, it can be concluded that nearly half of S. epidermidis strains isolated as true bacteremia agents exhibit slime activity.

In the present study, the frequency of slime production was 39.7% in CoNS evaluated as true bacteremia agents and 18.0% in CoNS evaluated as contaminants, and the difference between the two groups was statistically significant (p<0.05). However, contrary to our finding, Jones and colleagues [15] reported that slime production in CoNS isolated from blood cultures had a limited role in the differentiation between clinically significant and contaminant strains.

CoNS isolated from nosocomial infections, particularly S. epidermidis and S. haemolyticus, are usually resistant to multiple antibiotics, and more than 80% are resistant to methicillin (16). In a study conducted by Koksal et al. [14], methicillin resistance was detected in 70 out of 86 slime-producing CoNS (81.4%) and in 65 out of 114 non-slime-producing strains (57.1%). Similar frequencies were determined in our study: 82.6% and 48.6% in slime-producing and non-slime-producing CoNS, respectively. Statistical analyses of our results and those of others indicated that slime-producing CoNS are remarkably more resistant to methicillin than non-slime-producing strains.

In conclusion, the presence of slime activity in CoNS isolated from blood culture samples is supportive evidence that they are most likely agents of true bacteremia cases. Additionally, when planning treatment of infections occurring with these bacteria, antibiotic sensitivity test results should be considered because the frequency of methicillin resistance in slime-positive CoNS is high.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of this study.

Informed Consent: Written informed consent was not obtained due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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References


