

SPECIES DIVERSITY AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS* SPP. ISOLATES ASSOCIATED WITH CATHETER-RELATED BLOODSTREAM INFECTIONS IN PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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ABSTRACT

INTRODUCTION: The human body is colonized by a large number of different bacterial and fungal species, with most microorganisms inhabiting the skin, oral cavity, and the lower parts of the gastrointestinal tract.

AIM: The aim of this study was to investigate the species diversity of clinically significant *Staphylococcus* spp. isolates, obtained from blood cultures of patients with central venous catheter (CVC) following hematopoietic stem cell transplantation (HSCT) during the period January 2019–December 2020, as well as to test their susceptibility to a set of antimicrobials.

MATERIALS AND METHODS: A total of 21 non-duplicate clinically significant staphylococcal isolates from blood cultures of 17 patients with implemented CVCs following HSCT were obtained. The Bactec and Phoenix automated systems (BD, USA) were used for identification and antimicrobial susceptibility testing.

RESULTS: In the collected group of 21 isolates, 85.7% were coagulase-negative staphylococci (CoNS): *S. epidermidis*, n=12; *S. haemolyticus*, n=4; *S. hominis*, n=2, and 3 isolates were identified as *S. aureus*. Methicillin resistance was 85.7% and was detected only in the CoNS group. In decreasing order, the resistance rates in CoNS group were as follows: 100% for penicillin and cefoxitin >83.3% erythromycin >72.2% ciprofloxacin >61.1% gentamicin >44.4% for clindamycin and trimethoprim/sulfamethoxazole. No resistance to vancomycin, teicoplanin, and linezolid was found. *S. aureus* isolates demonstrated preserved susceptibility to all antimicrobials with exception to penicillin.

CONCLUSION: In the present study, CoNS were identified as the most common cause of catheter-related bloodstream infections (BSIs) in patients following HSCT, with *S. epidermidis* being the predominant species.

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All CoNS isolates were methicillin-resistant, but also exhibited reduced susceptibility to other antimicrobials and thus causing difficult-to-treat infectious complications in patients after HSCT.

Keywords: staphylococci, blood, infection, catheter, stem cell transplantation, resistance

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INTRODUCTION

The human body is colonized by a large number of different bacterial and fungal species, with most microorganisms inhabiting the skin, oral cavity, and the lower parts of the gastrointestinal tract. The resident microbial flora is part of the natural resistance of the human body and its major role is to protect the skin and mucous membranes from pathogenic microorganisms (1). The most common skin colonizers are the representatives of the genus *Staphylococcus*. Most staphylococcal species are included in the coagulase-negative staphylococcus group (CoNS) (*S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. saprophyticus*, *S. capitis*, etc.), microorganisms with weak pathogenic potential but with various virulence factors. Species like *Staphylococcus aureus* and *Staphylococcus lugdunensis*, often isolated from skin and associated with wound and blood infections, are known as coagulase-positive staphylococci because of their specific characteristic to produce the enzyme coagulase (2).

In immunocompetent individuals, CoNS are rare causative agents of infections, but in immunocompromised patients (patients with oncohematological diseases, after solid organ and stem cell transplantation, patients with high-dose and prolonged corticosteroid therapy, HIV infection), these organisms often demonstrate their pathogenic potential and lead to life-threatening complications. The bloodstream infections (BSIs) are the most important infectious complication in patients after hematopoietic stem cell transplantation (HSCT) and are mainly associated with the presence of a central venous catheter (CVC) or mucosal damage in these patients. Coagulase-negative staphylococci are among the most commonly recovered bacteria and responsible for 25% of all reported bacteremia cases (3). It has been suggested that the increasing number of these infections is linked to the biopolymer introduction in the medical practice and its use in high-risk individuals (4). The ability of CoNS to adhere to the artificial medical devices, mediated by the slime production of these bacteria, their main virulence factor, leads to the occurrence of BSI (5). Moreover, the slime layer around the bacteria prevents the penetration of the antibiotics (6,7). Another rising issue is the increasing resistance of CoNS to antimicrobial

agents, which further complicates the treatment process (3).

AIM

The aim of this study was to investigate the species diversity of clinically significant *Staphylococcus* spp. isolates, obtained from blood cultures of patients with CVCs following HSCT, hospitalized in St. Marina University Hospital in Varna during the period January 2019–December 2020, as well as to test the susceptibility of these isolates to a set of antimicrobial agents.

MATERIALS AND METHODS

Between January 2019 and December 2020, 968 blood cultures of 77 patients with implemented CVCs following HSCT were studied. The blood samples were incubated in the automated BACTEC system (BD, USA) for 5 days. The Phoenix automated system (BD, USA) was used for species identification and antimicrobial susceptibility testing. The results were reported according to EUCAST guidelines 2019/2020 (8). Methicillin resistance was interpreted according to the results of cefoxitin resistance.

RESULTS

A total of 363 positive blood cultures were detected (37.5%). Forty-five different non-duplicate microorganisms were recovered. Gram-positive bacteria were identified in 53.3% (n=24) and Gram-negative—in 44.4% (n=20). Only a single fungal isolate was found (2.2%). The most commonly isolated bacteria were representatives of genus *Staphylococcus* (46.7%), followed by *E. coli* (24.4%).

In this study a total of twenty-one clinically significant staphylococcal isolates were recovered from the blood samples of 17 HSCT patients, with 85.7% belonging to the CoNS group (*S. epidermidis*, n=12; *S. haemolyticus*, n=4; *S. hominis*, n=2). Only three isolates were identified as *S. aureus*. Among all isolates, *S. epidermidis* was the most commonly isolated species (57.1%). Resistance to penicillin was found in 90.5% (CoNS, n=18 and *S. aureus*, n=1). Methicillin resistance in the whole studied bacterial collection was 85.7% and was detected only in the representatives of the CoNS group.

In decreasing order, the resistance rates in CoNS group to a set of antimicrobial agents were as

follows: 100% for penicillin and cefoxitin; >83.3% erythromycin >72.2% ciprofloxacin >61.1% gentamicin >44.4% for clindamycin and trimethoprim/sulfamethoxazole. No resistance to glycopeptides (vancomycin and teicoplanin) and linezolid was found in the whole collection of 21 isolates.

The antimicrobial resistance levels of all studied isolates according to their species affiliation are presented in Table 1.

Table 1. Species diversity and antimicrobial resistance of 21 clinically significant staphylococcal blood isolates obtained from 17 patients with CVCs after HSCT (in %).

Blood Isolates n=21	P	FOX*	CIP	G	V	TEC	LZD	TSM	DA	ERY
<i>S. epidermidis</i> , n=12 (57.1%)	100%	100%	66.7% (n=8)	66.7% (n=8)	0%	0%	0%	23.8% (n=5)	14.3% (n=3)	42.9% (n=9)
<i>S. haemolyticus</i> , n=4 (19%)	100%	100%	75% (n=3)	50% (n=2)	0%	0%	0%	50% (n=2)	75% (n=3)	100% (n=4)
<i>S. hominis</i> , n=2 (9.5%)	100%	100%	100%	50% (n=1)	0%	0%	0%	50% (n=1)	100%	100%
<i>S. aureus</i> , n=3 (14.3%)	33.3% (n=1)	0%	0%	0%	0%	0%	0%	0%	0%	0%

Abbreviations: P, penicillin; FOX*, cefoxitin (indicator for methicillin resistance); CIP, ciprofloxacin; G, gentamicin; V, vancomycin; TEC, teicoplanin; LZD, linezolid; TSM, trimethoprim/sulfamethoxazole; DA, clindamycin; ERY, erythromycin.

DISCUSSION

In present-day transplant medicine, various artificial materials are used for different purposes: stem cell infusion, infusion of IV solutions and biological products, routine monitoring of laboratory markers. The implemented foreign devices are an ideal environment for growth of microorganisms that produce polysaccharide substances (slime), mediating their adhesion to the devices and subsequent biofilm formation. Coagulase-negative staphylococci, the major representatives of the skin microflora, are the most commonly isolated bacteria from the CVC lumen and respectively associated with foreign body-bloodstream infections. Their most important virulence factor is the ability for slime production. These bacteria often demonstrate reduced susceptibility to a number of antimicrobial agents. Thus, on one side, the resistance gene carriage and on the other—the slime formation associated with decreased penetration of antibiotics in the bacterial cell, lead to occurrence of difficult-to-treat infections (9, 10).

In our study, the CoNS isolation rate (85.7%) exceeded that of *S. aureus* (14.3%). In other studies which investigated the causative agents of bloodstream infections in patients after HSCT, similar results were reported (11–13).

In the tested isolates, the methicillin resistance was the highest (85.7%) and all CoNS demonstrated this type of resistance (MRS, methicillin-resistant staphylococci). No MRSA (methicillin-resistant

Staphylococcus aureus) were detected. Methicillin-resistant staphylococci are among the problematic to treat pathogens because they are resistant to all beta-lactams (incl. carbapenems) and very often demonstrate multiple drug-resistance profile. Along with the beta-lactam resistance, our CoNS isolates exhibited reduced susceptibility to other antimicrobial groups ranging from 44.4% for sulfonamides and lincosamides to more than 80% for macrolides. It is well known that methicillin resistance is more frequently documented in CoNS rather than *S. aureus*. Our results are in concordance with the data reported by other authors and support this statement (14). It is worrisome that a high percentage of the staphylococcal isolates demonstrate reduced sensitivity to macrolides, quinolones and aminoglycosides, agents usually preferred in infections caused by Gram-positive bacteria and are an alternative to beta-lactams. Many guidelines recommend prophylaxis or therapy with quinolones for patients who have undergone HSCT and are in the early stages of transplantation or when febrile neutropenia occurs (15). The increased resistance observed to these antimicrobi-

als would interfere with the preventive and treatment strategies in these patients.

A reasonable choice for empirical therapy of CoNS-associated bacteremia are the glycopeptide antimicrobials (vancomycin, teicoplanin), agents with preserved activity against MRS. In case of CVC colonization and inability to eradicate the bacteria causing the BSI, its replacement is recommended (16). In our study no resistance to the strategic glycopeptides and linezolid was found in all 21 isolates. However, there are already described cases of glycopeptide-resistant staphylococci (17).

In contrast to CoNS, *S. aureus* isolates in this study demonstrated preserved susceptibility to all antimicrobial with exception of penicillin. No methicillin-resistant isolates (MRSA) were found.

CONCLUSION

In the present study CoNS were identified as the most common cause of catheter-related bloodstream infections in patients following HSCT, with *S. epidermidis* being the predominant species. All CoNS isolates were methicillin-resistant. These results confirm that the representatives of genus *Staphylococcus* are a common cause of foreign body infections in this patient group. A serious problem is their constantly increasing antimicrobial resistance and ability to permanently colonize the artificial devices, causing difficult-to-treat infectious complications. The only option for definitive therapy remains the removal or replacement of the colonized CVC.

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