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Antibiotic susceptibility profile in clinical significant CoNS isolates from blood cultures

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Abstract

Coagulase-negative staphylococci (CoNS) are part of the normal flora of human skin. However, recent studies indicate that coagulase-negative staphylococci have emerged as a major cause of opportunistic infection. CoNS account for a significant proportion of nosocomial bacteremia cases related to the insertion and maintenance of intravascular catheters. The aim of this study was to analyze antimicrobial profile of significant CoNS isolates from blood in patients with clinical manifestation of sepsis hospitalized in University Children's Hospital, Clinic of Nephrology and Clinic of Surgery. Standard criteria (more than one blood culture, the same isolate from less two samples, positive clinical parameters for real sepsis) to categorize isolates as contaminants versus blood stream infection were used. For this analysis, only one CoNS isolate from each patient was included. Processing of blood cultures was done with the automated Bact/Alert system 3D (bioMerieux, France). The isolated strains were identified on device Vitek 2 and tested to find out their sensitivity and resistance to the *Staphylococcus* spp organism, (determination their MICs). From the period of three years (May 2014 to May 2016), a total of 11 094 blood cultures (set aerobes/anaerobes and pediatric bottles) in the Institute of Microbiology and Parasitology, University Clinical Center, Skopje, were investigated. A total of 1794 (16,2%) blood cultures were positive. From them, CoNS isolates were in 645 (485 strains with resistance to methicillin - MR), thus percent of staphylococcal methicillin resistance was 75,2%. A total of the 645 isolates, only 112 (17,4%), have been related with clinical signification. The most of clinical significant CoNS isolates (53), belong to *Staphylococcus epidermidis.* From the other species, the more frequent isolates were *Staphylococcus hominis* and *Staphylococcus haemolyticus*, 26 and 25 strains, respectively. All strains were resistant to penicillins (100%). The percent of resistance to oxacillin was very high, 100% in *Staphylococcus haemolyticus* and 96% in *Staphylococcus epidermidis* and *S. hominis*. The strains were 100% susceptible to vancomycin and tygerciclyne. Five strains of *Staphylococcus epidermidis* and one strain from *Staphylococcus hominis* were with intermediate susceptibility to teicoplanine (MICs value were 16 mg/l for all). The emergence of strains with intermediate susceptibility to teicoplanin indicates the danger of resistance of staphylococci to glycopeptides. In the future this would be a major therapeutic problem. The conclusion is that given the increasing multidrug resistance among staphylococci and the possible emergence of vancomycin-resistant strains, global strategies are needed to control emergence and spread of multiply resistant staphylococci.

Keywords: CoNS, sepsis, blood cultures, Bact/Alert, antimicrobial profile.

Introduction

Coagulase-negative staphylococci (CoNS) are part of the normal flora of human skin. Lacking coagulase, an enzyme-like protein that was traditionally associated with virulent potential of staphylococci, coagulase-negative staphylococci are usually considered low-virulent pathogens comparing to the well-known pathogenic coagulase-positive *Staphylococcus aureus* (Beker K, 2014). However, recent studies indicate that

coagulase-negative staphylococci have emerged as a major cause of opportunistic infection (Naomi P, 2002; Reimer L G, 1997). CoNS account for a significant proportion of nosocomial bacteremia cases related to the insertion and maintenance of intravascular catheters (Naomi P, 2002; Beker K, 2014). Coagulase-negative staphylococci (CoNS) are now also recognized as a major cause of nosocomial infective endocarditis in coronary care units (CCU) (Beker K, 2014; Cekovska Z, 2015; Voineagu Lavinia). Invasive CoNS sepsis can be recognized and requires specific antibiotic therapy.

The aim of the study

The aim of this study is to analyze antimicrobial profile of significant CoNS isolates from blood in patients with clinical manifestation of sepsis.

Materials and Methods

CoNS isolates from blood cultures in patients with major significance for real septic condition have been analyzed. Samples were from patients hospitalized in University Children's Hospital, Clinic of Nephrology and Clinic of Surgery (patients in Intensive Care Units, patients with medical devises; generally, patients with low immune defense).

Criteria used to categorize isolates as contaminants versus blood stream infection were the following (Weinstein M P, 1997):

1. Fever or signs of sepsis at the time of blood culture;
2. Isolation of the same potential skin contaminant from two or more blood cultures drawn on separate occasions within a 48-hour period and isolated from a patient with an intravascular access device inserted at least 48 hours before and physician institutes appropriate antimicrobial therapy;
3. Absence of any other possible site of infection.

Only one CoNS isolate from each patient in this analysis is included.

CoNS isolates that were suspected contaminants (according to some clinical and microbiological parameters - without signs of sepsis in the patient and isolated in only one blood culture) were excluded from this study and their antimicrobial profile are not analyzed.

Processing of blood cultures, the automated Bact/Alert system 3D (bioMerieux, France) were used. The assay is performed directly on positive blood culture specimens that are determined by Gram Stain as Gram Positive Cocci in Clusters (GPCC) or as Gram Positive Cocci in singles (GPC). Samples were collected and processed using standard microbiological protocols. The isolated strains were identified on device Vitek 2 on IDGP identification cards and the sensitivity test was perfomed on AST-P580 cards (Penicillin - P, oxacillin (cefoxitin - FOX), gentamycin - GM, tobramycin - TB, levofloxacin - LV, moxifloxacin - MX, clindamycin - CL, erythromycin - ER, tetracycline - TE, fosfomycin - FOS, rifampycin - R, cotrimoxazol - CO, teicoplanine - TEI, vancomycine - VA and tygecicline – TYG, were tested to find out their sensitivity and resistance to the *Staphylococcus* spp organism, (determination their MICs).

Results

In the three years period (may 2014 to may 2016), 11 094 blood cultures were investigated (set aerobes/anaerobes and pediatrics) at the Institute of Microbiology and Parasitology, University Clinical Center, Skopje. Of the total surveyed blood cultures, 1794 (16.2%) were positive. From them, CoNS 645 were isolates: 485 strains with resistance to methicillin (MR). Percent of methicillin resiatnce was 75.2%.

A total of the 645 isolates, only 112 have been with clinical signification (17.4%). It is very clear that the rest of CoNS isolates (82.6%) were contaminats.

The most of clinical significant CoNS isolates (53), belong to *Staphylococcus epidermidis* (Table and Figure 1).

Table 1. Different CoNS species isolated from blood cultures

A total of number	*Staphylococcus epidermidis*	*Staphylococcus hominis*	*Staphyloccus haemolyticus*	Other
112	53	26	25	8*

**Staphylococcus capitis* (2 strains), *Staphylococcus warneri* (3 strains), *Staphylococcus lugdunensis* (3 strains)

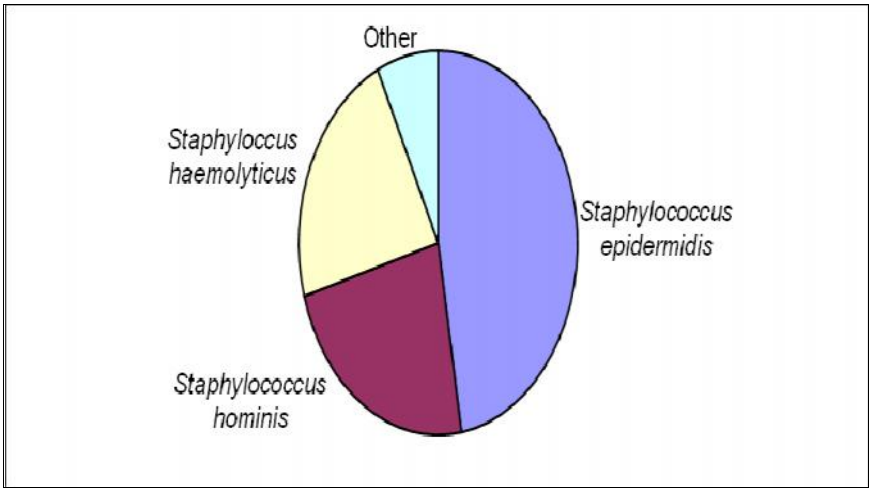

Figure 1. Different CoNS isolates

Table 2. Origin of the strains

Clinic	Number of isolated strains
Children's Hospital*	66
Nephrology	32
Neurosurgery	14

*80% from Intensive Care Units

Table 3. Resistant profile of *Staphylococcus epidermidis* isolates

Antibiotic	P	FOX	GM	TB	LV	MX	CL	ER	TE	FOS	R	CO	TEI	VA	TYG
Number	53	51	37	40	11	8	34	38	33	4	8	3	0*	0	0
Percent	100	96	69,8	75,47	20,7	15,1	64,1	71,7	62,26	7,5	15,1	5,7	0	0	0

Penicillin - P, cefoxitin - FOX, gentamycin - GM, tobramycin - TB, levofloxacin - LV, moxifloxacin - MX, clindamycin - CL, erythromycin - ER, tetracycline - TE, fosfomycin - FOS, rifampycin - R, cotrimoxazol - CO, teicoplanine - TEI, vancomycine – VA, tygecicline – TYG

*Resistance strain was not found, but five strains were with intermediate susceptibility to teicoplanine

Table 4. Resistant profile of *Staphylococcus haemolyticus* isolates

Antibiotic	P	FOX	GM	TB	LV	MX	CL	ER	TE	FOS	R	CO	TEI	VA	TYG
Number	25	25	24	25	17	14	18	21	14	24	2	16	0	0	0
Percent	100	100	96	100	68	56	72	84	56	96	8	64	0	0	0

Table 5. Resistant profile of *Staphylococcus hominis* isolates

Antibiotic	P	FOX	GM	TB	LV	MX	CL	ER	TE	FOS	R	CO	TEI	VA	TYG
Number	26	25	16	18	8	8	19	20	16	18	2	1	0*	0	0
Percent	100	96	61,54	69,2	30,7	30,7	73,1	76,9	61,5	69,2	7,7	3,84	0	0	0

*Resistance strain was not found, but one strain was with intermediate susceptibility to teicoplanine

Discussion

According to the literature data, these three CoNS species: *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus haemolyticus*, leading etiology of bacteremia and sepsis in immunocompromised hosts. In our case, the first three isolates were the same. (Table and Figure 1). Concerning to their origin, the most of them have been isolate from University Children's Hospital (80% from Intensive Care Units). The patients hospitalized in the other two departments were with one or more medical devices (intravascular catheters, urinary catheters, external or internal catheters).

Over the last decades, there has been an enormous increase and emergence of CoNS strains, particularly *S.epidermidis, S.haemolyticus* and *S.hominis*, resistant to more antibiotics, especially in nosocomial settings (Mack D, 2000; Becker K, 2014). Resistance to penicillin among these and the other coagulase-negative staphylococci (CoNS), approaches 90 to 95 percent. Resistance to methicillin and semisynthetic penicillins has been observed in more than 80 percent of CoNS isolates; these isolates are often resistant to multiple classes of antibiotics in addition to beta-lactams (Becker K, 2014).

In addition, it will be discussed for each of these three CoNS species individually.

Staphylococcus epidermidis

Staphylococcus epidermidis is a part of the human normal flora (approximately 65-90% of all staphylococci recovered from human aerobic flora). Consequently, it is a true opportunistic pathogen, as it requires a major breach in the host's innate defences. Among all CNS, *Staphylococcus epidermidis* strains represent the most frequent cause of nosocomial sepsis and the most common agents of infections with implanted medical devices (Naomi P, 2002, Cekovska Z, 2015)..

Those most susceptible to infection are intravenous drug users, newborns, elderly, and those using catheters or other artificial appliances. The organism produces a glycocalyx "slime" that acts as glue adhering it to plastic and cells, and also causes resistance to phagocytises and some antibiotics (Mack D, 2000). Rather, further strategies to inhibit biofilm formation will need to be explored to limit chronic catheter-related infections in all patients, especially in neonates.

Methicillin resistance/multiple drug resistance has been documented more often in disease causing strains of *S. epidermidis* than in skin colonizing strains (Weinstein M P, 1997). Most of these strains harbor mec A, the gene encoding the penicillin –binding protein PBP2a, which has decreased affinity for beta-lactam antibiotics (Mack D, 2000; Tenover FC, 1999).

In our study, all strains were resistant to penicillins (100%) and the percent of resistance to oxacillin was very high (96%) (Table 3). The percent of resistance to gentamycin, tobramycin, levofloxacin and moxifloxacin was 69.8%, 75%, 47%, 20.7% and 15.1%, respectively. Inducible clindamycine resistance was positive in 3 strains, but separately resistance to erythromycin and clyndamycine were 71.7% and 64.1%, respectively. The strains were 100% susceptible to vancomycin and tygerciclyne, but five strains were with intermediate susceptibility to teicoplanine (MICs value were 16 mg/l for all five – Table 3).

The emergence of strains with intermediate susceptibility to teicoplanin indicates the danger of resistance of staphylococci to glycopeptides. In the future this would be a major therapeutic problem.

Staphylococus haemolyticus

Staphylococus haemolyticus can be found on normal human skin flora and can be isolated from axillae, perineum, and ingunial areas of humans. *S. haemolyticus* is also the second most common coagulase-negative staphylocci presenting in human blood. Therefore, like other non-aureus staphylococci, its pathogenic characters were not well-studied until recently, when *S.haemolyticus* started emerging as a major cause of nosocomial infections (infections acquired during treatment at a hospital for another disease). Reported cases of infections caused by *S. haemolyticus* include septicemia (dysfunction of organ systems resulting from immune response to a severe infection), peritonitis (inflammation of the serous membrane lining abdominal cavity), and infections of urinary tract, wound, bone and joints (Becker K, 2014). In rare cases, *S. haemolyticus* has also been reported to cause infective endocarditis, inflammation of the heart (the endocardium), which might lead to severe complications such as heart failure or death. Common clinical symptoms of an *S. haemolyticus* infection are fever and an increase in white blood cell population (leukocytosis); signs and parameters witch are present in our patients. The bacteria can cause also meningitis (especially in neurosurgical patients), skin

or soft tissue infections and prosthetic join infections (David Souvenir, 1998).

Although *Staphylococcus haemolyticus* is relatively less virulent than some other staphylococci such as *S.aureus*, the ability of the species to acquire multi-antibiotic resistance has made it a serious threat to worldwide health care facilities. According the literature data, *S. haemolyticus* has the highest level of antibiotic resistance among the CoNS. Common antibiotics that are subject to resistance in *S. haemolyticus* include methicillin, gentamycin, erythormycin, and uniquely among staphylococci, glycopeptide antibiotics. The resistance genes for each type of antibiotic can be located on the chromosome (methicillin), on the plasmids (erythromycin) or on both chromosome and plasmids (gentamycin) (Archer G L, 1994).

In our study, *Staphylococus haemolyticus* strains were 100% resistant to beta-lactam antibiotics. The strains showed high level of gentamycin resistance (96%). All 25 strains were resistant to tobramycin. High level of resistance is found to quinolones: 68% of strains to levofloxacine and 56% to moxifloxacine. Inducible clindamycine resistance positive was in 4% of the cases, but separately resistance to erythromycin and clyndamycine were 84% and 72%, respectively. The strains showed 100% susceptibility to vancomycin, teicoplanine and tygerciclyne. The isolate with intermediate susceptibility to teicoplanine between *Staphylococcus haemolitycus* isolates in this investigation, is not found (Table 4).

Staphylococus hominis

In a certain study, *S. hominis* was calculated to account for 22% of the total staphylococci species isolated from individuals, second to *S. epidermidis* at 46% *S. hominis* is the predominant species on the head, axillae, arms, and legs (David Souvenir, 1998). *S. hominis* is normally found on human skin of usually harmless people, but it can sometimes cause infections in people with abnormally weak immune systems: first described in 1998, and was first implicated in causing bacteremia in 2002. Recently, a novel subspecies of *Staphylococcus hominis*, *S. hominis* subsp. *novobiosepticus* (SHN), was isolated from blood cultures and other clinical specimens responsible to caused infections. *S. hominis* subsp. *hominis* also is has been found in blood from children hospitalized in ICUs (Voineagu Lavinia, 2012). Similarities in some properties between *S. hominis* and several other species suggest a close relationship between *S. hominis* and *S. epidermidis, S. haemolyticus*, and *S. warneri*.

According the literature data, more strains of this species were resistant to methicillin and gentamicin, and most strains were resistant to erythromycin, clindamycin, chloramphenicol, trimethoprim/ sulfamethoxazole, and ciprofloxacin.

In our study, all *Staphylococcus hominis* strains were resistant to penicillins (100%) and the percent of resistance to oxacillin was very high (96%), like in *Staphylococcus epidermidis* strains. *Staphylococcus hominis* isolates showed 61%, 54% resistance to gentamycin and 69, 2% resistance to tobramycin. Percent of resistance to investigated quinolones were the same: 30.7% to levofloxacine and moxifloxacine. Inducible clindamycine resistance were positive in two cases, but separately resistance to erythromycin and clyndamycine were 76. 9% and 73. 1%, respectively. The strains were 100% susceptible to vancomycin and tygerciclyne. Intermediate susceptibility to teicoplanine between *Staphylococcus hominis* isolates in this analysis, was detected only in one strain (Table 5).

Other coagulasa negative species

The other coagulasa-negative staphylococci found from blood in our study were: two strains of *Staphylococcus capitis* (2 strains) and three of *Staphylococcus warneri* and *Staphylococcus lugdunensis*. *Staphylococcus lugdunensis* is unique among the CoNS by virtue of its susceptibility to a wide range of antimicrobials: our isolates showed that all tree strains were susceptible to all investigated antimicrobial agents (excepted one strain which was resistant only to penicillin).

The other staphylococci were with different antimicrobial profile, but their clinical signification is not so sure (according clinical condition of sepsis). In the case of isolation of these species, it is possible that high body temperature and fever due to other existing latent foci of infection (Kloos W E, 1994).

Conclusion

Now, the conclusion is that given the increasing multidrug resistance among staphylococci and the possible emergence of vancomycin-resistant strains, global strategies are needed to control emergence and spread of multiply resistant staphylococci.

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