

Original Article

Nosocomial coagulase-negative staphylococci in Belgrade: between Scylla and Charybdis

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Abstract

Introduction: Coagulase-negative staphylococci (CoNS) are increasingly resistant nosocomial pathogens. We aimed to analyze the prevalence of CoNS isolates in clinical settings, the evolution of antimicrobial resistance of CoNS, and antibiotic consumption in a hospital.

Methodology: This retrospective cohort study was carried out at a tertiary healthcare facility over 17 months. Identification of isolated cultures and antibiotic susceptibility testing were performed using the Vitek2 system. Of 1,217 isolates, 209 were obtained from 193 patients who had symptoms of nosocomial infections. Data were analyzed by descriptive statistics. Antibiotic consumption in the hospital is expressed in defined daily doses/100 patient days.

Results: Sixty-one percent of patients were admitted to the internal medicine ward, while others were admitted to the surgical ward. Forty-four percent of Gram-positive isolates were from wound swabs, and 26% were from blood. The predominant Gram-positive bacteria were CoNS. Antibiotic resistance of CoNS was highest against beta-lactam antibiotics, macrolides, and tetracyclines. Tigecycline, linezolid, and vancomycin produced the highest activities against CoNS in *in vitro* conditions, and consumption of linezolid and tigecycline increased in the same period.

Conclusion: There are just a few remaining therapeutic options for the treatment of CoNS according to our results; vancomycin, linezolid, and tigecycline might be considered as first-choice antibiotics, but such a hypothesis should be supported with a pharmaco-economic analysis. Unfortunately, novel antimicrobial agents are still unavailable and/or too expensive in developing countries. However, inappropriate use of those antibiotics may lead to the rapid development of resistant strains in the near future.

Key words: *Staphylococcus*; nosocomial infection; resistance; antibiotics.

J Infect Dev Ctries 2016; 10(9):907-912. doi:10.3855/jidc.7939

(Received 23 November 2015 – Accepted 08 February 2016)

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Introduction

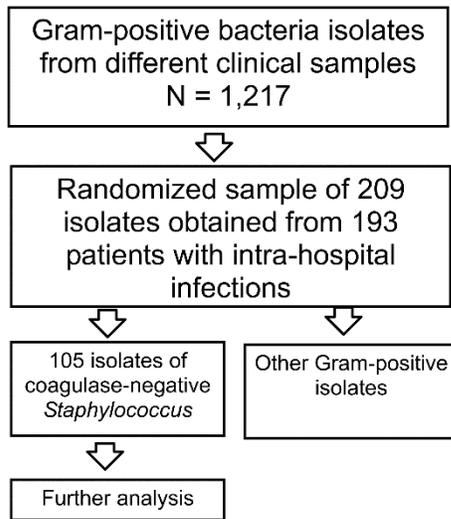
Healthcare-associated infections are more frequent in resource-limited settings than in developed countries [1,2]. Coagulase-negative staphylococci (CoNS) are increasingly resistant nosocomial pathogens. Once considered a low-virulence staphylococci, CoNS have become a major healthcare problem, especially in the intensive care unit (ICU) environment and in elderly and immunocompromised patients [3]. Serbia is an upper middle-income country with an inverted population pyramid and large potential target population for CoNS nosocomial infections [4]. National regulations, as well as local guidelines for the prevention of nosocomial infections, are still not fully applied in the healthcare system. Accordingly, nosocomial infections with CoNS might become life-

threatening both to critically ill patients and to healthcare systems in this developing country.

Methodology

The retrospective cohort study was carried out at the University Medical Center "Bezanijska kosa", Belgrade, Serbia, a 360-bed tertiary care center. Microbiological outcomes and data on antibiotic consumption were analyzed. Records of 1,217 Gram-positive isolates from the hospital microbiology laboratory during the 17-month period from June 2013 to November 2014 were analyzed. From the total pool of isolates, 209 isolates obtained from 193 patients with intra-hospital infections were randomly selected for further analysis (Figure 1). Identification of isolated cultures of bacteria and antibiotic susceptibility testing

Figure 1. Study design.



in the hospital microbiological laboratory were done using the Vitek2 system (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's recommendations.

Susceptibility of CoNS to the following antibiotics was tested: penicillin (98/105), amoxicillin (93/105), amoxicillin + clavulanic acid (93/105), ampicillin + sulbactam (83/105), cefaclor (96/105), cephalexin (89/105), cefuroxime (104/105), cefuroxime (76/105), cefotaxime (98/105), ceftazidime (87/105), ceftriaxone (95/105), cefepime (87/105), ertapenem (83/105), imipenem (99/105), meropenem (95/105), amikacin (75/105), gentamicin (92/105), tobramycin (47/105), ciprofloxacin (102/105), levofloxacin (104/105), ofloxacin (92/105), azithromycin (100/105), erythromycin (102/105), clindamycin (71/105), linezolid (83/105), teicoplanin (89/105), vancomycin (102/105), tetracycline (90/105), tigecycline (95/105), fusidic acid (105/105), sulfamethoxazole + trimethoprim (104/105), and piperacillin (20/105).

All isolates were obtained from patients admitted to the University Medical Center "Bezanijska kosa" > 48 hours before the antimicrobial surveillance test. They had symptoms and signs of nosocomial infections during the investigated period. Patients' demographic and epidemiological data were obtained. Patients' personal data were not used in the study, and the data could not be correlated with the patients. Data were analyzed by descriptive statistics. Hospital antibiotic consumption is expressed in defined daily doses (DDD)/100 patient days.

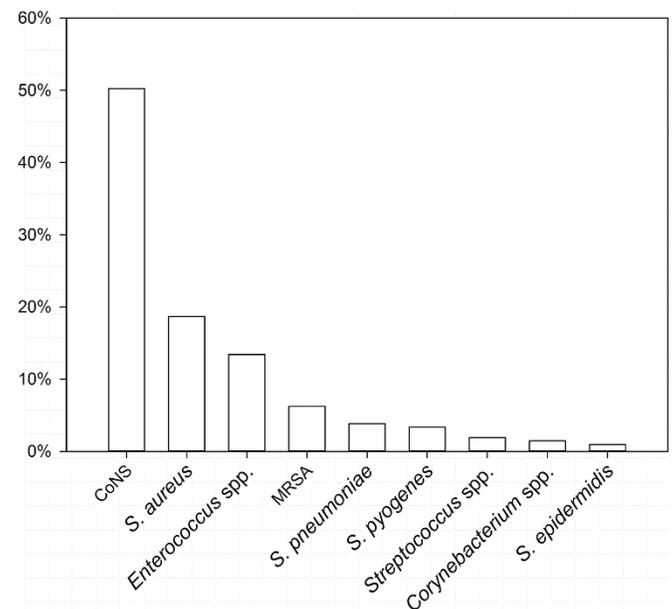
Results

Of 193 patients with intra-hospital infections, 117 (61%) were admitted to the internal medicine ward (i.e., hematology, cardiology, or pulmonology), while others were admitted to the surgical ward. Of 209 Gram-positive isolates, 91 were from wound swabs (44%), 55 from blood (26%), and 38 from the upper and lower respiratory tract (18%) (i.e., from nasal and pharyngeal swabs, endotracheal tube, or sputum). In addition, Gram-positive pathogens were isolated from skin, urine, and other samples as well.

The predominant Gram-positive bacteria was CoNS, which was isolated from 105 clinical samples (50%), followed by *Staphylococcus aureus* (18.5%), *Enterococcus* spp. (14%), and methicillin-resistant *S. aureus* (6%) (Figure 2). Other Gram-positive bacteria (e.g., *S. pneumoniae*, *S. pyogenes*, *Streptococcus* spp., *Corynebacterium* spp.) were isolated rarely (11.7%).

CoNS isolates exhibited similar distribution across the originating hospital ward (internal medicine, 68; surgery, 37), but distinct distribution across the originating biological sample compared to the total pool of Gram-positive isolates (blood, 48; wounds, 39). The samples collected from the patients with bloodstream CoNS infection included blood samples from peripheral veins (40/48), and swabs from central venous catheter (CVC) hubs (8/48).

Figure 2. Distribution of Gram-positive bacteria found in hospital isolates during the 17-month study period.



CoNS: coagulase-negative staphylococci; MRSA: methicillin-resistant *Staphylococcus aureus*

Mixed infections were detected in 4/48 blood samples (3 co-infections of CoNS with Gram-negative bacteria and 1 co-infection with Gram-positive bacteria), and 9/39 wound swabs (7 co-infections of CoNS with Gram-negative bacteria and 2 co-infections with Gram-positive bacteria).

The susceptibility of isolated CoNS to tested antibiotics is presented in Figure 3.

Antibiotic resistance of CoNS was highest against beta-lactam antibiotics, followed by macrolides and tetracycline. Tigecycline, linezolid, and vancomycin produced the highest activities against CoNS in *in vitro* conditions.

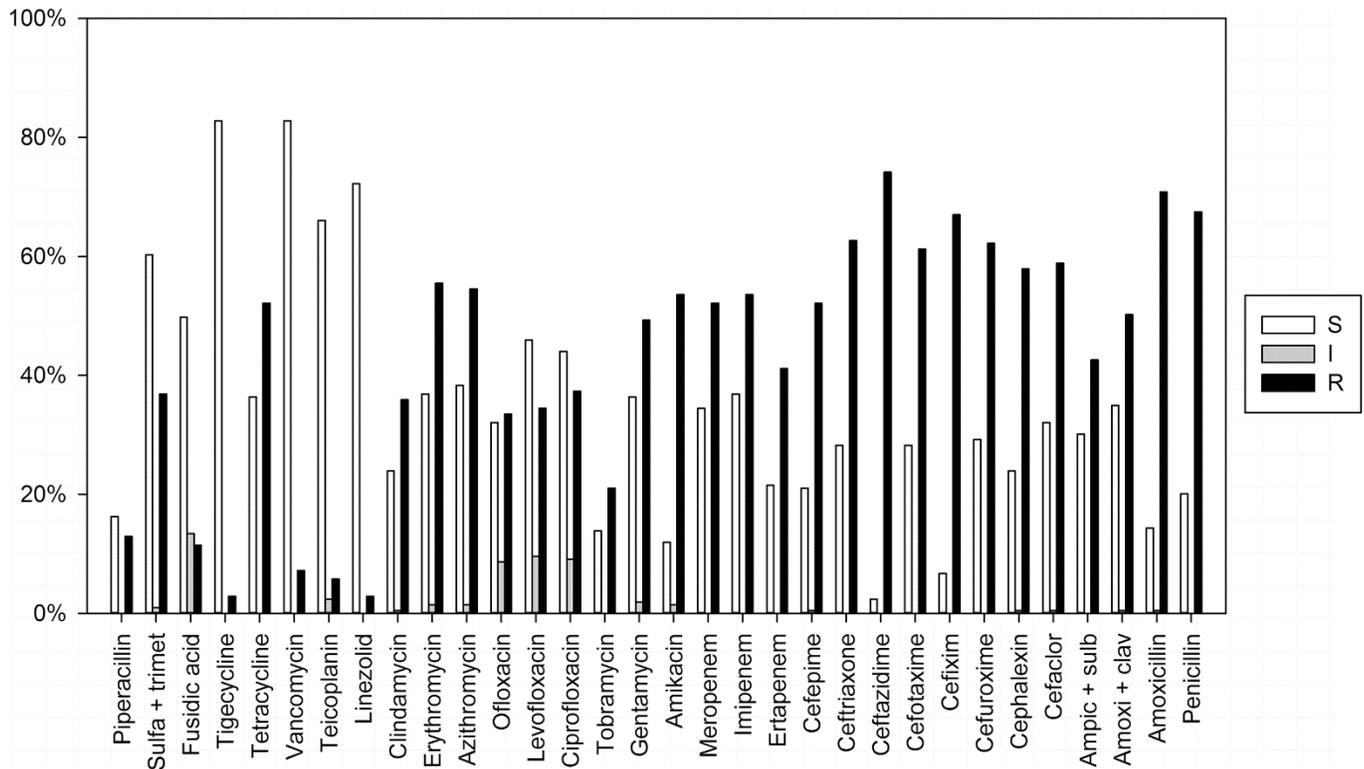
In 2013 and 2014, hospital antibiotic expenditures totaled around USD \$30,000 and \$27,000 per month, respectively. At the same time, expenditures on reserve antibiotics totaled around USD \$21,000 and \$20,000 per month, respectively. However, consumption of linezolid and tigecycline increased in the same period, from 0.05 and 0.76 to 0.125 and 1.20 DDD/100 patient days, respectively.

Discussion

There has been a dramatic epidemiological shift toward Gram-positive nosocomial pathogens during the last decades. Despite high false-positive blood culture rates, CoNS has become a leading cause of nosocomial bloodstream infections, especially in patients with comorbidities and those subjected to certain diagnostic and therapeutic procedures [5-10].

Almost two-thirds of our patients with isolated Gram-positive bacteria from various clinical samples were admitted to the internal medicine ward, mainly to the departments of hematology, cardiology, or pulmonology. It is well known that hematological diseases are among risk factors of infections in hospital settings. Also, respiratory infections account for the majority of serious nosocomial infections. Furthermore, the onset of pressure ulcers in long-term care facilities is a common risk factor of hospital infections [11]. On the other hand, surgical-site infections are described as the most frequent nosocomial infections worldwide [12]. However, in our patients, Gram-positive bacteria from wound swabs were mainly observed at the internal medicine ward. This might suggest that patients develop wounds as decubitus due to their extended stay

Figure 3. Sensitivity of coagulase-negative staphylococci to antibiotics (isolates from the hospital microbiology laboratory collected between June 2013 and November 2014). Sulfa + trimet: sulfamethoxazole + trimethoprim; ampic + sulb: ampicillin + sulbactam; amoxi + clav: amoxicillin + clavulanic acid.



S: sensitive; I: intermediate; R: resistant.

in a hospital setting and/or use of different medical devices (*e.g.*, catheters).

In our study, CoNS bloodstream infections were mainly confirmed by peripheral vein blood cultures. Such a method is sufficiently specific and accurate, with very high positive and negative predictive values [13]. The peripheral vein blood cultures could be combined with semi-quantitative conservative techniques to screen for catheter-related bloodstream infections, while quantitative blood cultures could be used as a confirmatory method.

With respect to mixed infections, our results fit into the range confirmed by other authors [14,15]. In mixed infections, CoNS might produce beta-lactamases and contribute to the failure of antimicrobial therapy.

Antibiotic resistance of Gram-positive bacteria is a major healthcare problem. Also, biofilms formed by CoNS make these infections difficult to treat [3]. According to our results, resistance of CoNS was highest against beta-lactam antibiotics, followed by macrolides and tetracycline. Vancomycin, linezolid, and tigecycline produced the highest activities against CoNS in *in vitro* conditions. Similar data were observed in five-point prevalence studies in Spanish hospitals from 1986 to 2002 in a large group of patients. These studies showed an overall increase in CoNS resistance to most antimicrobials, mainly to oxacillin, erythromycin, gentamicin, and ciprofloxacin [16]. On the other hand, there are novel antibiotics that have been developed to overcome resistance of CoNS (*e.g.*, linezolid, daptomicin, tigecycline, and quinupristin/dalfopristin) [17]. However, some of those antimicrobials (*e.g.*, daptomicin, quinupristin/dalfopristin and tedizolid) are not always available in developing countries due to their cost.

Studies have reported vancomycin resistance of CoNS. Vancomycin resistance is a consequence of biofilm production by CoNS [18]. According to our results, vancomycin is still one of the drugs of choice for the eradication of such resistant bacteria. Susceptibility of CoNS to vancomycin was tested in 102 clinical samples in hospital microbiological laboratory, and only 7 (6%) of isolated CoNS were resistant to vancomycin. Of note, almost all of those 7 clinical samples were from same hospital ward. Better epidemiological measures are obviously needed in such cases.

Linezolid is another option in the therapy of nosocomial CoNS infection, according to our results. Susceptibility of CoNS to linezolid was tested in 83 out of 105 clinical samples. Only CoNS isolated from one CVC (2% of the whole pool of investigated clinical

samples) was resistant to linezolid. Linezolid is a new agent, and resistance to linezolid is still rare. Our results are mostly in agreement with those of other reports. However, resistance of CoNS to linezolid has also been found. Such resistance is due to target modifications, such as mutations in the V domain of 23 S rRNA and ribosomal proteins such as L3 and L4 [19-22].

Tigecycline is the first member of a class of antibiotics called glycylcyclines. It is a broad-spectrum antibiotic with activity against many Gram-positive and Gram-negative bacteria [23]. In our research, tigecycline was one of the most effective antibiotics against CoNS. Only a few CoNS isolates were resistant to this antibiotic. There are clearly defined indications for tygecicline use; these include treatment of complicated skin and skin structure infections, as well as complicated intra-abdominal infections. According to our research, CoNS were isolated mostly from wound swabs (44%). Furthermore, tigecycline is important in the treatment of this type of intra-hospital infection. On the other hand, its inappropriate use (for example, treatment of respiratory and urinary infections) could trigger CoNS resistance to tigecycline.

There seems to be a bright future for the use of novel antibiotics, such as daptomicin, quinupristin/dalfopristin, dalbavancin, or tedizolid in our country and region. The majority of these antibiotics are still not approved by the national drug agency, and accordingly, resistance to them is very low. However, we could not be sure about the increasing trend of resistance to those antibiotics in the near future.

In-hospital consumption of the last remaining antibiotics that are effective against CoNS will inevitably rise within the next few years. Such a trend could be due to several factors, including increasing incidence of nosocomial CoNS infections, widespread resistance of CoNS to other antibiotics, and an aging population. Inappropriate antibiotic treatment of patients with CoNS bacteremia may cost up to USD \$200,000 per year (data from an American tertiary care hospital) [24]. Consumption of linezolid and tigecycline increased in our study despite the parallel decrease in overall hospital antibiotic expenditures. Recommended Centers for Disease Control (CDC) core elements of hospital antibiotic stewardship programs include, among others, appointing a physician leader responsible for program outcome, action, tracking (monitoring antibiotic prescribing), reporting, and education [25]. Medical Center "Bezanijska kosa" introduced such a system at the end of 2013. A clinical pharmacologist was appointed, local antimicrobial

resistance has been tracked, guidelines for antibiotic usage have been implemented, antibiotic monitoring has been conducted, and appropriate continuing medical education (CME) courses have been delivered for the staff. Preliminary results are promising, but long-term follow-up is needed.

These data about CoNS nosocomial infection frequency and its resistance have influence on the design of empirical therapy regimens. A similar study could be conducted periodically in order to keep medical professionals informed about local antimicrobial resistance. Such a study is a quick, useful, and easy tool in daily medical practice. Current local antimicrobial therapy guidelines should take into account the emerging role of vancomycin, linezolid, and tigecycline in the treatment of nosocomial CoNS infections, and this hypothesis should be supported with a pharmacoeconomic analysis.

Conclusions

Management of CoNS nosocomial infections is challenging issue with just a few remaining therapeutic options. Vancomycin, linezolid, and tigecycline might be considered as first-choice antibiotics, but proper cost-effectiveness analysis is needed to confirm such a hypothesis. Novel antimicrobial agents are still unavailable and/or too expensive in developing countries. However, inappropriate use of those antibiotics may lead to the rapid development of resistant strains in the near future.

Acknowledgements

This study was supported by the Scientific Research Grant from Ministry of Science of Montenegro, No. 01-1370 and 01-907/2, and by the Scientific Research Grant from the Ministry of Education and Science of Serbia, No. OI 175046. We are grateful to Dr. Mihail Basa for his technical assistance.

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Conflict of interests: No conflict of interests is declared.