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Investigation of Glycopeptide Susceptibility of Coagulase-Negative Staphylococci (CoNS) From a Tertiary Care Hospital in Gorgan, Northern Iran

Hamideh Mehri,[1] Roghaye Jahanbakhsh,[1] Fatemeh Shakeri,[2] Abdollah Ardebili,[1,3] Naser Behnampour,[4] Behnaz Khodabakhshi,[5] and Ezzat Allah Ghaemi[1,3,*]

[1]Department of Microbiology, Golestan University of Medical Sciences, Gorgan, Iran
[2]Department of Microbiology, Mazandaran University of Medical Sciences, Sari, Iran
[3]Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[4]School of Health, Golestan University of Medical Sciences, Gorgan, Iran
[5]Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan, Iran

[*]*Corresponding author*: Ezzat Allah Ghaemi, Department of Microbiology, Golestan University of Medical Sciences, Gorgan, Iran. Tel: +98-9113711770, Fax: +98-1732440225, E-mail: eghaemi@yahoo.com

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Abstract

Background: Coagulase-negative staphylococci (CoNS) are clinically important, especially in nosocomial and neonatal infections. The increasing emergence of glycopeptide-resistant CoNS has made these agents therapeutically challenging.
Objectives: We aimed to investigate the susceptibility patterns of CoNS to teicoplanin and vancomycin in Gorgan, northern Iran.
Materials and Methods: A total of 100 clinical samples were obtained from different wards of a hospital and screened for CoNS with standard microbiological and biochemical tests. Antibiogram testing was carried out for the detection of vancomycin-, teicoplanin-, and multidrug-resistant (MDR) species. The minimum inhibitory concentration (MIC) of vancomycin was determined using E-test strips. The presence of the *vanA* gene was investigated with PCR.
Results: Only 1% of CoNS (*Staphylococcus haominis*) showed resistance to vancomycin and 15% of these were intermediate-resistant to teicoplanin on the disc diffusion agar test. In addition, all isolates (100%) were negative for *vanA* on PCR and sensitive with E-test methods. The mean MIC value of vancomycin was 1.35 $\pm$ 0.29 μg/mL. *S. haemolyticus* and *S. epidermidis* showed the highest rates of MDR (50% and 24%, respectively). Additionally, CoNS isolated from blood (42%) and urine samples (30%) had the highest rates of MDR.
Conclusions: Vancomycin, but not teicoplanin, can be considered an effective antibiotic of choice for controlling infections caused by MDR CoNS in Gorgan, depending on the bacterial species.

Keywords: Coagulase-Negative Staphylococcus, Drug Resistance, Teicoplanin, Vancomycin

1. Background

The Staphylococcus genus includes at least 40 species. Of these, nine have two subspecies, one has three subspecies, and one has four subspecies (1). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora (2). Coagulase-negative staphylococci (CoNS) species make up a large and heterogeneous group of Gram-positive bacteria, into which more than 30 species have been introduced (3, 4).

In recent years, the importance of CoNS has increased, especially because of their major causative role in the nosocomial infections in both developed and developing countries (5, 6). The widespread use of immunosuppressive drugs, indwelling intravascular catheters, artificial tools, patient- and personnel-related factors, and health-care settings have been reported to be associated with the spread of these infections (7). Premature infants are at a high risk of CoNS infections (6).

Among the CoNS, *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus* are more common in nosocomial infections, most of which have gained resistance to methicillin, penicillin, and other antibiotics (8). The genes responsible for the multi-drug resistance (MDR) phenotype of CoNS are located on plasmids, which can be easily exchanged between the species. Indeed, despite continuing efforts, antibiotic resistance remains a major problem in controlling the CoNS infections (9). To achieve effective treatment, a variety of glycopeptide antibiotics, including vancomycin, teicoplanin, telavancin, ramoplanin, and decaplanin are primarily prescribed (10). Vancomycin is the most important

drug for treatment of the MDR *S. epidermidis* (11). However, resistance to vancomycin has been frequently reported among the staphylococci (12, 13). In 1987, Schwalbe and colleagues reported the first clinical isolate of vancomycin-resistant CoNS (13). Since then, many vancomycin-resistant or other glycopeptide-resistant CoNS isolated from clinical samples have been documented in different countries (14-18). Additionally, the emergence of resistance to teicoplanin has been reported (15). Therefore, the use of vancomycin and teicoplanin as appropriate therapeutic agents against CoNS infections is under debate (19).

2. Objectives

Because of the importance of CoNS, especially in nosocomial and neonatal infections, and the worldwide emergence of vancomycin-resistant strains, we aimed to determine the prevalence of teicoplanin- and vancomycin-resistant CoNS isolates from patients in a hospital in Gorgan, northern Iran, using both phenotypic and molecular methods.

3. Materials and Methods

3.1. Clinical Samples and Bacterial Isolates

From January 2013 to May 2014, a total of 100 clinical CoNS isolates were collected from patients hospitalized in distinct wards of Sayyad Shirazi hospital in Gorgan, Iran. The origins of the isolates were different clinical specimens, including blood, urine, eyes, tracheal aspirates, wounds, and catheters. Each specimen was cultured on blood agar and incubated at 37°C for 24 h. Identification of CoNS to the species level was done with Gram staining, then standard biochemical tests were performed, including cultivation in mannitol salt agar; oxidation-fermentation (OF); the Voges-Proskauer (VP) test; hydrolysis of L-pyrrolidonyl-beta-naphthylamide (PYR); nitrate reduction; acid production; coagulase, catalase, phosphatase, ornithine decarboxylase, and urease enzymatic tests; and resistance to deferoxamine, bacitracin, albamycin, and polymyxin B antibiotics (20, 21). The isolates verified as CoNS were stored in trypticase soy broth with glycerol at -20°C until being subjected to further experiments.

3.2. Antimicrobial Susceptibility Testing

Kirby-Bauer's disk agar diffusion method was carried out for the determination of CoNS susceptibility to 16 antimicrobial agents. Antibiotic discs were purchased from Rosco Diagnostica (Denmark) and included ampicillin (AMP, 10 μg), cephazolin (CFZ, 30 μg), cefotaxime (30 μg), ciprofloxacin (CIP, 5 μg), clindamycin (CLI, 2 μg), erythromycin (ERY, 15 μg), gentamicin (GM, 10 μg), nitrofurantoin (NIT, 300 μg), norfloxacin (NOR, 10 μg), oxacillin (OXA, 1 μg), penicillin (PEN, 5 μg), rifampin (RIF, 10 μg), cotrimoxazole (STX, 25 μg), teicoplanin (TEC, 30 μg), tetracycline (TE, 5 μg), and vancomycin (VAN, 30 μg). The test used a bacterial suspension with a turbidity adjusted equivalent to a 0.5 McFarland standard, and was performed on Mueller-Hinton agar plates (Merck, Germany) based on the Clinical & Laboratory Standards Institute (CLSI) guidelines (22, 23). *S. aureus*, strain COL, was used as a control. MDR of an isolate was defined as resistance at least to three different classes of antibiotics (24).

3.3. Detection of the vanA Gene

PCR assays were performed on CoNS isolates for detection of the gene encoding vancomycin resistance, *vanA*. Total DNA was extracted using the phenol/chloroform/isoamyl alcohol method as previously described (25). The primer pair, 5'-GGCAAGTCAGGTGAAGATG-3' and 5'-ATCAAGCGGTCAATCAGTTC-3', was used for amplification of fragments with lengths of 713 bp (26). The PCR thermal profile was comprised of enzyme activation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 5 minutes. The PCR products were subjected to electrophoresis in a 1.5% agarose gel containing ethidium bromide, then photographed with a UV transilluminator. *Enterococcus faecalis* PTCC 1237 was used as a positive control.

3.4. Vancomycin E-Test

The minimum inhibitory concentration (MIC) of vancomycin was determined using E-test strips, according to the manufacturer's guidelines (Bioanalyse, Turkey). Briefly, the bacterial suspension with a turbidity adjusted equivalent to a 0.5 McFarland standard was swabbed on Mueller-Hinton agar medium supplemented with 2% NaCl, then an E-test strip containing vancomycin-concentration gradient was placed on the medium and incubated at 37°C for 24 hours. Finally, the MIC was calculated as previously described (26).

3.5. Statistical Analysis

The data were analyzed using the statistical package for the social sciences (SPSS) software, version 16 (IBM Co., IL, USA). The chi-square test was performed for the categorical variables and ANOVA was used for numerical ones, and the results were expressed as frequency percentage and mean $\pm$ standard deviation (SD), respectively. P values of < 0.05 were considered statistically significant.

4. Results

As shown in Table 1, *S. haemolyticus* and *S. epidermidis* were the most prevalent CoNS isolates from different wards of the hospital (34% and 27%, respectively). *S. hominis* (12%), *S. saprophyticus* (9%), and other species (18%) appeared with less frequency. Of the CoNS isolates, 37% were detected in patients hospitalized in the internal medicine ward, 14% in the ICU, 21% in the pediatric ward, and 28% in other wards. The majority of isolates were recovered from blood and urine specimens (43% and 33%, respectively). Moreover, 69% of CoNS were isolated from females and 31% from males. The highest percentage of isolates (35%) was from patients aged < 20 years old.

Table 1. Prevalence of Coagulase-Negative Staphylococci According to Species, Patient Gender and Age, Hospital Ward, and Isolation Source

Variable	Frequency (%)
Bacterial species	
S. epidermidis	24 (24)
S. haemolyticus	37 (37)
S. hominis	12 (12)
S. saprophyticus	9 (9)
Other	18 (18)
Patient gender	
Female	69 (69)
Male	31 (31)
Patient age	
< 20	35 (35)
20 - 45	32 (32)
> 45	33 (33)
Hospital ward	
Internal medicine	37 (37)
ICU	14 (14)
Pediatrics	21 (21)
Unspecified	28 (28)
Isolation source	
Blood	43 (43)
Urine	33 (33)
Eye	8 (8)
Tracheal aspirate	8 (8)
Wound/abscess	2 (2)
Catheter	6 (6)
Total	100

4.1. Multi-Drug Resistance (MDR)

Out of 100 CoNS, 62 (62%) strains exhibited MDR and 38 (38%) were identified as non-MDR. As depicted in Table 2, there was a significant difference in MDR distribution among various CoNS species (P = 0.002); *S. haemolyticus* showed higher rates of MDR compared to the other CoNS, and *S. saprophyticus* had the lowest frequency among the MDR CoNS. There was no significant difference in frequency distribution of MDR CoNS isolated from different hospital wards or from different specimens (P > 0.05). However, MDR was seen at higher rates among the CoNS isolated from blood and urine specimens (42% and 30%, respectively).

4.2. Glycopeptide Susceptibility Pattern

Out of 100 CoNS, 99 (99%) strains were susceptible to vancomycin and one (1%) strain (*S. hominis*) showed resistance to this antibiotic, according the antibiogram test. However, all strains (100%) were revealed to be *vanA*-negative on PCR. In addition, out of 100 CoNS, 85 (85%) strains demonstrated complete susceptibility and 15 (15%) showed intermediate susceptibility to teicoplanin; none of the CoNS strains were resistant to teicoplanin. The mean inhibition zones of vancomycin and teicoplanin are presented in Table 3 in terms of species, patient gender and age, hospitalization ward, and CoNS isolation source. There is a significant difference between CoNS species regarding the mean inhibition zones of vancomycin (P < 0.001) and teicoplanin (P < 0.001); *S. epidermidis* exhibited the highest susceptibility to both antibiotics, and *S. saprophyticus* appeared to have the least. However, no significant differences were found in the inhibition zones of vancomycin and teicoplanin against CoNS based on gender, age group, hospital ward, and isolation source (P > 0.05). In addition, the overall mean inhibition zone of vancomycin was significantly higher than that of teicoplanin (P < 0.001), which indicates that CoNS might be more sensitive to vancomycin than to teicoplanin.

4.3. Vancomycin MIC Values

The MIC values for vancomycin were determined with the E-test as follows: 0.75 μg/mL in 3 strains (3.03%), 1 μg/mL in 36 strains (36.36%), 1.5 μg/mL in 56 strains (56.57%), and 2 μg/mL in 4 strains (4.04%). In fact, the MIC values for all CoNS were calculated to be $\leq$ 2 μg/mL, confirming 100% susceptibility of these bacteria to vancomycin. As shown in Table 4, there was a significant difference in vancomycin MIC values among the CoNS species (P = 0.001) and the isolation sources (P = 0.007), while no significant differences were found between MIC values based on patient gender and age or on hospital ward (P > 0.05).

Table 2. Prevalence of Multidrug Resistant (MDR) Coagulase-Negative Staphylococci According to Species, Hospital Ward, and Isolation Source[a]

Variable	Non-MDR	MDR	P Value
Bacterial species			0.002
S. epidermidis	9 (24)	15 (24)	
S. haemolyticus	6 (16)	31 (50)	
S. hominis	5 (13)	7 (11)	
S. saprophyticus	6 (16)	3 (5)	
Other	12 (31)	6 (10)	
Hospital ward			0.122
Internal medicine	18 (47)	19 (31)	
ICU	4 (11)	10 (16)	
Pediatrics	4 (11)	17 (27)	
Unspecified	12 (31)	16 (26)	
Isolation source			0.317
Blood	17 (45)	26 (42)	
Urine	14 (37)	19 (30)	
Eye	2 (5)	6 (10)	
Tracheal aspirate	2 (5)	6 (10)	
Wound/abscess	2 (5)	0 (0)	
Catheter	1 (3)	5 (8)	
Total	38 (38)	62 (62)	

[a]Values are expressed as No. (%).

5. Discussion

CoNS are considered a major cause of nosocomial infections, mainly occurring in immunocompromised patients and premature newborns, and are mostly associated with healthcare settings. Antibiotic resistance has been a growing issue in the treatment of CoNS infections (23, 27). The current study revealed that *S. haemolyticus* and *S. epidermidis* were the most prevalent CoNS from clinical isolates. This result, in line with many previous studies, confirms the predominance of these two species in various clinical samples compared to other CoNS species (28-31). In addition, *S. haemolyticus* and *S. epidermidis* have been reported to be among the most frequent CoNS species acquiring resistance to multiple antibiotics, which is in agreement with the results of the current study (3, 8). Indeed, we found *S. haemolyticus* and *S. epidermidis* among the strains with higher rates of MDR compared to the other CoNS. Furthermore, nearly consistent with Singh and colleagues (32) and Ghadiri and colleagues (33), 43% and 33% of the CoNS were from blood and urine specimens in our study, among which higher rates of MDR were detected.

Among all CoNS species, *S. haemolyticus*, *S. epidermidis*, *S. hominis*, and *S. warneri* have more common shown resistance to vancomycin (23). However, in the current study, only one CoNS isolate, belonging to *S. hominis*, exhibited resistance to vancomycin using the disk diffusion test, which was revealed on PCR not to possess the *vanA* gene. This could be indicative of the higher sensitivity of the latter method compared to the former, or may be due to technical errors related to either of these methods. Furthermore, 99% of the CoNS demonstrated susceptibility to vancomycin, and this finding is in accordance with previous investigations among other populations (29, 34-36). Although in our study, none of the CoNS showed resistance to teicoplanin, 15% had intermediate resistance to it. Moreover, similar to our findings, resistance to teicoplanin has been more frequently reported than to vancomycin (14, 15). Tacconelli et al. found that among 535 CoNS isolates, 20 (4%) and 1 (0.2%) strains were resistant to teicoplanin and vancomycin, respectively (15). These findings suggest vancomycin as an antibiotic of choice in cases of MDR to non-glycopeptide antibiotics.

The MIC value refers to the minimum concentration of an antibiotic that inhibits the growth of a certain microor-

Table 3. Inhibition Zone (mm) of Vancomycin and Teicoplanin Against Coagulase-Negative Staphylococci[a]

Variable	Vancomycin	P Value	Teicoplanin	P Value
Bacteria species		0.001		0.001
S. epidermidis	19.29 ± 1.55		18.04 ± 1.78	
S. haemolyticus	18.24 ± 1.14		15.38 ± 2.43	
S. hominis	18.00 ± 1.60		15.50 ± 2.32	
S. saprophyticus	17.56 ± 1.24		15.33 ± 2.29	
Others	19.28 ± 0.96		16.39 ± 2.14	
Patient gender		0.472		0.183
Female	18.52 ± 1.53		15.99 ± 2.54	
Male	18.74 ± 1.09		16.69 ± 2.11	
Patient age		0.528		0.117
1 - 20	18.49 ± 1.67		16.47 ± 2.47	
20 - 45	18.47 ± 1.39		15.48 ± 2.37	
> 45	18.82 ± 1.10		16.64 ± 2.36	
Hospital ward		0.295		0.095
Internal medicine	18.27 ± 1.59		15.50 ± 2.48	
ICU	18.93 ± 1.14		16.36 ± 2.95	
Pediatrics	18.57 ± 1.66		17.12 ± 2.90	
Unspecified	18.86 ± 0.97		16.39 ± 2.28	
Isolation source		0.424		0.083
Blood	18.74 ± 1.16		16.61 ± 2.51	
Urine	18.18 ± 1.55		15.24 ± 2.32	
Eye	19.00 ± 2.27		16.13 ± 1.36	
Tracheal aspirate	18.63 ± 1.30		16.88 ± 1.73	
Wound/abscess	19.50 ± 0.71		18.50 ± 2.12	
Catheter	18.83 ± 0.98		17.08 ± 3.23	
Total	18.59 ± 1.41		16.21 ± 2.43	

[a] Values are expressed as mean ± SD.

ganism (37). Vancomycin has been suggested as a treatment of choice for MDR CoNS (38). The CoNS with MIC values of ≤ 1 have been suggested to be highly susceptible to vancomycin, while those presenting with MIC values of ≥ 2 necessitate the use of non-vancomycin alternatives (such as daptomycin) in order to be efficiently eradicated (39-41). The mean MIC value for CoNS isolates in the current study was calculated as 1.35 ± 0.29 μg/mL, establishing sufficient susceptibility to vancomycin. *S. haemolyticus* and *S. saprophyticus* had lower MIC values for vancomycin compared to *S. epidermidis* and *S. hominis*, and therefore demonstrated a higher susceptibility to this antibiotic. In addition, we found that these MIC values were independent of patient gender and age and of hospital ward, while they significantly differed between CoNS from different specimens.

In conclusion, *S. haemolyticus* and *S. epidermidis* were the most prevalent among the CoNS in patients in Gorgan. In addition, it seems that CoNS isolates from Gorgan are fully susceptible to vancomycin, despite presenting a higher resistance to multiple non-glycopeptide antibiotics that are routinely prescribed. Thus, vancomycin can be considered an effective antibiotic of choice for controlling infections caused by CoNS depending on the species, but regardless of patient gender and age, type of disease, isolation source, and hospital ward. However, much more research is required to support this suggestion.

Table 4. Vancomycin MIC Values Among the Coagulase-Negative Staphylococcus Isolates

Variable	MIC (mean ± SD)	P Value
Bacterial species		0.001
S. epidermidis	1.48 ± 0.23	
S. haemolyticus	1.19 ± 0.27	
S. hominis	1.42 ± 0.29	
S. saprophyticus	1.19 ± 0.30	
Others	1.35 ± 0.29	
Patient gender		0.434
Female	1.30 ± 0.30	
Male	1.36 ± 0.28	
Patient age		0.425
1 - 20	1.33 ± 0.26	
20 - 45	1.26 ± 0.35	
> 45	1.36 ± 0.26	
Hospital ward		0.832
Internal medicine	1.29 ± 0.32	
ICU	1.30 ± 0.28	
Pediatrics	1.36 ± 0.23	
Unspecified	1.32 ± 0.31	
Isolation source		0.007
Blood	1.39 ± 0.29	
Urine	1.17 ± 0.26	
Eye	1.38 ± 0.23	
Tracheal aspirate	1.50 ± 0.27	
Wound/abscess	1.50 ± 0.22	
Catheter	1.25 ± 0.27	
Total	1.35 ± 0.29	

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Footnotes

Authors' Contribution: Study concept and design, Ezzat Allah Ghaemi; acquisition of data, Hamideh Mehri, Fatemeh Shakeri, and Roghaye Jahanbakhsh; statistical analysis and interpretation of data, Ezzat Allah Ghaemi, Hamideh Mehri, and Naser Behnampour; drafting of the manuscript, Hamideh Mehri and Abdollah Ardebili; critical revision of the manuscript for important intellectual content, Ezzat Allah Ghaemi and Behnaz Khodabakhshi; study supervision and administrative, technical, and material support: Ezzat Allah Ghaemi.

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References

1. Harris LG, Foster SJ, Richards RG. An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. *Eur Cell Mater.* 2002;**4**:39–60. [PubMed: 14562246].

2. Martinko JM, Madigan MT. In: Brock biology of microorganisms. 11 ed. Englewood C, editor. Prentice Hall; 2005.
3. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev.* 2014;**27**(4):870–926. doi: 10.1128/CMR.00109-13. [PubMed: 25278577].
4. Mayhall CG. Hospital epidemiology and infection control. 3 ed. Lippincott Wiliam and Wilkins; 2004. pp. 495–510.
5. Tan TY, Ng SY, Ng WX. Clinical significance of coagulase-negative staphylococci recovered from nonsterile sites. *J Clin Microbiol.* 2006;**44**(9):3413–4. doi: 10.1128/JCM.00757-06. [PubMed: 16954288].
6. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal sepsis due to coagulase-negative staphylococci. *Clin Dev Immunol.* 2013;**2013**:586076. doi: 10.1155/2013/586076. [PubMed: 23762094].
7. Bannerman TL. In: Manual of clinical microbiology. Murry P, Barron E, Jorgerson J, Pfaller M, editors. ; 2003. p. 384.Staphylococcus, Micrococcus and other catalase positive cocci that grow aerobically.
8. Xu Z, Mkrtchyan HV, Cutler RR. Antibiotic resistance and mecA characterization of coagulase-negative staphylococci isolated from three hotels in London, UK. *Front Microbiol.* 2015;**6**:947. doi: 10.3389/fmicb.2015.00947. [PubMed: 26441881].
9. Winn WC, Koneman EW. Koneman's color atlas and textbook of diagnostic microbiology. Lippincott williams and wilkins; 2006.
10. Paiva RM, Mombach Pinheiro Machado AB, Zavascki AP, Barth AL. Vancomycin MIC for methicillin-resistant coagulase-negative Staphylococcus isolates: evaluation of the broth microdilution and Etest methods. *J Clin Microbiol.* 2010;**48**(12):4652–4. doi: 10.1128/JCM.01182-10. [PubMed: 20861345].
11. Zhang Y, Fu Y, Yu J, Ai Q, Li J, Peng N, et al. Synergy of ambroxol with vancomycin in elimination of catheter-related Staphylococcus epidermidis biofilm in vitro and in vivo. *J Infect Chemother.* 2015;**21**(11):808–15. doi: 10.1016/j.jiac.2015.08.017. [PubMed: 26423688].
12. Sirichoat A, Wongthong S, Kanyota R, Tavichakorntrakool R, Chanawong A, Welbat JU, et al. Phenotypic Characteristics of Vancomycin-Non-Susceptible Staphylococcus aureus. *Jundishapur J Microbiol.* 2016;**9**(1):ee26069. doi: 10.5812/jjm.26069. [PubMed: 27099678].
13. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. *N Engl J Med.* 1987;**316**(15):927–31. doi: 10.1056/NEJM198704093161507. [PubMed: 3821839].
14. Krcmery VJ, Trupl J, Spanik S. Bacteremia due to teicoplanin-resistant and vancomycin-susceptible Staphylococcus haemolyticus in seven patients with acute leukemia and neutropenia receiving prophylaxis with ofloxacin. *Infection.* 1997;**25**(1):51–2. [PubMed: 9039542].
15. Tacconelli E, Tumbarello M, Donati KG, Bettio M, Spanu T, Leone F, et al. Glycopeptide resistance among coagulase-negative staphylococci that cause bacteremia: epidemiological and clinical findings from a case-control study. *Clin Infect Dis.* 2001;**33**(10):1628–35. doi: 10.1086/323676. [PubMed: 11595984].
16. Boneca IG, Chiosis G. Vancomycin resistance: occurrence, mechanisms and strategies to combat it. *Expert Opin Ther Targets.* 2003;**7**(3):311–28. doi: 10.1517/14728222.7.3.311. [PubMed: 12783569].
17. Palazzo IC, Araujo ML, Darini AL. First report of vancomycin-resistant staphylococci isolated from healthy carriers in Brazil. *J Clin Microbiol.* 2005;**43**(1):179–85. doi: 10.1128/JCM.43.1.179-185.2005. [PubMed: 15634969].
18. Iweriebor BC, Ramalivhana NJ, Hattori T, Okoh AI, Obi CL. Vancomycin resistant coagulase-negative Staphylococcal isolates from HIV positive patients in the Limpopo Province, South Africa. *J Microbiol Antimicrob.* 2013;**5**(2):18–24.
19. Valencia-Rey P, Weinberg J, Miller NS, Barlam TF. Coagulase-negative staphylococcal bloodstream infections: Does vancomycin remain appropriate empiric therapy?. *J Infect.* 2015;**71**(1):53–60. doi: 10.1016/j.jinf.2015.02.007. [PubMed: 25725152].
20. Jorgensen JH, Turnidge JD, Washington J. A. . In: Antibacterial susceptibility tests: Dilution and disk diffusion methods. 7 ed. Murray PR, Baron EJ, Pfaller MA, Tenover FYR, editors. Washington: American Society for Microbiology; 2005. pp. 221–36.
21. Monsen T, Ronnmark M, Olofsson C, Wistrom J. An inexpensive and reliable method for routine identification of staphylococcal species. *Eur J Clin Microbiol Infect Dis.* 1998;**17**(5):327–35. [PubMed: 9721961].
22. CLSI . Performance standards for antimicrobial susceptibility testing. 20 ed. ; 2010.
23. May L, Klein EY, Rothman RE, Laxminarayan R. Trends in antibiotic resistance in coagulase-negative staphylococci in the United States, 1999 to 2012. *Antimicrob Agents Chemother.* 2014;**58**(3):1404–9. doi: 10.1128/AAC.01908-13. [PubMed: 24342646].
24. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;**18**(3):268–81. doi: 10.1111/j.1469-0691.2011.03570.x. [PubMed: 21793988].
25. Shakeri F, Shojai A, Golalipour M, Rahimi Alang S, Vaez H, Ghaemi EA. Spa Diversity among MRSA and MSSA Strains of Staphylococcus aureus in North of Iran. *Int J Microbiol.* 2010;**2010** doi: 10.1155/2010/351397. [PubMed: 20862383].
26. Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of a vancomycin-resistant Staphylococcus aureus isolate from the respiratory tract of a patient in a university hospital in northeastern Iran. *J Clin Microbiol.* 2012;**50**(11):3581–5. doi: 10.1128/JCM.01727-12. [PubMed: 22933598].
27. Rahbar M, Mohammad-Zadeh M, Asl H, Azimi L, Lari A. Detection prevalence of inducible clindamycin resistance in Coagulase-Negative Staphylococci (CoNS) isolates in an Iranian 1000-bed tertiary Care Hospital Using D Test. *Health med.* 2012;**6**(8):2642–6.
28. Sivadon V, Rottman M, Chaverot S, Quincampoix JC, Avettand V, de Mazancourt P, et al. Use of genotypic identification by sodA sequencing in a prospective study to examine the distribution of coagulase-negative Staphylococcus species among strains recovered during septic orthopedic surgery and evaluate their significance. *J Clin Microbiol.* 2005;**43**(6):2952–4. doi: 10.1128/JCM.43.6.2952-2954.2005. [PubMed: 15956429].
29. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. *Microbiol Res.* 2009;**164**(4):404–10. doi: 10.1016/j.micres.2007.03.004. [PubMed: 17475456].
30. Pinheiro L, Brito CI, de Oliveira A, Martins PY, Pereira VC, da Cunha Mde L. Staphylococcus epidermidis and Staphylococcus haemolyticus: Molecular Detection of Cytotoxin and Enterotoxin Genes. *Toxins (Basel).* 2015;**7**(9):3688–99. doi: 10.3390/toxins7093688. [PubMed: 26389954].
31. Czekaj T, Ciszewski M, Szewczyk EM. Staphylococcus haemolyticus - an emerging threat in the twilight of the antibiotics age. *Microbiology.* 2015;**161**(11):2061–8. doi: 10.1099/mic.0.000178. [PubMed: 26363644].
32. Singh S, Banerjee G, Agarwal SK, Kumar M, Singh RK. Simple method for speciation of clinically significant coagulase negative Staphylococci and its antibiotic sensitivity/resistant pattern in NICU of tertiary care centre. *Biomed Res.* 2008;**19**(2):97–101.
33. Ghadiri H, Vaez H, Khosravi S, Soleymani E. The antibiotic resistance profiles of bacterial strains isolated from patients with hospital-acquired bloodstream and urinary tract infections. *Crit Care Res Pract.* 2012;**2012**.
34. Cuevas O, Cercenado E, Vindel A, Guinea J, Sanchez-Conde M, Sanchez-Somolinos M, et al. Evolution of the antimicrobial resistance of Staphylococcus spp. in Spain: five nationwide prevalence studies, 1986 to 2002. *Antimicrob Agents Chemother.* 2004;**48**(11):4240–5. doi: 10.1128/AAC.48.11.4240-4245.2004. [PubMed: 15504847].
35. Sheikh AF, Mehdinejad M. Identification and determination of coagulase-negative Staphylococci species and antimicrobial susceptibility pattern of isolates from clinical specimens. *Afr J Microbiol Res.*

2012;**6**(8):1669–74.
36. Reynolds R, Potz N, Colman M, Williams A, Livermore D, MacGowan A, et al. Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland 2001-2002: the BSAC Bacteraemia Resistance Surveillance Programme. *J Antimicrob Chemother.* 2004;**53**(6):1018–32. doi: 10.1093/jac/dkh232. [PubMed: 15128723].
37. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RJ, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant Staphylococcus aureus bacteremia. *J Clin Microbiol.* 2004;**42**(6):2398–402. doi: 10.1128/JCM.42.6.2398-2402.2004. [PubMed: 15184410].
38. Baddour LM, Wilson WR, Bayer AS, Fowler VJ, Bolger AF, Levison ME, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. *Circulation.* 2005;**111**(23):394–434. doi: 10.1161/CIRCULATIONAHA.105.165564. [PubMed: 15956145].
39. Garcia de la Maria C, Cervera C, Pericas JM, Castaneda X, Armero Y, Soy D, et al. Epidemiology and prognosis of coagulase-negative staphylococcal endocarditis: impact of vancomycin minimum inhibitory concentration. *PLoS One.* 2015;**10**(5):0125818. doi: 10.1371/journal.pone.0125818. [PubMed: 25961578].
40. Rybak MJ, Lomaestro BM, Rotschafer JC, Moellering RJ, Craig WA, Billeter M, et al. Therapeutic monitoring of vancomycin in adults summary of consensus recommendations from the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Pharmacotherapy.* 2009;**29**(11):1275–9. [PubMed: 19873687].
41. Garcia-de-la-Maria C, Marco F, Armero Y, Soy D, Moreno A, del Rio A, et al. Daptomycin is effective for treatment of experimental endocarditis due to methicillin-resistant and glycopeptide-intermediate Staphylococcus epidermidis. *Antimicrob Agents Chemother.* 2010;**54**(7):2781–6. doi: 10.1128/AAC.01011-09. [PubMed: 20421394].