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Clinical and Resistance Pattern of Coagulase Negative Staphylococci

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Abstract

Background and Objectives: Coagulase-negative staphylococci are commensal bacteria inhabiting human. Isolation of these bacteria from healthcare-associated and community infections is increasingly observed with resistance response to many antibiotics. The study aimed to identify the clinical and antimicrobial resistance pattern of coagulase-negative staphylococci isolated from various healthcare-associated and community-acquired infections.

Methods: This cross-sectional study was conducted in Shar Teaching Hospital and San Clinical Laboratory for a period of one year starting from 1st of November 2019.Isolated coagulase-negative staphylococci were collected from various hospitals and clinical laboratories. The isolate species were confirmed by several tests including VITEK® 2. VITEK® 2 systems was also used to determine the susceptibility to different antibiotics including cefoxitin, oxacillin gentamicin, linezolid, and glycopeptide agents' teicoplanin and vancomycin which were tested also by E-test.

Results: Coagulase-negative staphylococci were isolated from urine specimens (23%), followed by blood samples (20%), sterile body fluids (12%), and others. From one hundred isolates, the most common species of coagulase-negative staphylococci were Staphylococcus epidermidis (57%), Staphylococcus haemolyticus (24%), Staphylococcus hominis (8%), and others. The isolates showed high degree of resistance to benzyl penicillin (92%), oxacillin (85%), and cefoxitin (83%). No resistance to linezolid, tigecycline, and glycopeptides was detected.

Conclusion: Staphylococcus epidermidis and Staphylococcus haemolyticus were the main coagulase-negative staphylococci isolates from several healthcare-associated infections such as bloodstream and urinary tract infection. Most of the isolates showed resistance responses to benzyl penicillin, oxacillin, and cefoxitin, while they were susceptible to glycopeptides, linezolid, and tigecycline, but intermediate response to glycopeptide was also observed.

Key words: Antimicrobial resistance, Coagulase negative Staphylococci, Glycopeptides.

Introduction

Coagulase-negative staphylococci (CoNS) are commensal bacteria inhabiting the anterior nares and the skin of humans.[1] They include Gram-positive aerobic cocci that lack coagulase enzyme, hence called CoNS, in contrast to coagulase Producing-Staphylococcus aureus which is a constant human pathogen.[2] Although CoNS are considered as harmless; over the last few years there has been an increased incidence of healthcare-associated and community-acquired infections caused by CoNS.[3] Currently, the genus Staphylococcus compromise at the minimum 40 species of Gram-positive catalase-positive cocci, including in recent time, the proposed S. microti. Among the CoNS subsets, 18 species have been

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isolated from humans, but, until now, only five species of CoNS have frequently been associated with nosocomial spread. These are; S. epidermidis, S. haemolyticus, S. warneri and S. hominis, and S. lugdunensis.[4] Coagulase-negative staphylococci have an established role in infections of the bloodstream, urinary tract, surgical sites, prosthetic devices and shunts.[3] Coagulase-negative staphylococci infections mainly result from hospitalization.[5] Antimicrobial resistance has always been an important problem challenging the treatment of CoNS infections. CoNS species associated with human infections are usually resistant to β-lactams as well as other antibiotics, including aminoglycosides, quinolones, macrolides and tetracyclines.[6,7] Antibiotic resistance is mainly due to genes carried on a plasmid such those found in methicillin-resistant CoNS strains.[8] Resistance to glycopeptides can also be expressed heterogeneously by methicillin-resistant strains of both S. aureus (MRSA)[9] and CoNS.[10] About 80–90% of CoNS isolates associated with hospital infections were found to be methicillin-resistant coagulase-negative staphylococci.[11] The distinction between oxacillin-susceptible and oxacillin-resistant strains is a useful clue for proper use of vancomycin in the hospital setting and to keep it as a reserve drug for serious infections caused by oxacillin-resistant strains.[12] Vancomycin is one of the most commonly used cell wall-active agents that retains activity, and is an alternative drug of choice for treatment of infections with resistance strains.[13] Wide spread use of glycopeptides has led recently to emergence of CoNS isolates with decreased susceptibility of glycopeptides, displaying a minimum inhibitory concentration (MIC) of 4-8 mg/L for vancomycin, and MIC 8-16 for teicoplanin.[1] The MICs of teicoplanin have a tendency to be distributed over an increasingly wide range, while vancomycin MICs have remained more stable.[10] Few studies detected vancomycin resistance in staphylococci.[14] Reduced susceptibility to glycopeptides has been reported more commonly among S. haemolyticus than among S. epidermidis .[10, 15] The aim of the study is to identify the clinical and antimicrobial resistance pattern of different coagulase-negative staphylococci isolated from various healthcare-associated and community-acquired infections.

Materials and methods

This cross-sectional study was conducted in Shar Teaching Hospital and San Clinical Laboratory for a period of one year starting from 1st of November 2019. Presumptive CoNS isolates from various clinical samples were collected. The isolates were from patients admitted to hospital and patients attending outpatient departments and healthcare laboratories. Coagulase-negative staphylococci were regarded a significant cause of bacteremia when the same CoNS strain was isolated two or more times from blood culture sample within five days; when one blood culture sample yielded growth of CoNS, then at least two clinical parameters was taken into consideration; body temperature >38°C or <36°C, elevated CRP, or systolic blood pressure <90 mmHg.[12] Automated blood culture system (BACT/ALERT® 3D, bioMerieux) was used to detect growth in blood samples. The isolates were and transported and processed the same day by cultivating them on blood agar (Condalab), incubated at 37°C for 18-24 hours. The cultivated isolates were confirmed based on colony characteristics, Gram stain features, positive catalase test and negative tube coagulase test.[16] CoNS species were identified with VITEK® 2 system (bioMerieux) using VITEK®2 GP ID card.[12] Glycerol isolates were stocked according to Khosravi et al. and stored at -80°C for further uses.[17] Analysis of susceptibility to antimicrobial agents was performed according to broth microdilution methods as described by the National Committee for Clinical

Laboratory Standards by using the VITEK® 2 AST-P592, and AST-P580 cards. The following antimicrobial were tested; benzylpenicillin, oxacillin, cefoxitin gentamycin, moxifloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, tigecycline, rifampicin, and trimethoprim/sulfamethoxazole.[18]Standard E-test was performed on Mueller Hinton agar (Condalab) according to NCCLS.[18] Standard E-test, is a gradient of antibiotic concentrations on a plastic strip, it is used to determine the Minimum Inhibitory Concentration (MIC) of that antibiotic.[19] E-test strips for vancomycin and teicoplanin 0.016–256 mg/L (Liofilchem®) were used. Agar plates were incubated at 35°C, the test was read after 24 hours and MIC was determined. Inducible clindamycin test for CoNS isolates was analyzed based on VITEK®2 system using standard AST-GP cards in accordance with the manufacturer's protocol. Data entry was done using Microsoft excel and statistical analysis was performed using stata software 12; (T test, chi square) tests was performed. The research was approved by ethical committee of Kurdistan Higher Council of Medical Specialties.

Results

In this study one hundred confirmed CoNS isolate were proceeded and tested. Fifty-five of the isolates were taken from patients admitted to hospital and 45 isolates were from outpatient clinics. Forty-eight isolates were from male patients aged from four to 77 years, while 52 isolates were from female patients aged from one to 75 years Table (1). All the patients from the hospitalized group (n=55) had medical interventions, while in the outpatient group, 6 out 45 (15.3%) had intervention (intravenous cannula). CoNS were isolated from urine specimens (23%), followed by blood samples (20%), sterile body fluids (12%), tip of the central venous catheter, other specimens Table (1).

Table (1): Patients characteristics and source of CoNS isolates.

Variable		n=100	Inpatient (n=55)	Outpatient (n=45)	p-value*
Sex and age	Male	48	29	19	0.2955
	age (4-77 y), x̄ 45.1 y				
	Female	52	26	26	
	age (1-75 y), x̄ 42.03 y				
Antibiotics used	Yes	55	36	19	< 0.001
	No	45	9	36	
Intervention	Yes	61	55	6	< 0.001
	No	39	0	39	
Specimen	Urine	23	3	20	
	Blood	20	20	0	
	Sterile body fluid	12	1	11	
	Vascular Catheter	11	11	0	
	Wound swab	9	4	5	
	CSF	7	7	0	
	High vaginal swab	5	0	5	
	Respiratory	5	5	0	
	Others**	8	4	4	

* Chi-Square test

** Other samples included ear swab, pus, and external ventricular drain shunt.

Table (2) shows the isolated species of CoNS from clinical specimens. S. epidermidis follows by S. haemolyticus were the commonest isolates. S. epidermidis was isolated from 9 (45%) of blood specimens and from 9 (75%) of sterile body fluids specimens and 7 (77.7%) of wound swabs. S haemolyticus, was isolated from 3 (15%) of blood and 12 (52.1%) of urine samples. The only isolate of S. warneri was from a CSF sample.

Table (2): The isolated species of CoNS from different clinical specimens (n=100).

CON species	Specimens No.									
	N	Urine	Blood	Sterile fluid	Vascular Catheter	Wound swab	CSF	High vaginal swab	Respirat. samples	Others
S. epidermidis	57	7	9	9	8	7	5	3	4	5
S. haemolyticus	24	12	3	1	2	2	1	1	1	1
S. hominis	8	0	6	1	1	0	0	0	0	0
S. lugdunensis	4	2	0	0	0	0	0	0	0	2
S. sciuri	3	1	0	1	0	0	0	1	0	0
S. vitulinus	3	1	2	0	0	0	0	0	0	0
S. warneri	1	0	0	0	0	0	1	0	0	0
Total	100	23	20	12	11	9	7	5	5	8

The resistance response of CoNS isolates is shown in Table (3). Among all CoNS spp., resistance to benzylpenicillin and oxacillin were 92% and 85% respectively while most effective agents were glycopeptides, linezolid, and tigecycline. Methicillin-resistance strains of CoNS species were concluded from oxacillin resistance and positive cefoxitin screen test. [17, 20] From the 100 isolates, 83 samples were methicillin-resistant

Table (3): Antimicrobial resistance response of CoNS isolates tested by VITEK® 2 systems.

Tested antimicrobial agent	S. epidermidis (n=57)	S. haemolyticus (n=24)	S. hominis (n=8)	S. lugdunensis (n=4)	S. sciuri (n=3)	S. vitulinus (n=3)	S. warneri (n=1)	Total resistance of CoNS
Benzylpenicillin	53(92.9%)	21(87.5%)	8(100%)	4(100%)	3(100%)	2(66.6%)	1(100%)	92
Oxacillin	49(85.9%)	21(87.5%)	8(100%)	1(25%)	3(100%)	2(66.6%)	1(100%)	85
Cefoxitin screen	48(84.2%)	21(87.5%)	8(100%)	0	3(100%)	2(66.6%)	1(100%)	83
Gentamicin	20(35%)	16(66.6%)	7(87.5%)	0	0	0	1(100%)	44
Moxifloxacin	9(15.7%)	7(29.1%)	6(75%)	1(25%)	0	1(33.3%)	0	24
Erythromycin	42(73.6%)	18(75%)	7(87.5%)	3(75%)	2(66.6%)	1(33.3%)	1(100%)	74
Clindamycin	20(35%)	13(54.1%)	5(62.5%)	3(75%)	0	1(33.3%)	1(100%)	43
Linezolid	0	0	0	0	0	0	0	0
Teicoplanin	0	0	0	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0	0
Tetracycline	38(66.6%)	14(58.3%)	8(100%)	2(50%)	3(100%)	0	0	65
Tigecycline	0	0	0	0	0	0	0	0
Rifampicin	12(21%)	6	2(25%)	0	0	0	1(100%)	21
Trimethoprim/ sulfamethoxazole	19(33.3%)	10(41.6%)	1(12.5%)	0	0	0	0	30

Table (4) compares glycopeptide susceptibility of all CoNS species as evaluated by both VITEK ®2 system and E-test. All strains of CoNS 100% were susceptible to vancomycin by VITEK® 2 system, while only one strain showed an intermediate susceptibility by E-test. For teicoplanin, susceptibility was 96% by VITEK® 2 system and 98% by E-test, while 4% and 2% were having an intermediate susceptibility by VITEK® 2 system and E-test respectively.

Table (4): Vancomycin and teicoplanin susceptibility of CoNS tested by VITEK® 2 and E-test.

Method	Response and MIC (mg/L)	Vancomycin	Teicoplanin
	Resistant		
VITEK 2 test	Vanc. >32 mg/L	0	0
E-test	Teico. >32 mg/L	0	0
	Intermediate		
VITEK 2 test	Vanc. 8-16 mg/L	0	4
E-test	Teico. >16 mg/L	1	2
	Susceptible		
VITEK 2 test	Vanc. < 4 mg/L	100	96
E-test	Teico. < 8 mg/L	99	98
Total		100	100

For inducible clindamycin test, only 11 CoNS isolates showed a positive result; five S. haemolyticus, three S. epidermidis, two S. hominis, and one S. sciuri.

Discussion

Although CoNS lack many cell-bound and secreted virulence factors possessed by S. aureus; yet, CoNs are capable of adhering to prosthesis and catheter surfaces, and have the ability to form biofilm. Add to that, with the widespread use of antibiotics, CoNS became one of the main pathogens causing community and healthcare-associated infections.[21] The CoNS species from this study were isolated among all ages and both sexes; mainly from healthcare-associated infections, but also from different community-acquired infections. CoNS were isolated from a range of infections including genitourinary tract infection, bloodstream infection, catheter-related infections, wound infection, respiratory tract infection, and CNS infections. Among hospitalized patients, bloodstream and central venous catheter infections were most common infection; this was observed by Nasaj ET al.[8] where CoNS was isolated from blood was (43%) followed by urine (36.3%), and vascular catheter (14.3%). This was also observed by Aher study where the majority of CoNs isolates were obtained from blood cultures followed by urine samples (21%).[22] The reason for such infections by CoNS is the predisposing factors such as medical devices, interventions, immune status, or critically ill patients, affinity of CoNS to prosthetic and plastic surfaces, and ability to form biofilm to establish infection. Large numbers of CoNS were also isolated from outpatient clinics, mostly from urinary tract infection, sterile body fluid, wound and vaginal swab; our observation was similar to what found by Khosravi ET al.[17] In our study, CoNS were isolated from various hospital wards and from outpatient clinics. Among the hospitalized patients, CoNS isolates were obtained from ICU patients (32.7%), nephrology (30.9%), and surgical ward (18.1%). Least was from internal medicine and pediatric wards. This is comparable to Bhatt et al. study[23] but in variance with Raina et al. study where CoNS mainly isolated from pediatric ward patients (20%), then ICU patients (18.3%).[12] This may be explained

by difference in the patient's status, medical interventions and the quality of healthcare service. Species identification of CoNS showed that S. epidermidis then S. haemolyticus constituted were the commonest isolates. Similar results were shown previously by Usha et al.[24] This was also observed by Bora et al., where they concluded the variation in different CoNS species may be determined geographically,[23] but this may be due to variability human microbiota including CoNS and to various hospital settings owing to differences in antimicrobial pressure. In our study we had not documented any S. saprophyticus; this may be due to that we used VITEK® 2 to identify the species of CoNS in contrast to previous works using colony characteristics and other tests. CoNS commonly develop resistance to multiple antibiotics and serve as reservoir for resistance genes that can be spread to other pathogens;[25] the resistance of β-lactam antibiotics in CoNS is by expression of the mecA gene is an example.[26] In our study, the highest resistance rate among the CoNS isolates was against benzylpenicillin similar to Khan et al. study showing the highest resistance was exhibited to penicillin (99.5%) and ampicillin (99%).[27] The CoNS species in our study showed high resistance to oxacillin except for S. lugdunensis where only 25% were resistant to oxacillin, this was similar to studies done by[3, 27]where all the isolates of S. lugdunensis were sensitive to penicillin. This study demonstrated high prevalence of cefoxitin resistance (83%) with oxacillin resistance indicating possible methicillin resistance. Cefoxitin resistance less than among our isolates was observed in a study from Iran[17] where resistance reached 65.6% (n=82), and to a previous study in which only 50.4% of their CoNS strains were reported to be cefoxitin resistant.[5] This increasing resistance is due to extensive antibiotic use especially in our locality and accumulation of resistance genes over time. None of our CoNS strains were resistant to vancomycin, teicoplanin, linezolid, and tigecycline. Resistance to these agents is very low or rare. In our study, only one strain showed intermediately susceptibility to vancomycin (E-test) while for teicoplanin, intermediate susceptibility was observed to be 4% by VITEK® 2 system and 2% by E-test, indicating the development of resistance. Heterogeneous glycopeptide-intermediate staphylococci (hGIS) were previously observed over decades with E-tests[10] and intermediate response is documented in this study and this may precede future resistance to glycopeptides. As founded, glycopeptides must be kept as last choice for multi-resistant staphylococci and enterococci. Inducible clindamycin test of the CoNS isolates was also performed by VITEK® 2 system.[28] In our study 11 CoNS isolates showed inducible clindamycin test, indicating the spread of inducible clindamycin resistance in our area and the need for this test if clindamycin is chosen for treatment.

Conclusion

Staphylococcus epidermidis and Staphylococcus haemolyticus were the main CoNS isolated from several healthcare-associated infections such as bloodstream and urinary tract infection. CoNS were also reported from community-acquired infections. Most of the isolates showed resistance to benzylpenicillin, oxacillin, and cefoxitin; while they were susceptible to glycopeptides, linezolid, and tigecycline, but intermediate response to glycopeptide was also observed

Conflicts of interest

The author reports no conflicts of interest.

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