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Full Length Research Paper

Identification and determination of coagulase-negative *Staphylococci* species and antimicrobial susceptibility pattern of isolates from clinical specimens

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Coagulase negative S*taphylococci* (CoNS) are the important agents in nosocomial infection. Recently, resistant of CoNS against antimicrobial agents is increasing. The aim of this study is determination of species of CoNS, and antimicrobial susceptibility pattern of species obtained from clinical specimens. Clinical specimens from different organism were collected. Laboratory tests included: culture, Gram stain, coagulase test, biochemistry tests, and antimicrobial susceptibility pattern were performed by standard methods. 201 *Staphylococci* were isolated from varying infected organ, of which 134(66.7%) were identified as proven CoNS that distributed among 16 species. The majority of species of CoNS was *Staphylococcus epidermidis* (19.4%) and the lowest *Staphylococcus auricularis* (0.74%), *Staphylococcus caprae* (0.74%) and *Staphylococcus intermedius* (0.74%). Also majority of CoNS related to organ was obtained from urine specimens (51.5%). The antimicrobial susceptibility patterns showed that the most resistant of the CoNS belonged to oxaciline (94.02%) and least resistant belong to vancomycine (20.89%). Among of the CoNS species, *S. epidermidis* (96.15%) have had higher resistant to oxacillin. *S. epidermidis* had the highest frequency among the CoNS which were isolated from clinical specimens, and majority of CoNS were isolated from urine specimens, the higher resistant of CoNS belonged to oxaciline and the lowest to vancomycine.

Key words: Coagulase negative Staphylococci (CoNS), antimicrobial susceptibility pattern, clinical specimens.

INTRODUCTION

Coagulase-negative S*taphylococci* (CoNS) are the most important pathogens in infectious disease. CoNS are reported as the third most widespread causative agent of nosocomial infections (Murray et al., 2003; Mayhall, 2004). CoNS are important and frequently encountered pathogens in hospital surroundings, and they account as a majority of all nosocomial infections (Von Eiff et al., 2001). Infection is the major complication associated with the use of foreign bodies such as catheters. Based on the type of device and its insertion site, dissimilar infection syndromes create with CoNS for example: Peritonitis, Septicemia, endocarditis, and Ventriculitis (Heilmann and Peters, 2000). These bacteria usually infect immuno-compromised patients, such as premature newborns and patients with leukemia or other malignant diseases who acquired neutropenia after receiving cytotoxic agents (Peters et al., 1995; Souvenir et al., 1998). Only 16 coagulase-negative species have been found in specimens of human origin. Among the CoNS, *Staphylococcus epidermidis* principal cause of infection, chiefly in hospitalized patients with indwelling foreign bodies and in immunocompromised patients (Piette and Verschraegen, 2009). *S. epidermidis* has caused some cases of bacteremia, surgical wound infections (Livermore, 2000; Rupp et al., 1994), conjunctivitis and

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keratitis, (Kamalarajah and Best, 2002), also osteomyelitis, wound infection, otitis media, endophthalmitis, UTI, was reported (Heilmann and Peters, 2000; Peters et al., 1995). *Staphylococcus saprophyticus* was often regarded as a more important opportunistic pathogen than *S. epidermidis* in human urinary tract infections (UTIs), particularly in young sexually active females. It was considered to be the second most common cause of acute cystatitis or pyelonephritis in these patients (Raz et al., 2005).

Recently use of broad-spectrum antibiotic for treatment infections lead to CoNS bacteria increasing the development of antibiotic resistance. In resulting large amount of nosocomial isolates of CoNS became resistant to various antibiotics (Shubhra et al., 2008) and it was showed that 80 to 90% of the CoNS strains isolated from human specimens create beta-lactamase (Peters et al., 1995; Diekema et al., 2001). *S. epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Staphylococcus hominis*, and *S. saprophyticus* among the CoNS species were found to have elevated levels of resistance to a variety of antibiotics (York et al., 1996).

Regard too many infections due to coagulase-negative *Staphylococci*, and increasingly resistant to antibiotics agents, the present study was conducted to identify frequency of CoNS species isolated from human specimens, and determination of susceptibility pattern of these species to antibacterial agents.

MATERIALS AND METHODS

Study population and collection of clinical specimens

A descriptive cross-sectional study was carried out from 2005 to 2009. Clinical specimens were obtained from patients who admitted at several wards of the Golestan hospital in Ahvaz, Iran South Western region of Iran (this hospital is a major referral center in our region). Criteria of selection of subjects were based on clinical assessment by the attending physician. All cases that recognized as suffering from staphylococcal infection, including; sex, age, and clinical specimens were evaluated. The age ranging was one month up to > 75 years old (The median age was 43.47 years) that divided in to 6 groups of age included: group 1 (<14 years), group 2 (15-29 years), group 3 (30-44 years), group 4 (45-59 years), group 5 (60 - 74 years), and group 6 (>75 years). Specimens were collected from Blood, Tracheal tube, body fluids discharge Sputum, Ear, throat), urine (midstream method), and wounds.

Isolation and identification of CoNS

The blood sample were inoculated into Trypticase Soy Broth (Merck, Germany) and incubated at 37°C for 48 h. Growth positive, were sub cultured on Blood agar plates, and incubated at 37°C under candle jar condition.

Urine samples cultured on to Mueller-Hinton (Merck, Germany), Blood agar and MacConkey agar. Urine specimens were considered clinically significant, when colony counts were more than 100,000 CFU/ ml after overnight incubation at 37°C condition. Also other specimens (Tracheal tube, body fluids discharge and wounds) were cultured on blood and MacConkey agar plates (Himedia, India and Merck, Germany) and incubated at 37°C for 24 to 48 h. CoNS were identified by colonies morphology on blood agar plate, gram stain, Catalase test, tube test coagulase reaction (Forbes et al., 2007).

The following characteristics were determined by, susceptibility tests to novobiocin (5 µg/disk), voges prosquer, nitrate reduction test, urea test, and resistant to polymyxin B, bacitracin (0.04 U/disk) test, also acid production tests from maltose, xylose, rafinos, lactose, sucrose, trehalose, fructose, mannitol, beta golactos and mannose were done (Jorgensen et al, 2005; Koneman et al., 1997).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was completed with disks (Padtan Teb, Iran and Haimedia, India Company) of Oxacilin (20 µg), Vancomycin (30 µg). Cephalexin (30 µg), Clindamycin (2 µg), Erythromycin (15 µg), Tetracycline (30 µg), Streptomycin (10 µg), novobiocin (5 µg), and Penicillin (10 µg), by Disk diffusion (Kirby Bauer's) technique according to CLSI strategy on Mueller-Hinton agar (Merck, Germany) (Forbes et al., 2007; NCCLS, 2002). Statistical tests were chi-square tests of independence. The distribution of these averages was compared between groups by the Mann-Whitney (Wilcoxon rank sum) test. All tests were two tailed. *P* values of less than 0.05 were regarded as significant.

RESULTS

Based on culture, Gram stain, catalase, coagulase and biochemistry tests, 201 *Staphylococci* were isolated from varying infected organ, of which 134(66.7%) were identified as proven CoNS that distributed among 16 species (Table 1).

According to age, majority of CoNS species (19.65%) were isolated from one month up to 44 years nearly equal for each groups, and minority (6.7%) were isolated from >75 years group of age.

Among the CoNS, 77(57.5%) belonged to males and 57 (42.5%) belonged to females. Based on susceptibility to novobiocin 92(68.7%) were sensitive to novobiocin and 42(31.3%) resistance to it. (Table1).

The most isolated CoNS were obtained from urine (51.5%) specimens and followed by blood (25.4%), thracho tube (9.7%), Bodies fluid discharge (8.2%), and wounds (5.2%) respectively (Table 2).

As shown in Table 2, the predominant species (19.4%) of CoNS was *S. epidermidis,* and the majority of them were isolated from urine 12(17.4%). Therefore there was statistically significant deference between *S. epidermidis* and other CoNS species were isolated from urine specimens (P= 0.018). Also there was statistically significant deference between *S. epidermidis* and other CoNS species were isolated from blood *specimens* (P= 0.003).

S. saprophyticus (12.7%) after *S. haemolyticus* (14.9%) the third agent of CoNS that isolated from clinical specimens. *S. saprophyticus* (15.9%) after *S. epidermidis* (17.4%), was an important pathogen of CoNS were isolated from urine (Table 2), and the mostly isolated of

Table 1. Frequency of CoNS species and their susceptibility to Novobiocin were isolated from clinical specimens.

Staphylococci species (CoNS)	Novobiocin		Total No (%)
	Sensitive No (%)	Resistant No (%)	
S. epidermidis	26(29.4)	-	26(19.4)
S. haemolyticus	20(14.9)	-	20)(14.9)
S. saprophyticus	17(12.7)	-	17(12.7)
S. cohnii. spp	-	11(8.2)	11(8.2)
*S. capitis.*spp	11(8.2)	-	11(8.2)
S. xylosus	-	8(6)	8(6)
S. lugdunensis	10(7.5)	-	10(7.5)
S. hominis	8(6)	-	8(6)
*S. schleiferi.*spp	3(2.2)	-	3(2.2)
S. sciuri	-	2(1.5)	2(1.5)
S. simulans	8(6)	-	8(6)
S. warneri	3(2.2)	-	3(2.2)
S. arlettae	-	4(3)	4(3)
S. auricularis	1(0.74)	-	1(0.74)
S. caprae	1(0.74)	-	1(0.74)
S. intermedius	1(0.74)	-	1(0.74)
Total	92(68.7)	42(31.3)	134(100)

Table 2. Frequencies of CoNS species were isolated from different organs.

Staphylococci species (CoNS)	Urine No (%)	Blood No (%)	Tracheal tube No (%)	Bodies fluid discharge No (%)	Wound No (%)
S. epidermidis	12(17.4)	9(26.5)	1(7.7)	2(18.2)	2(28.6)
S. haemolyticus	9(13)	4(11.8)	2(15.4)	3(27.3)	2(28.6)
S. saprophyticus	11(15.9)	3(8.8)	1(7.7)	2(18.2)	0(0)
S. cohnii. spp	10(14.5)	0(0)	1(7.7)	0(0)	0(0)
*S. capitis.*spp	5(7.2)	3(8.8)	0(0)	2(18.2)	1(14.3)
S. xylosus	5(7.2)	2(5.9)	1(7.7)	0(0)	0(0)
S. lugdunensis	8(11.6)	1(2.9)	1(7.7)	0(0)	0(0)
S. hominis	2(3)	4(11.8)	1(7.7)	1(9.1)	0(0)
S. schleiferi. spp	0(0)	3(8.8)	0(0)	0(0)	0(0)
S. sciuri	0(0)	1(2.9)	1(7.7)	0(0)	0(0)
S. simulans	2(3)	1(2.9)	3(23)	1(9.1)	1(14.3)
S. warneri	0(0)	2(5.9)	0(0)	0(0)	1(14.3)
S. arlettae	3(4.3)	0(0)	1(7.7)	0(0)	0(0)
S. auricularis	0(0)	1(2.9)	0(0)	0(0)	0(0)
S. caprae	1(1.4)	0(0)	0(0)	0(0)	0(0)
S. intermedius	1(1.4)	0(0)	0(0)	0(0)	0(0)
Total	69(51.5)	34(25.4)	13(9.7)	11(8.2)	7(5.2)

S. saprophyticus was obtain from females (P=0.012). In the other hand we indicated that *S. epidermidis* more than *S. saprophyticus* were isolated from male urine.

The results of antibacterial susceptibility pattern of CoNS species were shown that the majority of the resistance of CoNS were against to oxacillin (94.02%) and the minority of resistance were to Vancomycin (20.89%) (Table 3).

DISCUSSION

Coagulase-Negative *Staphylococci* species formerly known as contaminants bacteria, but are now as important possible pathogens with the augment in number of sternly incapacitated patients. Thirty nine species of CoNS are recognized (Euze, 2007), but only 16 of these species had isolated from commonly or

Table 3. Number of antibiotics resistance pattern of CoNS species were isolated from clinical specimens.

Antibiotics CoNS	AM No.	E No.	S No.	TE No.	CC No.	P No.	AMX No.	CF No.	OX No.	VA No.
S. epidermidis	23	18	17	15	13	21	19	15	25	6
S. haemolyticus	20	17	11	12	14	19	17	14	19	3
S. saprophyticus	13	9	10	13	4	13	10	8	17	4
S. cohnii. spp	10	10	8	9	9	11	5	8	11	2
*S. capitis.*spp	9	9	7	9	7	9	7	8	11	4
S. xylosus	8	6	7	7	7	6	2	6	8	2
S. lugdunensis	7	5	4	3	2	6	6	4	8	0
S. hominis	7	5	4	6	4	7	6	4	5	1
*S. schleiferi.*spp	3	1	3	2	3	2	3	2	2	1
S. sciuri	2	1	1	1	1	1	1	1	2	0
S. simulans	6	5	8	6	7	8	5	7	8	4
S. warneri	3	2	2	1	3	3	2	2	3	1
S. arlettae	4	4	3	2	3	3	1	2	4	0
S. auricularis	1	1	1	1	1	1	1	1	1	0
S. caprae	1	1	1	1	1	1	1	0	1	0
S. intermedius	1	0	1	1	1	1	1	1	1	0
Total	118	94	88	89	80	112	87	83	126	28
%	88.05	70.14	65.67	66.41	59.7	83.58	64.92	61.94	94.02	20.89

Ampicillin (AM), Erythromycin (E), Streptomycin (S), Tetracyclin (TE), Clindamycin (CC), Penicillin (P), Amoxicillin (AMX), Cephalexin (CF), Oxacillin (OX), Vancomycin(VA).

hospitalize infectious patients. The majority of CoNS that isolated from clinical sample is *S. epidermidis*. The frequency of the *S. epidermidis* that isolated from infected organs was reported 50% up to 70% by many of investigator (Kawamura and Best, 1998; Del' Alamo et al., 1999; Cuevas et al., 2004; Sivadon et al., 2005; Singhal et al., 2006; Mohan et al., 2002; Koksal et al., 2009). In present study, 134 CoNS were isolated from various clinical sources including wounds, blood, urine, thracho tube and bodies fluid discharge, that distributed in 16 species. Our data were shown that the majority species of CoNS were novobiocin sensitive, also the predominant species of CoNS belonged to *S. epidermidis* (19.4%), but isolated of this organism in many studies were higher than our finding (Akinkunmi and Lamikanra, 2010; Kawamura and Best, 1998; Del'Alamo et al.,1999; Cuevas et al., 2004; Sivadon et al., 2005; Singhal et al., 2006; Mohan et al., 2002; Koksal et al., 2009), this difference probably effected by geographic situation (our region have hot and humidly climate).

Fifteen percent of all CoNS were belonged to *S. haemolyticus* and it was the second mostly isolated in our data. This finding nearly consistence to previous studies (Akinkunmi and Lamikanra, 2010; Kawamura and Best, 1998; Del'Alamo et al.,1999; Cuevas et al., 2004; Sivadon et al., 2005; Singhal et al., 2006; Mohan et al., 2002; Koksal et al., 2009; Gatermann et al., 2007). *S. saprophyticus* (12.7%) was the third mostly isolated, and *S. auriculans, S. caprae, S. intermedius* had have the lowest rate of isolation among CoNS species in our study, on the other hand Kawamura et al. (1998) reported, *S. saprophyticus* was fifth step of their isolated, that this difference probably were belong to the obtained the specimens from different organ. Kawamura and Best were shown that *S. caprae* with high frequency (14%) exist in Japan (Kawamura and Best, 1998), but in our finding was lower, this difference may be due to the culture of Japan's population and probably belonged to the method of the isolation (method of Kawamura and Best, was DNA hybridization).

S. cohnii (8.2%) *S. capitis* spp (8.2%) and *S. lugdunensis* (7.5%) were considered as the important isolated of CoNS in our data. The higher rate of *S. cohnii* (6%) were reported by Del' Alamo et al., (Del' Alamo et al., 1999), and S. *capitis* with 10% frequency and *S. lugdunensis* with 13% was reported by Singhal et al., (Singhal et al., 2006). *S. lugdunensis* with 9% frequency in Turkey was reported by Koksal et al., (Koksal et al., 2009).

The highest rates of *S. saprophyticus* (90.9%) were isolated from female's urine specimens in our data. This is similar to the findings of other workers who reported isolation of *S. saprophyticus* from urine was related to sex (De Paulis et al., 2003; Huebner and Goldmann, 1999).

Our study indicated that the group of 30-44 of age high risk group to acquisition CoNS infections, and *S. epidermidis, S. haemolyticus*, *S. saprophyticus* accounts the majority of infections caused by CoNS in this group, therefore infections caused by CoNS species in this

groups of age should be reconsider.

In our finding 25.4 and 5.2%, of CoNS were isolated from blood culture and wounds respectively, and *S. epidermidis* had the upper most of isolated 9(26.5%) among other CoNS species from blood culture. Isolation of *S. epidermidis* form wounds in Archer reported (Archer, 2000) were more than our finding, this difference may be affected by method of collection of specimens, because *S. epidermidis* were considered as commensally organisms of human skin (Archer, 2000).

Froggatt et al. (1989) statement that the most common source of isolated *S. haemolyticus* was wounds, followed by urine, blood, and other sources, Our finding confirm Froggatt et al reported, because we observed that mostly isolated of *S. haemolyticus* was belonged to wounds (28.6%) and body fluids (27.3%) specimens.

Susceptibility pattern of CoNS species against antibacterial agents in our finding showed that the majority of the resistance of CoNS were belonged to beta lactam groups, and the mostly of this resistance were against oxacillin (94.02%), and lowest to vancomycin. Oxacillin resistances Coagulase negative *Staphylococci* in different parts of Europe were reported between 70% and 80%, and similar also reported from the United States, Canada and Latin America (Hanberger et al., 2001; Vincent, 2000; Diekema et al., 2001). Maximum CoNS resistance to ampicillin (89%) followed by cefotaxime (59%) was reported by Goyal et al., (2006), which our data is compatible to their findings findings. Resistance to Erythromycin, Clindomycine and Vancomycin in our data was more than several studies (Akinkunmi and Lamikanra, 2010; Mohan et al., 2002; Goyal et al., 2006; Agvald-Öhman et al., 2004; Ferreira et al., 2002). This increasing resistance to Vancomycin in our region probably affected by increasing the use of this antibiotic for treatment of CoNS or other infections.

Our data show that S*. lugdunensis, S. sciuri, S. arlettae, S. auricularis, S.* caprae and *S. intermedius* had have the lowest resistant against vancomycin, and S. *epidermidis* had the highest resistance against vancomycin and oxacillin. The rate of *S. epidermidis* resistance against oxacillin in our study accordance with Pauline (Pauline and Akgun, 1998) reported. The highest resistance rates of CoNS against oxacillin were shown in CoNS that isolated from wounds (100%) and blood (97.05%) specimens in our study, and it was nearly similar to Svetlana Kozitskaya reported (Kozitskaya et al., 2004).

Conclusion

We concluded that 2/3 of all clinical isolation of *Staphylococci* were CoNS species, which the majority of them belonged to *S. epidermidis. Also S. epidermidis* were isolated with high percentage from urine, blood and wound than other CoNS species, also this organism had have the highest resistance against oxacillin and vancomycin among other CoNS species.

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