Identification and Antimicrobial Susceptibility Pattern of Clinically Significant Coagulase-negative Staphylococci

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Aims: This study was undertaken to determine the prevalence and pathogenicity of coagulase-negative staphylococci in clinical samples and to study the antibiotic-sensitivity pattern of the coagulase-negative isolates.

Methods: A prospective study was conducted over a period of two years on patients admitted in the Sher-i-Kashmir Institute of Medical Sciences. Blood, urine, pus, catheter tips, cerebrospinal fluid and peritoneal fluid samples of patients who fulfilled the criteria for being labeled as nosocomial were cultured.

Results: One hundred and six strains of coagulase negative staphylococci were isolated from the samples and among them 90 isolates were identified as *Staphylococcus epidermidis* (84.90%). Most of the coagulase-negative staphylococci isolates were resistant to penicillin, cephalosporins and fluoroquinolones. Methicillin resistance was found in a significant number of coagulase-negative isolates. All the isolates were found to be sensitive to vancomycin.

Conclusions: The results of the study emphasized that isolation of coagulase-negative staphylococci should be viewed with serious concern and accurate species identification and antimicrobial susceptibility testing should be performed in all cases.

Keywords: antimicrobial susceptibility testing; coagulase-negative staphylococci (ConS); nosocomial pathogens.

INTRODUCTION

Coagulase-negative staphylococci (ConS) are commensals commonly found on human skin and mucous membranes. They have the potential to cause serious human disease due to their ability to become pathogenic when alterations in the integument allow them to enter into the body. ConS had long been regarded as harmless skin commensals and dismissed as culture contaminants.[1-3] Their role as pathogens was increasingly suspected and studied and they are now regarded as well established agents of infection. The resistance of these organisms to a wide-range of antimicrobial agents is well documented. The increasing incidence of infections by these bacteria can be attributed to their affinity for the foreign materials that are integral to modern medicine.[4] The increasing use of prosthetic devices, intravascular catheters, and other invasive technologies in patients who are sick, more immunosuppressed, and at the extremes of life has brought ConS to the forefront of nosocomial pathogens. The isolation of ConS should be viewed with serious concern and accurate species identification and antimicrobial susceptibility testing should be performed in all cases. Also the increasing significance of ConS as one of the leading nosocomial pathogens needs to be recognized.

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METHODS

This study was conducted in the Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences, Soura, India, from June 2001 to June 2003. The institutional review board approved the study protocol, informed consent document and procedures. Patients who fulfilled the criteria for being labeled as nosocomial (i.e. who developed an infection after 48 hours of hospital admission) were included in the study.

Blood samples were collected from patients with septicemia, patients with central venous catheter, peripheral intravenous and other catheters, patients from surgical, medical and neonatal intensive care units, immunocompromised patients and patients with

prosthetic valve endocarditis. Intravascular catheter segments, catheter tips or central venous catheter hubs were collected from patients on intravenous or other catheters. Aspirates were collected from joint spaces in patients with prosthetic implants and CSF from patients with ventriculoperitoneal shunt and patients with epidural catheter. Peritoneal fluid was taken from patients with chronic ambulatory peritoneal dialysis (CAPD) and pus from patients with surgical site infections and diabetic patients with ulcers or abscesses. Urine samples were collected from patients with indwelling urinary catheters and young sexually active females with recurrent urinary tract infections and vascular graft material or swab were also collected.

Clinical samples were processed on blood agar, MacConkey agar, nutrient agar, mannitol salt agar and cooked meat broth. Each specimen was plated on blood agar and other media as indicated under all aseptic conditions and incubated at 37°C for 24 hours and subsequently for 48 hours. In addition culture of intravenous catheter segment was performed according to the semi-quantitative method of Maki et al.[5] Catheter segments were cultured after rolling over a blood agar plate and incubated for 18 hours at 37°C after which the number of colony forming units (cfu) was counted. Cultures yielding more than 15 cfu were associated with catheter-related bacteremia.

The coagulase- negative staphylococci were identified by the colony appearance on blood agar, nutrient agar and MacConkey agar. On mannitol salt agar, ConS formed small orange colonies surrounded by red or purple medium. Gram staining was done followed by catalase test. Slide coagulase test was done to detect the bound coagulase or clumping factor. Tube coagulase test was done to confirm the findings of the slide coagulase test. Once an organism was confirmed as ConS, it was further characterized by the scheme recommended by the sub-committee on the taxonomy of Staphylococci and Micrococcus. Antimicrobial sensitivity of ConS was done by Stokes disc diffusion method. Minimum inhibitory concentration (MIC) was determined by broth dilution method. Control strain used for performing MIC was Staphylococcus ATCC 29213.

RESULTS

Out of a total of 3100 nosocomial infections studied, 106 cases were due to coagulase- negative staphylococci infections, with a prevalence of 3.42%. Table 1 shows the distribution of the 106 isolates from various sources.

Table 1. Distribution of 106 isolates of coagulase negative staphylococci from various sources (n=106).

Specimen	Number (%)
Blood	34 (32.07)
Urine	26 (24.53)
Catheter tips/drain tips/ IV cannulas	14 (13.21)
Pus	12 (11.33)
Cerebro-spinal fluids	10 (9.43)
Peritoneal fluid	10 (9.43)

There was a significant difference in prevalence of various clinical groups (p value = 0.0342)._The incidence of infection was higher in males (65%) than in females (35%). The association between ConS infection and sex was statistically significant.

Most of the isolates were from catheterized patients, patients with intravascular prosthetic devices, patients in intensive care units and postoperative wards. The prevalence was 3.77% in nosocomial bacteremias, 3.25% in nosocomial urinary tract infections, 2% in wound infections, 2.50% in meningitis and 5.00% in peritonitis (Table 2).

Table 2. Prevalence of coagulase-negative staphylococci infections in clinical samples collected from various sources in department of microbiology, SKIMS, Soura.

Infection	Specimen	Cases	ConS isolates	Rate (%)
Bacteremia	Blood	900	34	3.77
Urinary tract infections	Urine	800	26	3.25
Wound infections	Pus	600	12	2.00
Meningitis	CSF	400	10	2.50
Peritonitis	Peritoneal fluid	200	10	5.00
On i.v lines/in-dwelling devices	i.v tips/ drain tips	200	14	7.00

Ninety out of the 106 isolates were identified as *Staphylococcus epidermidis* (84.90%) and 16 as

Staphylococcus saprophyticus (15.09%).

There was an association of ConS with underlying clinical disorders. 43% of bacteremias due to ConS were associated with the use of central venous catheters and prosthetic devices, 22% of the isolates were from patients with underlying leukemia or lymphoma, 20% from patients admitted to neonatal intensive care unit and 8% patients either had diabetes or chronic renal failure. 60% of isolates recovered from peritoneal fluid were obtained from patients on chronic ambulatory dialysis.

Tables 3 and 4 show the antibiotic sensitivity and resistance pattern of ConS strains isolated from various clinical samples. The most effective antibiotic agents used against the 106 coagulase-negative staphylococci were vancomycin (100%) and methicillin (83.96%) followed by amikacin (70%), gentamycin (66.1%), norfloxacin (64.15%) and ciprofloxacin (61.3%) cefotaxime (58.49%) and cephalexin (57.5%).

Table 3. Antibiotic sensitivity and resistance pattern of coagulase-negative isolates.

Antimicrobial Agent	Antibiotic content in disc (µg)	No. of sensitive strains (%)	No. of resistant strains (%)
Penicillin-P	10 µg	10 (9.4%)	96 (90.1%)
Amoxycillin-A	30 µg	16 (15.1%)	90 (84.9%)
Gentamycin-G	10 µg	70 (66.1%)	36 (33.96%)
Amikacin-Ak	30 µg	74 (70%)	32 (30.18%)
Ciprofloxacin-C	5 µg	65 (61.3%)	41 (38.67%)
Cephalexin-Cp	30 µg	61 (57.5%)	45 (42.45%)
Cefotaxime-Ce	30 µg	62 (58.49%)	44 (41.5%)
Norfloxacin-Nf	10 µg	68 (64.15%)	38 (35.84%)
Methicillin-M	5 µg	89 (83.96%)	17 (16.03%)
Vancomycin-V	30 µg	106 (100%)	-

Table 4. Distribution of resistance pattern of 106 coagulase-negative isolates from various clinical samples.

S.No	Specimen	No. of isolates	Penicillin	Amoxycillin	Gentamycin	Amikacin	Ciprofloxacin	Cephalexin	Cefotaxime	Norfloxacin	Methicillin
1.	Blood	34	31 (91.1)	29 (85.2)	9 (26.4)	8 (23.5)	10 (29.4)	12 (35.2)	11 (32.3)	10 (29.4)	3 (8.8)
2.	Urine	26	24 (92.3)	23 (88.4)	4 (15.3)	3 (11.5)	5 (19.2)	6 (23.0)	7 (26.9)	5 (19.2)	3 (11.5)
3.	Catheter tips etc.	14	12 (85.7)	10 (71.4)	5 (35.7)	5 (35.7)	6 (42.8)	7 (50)	6 (42.8)	5 (35.7)	2 (14.2)
4.	Pus	12	11 (91.6)	10 (83.3)	5 (41.6)	4 (33.3)	6 (50)	7 (58.3)	8 (66.6)	8 (66.6)	4 (33.3)
5.	CSF	10	9 (90)	8 (80)	5 (50)	5 (50)	7 (70)	8 (80)	7 (70)	5 (50)	3 (30)
6.	Peritoneal Fluid	10	9 (90)	10 (100)	8 (80)	7 (70)	7 (70)	5 (50)	5 (50)	5 (50)	2 (20)
	Total	106	96	90	36	32	41	45	44	38	17
	Percentage		(90.1)	(84.9)	(33.9)	(30.1)	(38.6)	(42.4)	(41.5)	(35.8)	(16.0)

Note: Figures in parentheses show percentages

The least sensitive antimicrobial agents were penicillin (9.4%) and amoxycillin (15.1%). Among the ConS isolates from blood 91.7% were resistant to penicillin and 85.29% were resistant to amoxycillin. Similarly 35.29%, 32.35%, 29.4%, 29.4%, 26.24%, 23.5% and 8.82% were resistant to cephalexin, cefotaxime, norfloxacin, ciprofloxacin, gentamycin, amikacin and methicillin respectively. Among the ConS isolated from urine, 92.5% were resistant to penicillin and similarly 88.46%, 26.92%, 23.07%, 19.23%, 19.23%, 15.38%, 11.53%, 11.53%, 23.5% and 8.82% were resistant to amoxycillin, cefotaxime, cephalexin, ciprofloxacin norfloxacin, gentamycin, amikacin and methicillin, respectively. In those isolated from catheter tips, 85.7% strains of were resistant to penicillin and similarly 71.42%, 50%, 42.85%, 42.85%, 35.71%, 35.71%, 35.71% and 14.28% were resistant to amoxycillin, cephalexin, cefotaxime, ciprofloxacin, amikacin, gentamycin, norfloxacin and methicillin respectively. Among the strains isolated from pus samples, 91.66% were resistant to penicillin and similarly 83.33%, 66.6%, 66.6%, 58.33%, 50%, 41.66%, 33.33%, and 33.33% were resistant to amoxycillin, cefotaxime, norfloxacin, cephalexin, ciprofloxacin, gentamycin, amikacin, and methicillin, respectively. In the strains isolated from cerebrospinal fluid 90% were resistant to penicillin and similarly 80%, 80%, 70%, 70%, 50%, 50%, 50% and 30% were resistant to amoxycillin, cephalexin, cefotaxime, ciprofloxacin, norfloxacin, gentamycin, amikacin, and methicillin, respectively. Hundred percent of strains of the ConS isolated from peritoneal fluid were resistant to amoxycillin and similarly 90%, 80%, 70%, 70%, 50%, 50%, 50% and 20% were resistant to penicillin, gentamycin, amikacin, ciprofloxacin, cephalexin, cefotaxime, norfloxacin, and methicillin, respectively.

DISCUSSION

Coagulase-negative staphylococci, which were earlier dismissed as contaminants, have now emerged as important potential pathogens. They have become increasingly resistant to antibiotics- the most recent threat being the emergence of strains with moderate levels of resistance to vancomycin.[6]

In the present study, the prevalence of ConS infection in Sher-i-Kashmir Institute of Medical Sciences was 3.42 %. The prevalence was 3.77% in nosocomial bacteremias, 3.25% in nosocomial urinary tract infections, 2% in wound infections, 2.50% in meningitis and 5.00% in peritonitis. Sidebottom et al[7] in 1988 also noted a prevalence of 4.4% during their 15-years study on the blood stream isolates of ConS in neonatal intensive care patients. Edgeworth et al[8] reported a prevalence of 3.7 % in patients of bacteremia. Ninety out of the 106 isolates were identified as *Staphylococcus epidermidis* (84.90%) and 16 as *Staphylococcus saprophyticus* (15.09%). Akatov et al[9] in 1981 isolated 55.1% *S. epidermidis* species and 13.5% S. *saprophyticus* species in their study of ConS isolates from clinical specimens.

The results of earlier studies[10-12] conducted on blood samples are consistent with our study with rates of isolation ranging from 33.7% to 51.1%. Our results are also in accordance with those of Pal and Ayyagari[13] who found that the most common source of ConS from various clinical specimens was blood (37.35%), followed by urine (22.6%) and cerebrospinal fluid (20%). In our study, 43% of bacteremias due to ConS were associated with the use of central venous catheters and prosthetic devices, 22% of the isolates were from the patients with underlying leukemia or lymphoma, 20% from the patients admitted to neonatal intensive care unit and 8% patients either had diabetes or chronic renal failure. These results are in accordance with the findings of other studies.[14-16] Also, according to Smith et al[17] 69% of the infections due to ConS were associated with the use of prosthetic devices. Stillman et al[18] reported increased rates of coagulase-negative staphylococcal blood stream infections and 64% of these were found in critical care areas. The EPIC[19] study in Europe gave a prevalence rate of 44.9% in intensive care units, which was associated with the increasing use of intravascular catheters.

An important aspect of the emergence of coagulase-negative infections is the development of resistance to antimicrobial agents. The most effective antibiotic agents used against the 106 ConS were vancomycin (100%) and methicillin (83.96%) followed by amikacin (70%), gentamycin (66.1%), norfloxacin (64.15%) and ciprofloxacin (61.3%) cefotaxime (58.49%) and cephalexin (57.5%). The least sensitive antimicrobial agents were penicillin (9.4%) and amoxicillin (15.1%).

These results are consistent with those of Richardson et al[20] who concluded that 80-85% of clinically significant ConS isolated in their study were resistant to penicillin and more than 30% were resistant to methicillin. Refsahl and Anderson[14] in 1992, noted methicillin resistance in 30.5% of the ConS. Our findings are similar to other studies[2,3] conducted in India. Pal and Ayyagari[13] stated that 14.6% of their ConS isolates were resistant to methicillin while vancomycin was effective against all the isolates. Also Deepak et al[21] showed more than 80% of their ConS isolates were resistant to penicillin, 75% to ampicillin and 79% to trimethoprim.

MIC was determined for multi-drug resistant strains. MIC for penicillin, amoxycillin and amikacin was 64-256µg/ml, for gentamycin 32-128 µg/ml, 32-256 µg/ml for cephalexin and cefotaxime, 16-128 µg/ml for ciprofloxacin, 16-64 µg/ml for norfloxacin and MIC for methicillin was 16-32 µg/ml. Ponce de et al[22] found MIC of more than 64 µg/ml for multi-drug resistant strains of ConS for penicillin, methicillin, cefotaxime, amikacin, gentamycin, rifampicin and vancomycin while rest of the strains had an intermediate resistance with MIC's of 16-32 µg/ml.

Richardson et al[20] noted that the MIC of the various ConS isolates was more than 256 µg/ml for gentamycin, tobramycin and kanamycin. Also, Foleno et al,[23] during their study of ConS found that the MIC for ciprofloxacin, norfloxacin and enoxacin was between 64-128µg/ml.

CONCLUSIONS

Coagulase-negative staphylococci are currently among the most common cause of infections in hospitalized patients. Increasing resistance to antibiotics would indicate that their prevalence will continue to rise. Although the incidence of methicillin-resistant *Staphylococcus epidermidis* strains and other methicillin resistant staphylococci varies from hospital to hospital, a steady increase has been observed and often exceeds 50%. Coagulase-negative methicillin-resistant staphylococci are a major cause of medical device-associated infections, especially in immunocompromised patients, and the excessive use of vancomycin in the empirical treatment is complicated by the emergence of multi-drug resistant strains. The results of the present study emphasize that isolation of ConS should be viewed with serious concern and accurate species identification and antimicrobial susceptibility testing should be performed in all cases.

DISCLOSURE

The authors report no conflicts of interest in this work.

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