CHARACTERISATION OF COAGULASE-NEGATIVE STAPHYLOCOCCI (CoNS) ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

BACKGROUND

Species of CoNS have important traits and are frequently associated with various kinds of infections that are manifested clinically. The present study was undertaken to determine the bacterial profile of Urinary Tract Infections (UTI) caused by CoNS and to assess their antibiotic susceptibility pattern.

MATERIALS AND METHODS

The general principles of collection, transport and storage of specimens are applied. 500 fresh midstream urine samples studied which were collected over a 10-month period from the women and men with a complaint of dysuria who visited the Medical, Surgical and Gynaecological Outpatient Departments of MGM Hospital, Warangal were included in the study. All clinical specimens were processed as per the standard laboratory procedures. Antibiotic susceptibility patterns were determined by Kirby Bauer's disc diffusion method as per CLSI guidelines.

RESULTS

Out of 500 urine samples tested, 90 (18%) isolates were coagulase-negative staphylococci and the predominant species was S. saprophyticus. Antibiotic susceptibility testing showed Penicillin resistance of 80%, Amoxicillin resistance of 82.35% and Cephalexin resistance of 58.33%, while majority isolates were sensitive to Vancomycin, Gentamicin and Co-trimoxazole.

CONCLUSION

The results of the present study show that among the species of CoNS, S. saprophyticus is a major pathogen causing urinary tract infections. It should be supported by antimicrobial susceptibility testing, to prevent the spread of resistant isolates and to promote rational prescribing practices of the antibiotics in that region.

KEY WORDS

Coagulase-Negative Staphylococci, Urinary Tract Infection, Antibiotic Susceptibility.

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BACKGROUND

A large number of Staphylococcus species distinct from Staphylococcus aureus comprise the group known as Coagulase Negative Staphylococci (CoNS), so named as they lack the ability of the production of the enzyme coagulase. There are more than 45 recognised species of coagulase-negative staphylococci (CoNS). CoNS are gram-positive cocci that divide in irregular "grape-like" clusters and are unable to produce coagulase and thus to coagulate the rabbit plasma.[1] Species of CoNS that have important traits and frequently associated with various kinds of infections that are manifested clinically (urinary tract infections, biomaterial based prosthetic device infections, skin and soft-tissue infections, bacteraemia, endocarditis). In clinical practice urinary tract infections are commonly seen, which may be symptomatic or asymptomatic and either type of infections can result in serious sequelae if left untreated. Different microorganisms including fungi and viruses can cause UTIs, but bacteria are the major causative organisms and are responsible for more than 95% of UTI cases. Among the bacteria, Escherichia coli is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections followed by Klebsiella and coagulase negative staphylococci. Following are some clinically important species of coagulase negative staphylococci: S. epidermidis, S. saprophyticus, S. haemolyticus, S. capitis, S. hominis, S. auricularis, S. warneri, S. lugdunensis, S. intermedius, S. schleiferi and S. simulans. Anyone of the species of CoNS may be involved in human urinary tract disease. Historically, CoNS have been considered as saprophytes with little pathogenic potential, but can produce serious human infections under appropriate conditions, especially in debilitated and immunocompromised patients. Similarly, coagulase negative staphylococci are also implicated as the main causative agents of nosocomial infections.

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Objectives

The main objectives of the study are to determine the bacterial profile of Urinary Tract Infections (UTI) caused by CoNS and to assess their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Study Design

Descriptive study.

500 fresh midstream urine samples studied which were collected over a 10-month period from the women and men with a complaint of dysuria attended to the Medical, Surgical and Gynaecological outpatient departments of MGM Hospital, Warangal. The general principles of collection, transport and storage of specimens are applied, and all the clinical specimens were processed as per the standard laboratory procedures.[2] The specimens were examined microscopically and by quantitative culture. A dip-inoculum culture method with MacConkey and CLED medium was used and incubated at 37.8^{0}C for 24 hours and for 48 hours in negative cases. A specimen was considered positive for UTI if a single organism was cultured at a concentration of 10^5 CFU/mL, or when a single organism was cultured at a concentration of 10^4 CFU/mL and ≥ 5 leukocytes per high-power field were observed on microscopic examination of the urine. Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram positive cluster forming bacteria which are catalase positive, oxidase negative, bacitracin resistant, furazolidone sensitive and fermentative by the Oxidation-Fermentation (OF) test are identified as staphylococcal. The staphylococci strains were subjected to slide and tube coagulase test and those strains, which are negative by both methods are identified as coagulase negative staphylococci (CONS).

The identification of all the species of CONS isolated from the samples was done by using various standard biochemical tests like Ornithine decarboxylase test, Urease test, trehalose, lactose and Mannitol fermentation tests, the Voges-Proskauer (VP) test and the Novobiocin and Polymyxin B susceptibility tests. Antibiogram of isolates was performed using Kirby-Bauer[3] method (Clinical and Laboratory Standards Institute- CLSI guidelines). Antimicrobial agents tested were Penicillin, Amoxicillin, cephalexin, Erythromycin, ofloxacin, Gentamicin, Vancomycin and Co-trimoxazole.

RESULTS

Total No. Processed	Coagulase-Negative Staphylococci No.	%
500	90	18

Table 1. Shows out of 500 samples tested 90 (18%) were found to be Coagulase-Negative Staphylococci

Total No.	Male	%	Female	%
90	32	36	58	64

Table 2. Shows Sex Wise distribution of Coagulase Negative Staphylococci out of 90 Isolated Strains

Sl. No.	Species of Coagulase Negative Staphylococci	Isolated	%
1	S. saprophyticus	72	80
2	S. epidermidis	13	14
3	S. haemolyticus	5	6
4	S. warneri	-	
5	S. hominis	-	
6	S. capitis	-	
7	S. simulans	-	
	Total	**90**	

Table 3. Shows Species Wise distribution of Coagulase-Negative Staphylococci

Sl. No.	Antibiotic	S. saprophyticus	S. epidermidis	S. haemolyticus
1	Penicillin	57 (80%)	6 (46.15%)	2 (40%)
2	Amoxicillin	38 (52%)	6 (46.15%)	1 (20%)
3	Cephalexin	42 (58.33%)	5 (38.46%)	2 (40%)
4	Erythromycin	31 (43.05%)	4 (30.76%)	2 (40%)
5	Ofloxacin	14 (19.4%)	4 (30.76%)	3 (60%)
6	Gentamicin	18 (25%)	8 (61.53%)	3 (60%)
7	Vancomycin	11 (15.27%)	3 (23.07%)	1 (20%)
8	Co-trimoxazole	24 (33.33%)	6 (46.15%)	4 (80%)
	Total	**72**	**13**	**5**

Table 4. Shows Antibiotic Resistance in relation to species. Total number of Drugs used was 8. They were Penicillin, Amoxicillin, Ofloxacin, Cephalexin, Gentamicin, Erythromycin, Co-Trimoxazole and Vancomycin

DISCUSSION

The role of some gram-positive, catalase-positive, coagulase-negative cocci as pathogens of the urinary tract is increasing. Such infections were found typically among women in the outpatient health service.

Pereira (1962)[4] and Mitchell (1964)[5] established one variety of coagulase-negative staphylococcus as a primary pathogen of the normal female urinary tract. Gallagher, Montgomerie and North (1965)[6] found that 'coagulase-negative staphylococci,' at 14% were the second most common pathogen in bacteriologically proven cases of urinary tract infection in general practice. These have been shown by several workers to be a common cause of primary urinary infection, especially in young females (Mabeck,[7] 1969; Kerr,[8] 1973; Maskell,[9] 1974; Meers,[10] 1974) subsequently named Micrococcus subgroup 3 and now renamed Staphylococcus saprophyticus biotype 3 (Buchanan and Gibbons, 1974).[11] No matter what the taxonomic outcome, it is clear that there exists a discrete group of Gram-positive, catalase-positive, coagulase-negative, novobiocin-resistant cocci with a special predilection for the urinary tract of apparently normal women, in whom they cause an acute inflammatory process second in frequency only to Escherichia coli. This organism so called Staphylococcus saprophyticus is cultured frequently from the genitourinary mucosa of young sexually active women, which is identified by clinical microbiology owing to its novobiocin resistance. Novobiocin resistance is rarely found among other species of coagulase negative Staphylococci that are grown from the urine. S. saprophyticus colonises the bladder and urethral epithelium by different types of adhesions which include hemagglutinins with autolytic and adhesive properties, surface-associated lipase that forms fimbria-like surface appendages. All these help in maintenance of tight adherence to the surfaces. The reasons for high survivability of S. saprophyticus inside the urinary tract is in part due to the adhesions anchored within the cell wall, allowing the organism to effectively adhere and colonise the uroepithelium, together with urease which contributes to the persistent growth of the infection. Some strains of S. saprophyticus have the ability to create biofilms, increasing

their virulence especially in patients with catheters. Sellin et al (1975)[12] have shown that these organisms are only rarely found in the normal flora of the genitourinary tract of women. Wallmark et al (1978)[13] studied 661 urinary specimens, out of which 173 (26.17%) were S. saprophyticus. Studies of female outpatients in Sweden and at the University of Florida and Washington found S. saprophyticus to be the cause of 3,230 and 11 percent of urinary tract infections respectively, second to E. coli the cause of 65 to 85 percent of infections (Jordan et al 1980).[14] S. saprophyticus has been shown now clearly as one of the causes for dysuria-pyuria syndrome both in males and females.

Another organism that belongs to the group of Coagulase-Negative Staphylococci (CoNS), which was isolated from urine samples was S. epidermidis. S. epidermidis, the most frequently isolated species from human epithelia, predominantly colonising the axilla, head and nares as part of the normal commensal skin flora. Their ability to produce adhesion factors and withstand high salt concentrations is important to colonise human tissues. S. epidermidis has emerged as an important opportunistic human pathogen and clearly causes infection in patients with impaired immune system such as patients receiving immune-suppressive therapy, preterm infants, AIDS patients and drug abusers. It occupies the first position among the causative agents of nosocomial infections and also accounts for more than 50% of the late-onset sepsis episodes in neonates. Biofilm formation is the most important virulence factor of S. epidermidis. Its importance is rising mainly due to concurrent advances in medical practice with more people undergoing and surviving intensive care treatment with the increased use of indwelling medical devices such as arteriovenous shunts, contact lenses, urinary catheters, vascular catheters, prosthetic (orthopaedic) devices, peritoneal dialysis catheters, heart valves and vascular grafts. The increased survival of patients with a compromised immune system such as preterm neonates and HIV patients is among the other reasons of reporting of increased frequency of S. epidermidis infection. Although S. epidermidis infections only rarely develop into life-threatening diseases, they significantly increase morbidity in the affected groups. The fact that they are extremely difficult to treat represent a serious burden for the public health system.

Five isolates of the present study were to be S. haemolyticus, which is also an important nosocomial pathogen and is one of few coagulase negative staphylococcal species that colonies the urethra or periurethra of males and females causing urinary tract infection. Like other CoNS it tests negative for coagulase, ornithine decarboxylase, phosphatase, urease, DNAse and oxidase. S. haemolyticus is the second-most clinically isolated CoNS next to the S. epidermidis and also often associated with the insertion of medical devices such as prosthetic valves, cerebrospinal fluid shunts, orthopaedic prostheses, urinary dialysis and intravascular catheters.[15] Strains of S. haemolyticus produce a hemolysin, cytolysin and enterotoxin and able to form biofilms and often shows highest level of antibiotic resistance among the CoNS, which makes these infections difficult to treat. Peggy et al (1980)[16] studied 68 strains of coagulase-negative staphylococci from urinary samples. From there, they identified 64 to be S. saprophyticus and 2 to be S. epidermidis. There are also many reports of the cases of UTIs caused by other species of CoNS. For example, S. lugdunensis which is commonly found on the human skin, especially of the perineal region has a considerable potential as an opportunistic pathogen in causing UTI (Casanova-Roman et al in 2004).[17] The clinical course and virulence of infections due to S. lugdunensis are known to resemble that of infections due to S. aureus (Frank et al, 2008).[18]

In the present study, 90 (18%) coagulase-negative staphylococci isolated from 500 urine samples and the predominant species was S. saprophyticus, 72 out of 500 (14.4%) of urine samples. S. epidermidis isolates were 13 (2.6%) out of 500 urine samples and the S. haemolyticus isolates were 5 (1%). The highest prevalence of UTI in this study was found among the women between the age ranges of 26 - 30 years with a frequency of 64%. This pattern is similar to the observation of Mazzulli et al (2001),[19] who reported that young sexually active women were particularly prone to UTIs with an incidence of approximately 0.5 episodes per person per year. The present study correlated well with the above studies and references. In vitro susceptibility testing was done to these antibiotics: Penicillin, Amoxicillin, cephalexin, Erythromycin, ofloxacin, Gentamicin, Vancomycin and Co-trimoxazole. Multidrug resistance has been observed. Table No. 4 shows the resistant pattern of S. saprophyticus and other isolated coagulase-negative staphylococci to the above-mentioned antimicrobials.

CONCLUSION

The findings and results of the study indicate that urine culture is necessary for a definitive diagnosis of UTI and that empirical therapy should only be done by specialist physicians in cases where it is absolutely necessary. This study has been able to establish that among the species of CoNS, S. saprophyticus is the most prevalent urinary tract pathogen causing urinary tract infection especially in females.

Physicians and microbiologists must be aware that CoNS is an important cause of UTIs and apart from S. saprophyticus there are other species of CoNS (S. epidermidis, S. haemolyticus and S. warneri) that may be involved in urinary tract disease. Among the 72 isolates of S. saprophyticus, Penicillin resistance is 80% and Amoxicillin resistance is 82.35%. Ofloxacin resistance is 19.4%, Cephalexin resistance is 58.33%, Gentamicin resistance is 25%, Erythromycin resistance is 43.05%, Cotrimoxazole resistance is 33.33 % and Vanocomycin resistance is 15.27%. The resistance pattern to antibiotics may be explained in part by different local antibiotic practices and the influence of excessive and/or inappropriate antibiotic use, particularly broad-spectrum agents that were prescribed empirically. Reducing the number of prescriptions of a particular antibiotic can lead to a decrease in resistance rates. The greater mobility of individuals worldwide has also contributed to the spread of resistant isolates and the expansion of antibiotic resistance. The conclusion therefore seems that the choice of antimicrobials to be used in therapy should be supported by antimicrobial susceptibility testing. It also helps in preventing the spread of resistant isolates. On the other hand, the determination of organism and the antibiotic resistant pattern at periodic intervals helps the clinicians to be aware of the pathogens and also to develop rational prescribing practices of the antibiotics in that region.

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