INTRODUCTION

Breach of human skin barrier and utilization of prosthetic devices has led to an increase in the rate of infections with several organisms. One such group of organisms that has been a subject of much concern for the past decade is Coagulase-negative Staphylococci (CONS). Once considered relatively avirulent and probably a contaminant when isolated from clinical specimens, this class of organisms is now becoming an increasingly recognized as a cause of clinically significant infections. This group of organisms is recognized as the most common cause of blood stream infections in hospitalized patients, accounting for nearly one-third of all cases. CONS is the most commonly cultured intraocular pathogen, and accounts for an average of 40% postoperative and posttraumatic endophthalmitis cases. CONS is also responsible for up to 13% of urinary tract infections in inpatient settings. Resistance profile to antimicrobials for this group of pathogens is also alarming. It shows 69% resistance to Ampicillin, a commonly prescribed antibiotic for urinary tract infection (UTI). Inappropriate antibiotic prescription has led to an increase in antimicrobial resistance more so in the hospital environment.

The objective of this research was to determine the frequency of CONS in urinary tract infections, the sensitivities of the isolates to antimicrobial agents, and the association between biofilm production and antibiotic resistance for this group of organisms.

METHODOLOGY

Urine specimens, suggestive of urinary tract infection, were identified at Dr. Essa Laboratory over one-year period from January 2009 to January 2010. Specimens were further processed and studied at Immunology and Infectious Disease Research Laboratory (IIDRL), Microbiology Department, University of Karachi. Patients with symptoms suggestive of UTI, including dysuria, urgency or burning micturition were included in the study. Patients who were getting urinalysis for other indications including but not limited to proteinuria, hematuria, microalbuminuria, myoglobinuria, were
excluded from the study. Informed consent was obtained from all the patients. UTI was defined as detection of 10^5 cfu/ml. The isolates were processed at Immunology and Infectious Disease Research Laboratory (IIDRL), Microbiology Department, University of Karachi. Strict adherence to aseptic technique and standard collection protocols were observed taking first mid-stream urine (MSU) sample and using sterile containers for collection.

After standard microbiological procedures, e.g. Gram staining, oxidase, catalase, biochemical characterization was performed. Colonies were isolated by streaking the specimen onto culture media. The isolate was investigated phenotypically using commercially available API-20 system. The strip was incubated for 18 - 24 hours at 35 - 37°C, after which the results were read and interpreted. Genotypic investigation of the isolates was performed using PCR 16s r RNA gene sequencing. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method was employed for the screening of extracellular products.

The break point MICs were performed according to Clinical and Laboratory Standards Institute (CLSI) recommended standards. Minimum inhibitory concentration (MIC) of antibiotics was estimated by microtiter well plate method. Using 96-well microtiter plate format, bacteria were inoculated into a liquid growth medium in the presence of different concentrations of an antimicrobial agent. Growth was assessed after incubation period and the MIC value was read.

Scanning electron microscopy technique was used to visualize biofilm formation on indwelling catheters. A loop-full of isolate was taken from 24 hours old culture grown on Mueller Hinton agar and inoculated in a 5 ml plastic tube containing 3 ml Trypticase soy broth (TSB) for 24 hours. Moreover, 2 ml of 20% glucose was added and incubated for a further 24 hours period. The tube was decanted after the required incubation and 5 ml of 99% methanol was added as a fixative for 15 minutes at room temperature. After fixation, the specimens were rinsed gently in several changes of distilled water to remove excess fixative, followed by 0.1% crystal violet staining for 20 minutes. The tubes were briefly rinsed with distilled water to remove crystalline artifacts. Specimen was next air dried for 24 hours. Small square pieces of the stained slime layers were then mounted on copper stubs with the help of double-sided tapes. The sample was then coated with gold film by SEM JEOL JFC-1500 Quick auto sputter coater up to 300 Armstrong. Sample was then placed in sample chamber of SEM JEOL JSM-6380A scanning electron microscope and the scanning done under different magnification, acceleration, and voltage. Chi-square test was used for analysis with p-value of < 0.05 considered as statistically significant.

Figure 1: Relative frequency of isolates of Coagulase-negative staphylococcus identified from urine sample (N=220)

Figure 2: Antibiotic resistance profile of Coagulase-negative staphylococci.

Following unique accession numbers were given to DNA of the organisms when submitted to NCBI, Nucleotide database: S. hemolyticus - FJ394023 (catheter), S. saprophyticus - FJ379935 (urine), S. hominis - FJ429102 (urine) and S. sciuri - FJ440721 (urine).

RESULTS

CONS were the cause of 56 out of 1866 outpatient (3%) and 164 of 1261 inpatient (13%) urinary tract infections (p < 0.001). Two hundred and twenty CONS isolates were identified. These included S. saprophyticus (68 strain, 31%), S. epidermidis (42 strains, 19%), S. haemolyticus (33 strains, 15%), S. hominis (24 strains, 11%), S. xylosus (18 strains, 8%), Coagulase-negative S. aureus (13 strains, 6%), S. lugnunensis and S. sciuri (9 strains, 4% each) and S. chromogenes (4 strains, 2%).

Resistance profile of the CONS strains against 8 antibiotics assayed is presented in Figure 2. All isolates were sensitive to Vancomycin and Linezolid. Resistant to Ampicillin was 152 isolates (69%). One hundred and seven were resistant 83 isolates (37.5%) to Methicillin isolates (53%), to Ciprofloxacin 69 isolates (31.2%) to Amoxicillin Clavulanate; and to 37 isolates (16.9%) Fusidic acid. However, Teicoplanin showed a favourable profile with a resistance of only 13 isolates (5.9%).
DISCUSSION

The present results showed that Coagulase-negative Staphylococcus (CONS) was a significant uropathogenic entity, especially in the inpatient setting. These findings were consistent with previous studies that reported CONS to be the cause of 2 - 8% of outpatient and 2 - 13% of inpatient urinary tract infection (UTI).\textsuperscript{3,4} Until the last decade, presence of CONS in a urine specimen was regarded as a contaminant and its clinical significance as a pathogen was questioned. Numerous studies recognized the rising incidence of urinary tract infections secondary to CONS and dismissed the idea of CONS being a culture contaminant. Micheal et al. in his 6-year study on 205 intensive care units reported that CONS was the cause of nearly 40% of the blood stream infections.\textsuperscript{6} Kunin et al. pointed out that gram-positive bacterium populated catheter surfaces more densely and more commonly than did gram-negative bacteria and CONS represented 50% of all gram-positive variety.\textsuperscript{7}

The importance of the role of CONS in urology is due to their great capacity to colonize catheters and most prostheses. The particular organization of these bacteria into a conglomerate called biofilm is responsible for prosthetic infections, which can impair the renal function. In this study, the authors employed scanning electron microscopy for visualization of biofilm formation. Biofilm is merely microbial cells adherent to a surface enclosed in extracellular polymeric substance matrix and featuring unique structure, physiology and expression of gene.\textsuperscript{8} Formation of biofilm requires adhesion followed by accumulation and proliferation, resulting in the creation of multi-cell layered clusters on the surface. Adhesion is dependent on surface structures otherwise known as extracellular proteins of bacteria including SSP-1, SSP-2, teichoic acid, AtIE, MSCRAMMS among many others.\textsuperscript{8} Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of extracellular proteins provides a rapid, reproducible and discriminative method for characterization of Staphylococci on the basis of protein bands, specific to each species and strain.\textsuperscript{9} The significance of CONS caused biofilm infections is partly a consequence of significantly reduced susceptibility to antimicrobial agents, in a more frequent exchange of plasmids in the biofilm and as well as the risk of bacterial cell embolization.\textsuperscript{8} Our results showed that biofilm producing strains showed high level of resistance against antimicrobials. It is important to detect biofilm-producing bacteria in order to implement an appropriate antimicrobial therapy at an early stage.

Resistance to antimicrobial agents in the CONS group is widespread and is seemingly rising.\textsuperscript{5} This study showed that S. hemolyticus had the most resistant antibiotic profile; whereas S. saprophyticus showed the highest sensitivity to all antimicrobial agents, which is consistent with many previous studies.\textsuperscript{10} These results of antimicrobial resistance profiles were comparable to those found by Namrata et al., Maria et al. and Annie et al.\textsuperscript{10-12}

Resistance profile against Trimethoprim-Sulfamethoxazole (TMP-SMX), which is one of the first line empirical therapy for uncomplicated UTI, is favourable. However, Mikoajczyk et al. in a two-year study concluded that co-trimoxazole resistance was demonstrated more frequently and was very high among Methicillin resistant coagulase-negative staphylococci (52 - 63%) and even higher than among Methicillin resistant Staphylococcus aureus (MRSA).\textsuperscript{13}

Ampicillin is often prescribed as an empiric therapy in case of a suspected urinary tract infection. Resistance against Ampicillin was of the order of 69% in this study. These findings were similar to those of Namrata et al. though ampicillin resistance reported by aforementioned author was slightly lower.\textsuperscript{10} Goel et al. pointed out that clavulanate potentiated amoxicillin was found to be highly active against penicillin, ampicillin and amoxicillin resistant organisms.\textsuperscript{14}

Macrolides were another group of agents that had resistance of very high order. Zerin et al. in his study on macrolide and streptogramin susceptibility in Staphylococci showed that erm(C)-associated macrolide resistance was the most prevalent gene in CONS isolates.\textsuperscript{15} He also reported an incidence of 64% for erm(C) in Methicillin-resistant coagulase negative and 71% in MRSA. A study from Belgium reported Methicillin resistance to be at as high as 55 - 75%, particularly the nosocomial isolates.\textsuperscript{16} The present results confirmed the previous findings with Methicillin resistance of 53%. Martins et al. in his study from Brazil pointed out that Methicillin resistance was encoded by the mecA gene, which was inserted in the SCC mec cassette.\textsuperscript{17} Methicillin resistance has been a topic of great concern and various studies were performed in an effort to better understand its resistance phenomenon and pattern. A study from Turkey showed that the difference between Methicillin sensitive and resistant Staphylococci in terms of the rates of resistance against other antibiotics was found statistically significant with the exception of Fusidic acid (p < 0.05).\textsuperscript{18} For that study the resistance rates of isolates for Fusidic acid were very low.\textsuperscript{18}

In this study, all isolates were sensitive to Vancomycin among the 18 antibiotics tested. Gill et al. presented similar findings in his study.\textsuperscript{19} However, some reports have suggested the emergence of decreased susceptibility to Vancomycin in S. epidermidis. Denis O Garret National Center for Infectious Diseases and Centers for Disease Control and Prevention, reported the first case of Vancomycin resistant blood stream infection associated with S. epidermidis in 1996.\textsuperscript{20} In United States, CDC reported the first case of VRSA (Vancomycin resistant S. aureus) in 2002; until then 8
cases of VISA (Vancomycin intermediate \textit{S. aureus}) were already documented.\textsuperscript{21} Hare \textit{et al.} from India presented a study, which revealed the first time emergence of VISA/VRSA from this part of world. In his study, out of 783 \textit{S. aureus}, 2 \textit{S. aureus} strains were vancomycin and teicoplanin resistant; 6 strains of \textit{S. aureus} were vancomycin intermediate and 2 strains were teicoplanin intermediate. Out of the 898 CONS tested, 1 CONS strain was resistant to vancomycin and teicoplanin and 2 CONS strains were intermediate to vancomycin and teicoplanin. None of these isolates demonstrated vanA/vanB gene by PCR and all were mecA PCR positive. Saravolatz \textit{et al.} pointed out that Ceftraroline may represent a bactericidal treatment option for infections caused by these pathogens.\textsuperscript{23} The emergence of VRSA underscores the need for programmes to prevent the spread of antimicrobial-resistant microorganisms and control the use of antimicrobial agents in healthcare settings.

This study emphasized on the acceptance of \textit{Coagulase-negative Staphylococci} as a pathogen involved in UTI. Species identification could be of benefit for both epidemiological as well as patient care purposes. Maintenance of the ongoing practices of antibiotic prescription will likely further augment the peaking trends of antibiotic resistance. There is a need to conduct more studies on this subject. The findings gathered from such studies should be helpful for planning safer habit promotion programmes.

CONCLUSION

CONS are a potential uropathogens, with capability of slime production and resistance to common empirical prescriptions. This also warrants formulation of an appropriate antibiotic policy that covers CONS.

REFERENCES

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