MEDICAL CHANNEL

Original Article

COAGULASE NEGATIVE STAPHYLOCOCCU (CoNS) IS THE CONTAMINANTS IN THE CLINICAL SPECIMENS

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ABSTRACT

Backgrounds: Coagulase negative staphylococcus often formed as contaminants in clinical specimens e.g. in Swab from the skin, nose, throat, wounds, burns and bedsores, generally their presence is not clinically significant but they sometimes act as opportunistic pathogen and cause infection in the urinary tract or in debliated or immunodeficient subjects, more serious bacteriaemic infection. All staphylococci do not form enterotoxin. In some circumstances, however, coagulase negative staphylococcus (CoNS) may have a pathogenic role, they may cause peritonitis in patients on peritoneal dialysis, chronic septicemia or endocarditis in patients having heart surgery or fitted with an artificial heart valve, meningeal or bacteriamic infection in patients fitted with a ventriculovenous, cerebrospinal fluid shunt and septicemia in immunosuppressed and immunodefective patients. This study was aimed to provide the first insight into distribution of CoNS from infected wounds, to identify the common strains/species of coagulase negative staphylococcus (CoNS) in infected wound and antimicrobial susceptibility testing of coagulase negative staphylococcus (CoNS) isolated from the surgical wards. Wide variety of antibiotic have been used for the treatment of staphylococcal infections and at the same time each family of drugs were introduced, most strains were sensitive to them. Strains resistant to a variety of drugs, drug resistant strains are commonly present in hospital and may present a difficulty in therapy,Where the empericle treatment is only choice. Isolates of coagulase negative staphylococci from hospital sources show a greater frequency and wider spectrum of resistance to different antibiotics other than outside the hospital.

Method: In this total study hundred and fifty specimens were taken from surgical ward from June 2006 to December 2006 (75 from wound and 75 from skin), all the specimens were processed according to standard methods.

Results: 58 (39%) gram negative rods were isolated 29 (19%) gram positive cocci were isolated. 13 (9%) coagulase negative staphylococcus (CoNS) were isolated among these; 8 (62%) S.epidermidis were isolated, 3 (showed as under. 23%) S.hicus and 2 (15%) S.auricularis were isolated

Conclusion: It has been found from the study that coagulase negative staphylococci (CoNS) are the most common nosocomial organisms, found in hospital: S.hicus and S.auricularis were also isolated other than the S.epidermidis which is most common commensels of the skin and resistant to many antibiotics while vancomycin and tobramycin found the most effective antibiotic.

Key words: Coagulase negative staphylococcus (CoNS), S.epidermidis, S.hicus, S.auricularis.

INTRODUCTION

Staphylococci that do not produce coagulase are coagulase negative staphylococci (CoNS). The most clinically significant species in this group are S.epidermidis and S.saprophyticus. Most of these species have been isolated from humans usually found inhabitants the skin and mucous membranes. Certain species are found in very specific sites. Other have isolated from animals and animal products[1].

The cluster forming gram positive cocci of medical interest belong to the genera Staphylococcus, micrococcus and peptococcus. Of these genera only the Staphylococcus and peptococcus are commonly present as parasites in man[2]. Coagulase negative staphylococcus often formed as contaminants in clinical specimens in swab from the skin, nose, throat, wounds, burns and bed sore, generally their presence is not clinically significant but they some times act as opportunistic pathogens and cause infection in urinary tract or in debliated or immunodeficient subjects, more seriously bacteremic infection. Most strains form smooth, yellow colonies fairly similar to those of S.aureus. Some CoNS, grow so poorly under the anaerobic condition that they are liable to be misidentified as micrococci.

All the non aureus staphylococci may be termed as albus staphylococci[2]. Coagulase negative staphylococci do not form enterotoxin. In some circumstances, however CoNS may have some pathogenic role they may cause, peritonitis in patients on perotonial dialysis, chronic septicemia or endocarditis in patients having heart surgery or fitted with an artificial heart valve, meningeal or bacteremic infection in patients fitted with ventriculovenous cerebrospinal fluid shunt and septicemia in immunosuppressed and immunodeficient patients[2].

Staphylococci are widely spread in various niches such as clinical environment and food plants. Thirty six validated described species including 21 subspecies belong to staphylococci genus has been identified. Some strains are used for the technological abilities, and other are with diseases in human or animal[3].

Staphylococci are nosocomial pathogens associated with multiple antimicrobial resistance.For many years. Staphylococcus aureus was the only species recognized as an important human pathogen, whereas the Coagulase negative staphylococcus were viewed mostly as clinically non relevant contaminants. Recently the importance of CoNS strains as a major cause of nosocomial infections, mainly associated with the use of prosthetic and indwelling devices and immunocompromised patients, began to be ascertained. Although Staphylococcus epidermidis accounts for the majority of infection caused by CoNS, many other species have been identified in association with human infection. The emergence of CoNS as human pathogens and reservoirs of antimicrobial resistance determinants requires their rapid and variable identification in order to have an early prediction of the potential pathogenecity or antibiotic susceptibility of each clinical isolates and to clarity the clinical significance of each species[4].

Coagulase negative staphylococci (CoNS), which are part of the normal skin flora, have emerged as predominant pathogens in hospital acquired infections. They are associated with the presence of foreign bodies, such as prosthetic valves, cerebrospinal fluid shunts, and orthopedic prostheses, as well as intravascular, urinary, and dialysis catheters. Therefore, it has become increasingly important to accurately identify these isolates to the species level, in order to define the clinical significance of these bacteria, to carry out a proper epidemiologic surveillance, and to manage patients infected with CoNS in case of relapse (Poyart et al., 2001).

The risk of postoperative infection is not the same for all patients. The traditional infection risk protocol, first developed in the early 1960s, divided surgical procedures into 4 classes clean (Class I), clean-contaminated (Class II), contaminated (Class III), and dirty (Class IV), each with a different risk of infection. Later studies identified ranges of risk for each class: clean (1-5% infection risk), clean contaminated (3-11%), contaminated (10-17%), and dirty (27%) (Nichols and Louisiana, 1998).

Typing is usually indicated and the detected source of an outbreak of staphylococcal food poisoning or nosocomial infections. A variety of techniques have been used, including serological typing, biochemical reactions (biotyping), antibiotic susceptibility patterns, bacteriophage typing, and plasmid profiles. Each of these has its practical limitations. It must be recognized, however, that susceptibility patterns can be strongly influenced by the scope of antibiotics use within a given a locality, so that results can vary considerably from one community to another. Thus, antibiograms are of most used within a confined area rather than on a larger geographical scale. Susceptibility profiles may even change within an institution over time or at different locations within the institutions, thus making strain identification more difficult. On occasion, clonal variation in plasmid profile may be observed within a strain. This is most often noted by addition or deletion of a specific plasmid or modification resulting in a change of plasmid size. Many of the staphylococcuss species demonstrate complex plasmid profile, thus, allowing for considerable variation in profile between strains. Some strains may carry as many as 5-10 different plasmids (Kloos and Jorgensen, 1985).

Establishing the clinical significance of usual human pathogens may require repeated isolation of the same species from the same specimen source. In fact, algorithms proposed for the workup of CoNS from multiple blood cultures include timely identification of the species involved. Additionally, complete species identification is suggested for CoNS isolates from other normally sterile sites, such as joint or cerebral fluid, when these infections are considered clinically significant (Skow et al., 2005).

AIM OF STUDY

This study was aimed to provide the first insight into the distribution of CoNS from infected wound, skin around the wound of patients, to identify the common strains/species of CoNS and antimicrobial susceptibility pattern of CoNS isolated from the surgical wards of JPMC, (Tertiary care hospital) Karachi, Pakistan.

METHOD

In this study 150 specimens were taken from surgical wards of JPMC, Karachi, Pakistan from June 2006 to December 2006. 75 samples from wound, 75 samples from skin around the wound of the patients, all the specimens were put in brain heart infusion broth, and were incubated at 37ºC for 1-2 hours and then inoculated on blood agar and MacConkey agar, all media were incubated for 24 hours at 37C, plates were examined preliminary, identification of Coagulase negative was done on the basis of colony, morphology and culture characteristics on blood agar and MacConkey agar along with gram reaction (gram positive), catalase positive and Coagulase negative with the Coagulase tube method. After identifying the CoNS, further diagnostic biochemical tests were applied for identification of different species of CoNS.

Identification of Staphylococcus epidermidis

Isolates were inoculated in urea broth for 24 hours at 37°C turned in pink color shows urease positive. Isolate were inoculated on Muellar- Hinton agar by making lawn, novobiocin disc was applied and plate was incubated at 37°C for 24 hours. The result was sensitive to Novobiocin by forming zone of inhibition. Isolates were inoculated in Nitrate broth for 24 hours at 37°C turned in

red color shows Nitrate positive. **?** Isolates were inoculated on agar media containing maltose sugar for 24 hours at 37°C, yellow color of medium shows maltose positive. **?** Isolates were inoculated on agar media containg trehalose sugar and were incubated for 24 hours at 37°C medium was colorless. No color change shows trehalose negative. Resulting the isolates were Staphylococcus epidermidis (Kools and Jorgensen, 1985).

Identification of Staphylococcus hicus

Isolates were inoculated in urea broth for 24 hours at 37°C turned in pink color shows urease positive. The isolates were inoculated on Muellar Hinton agar by making lawn, Novobiocin disc was applied and plate was incubated for 24 hours at 37°C the result was sensitive to novobiocin by observing zone of inhibition. Isolates were inoculated in Nitrate broth for 24 hours at 37°C, no red color was seen and for further confirmation 4 drops of reagent A and reagent B were added resulting no color change. A pinch of zinc dust was added resulting no color change. Result is nitrate positive. Isolates were inoculated on agar media containing Maltose sugar for 24 hour at 37°C, colonies were colorless result was maltose negative Isolates were inoculated in b-glactose broth, no yellow color occurred in medium. Resulting isolates were Staph. Hicus (Kools and Jorgensen, 1985).

Identification of Staphylococcus auricularis

Isolates were inoculated in urea broth and was incubated at 37°C for 24 hours. No color change of broth shows, urease negative. Isolates were inoculated on agar media containing Maltose sugar and was incubated at 37°C for 24 hours. Yellow color of medium shows Maltose positive. Isolates were inoculated on agar media containing Rafinose sugar and was incubated at 37°C for 24 hours. Colonies were colorless result was Rafinose negative. Isolates were inoculated on Muellar- Hinton agar by making lawn, novobiocin disc was applied and plate was incubated for 24 hours at 37°C, the result was sensitive to novobiocin by forming zone of inhibition. One drop of oxidase was applied on filter paper, organisms were emulsified with sterile wood stick, no color change occurred, the result was cytochrome negative. Resulting isolates were Staphylococcus auricularis (Kools and Jorgensen, 1985).

RESULTS

58 (39%) gram negative rods were isolate 29 (19%) gram positive cocci were isolated.

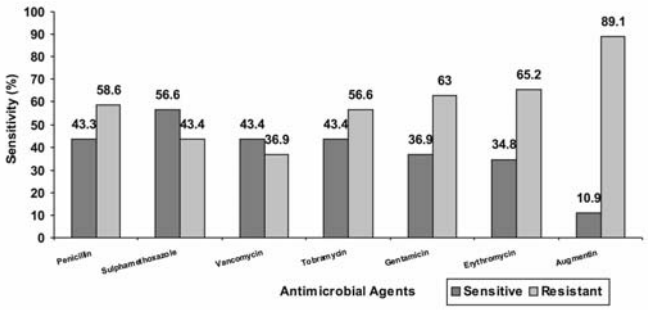
13 (9%) coagulase negative staphylococcus (CoNS) were isolated among these; 8 (62%) S.epidermidis were isolated, 3 (23%) S.hicus and 2 (15%) S.auricularis was isolated.While sensitivity pattern showed That Penicilin 19(43.3%), Sulphamethaxazole Trimethoprim 26(56.6%), Vancomycin 29(43.4%), Tobramycin 17(36.4%), Gentamycin 17(36.4), Erythromycin 16(34.8%), Augmentin 5(10.9), showed sensitive to these antibiotics.And Resistant pattern Showed that Peniclin.27(58.6), Sulphamethaxazole Trimethoprim 20(43.4), Vancomycin 17(36.9), Tobramycin 26(56.6), Gentamycin 29(63%), Erythromycin 30(65.2%) and Augmentin 41(89.1%) were found resistant to these antibiotics.

Isolates of coagulase negative staphylococcus (CoNS) shows a greater frequency and a wider spectrum of resistance to different antibiotics like penicillin, co-trimoxazole, gentacin, erythromycin, augmentin, which are generally used and reliable while vancomycin, tobramycin were found the most effective antibiotics as shwon table 1 and Graph 1.

Sensitivity pattern CoNS sensitivity pattern were performed in vitro disc diffusion method, McFarland 0.5 standardized suspension of bacteria was swabbed on the surface of Mueller Hinton agar plate, a paper disc of pencillin (10 IU), co-trimoxazole-trimethoprim (25mg), Vancomycin (30mg), tobramycin (10mg), erythromycin (15mg), augmentin (30mg) and gentamicin (10mg) were placed

FIGURE 1

ANTIMICROBIAL SENSITIVITY PATTERN OF VARIOUS COAGULASE NEGATIVE STAPHYLOCOCCUS (CONS) SPECIES ISOLATED



TABLE

ANTIMICROBIAL SENSITIVITY PATTERN OF VARIOUS COAGULASE NEGATIVE STAPHYLOCOCCUS (CONS) SPECIES ISOLATED

Organism isolated	P (10U)	SXT (25mg)	VA (30mg)	TN (10mg)	CN (10mg)	E (15mg)	AUG (30mg)
Total CoNS (13)							
Sensitive	19 (43.3%)	26 (56.6%)	29 (43.4%)	17 (43.4%)	17 (36.9%)	16 (34.8%)	05 (10.9%)
Resistant	27 (58.6%)	20 (43.4%)	17 (36.9%)	26 (56.6%)	29 (63%)	30 (65.2%)	41 (89.1%)

Key:P= PenicillinSXT= Sulphamethoxazole/trimethoprim VA=Vancomycin

TN=Tobramycin CN=GentamicinE=ErythromycinAUG=Augmentin

onto the inoculated surface within 15 minutes, after over night incubation the diameter of the zones produced by antimicrobial inhibition of bacterial growth were measured and the size of zone was inversely proportional to minimum inhibitory concentration of the organism and the isolates were interpreted as either susceptible or intermediate or resistant to particular drug according to the criteria.

DISCUSSION

Hospital provides reservoirs of micro-organisms, the organism multiply and show resistance to antimicrobials. Although hospital staff and patients and attendants who are attending the patients are considered the most important source of nosocomial infection there is growing evidence from the study that the colonized hospital environment is also of substantial importance. This study was aimed to provide the first insight into the isolation of coagulase negative Staphylococcus from wounds, skin around the wound, and was also ascertained the sensitivity pattern from surgical wards 2 and 3 of Jinnah Postgraduate Medical Centre, Karachi. As CoNS often found as contaminants in clinical specimens e.g. swab from skin, nose, throat, wounds, burns and bed sore, generally their presence is not clinically significant as they are normal flora of skin by habitants, but they some times act as opportunistic pathogen and cause infection in the urinary tract or in debilitated or immunocompromised subjects, more serious bacteriamic infection. All the non-aureus Staphylococci may be termed as albus Staphylococci. CoNS do not form enterotoxin. In some circumstances, however, CoNS may have pathogenic role, they may cause peritonitis in patients on peritoneal dialysis, chronic septicemia or endocardittis in patients having heart surgery or fitted with an artificial heart valve, meningeal or bacteremic infection, patients fitted with a ventriculovenous cerebrospinal fluid shunt and septicemia in immunosuppressed and immunodeffective patients. As far a frequency of nosocomial transmission of CoNS are concerned, transmission of bacterial species to patients may occur via close contact with hospital environment, staff and unhygienic condition of Lenin, is likely a great importance in hospital setting. The study was aimed to isolate the CoNS and their sensitivity pattern from wound as well as to emphasize on normal commensal area such as skin around the wound, staff attending the patient and Lenin which are very important source of CoNS, which spread within the hospital environment. This study was aimed to provide the first insight into distribution of CoNS from infected wound, skin around the wound, to identify the common strains/species of CoNS and antimicrobial susceptibility testing of CoNS isolated from surgical wounds. Different strains were found resistant to a variety of drugs, multi drug resistant were also found, which may cause the difficulty in therapy. Different isolates of CoNS from ward 2 and 3 of JPMC shows a greater frequency and wider spectrum of resistance to different antibiotics was observed.

It has been learent from the study that the CoNS are the most common nosocomial organisms found in surgical wards of JPMC, Karachi, Pakistan. Hence different type of CoNS species were isolated, *S.epidennidis* were the predominant among them, which coincide the study conducted by Manikandan et al.[9]; Fereira et al.[8] and CDC. In their study the Staphylococcus epidermidis were the predominant CoNS isolates. In my study the number of CoNS with emergence of different species are the coagulase positive staphylococcus, Similar findings were reported by the Dobbins et al.[10] who declared that coagulase negative staphylococcus remain the most frequent pathogens, suggesting the prevalence and emergence of CoNS of different species in the surgical wards. These CoNS may emerge as pathogenic although all the isolated organisms are human inhabitant excluding *S.hiycus* which is animal inhabitant, out of 4 *S.hiycus* isolates, found 75% from wound and 25% from the skin around the wound, is the alarming situation that the animal inhabitants are found from the surgical wards. While the antimicrobial sensitivity pattern of various CoNS isolates was different as stated by the *CDC*[5] says "that resistance pattern of CoNS may differ between hospitals and wards" while in my study, the Isolates regarding antibiotic sensitivity pattern shows that no antibiotic found 100% sensitive to any organism isolated during the study even on methicillin resistant staphylococcus that is vancomycin. The same was supported by the study conducted by Mohanty et al.[11] who also says that the data collected in his study suggest that multi drug resistance is a common problem in hospital pathogens, which suggest that the improvement in the techniques of antibiotic prophylaxis have reduced the incidence of postoperative bacterial infection in the last decades, but inappropriate usage may be harmful when given unnecessarily or for a prolonged period, and preoperative antibiotic prophylaxis may lead to the development of antibiotic resistant strains and thus causes an increase in infection. Knowledge of the most frequent causative organisms in various categories will help direct appropriate initial therapy. Hence combination therapy is recommended as the initial empiric treatment.

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