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Comparative Study on Antibiograms of Coagulase Positive and Negative *Staphylococci* Isolated from Various Samples

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Coagulase positive *Staphylococci* (COPS) were serious pathogens for many decades but coagulase negative *Staphylococci* (CONS) were thought to be laboratory contaminants or commensals and were not considered important in pathological studies. Currently CONS have also emerged as potential pathogens causing various diseases ranging from mild infections like eye and wound infections to fatal diseases like UTI, endocarditis, polymer associated infections etc. Multi-drug resistant strains of both coagulase positive and negative *Staphylococci* have been developed. Comparative study of their antibiograms hence has become indispensable. All the multidrug resistant strains are found to be sensitive for linezolids and chloramphenicol.

Key words: Multi drug resistant *Staphylococci*, Coagulase, Antibiogram.

Resistance to antibiotics is becoming a difficult problem in managing the infections caused by *Staphylococcus sp*, particularly multidrug resistant species. Methicillin-resistant (MRSA) and vancomycin-intermediate resistant (VISA) strains have emerged, which are also frequently resistant to multiple classes of antibiotics. There is therefore a need to discover and develop new approaches for combating *Staphylococci*. *Staphylococci* are gram positive cocci in clusters and are the most common cause of localized suppurative lesions and persist in the environment of predominant members of the normal skin flora of human beings. One among the important virulence factors is coagulase enzyme that classifies *Staphylococci* into two broad groups namely coagulase positive *Staphylococci* (COPS) and coagulase negative *Staphylococci (*CONS). Coagulase enzyme along with coagulase reacting factor (CRF) which is present in plasma produces staphylothrombin as a stoichiometric, non-covalent complex with prothrombin, that converts fibrinogen into fibrin. The important pathogenic *Staphylococci* include *Staphylococcus aureus, Staphylococcus epidermidis, S. saprophyticus, S. lugdunensis, S. hominis* and *S. haemolyticus,* which cause suppurative lesions in humans. They can also spread through the blood and cause meningitis, endocarditis, bacteremia, septicemia, pyemia, exfoliative disease. In -dwelling foreign bodies like intravascular line, vascular graft materials, hemodialysis shunts, prosthetic valve, pacemaker implants and artificial joints are the usual sources of polymer associated infections caused by CONS.

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Staphylococci are very important pathogens. They produce an array of extracellular and cell-bound proteins which are potentially important in pathogenesis. Coagulase is one such protein which has the ability to stimulate the clotting reaction of plasma. As far as virulence factors are concerned, production of slime plays an important role in pathogenesis. CONS are characterized by their ability to adhere to and grow on solid surfaces and subsequently produce polysaccharide slime, which is antiphyagocytic in nature. The extra cellular slime substance (ESS) may protect the bacteria against immunological host defense mechanism and antimicrobial therapy. The attachment of CONS to the polymer surface may be mediated via hydrophobic interaction and electrostatic bonding [1]. Depending on the kind of device and its insertion site, different infection syndromes generate a variety of clinical presentations [2, 3]. Fifty-seven percent of the study isolates were clinically significant, predominantly causing community-acquired soft tissue infection. There were species-related differences in virulence, with 91% of *Staphylococcus lugdunensis* isolates clinically significant compared with 11% of *S. haemolyticus* isolates[4]. *S. epdiermidis* is predominant in head and trunk whereas *S. hominis* in predominant in arms and legs. *S. epidermidis* is the most common pathogen isolated from wound infections. In one study, among 192 strains of CONS isolated from drain tips / catheter tips, intravenular cannuals, blood, skin aural swabs etc, 82.3% (158/192) were *S. epidermidis*, especially 4% (32/34) of isolates were from pus[5].

About 205 isolates of CONS from wounds and body fluids were studied (blood, urine, pleural and peritoneal fluids, etc.). *S. epi*dermidis accounted for 81%. The frequencies of other species were: *S. haemolyticus* (6%), *S .hominis* (5%), *S. capitus* (4%), *S. warneri* (3%), and others (1%). The most common source of the clinically relevant CONS isolates was from wounds [3].Urinary tract infections by CONS usually affect women than men. *S. saprophyticus* accounts for up to 42% of all UTIs in women of 16-35 yrs of age. CONS causes polymer associated infections. *S. epidermidis* is responsible for 50%-70% of catheter related infections. Polymer associated infections is mainly due to biofilm formation by CONS. In a study of 89 Staphylococcal isolates recovered from patients with bacterial endocarditis,[6] four isolates from patients with native valve endocarditis were *S. ludgunensis*. About 40%-50% of cases of prosthetic valve endocarditis are due to CONS particularly *S. epidermidis*[3] . In humans about 20% of all bacterial infections are caused by *Staphylococci.* As the Staphylococcal sensitivity patterns have changed, the aim of this study is to investigate the current susceptibility of the *Staphylococcal isolates from various samples.* In an investigation against resistant gram positive bacterial infections, linezolid was effective against many parameters. When fifteen patients who had renal failure, recent liver transplantation or surgery, cancer, endocarditis or human immunodeficiency virus infection along with infections due to two methicillin – resistant *Staphylococcus sp.* which showed adverse reactions to vancomycin, were treated with linezolids (600 mg every 12 h for 5-42 days). They showed improving results. It appears that administration of linezolid, in conjunction with surgical intervention or device removal, is an effective treatment for resistant gram – positive bacterial infections[7]. During the examination about the activity of, a novel cephalosporin, against methicillin - resistant S*taphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates, was found to be highly sensitive. Therefore, cephalosporin may provide an alternative treatment for multidrug resistant *Staphylococci*. The sensitivity profile exhibited was similar to those of linezolids[8].

Oxazolidinones have become reliable clinical antimicrobial agents to be utilized for infections caused by mutidrug resistant gram positive cocci, especially vancomycin-resistant *Enterococci* and methicillin-resistant *Staphylococci.* Linezolid sensitivity (MIC > / = 8 microg / ml) was confirmed by alternative susceptibility testing methods (Etest, AB BIO Disk, Solna, Sweden; disk diffusion method) and target mutation characterization by PCR and sequence analysis[9]. The leader 2004 surveillance initiative was undertaken to obtain current and representative data on the activity of linezolid against key species, including isolates during 2004 and included 2,872 *S. aureus*, 496 coagulase-negative *Staphylococcus* (CONS), 428

*Enterococcus faecalis,*196 *Enterococcus faecium,* and 422 *Streptococcus pneumoniae* isolates. All *S. aureus* isolates (54.2% oxacillin resistant) were susceptible to linezolid[10]. Glycopeptides such as vancomycins are the treatment of choice for infections due to methicillin-resistant *Staphylococcus aureus.* This study describes the identification of high-level vancomycin-resistant *S. aureus (VRSA)* isolates in a polymicrobial biofilm within an indwelling nephrostomy tube in a patient in New York. VRSA isolates were also resistant to aminoglycosides, fluoroquinolones, macrolides, penicillin, and tetracycline but remained susceptible to chloramphenicol, linezolid, rifampin, and trimethoprim - sufamethoxazole[11]. The development of intensive care units, paralleled by the increasing use of prophylactic antibiotics in extensive surgery, has led to situation in which patients whose own flora is depleted, may acquire hospital strains of *S. epidermidis* from staff, other patients and possibly from the environment[12]. In a study of performing antibiotic susceptibility testing for CONS against various antibiotics[13], it was known that from 96 CONS strains, 48 types of multiple drug resistance pattern was observed. In a study[14], among 192 strains of CONS, 90% were resistant to penicillin and 50% were resistant to cephalexin and ciprofloxacin. All isolates were sensitive to vancomycin.

MATERIALS AND METHODS

About 120 samples were collected. 41 wound swabs from accident / trauma cases, postoperative wound infections, 31 midstream urine samples from patients with various clinical symptoms of urinary tract infections, 18 blood samples, 10 corneal scrapings were collected from both sex including children and 12 umbilical swabs from the neonates with umbilical sepsis. Totally, 112 samples showed positive for *Staphylococci.* Identification of coagulase positive and negative *Staphylococci* were done by performing coagulase test. Species identification was done by various biochemical tests (VP test, urease, nitrate reduction and carbohydrate fermentation (glucose, lactose, sucrose, mannitol and maltose)test) Antibiotic resistance and sensitive patterns of both COPS and CONS were studied by antibiotic sensitivity test(Kirby Bauer method). Antibiotics used were amoxycillin, ampicillin ceftazidime caphalexin, caphazolin, cephotaxime, chloramphenicol, ciprofloxacillin, clindamycin, cloxacillin, cotrimaxazole, erythromycin, linezolid, methicillin, novobiocin, penicillin, tetracycline and vancomycin.

RESULTS AND DISCUSSION

Among 120 samples, 112 samples were positive for *Staphylococci* and among 112, 74 were coagulase positive *Staphylococci* and 38 were coagulase negative *Staphylococci*. From 41 wound samples, 33 were COPS and 8 were CONS.Within 31 urine isolates,18 were CONS and 13 were COPS. Among 18 blood samples, 14 were COPS and 4 were CONS.10 COPS and 2 CONS were isolated from umbilical isolates. 4 COPS and 6 CONS were from corneal scrapings. Species level identification was done by performing biochemical tests(VP test, urease test, nitrate reduction test, carbohydrate fermentation tests (glucose, lactose, sucrose, mannitol, maltose). Among wound isolates 6 strains were *S. epidermidis*, 2 were *S. hominis* and 33 were *S. aureus*. In urine, 9 were *S. saprophyticus*, 6 were *S. epidermidis*, 3 were *S. lugdensis* and 13 were *S. aureus*. From blood samples, 2 were *S. epidermidis*, 2 were *S. hominis* and 14 were *S. aureus*. In umbilical isolates, 1 was *S. epidermidis*, 1 was *S. hominis* and 10 were *S. aureus*. 6 strains of *S. epidermidis* and 4 strains of *S. aureus* were identified from corneal scrapings.

Among CONS, *S. epidermidis* was found to be a frequent strain (56%). *S. saprophyticus* was frequent among UTI strains (50%) followed by *S. epidermidis* (33%). Though the incidence rate of COPS is comparatively higher, in cases of urine and corneal samples, the incidence rate of CONS is higher than COPS. The reason might be due to the usage of catheters or other synthetic polymers and artificial lenses respectively as CONS are potential pathogens in causing polymer associated infections. Antibiograms of both COPS and CONS were performed with 18 antibiotics. Antibiogram of COPS exhibited the following: *S. aureus* strains from wound were sensitive to linezolid, chloramphenicol, vancomycin, novobiocin, tetracycline, erythromycin, and cotrimoxazole. Urine isolates were sensitive to linezolid, vancomycin, and novobiocin. *S. aureus*

strains from blood were quite strong multi-drug resistant strains and were sensitive only to linezolid and chloramphenicol. Umbilical isolates were found to be sensitive to linezolid, chloramphenicol and novobiocin. Corneal strains were sensitive to linezolid, chloramphenicol, vancomycin, novobiocin, tetracycline and erythromycin. Antibiogram of CONS exhibits the following results. CONS strains isolated from wound, blood and corneal samples, irrespective of their species differences, were found to be sensitive to linezolid, chloramphenicol, vancomycin, and novobiocin. CONS from urine, showed the same pattern except for their resistance against novobiocin, but they were sensitive to ciprofloxacin. Umbilical isolates showed sensitive pattern to linezolid, chloramphenicol, and novobiocin. All the strains (both CONS and COPS) showed maximum resistance to penicillin, methicillin, cloxacillin, ceftazidime, cephotaxime and ampicillin. All the strains (both CONS and COPS) were highly sensitive to linezolid and chloramphenicol. The prevalence of multidrug resistant strains of both coagulase positive and negative *Staphylococci* and their increased frequency of incidence has posed a major threat in treating patients especially with poor immunity. Hence, periodic study of their antibiotic sensitivity pattern is very indispensable. From this study, it is determined that Linezolid could be recommended as an effective drug followed by chloramphenicol, against infections caused by both coagulase positive and negative *Staphylococci*.

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