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RESISTANCE PATTERN OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* AGAINST FIVE GROUPS OF ANTIBIOTICS

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Abstract: Among the samples received in pathology laboratory, Pakistan Institute of Medical Sciences, Islamabad, 5069 samples had bacterial growth, among these 2580 (51%) samples were Gram-positive cocci and 1688 were *Staphylococcus aureus* during a period of two years. Out of these Gram-positive cocci 56% were resistant to penicillin group, 27% were resistant to cephalosporin group, 22% were resistant to aminoglycoside group, 15% were resistant to quinolone group and 31% were resistant to other antibiotics (cotrimaxazole, erythromycin, aztreonam, vancomycin, nitrofurantoin and meropenam). Antibiograms of Gram-positive cocci were determined against various antibiotics by disc diffusion method. The rate of resistance to most of the antibiotics such as ampicillin, piperacillin, carbenicillin, penicillin, cephradine, cefotaxime, oxacillin, ceftriazone, amikacin, ceclor, ofloxacin, pefloxacin, ciprofloxacin, cotrimexazole (septran), gentamicin, meropenem, ceftazidime, erythromycin, tobramycin, enoxacin was higher when tested against the isolates collected from pus as compared to those from blood and urine. Antibiotic resistant strains were more prevalent in pus samples than the other clinical isolates (blood and urine). The randomly selected 155 strains of *Staphylococcus aureus* when tested against five groups of antibiotics showed resistance rate against ampicillin (92%), cephradine (60%), and gentamicin (58%). However intermediate resistance was found in case of vancomicin (38%), in hospitalized and non-hospitalized patients.

Keywords: Gram-positive cocci, prevalence, antibiotics, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is one of the most versatile nosocomial (i.e. acquired in hospital) and dangerous human pathogen since publication of its role in sepsis by Ogston in 1880 and 1882 [Lowy 1998]. In spite of the introduction of antimicrobial agents and improvements in the frequency and morbidity of staphylococcal diseases in the twentieth century, staphylococci have persisted as an important hospital and community pathogen. They are responsible for more than 80 percent of the supportive diseases encountered in medical practice and are second only to *E. coli* as a cause of hospital acquired infections. The widespread use of penicillin in 1950's saw the spread of penicillin resistant *S. aureus* in hospitals. After this time methicillin and its derivatives became the drugs of choice for the treatment of infections caused by this organism. In the meantime methicillin-resistant staphylococci were reported from USA and Italy. Even before methicillin was widely used, as a strain of *S. aureus* with natural resistance to this antibiotic was identified by Jevons in 1961. Thereafter, methicillin-resistant *S. aureus* emerged as a major pathogen

worldwide. Several outbreaks were documented in many parts of the world caused by MRSA. These `epidemic` strains were labelled `E` MRSA [Kelkar 2002].

Vancomycin has long been considered the antibiotic of last resort against serious and multi-drug-resistant infections caused by Gram-positive bacteria. However, vancomycin resistance has emerged, first in enterococci and, more recently, in *S. aureus* [Boneca and Chiosis 2003]. Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. Few strains are resistant to all clinically useful antibiotics except vancomycin. Some workers have reported however the presence of vancomycin resistant strains [Shakibaie *et al.* 2002].

The present study was designed to investigate antibiotic resistance pattern against Gram-positive cocci and *Staphylococcus aureus* isolated from various clinical samples.

MATERIALS AND METHODS

Staphylococcus aureus isolates employed in this study was conducted at Pakistan Institute of Medical Sciences, Islamabad, Pakistan during a period of two years. The sensitivity pattern of Gram-positive cocci isolated from the samples brought to Pathology Laboratory was determined against commonly used antibiotics using disc diffusion method, at the hospital's laboratory. Samples comprised of blood, pus and urine, from out door patients (OPD) as well as indoor patients from different wards of the hospital. Out of 5069 growth positive samples, 2580 were Gram-positive cocci, out of these 155 were identified as *S. aureus* for further study against five groups of antibiotics.

SAMPLE PROCESSING

Blood samples were collected from patients visiting OPD, different wards and were brought to the pathology laboratory. Blood samples were processed in Brain Heart Infusion (BHI) broth (CM225-OXOID), and growth positive, were sub cultured on Blood Agar (CM55 and SR50-OXOID) and MacConkey Agar (CM7-OXOID) plates, and incubated for 24 hours at 37°C. In case of pus samples were directly inoculated on Blood Agar (CM55 and SR50-OXOID) and MacConkey Agar (CM7-OXOID) and incubated for 24 to 48 hours at 37°C. Urine samples were cultured on Cystine-Lactose-Electrolyte Deficient (CLED) medium (CM301-OXOID). These plates were incubated for 24 to 48 hours at 37°C. Then the bacterial growth was sub cultured on Blood Agar and MacConkey Agar.

IDENTIFICATION, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL STRAINS

Isolated colonies, after purification, were initially Gram stained. By using Bergey's Manual of Determinative Bacteriology (9^{th} edition), the isolates were biochemically characterized and identified up to species level.

IDENTIFICATION OF ISOLATES

Bacterial isolates were inoculated on sheep's blood agar and moist opaque shinny pale yellow to golden orange color were selected for various biochemical tests. Identification tests performed include Gram-staining, β-haemolysis, catalase test and DNase test to identify the pathogenicity of *S. aureus* [Collin *et al*. 1995].

DISC DIFFUSION METHOD

The disc diffusion test was done for each isolates and the Mueller Hinton Agar was used as growth media with 4% sodium chloride. The Mueller Hinton Broth was prepared and 5ml of broth medium was dispensed. The 155 already identified clinical isolates were inoculated in sterilized 5 ml Broth test tubes and placed in incubator overnight [DeLencastre *et al*. 1994]. The turbidity of broth cultures was adjusted according to 0.5 McFarland standards. The ten microlitre inoculum were poured onto the Mueller Hinton agar plates (MH:Difco) with 4% NaCl [NCCLS 1993] and spread with glass spreader. The plates were allowed to dry before applying the discs. Then the discs of given potency were placed on the inoculated plates [NCCLS 1997]. Then plates were placed in incubator at 35^{o}C for 16 to 18 hours. After 16 to 18 hours of incubation, the plates were examined and the zones of inhibition were measured.

RESULTS

This study was conducted during a period of two years on clinical isolates from indoor and outdoor patients in order to determine the prevalence of clinically significant Gram-positive cocci at the Pakistan Institute of Medical Sciences (PIMS) Islamabad, Pakistan. A total of 5069 growth positive samples, received in the pathology laboratory of PIMS for identification and sensitivity tests, which comprised of pus, urine and blood.

RESISTANCE PATTERN AGAINST ANTIBIOTICS

With regard to the prevalence of Gram-positive cocci from hospitalized and non-hospitalized patients, only 2580 yielded growth Gram-positive cocci, with 1688 being *Staphylococcus aureus.* Therapeutic use of these five groups of antibiotics, namely cephalosporins, aminoglycosides quinolones, penicillin and other antibiotics. The overall percentage towards different groups in the order of resistance being antibiotics resistance, penicillins (56%), other antibiotics (31%), cephalosporins (27%), aminoglycosides (22%) and quinolones (17%), and the group of the members of other antibiotics include cotrimaxazole, erythromycin, aztreonam, vancomycin, nitrofurantoin and meropenam (Fig. 1).

SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS*

The susceptibility of 155 strains of *Staphylococcus aureus* were assessed against various antimicrobial agents by disc diffusion method. Vancomicin

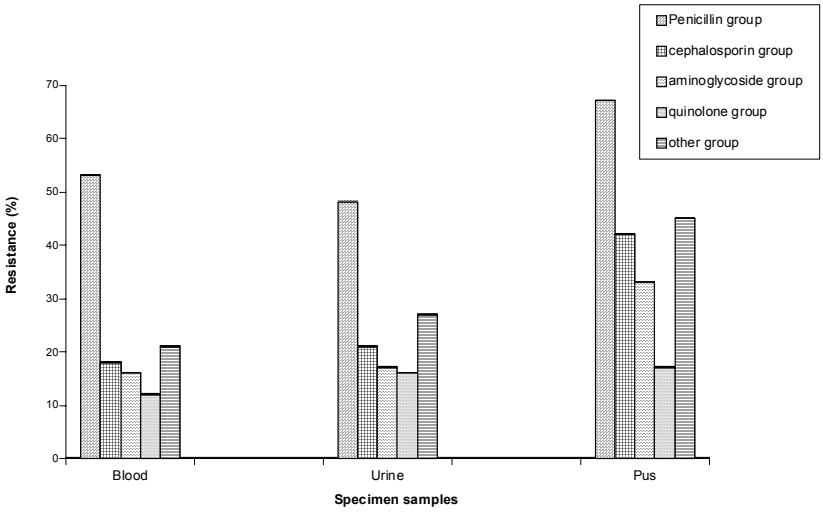

Fig.1 Percentage resistance of Gram Positive Cocci against various antibiotics applied during two years

was found to be most effective drug against these clinical isolates. There was no resistant isolate against vancomicin and only 59 isolates had intermediate sensitivity. Gentamicin was the second most effective drug against these isolates, with 41.94% sensitive strains. Ciprofloxacin and cephradine were found to be effective anti-microbial agents against *S.*

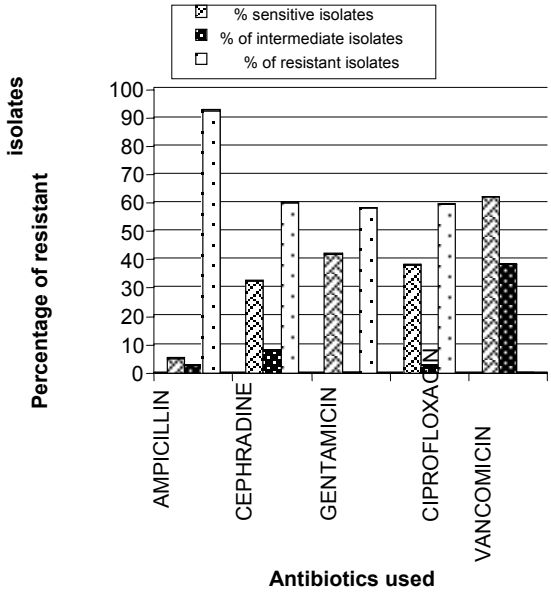

Fig. 2. Resistance pattern of *S. aureus* against five groups of antibiotics.

aureus exhibiting 38.06% and 32.27% sensitivity respectively, whereas ampicillin demonstrated only 5.16% sensitivity. The order of resistance of *Staphylococcus aureus* against five groups of antibiotics was ampicillin (92.26%), cephradine (60%), ciprofloxacin (59.35%), gentamicin (58.06%) and vancomicin (38.06%) an intermediate resistance. Oxacillin resistance for these isolates was 61.29%. Most of the oxacillin resistant strains were intermediate resistance towards vancomycin (Fig. 2).

DISCUSSION

Staphylococcus aureus is recognized as an important bacterial pathogen contributing towards hospital infection, globally. *Staphylococcus aureus* causes localized infection spreading into the blood stream [Espersen 1995]. Despite the use of potent antibiotic still high mortality exist in case of *Staphylococcus aureus* infection.

In the present study, *in-vitro* culture sensitivity pattern was assessed for Gram-positive cocci from blood, urine and pus and a high resistance was recorded against ampicillin and penicillin followed by cotri-mexazole, cephradine, gentamicin, amikacin, and oxacillin. Siddiqui *et al*. [2002] reported a similar antibiotic sensitivity pattern of (*Staphylococcus aureus),* which were isolated from pus wound and ear pus, against various antibiotics as amikacin, gentamicin and methicillin. They also reported more than 20% isolates from blood were resistant to penicillin, ampicillin, cephradine, cefataxime, ceftriazone, gentamicin, aztreonam, erythrocin, cotri-mexazole, enoxacin and more than 50% resistance was observed in case of penicillin, ampicillin, cotri-mexazole and erythrocin. Mahmood and co-workers [2001] reported 29% resistant isolates of *Staphylococcus aureus,* against cefalexin, and second more than 20% against generation cephalosporins.

In case of cephradine, ofloxacin and cotri-mexazole there was a non-significant ($p>0.05$) increase of resistance of isolates from blood. More than 30% urine isolates were resistant against ampicillin, penicillin, cephradine and cotri-mexazole and more than 30% pus samples were resistant to ampicillin, carbenecillin, penicillin, cephradine, cefotaxime, ceftriazone, ofloxacin, peflacin, cotri-mexazole, gentamycin, meropenem, erythrocin, oxacillin and enoxacin. These results are in accordance with the work of Shi *et al.* [1998] who also found high resistance against various antibiotics in clinical isolates from various specimens. The present findings are further substantiated by the work of Cheong *et al.* [1995] who isolated 18.4% *Staphylococcus aureus* from wound, pus and swabs which were also resistant to methicillin (91%), penicillin (23%), tetracyclines (23%) and erythromycin (13%).

Based on results of culture sensitivity, ampicillin was found to be the most effective (92%), when compared to the other four groups of antibiotics. Vancomicin established 38% intermediate resistance. Fish *et al*. [1995] reported comparable finding with regard to the present study, for eight

classes of antibiotics. The lower rates of resistance with imipenem (cilastalin), aztreonam and in combination therapy but high rates of resistance with penicillin, aminoglycoside, monotherapy and fluoro-quinolones was observed in the work of Fish *et al*. [1995]. Antibiotic sensitivity pattern in disc diffusion test with the different Gram-positive cocci isolated during 1998 and 1999 as well as previous antimicrobial therapy prescribed for the IDP and OPD patients, was compared to evaluate the trend of usage of different antibiotic groups such as penicillin, cephalosporins, quinolones, amimoglycosides and other antibiotics representing miscellaneous group.

Mirrett *et al*. [2001] found coagulase negative Staphylococci, in blood cultures, which were the frequent cause of true infection. Gentamicin has been used since early 1970s. Lemaitre *et al*. [1998] reported an unusual heterogeneous resistance in USA to methicillin and tobramycin but susceptibility to gentamycin isolates from pus. The present study reports highest percentage of *Staphylococcus aureus* from Pitie-Salperilriere hospital in France more than 45%. Results of the present study are in line with the work of Lemaitre *et al*. [1998].

Although cephalosporin was largely prescribed and used in antimicrobial therapy but penicillin was found to be the most effective against all isolates when tested in antibiotic sensitivity test. Namias *et al.* [1998] also reported *Staphylococcus aureus* isolates from ICU, with similar antibiotic resistance pattern, which was 30% to gentamicin 52% cefoxitin (2^{nd} generation) 26% ceftazidime. Fridkin *et al*. [1999] found 24% vancomycin resistant strains among 108 strains from ICUs of Jackson Memorial hospitals in USA.

Moreover, when low doses of antibiotics are used against bacteria, they inhibit the growth of susceptible bacteria, leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells then eventually affecting other cells [Craig 1998].

The study documents the importance of *Staphylococcus aureus* as important Gram-positive pathogen and increasing resistance in commonly used antibiotics. Although the high cost and inappropriate use of antibiotics have been documented and the long courses of prophylactic antibiotic may lead to increased resistance to antimicrobials, increased incidence of drug reactions, and increased dollar costs [Namias *et al.* 1999].

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