Staphylococcal resistance against five groups of life saving antibiotics in the year 2003-2005

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Abstract: In the year 2003 to 2005 a prospective study was conducted to find out the predominance of *Staphylococcus (Staphylococcus aureus*) resistance pattern in opposition to five life saving antibiotics as these are the sole agents to treat critically ill patients in hospitals. During the period of two years almost 2500 samples of bacterial culture were taken from different pathological laboratories and hospitals in Karachi. Among these 1500 were Gram positive cocci and 1000 samples were identified as *Staphylococcus aureus*. Life saving antibiotics were taken from five different groups and by mean of disk diffusion technique antibiogram of *Staphylococcus aureus* against these antibiotic were determined. During the course of study imipenem showed 11%, amikacin exhibited 58%, cefipime showed 31%, vancomycin and piperacillin/tazobactam displayed 24% resistance against *Staphylococcus aureus*. Imipenem was found to be most effective against *Staphylococcus aureus*.Resistance to other antibiotics developed quickly in *Staphylococcus aureus* collected from clinical areas where these antimicrobial agents are extensively used.

Keywords: Antibiotic, resistance, *Staphylococcus aureus.*

INTRODUCTION

There are various risk factors which are responsible for causing nosocomial and infections of general public or community. Mainly they are originated from those organisms having antibiotic resistant properties. Most important factors include previous treatment with broad spectrum antibiotics, judicious and irrational use of antibiotics resulted in the development of resistance among organisms. They suppressed normal flora and resulted in developing resistance in microorganism against antibiotics used (Liu, H: 2001). After publication of *Staphylococcus aureus* role in sepsis between the year 1880 to 1882,it become considered as most adaptable nosocomial and hazardous human pathogen (Lowy F.D 1998).*Staphylococci* was considered as a significant pathogenic organism in the 20th century both in hospitals and in general public. The major epidemiologic, microbiologic and clinical characteristics of vancomycin resistance has been reviewed in both coagulase-negative *staphylococ*ci and *Staphylococcus aureus* (Arjun Srinivasan,*et al.,* 2002). It has been found that nearly all epidemiological surveys focused on amikacin resistance in Gram-negative bacteria (Betts *et al.,* 1984, Gerding *et al.,* 1985 Moody *et al.,* 1982, Price *et al.,* 1981, Wielunsky *et al.,* 1983). The addition of tazobactam with piperacillin increase the net bacterial killing which was not found with piperacillin alone against *Staphylococcus* (Strayer *et al.,* 1994). A variety of antibiotics including vancomycin become ineffective against various hospital strains of *Staphylococcus aureus*. There are various strains of *Staphylococcus aureus* which are resistant to different antibiotic extensively used in hospitals except vancomycin but some reports showed presence of resistance in various *Staphylococcal* strains against vancomycin (Shakibaie *et al.,* 2002). This prospective study design was undertaken to obtain the prevalence of resistance of *Staphylococcus aureus* to imipenem, amikacin, cefipime, vancomycin and piperacillin/ tazobactam during the year 2003-2005.

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MATERIALS AND METHODS

Predominance studies

In the two years study period different hospitals of Karachi were surveyed where these antibiotics were widely used. During the course of study 2500 clinical samples were collected and among them 1500 samples were catalase positive Gram-positive cocci and 1000 were coagulase positive *Staphylococcus aureus*. Samples collected from urine, pus and blood from outdoor as well as indoor patients (INP) from different hospitals. One hundred and thirty samples from these were selected randomly for further assessment of resistance in opposition to five groups of antimicrobial agents (table 1). All media used during study were supplied by Oxoid UK, Inc.

Microbiology/sample procurement

The culture swabs were collected from different patients at weekly intervals and were safely transported to the microbiology laboratory. The samples from blood were immediately processed in Brain Heart Infusion (BHI)

broth. Then Gram-positive organisms were sub-cultured on Macconkey agar and blood agar plates. These plates were incubated at 37˚C for 24 hours. Blood agar and MacConkey agar were used for direct inoculation of pus samples and incubated at 37˚C for 24-48 hrs while Cystine-Lactose-Electrolyte Deficient medium was used for culturing urine samples and were incubated for 24-48 hrs at 37˚C, then the observed microbial growth was subcultured on blood agar and MacConkey agar. After a defined period of incubation, the colonies characteristic of Staphylococci were Gram-stained and then tested for catalase production. Those colonies which were large and well isolated consisted of catalase-positive Gram-positive cocci, were further evaluated for coagulase production by mean of commercial tube coagulase test. After verification coagulase-positive isolates were considered to be *Staphylococcus aureus* whereas coagulase-negative isolates were not further evaluated and were referred as coagulase-negative Staphylococci.

Disk diffusion method

Antimicrobial agent susceptibilities pattern was observed using agar disk diffusion technique by Kirby Bauer. The method used was described in the Clinical Laboratory Standards Institute (CLSI) Performance Standards M7-A using Mueller-Hinton agar and broth. The medium restraining antimicrobial agents were quality controlled daily with *Staphylococcus aureus* ATCC 29213. After specified incubation period the plates were scrutinized to measure the zone of inhibition and then compared with CLSI susceptibility of *Staphylococcus aureus.*

RESULTS

In the two year study period about 2500 samples were collected from inpatients and out patients then after identification by means of Gram staining, β-haemolysis, catalase test and coagulase production the receptiveness of 130 strains of *Staphylococcus aureus* were evaluated against five life saving antibiotics as shown in table 1 by disk diffusion method.

Table 1: Life saving antibiotics, their names, classify-cations and disk contents

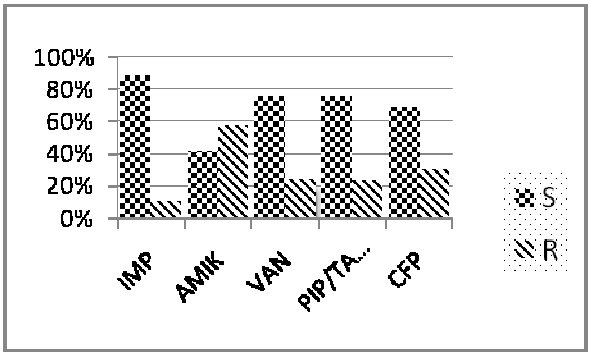
Generic Name	Classification	Disk Content (µg)
Imipenem/Cilastatin	carbapenem	10
Amikacin	aminoglycoside	30
Vancomycin	glycopeptide	30
Piperacillin/ Tazobactam	broad-spectrum penicillin	100/10
Cefipime	Cephalosporin (4^{th} gen)	30

During the course of study the percentage of patients colonized with antibiotic resistant *Staphylococcus aureus* was shown (table 2). Imipenem was found to be most effective antibiotic against *Staphylococcus aureus* showed 89% sensitivity. Vancomycin and Piperacillin/Tazobactam were equally effective (76% sensitivity).In the year 2003 to 2005 amikacin resistant *Staphylococcus aureus* was higher as compared to others and high prevalence of colonization (58%) occured. However 31% of *Staphylococcus aurous* were resistant to Cefipime.

Table 2: Percentage of antibiotic resistant colonized patients and isolates

Antibiotics	No. of isolates(% pt, colonized resistant to *S. aureus*)
Imipenem/cilastatin	14(11)
Amikacin	75(58)
Vancomycin	31(24)
Piperacillin/Tazobactam	31(24)
Cefipime	40(31)

Therefore imipenem was considered as most effective (89% sensitivity), Vancomycin and Piperacillin/ Tazobactam were second most effective (76% sensitivity), cefipime exhibited 69% sensitivity and amikacin demonstrated 42% sensitivity against *Staphylococcus aureus* (Graph 1).



Graph 1: *Staphylococcus aureus* susceptibility pattern against five antibiotics of different groups.

DISCUSSION

The basic objective of the study was to evaluate the *Staphylococcus aureus* susceptibility pattern against five life saving antimicrobial agents. *Staphylococcus aureus* responsible to cause localized infection diffusing upto blood stream. In case of *Staphylococcus aureus* regardless of use of very effective antimicrobial agents through out the world a high mortality or death rate still exist globally (Espersen F.1995). Therefore the practical concern is to focus not only over high rate of resistance but also that they may be more difficult to treat and can create serious global threat.

Wide spread antibiotic resistance developing in

Staphylococcus aureus was previously reported. Parker *et al.,* reported that incidences of beta-lactamase production was consistently been found to be over 80% in all parts of world (Parker *et al.,* 1990). Bozdogan *et al.* and Houghes *et al.,* isolated *staphylococcus aureus* strains at Hershey Medical Centre which was resistant to amikacin (Bozdogan *et al.,* 2003, Houghes.*et al.,* 2004). One of the major factor that favour expansion of vancomycin resistance in *Staphylococcus aureus* was high frequency of mutation as was indicated by Franziska Schaff *et al.,* (Franziska Schaff *et al.,* 2002). The addition of tazobactam with piperacillin and its impact against *Staphylococcus aureus* was investigated by Strayer *et al.,* and showed net bacterial killing which was not seen with piperacillin alone (Strayer *et al.,* 1994).

We focused to find out an in vitro culture sensitivity pattern of Gram-positive cocci from blood, urine and pus in this study and resistance rate was observed against five life saving antibiotics. Based on our finding, it appeared that amikacin resistance developed quickly although this resistance did not develop considerably against Gram-negative bacteria with its extensive use (Betts *et al.,* 1984, Gerding *et al.,* 1985, Moody *et al.,* 1982, Wielunsky *et al.,* 1983). The development of resistance in microbes against antibiotics is an increasing public health problem. The most probable factor considered responsible for the appearance of antibiotic resistant bacteria is the selection pressure for the use of antibioticsin hospitals. Friedland *et al.,* reported that amikacin resistance is related to more extensive usage of aminoglycoside in neonatal units (Friedland *et al.,* 1992).

It is of interest that *staphylococcus aureus* did not appeared too much resistant against imipenem and as compared to other groups of antibiotics it appeared to be most effective (89% sensitivity) antimicrobial agent as was previously reported by Fish *et al.,* and a lowest rate of resistance was observed with imipenem compared with eight classes of antibiotics (Fish *et al.,* 1995).

The current core issue was about the antibiotic vancomycin which was considered as the drug of "last resort" for many infections and the rate at which vancomycin resistance has multiply through enterococci has driven the researcher to apply the word "crisis" when discussing about the prospect of vancomycin challenging *Staph* (Ricki Lewis 1995). In the current study 24% resistance for vancomycin and piperacillin/tazobactam was observed and that might be due to extensive use of the combination in our hospitals as life saving antibiotics. Cefipime also showed 31% resistance which is also much higher. There are various possibilities for increasing development of resistance and it might be due to a decline in permeability, any chemical alteration in antimicrobial agents or any amendment in the affinity of the objective position. Resistance could either be endogenously arisen by mean of mutation or exogenously by transfer of R-factor (Hamilton-Miller, 1990).

CONCLUSION

The study documented the importance of *Staphylococcus aureus* and increasing resistance against it in commonly used antibiotics leading to higher cost and increased rate of drug reactions and we believe this deserve further study with an emphasis on optimal antibiotic utilization and frequent monitoring of bacterial spectrum, towards goal of protecting our patient from resistant bacteria.

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