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

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The present research was conducted to study the susceptibility and resistance pattern of *Staphylococcus aureus* isolates against various brands of commonly used antibiotics. A total of 50 bacterial isolates were obtained from various clinical specimens submitted to the clinical laboratory of Pakistan Institute of Medical Sciences Islamabad (PIMS). Out of 50 isolates 30 (60 %) were identified as coagulase positive *Staphylococcus aureus*. These isolates were tested for susceptibility pattern 19 (63%), these isolates were resistant to penicillin, 17 (57%) to ampicillin, 19 (63%) to cloxacillin, 20 (67%) to streptomycin, 11 (37%) to clindamycin, 16 (53%) to lincomycin and 19 (63%) to neomycin. These isolates had MIC range of penicillin and lincomycin (<0.5 to >512 µg/mL), ampicillin, cloxacillin and clindamycin (0.5 to 256 µg/mL), streptomycin (4 to >512 µg/mL) and neomycin (2 to 256 µg/mL). Clindamycin was found to be the most effective among these antibiotics tested in the study against these clinical isolates.

Keywords: *Staphylococcus aureus*, PIMS (Pakistan Institute of Medical Sciences) and MIC (Minimum Inhibitory Concentration).

Staphylococcus aureus recognized as an important pathogen in human disease. *S. aureus* is a common cause of community–acquired infections, including endocarditis, osteomyelitis, septic arthritis, pneumonia, and abscess (McKenney *et al.,* 1999; Van de-Bergh and Verbrugh, 1999). The spread of antimicrobial resistance has become a public health priority. The use of antimicrobials for nosocomial infections in hospitals were in the intensive care units (ICU) areas, observed the highest rate of resistance than the outpatients, due to the increased use of antibiotics in these areas (Fridkin, *et al*., 1999). *S. aureus* is recognized as one of the most important bacterial pathogen seriously spreading the hospital infection all over the world and the experiments show that the *S. aureus*, resistant strains survive longer than susceptible strains (Wagenvoort and Penders, 1997). In view of past problems of bacterial resistance novel approaches in antimicrobial resistance are badly needed. Development of novel "classic" antimicrobial

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Table 1
Minimum inhibitory concentrations of seven antibiotics in µg/mL against 30 isolates of *Staphylococcus aureus*

Isolate No.	PN	AP	CX	CC	L	S	N
77	<0.5	0.5	<0.5	4	16	32	8
467	2	64	8	16	16	32	16
3	256	32	4	32	4	16	8
517	<0.5	16	1	16	8	16	32
168	1	64	0.5	2	4	128	32
428	128	128	16	0.5	0.5	64	32
477	0.5	32	32	1	0.5	64	128
72	128	256	128	1	1	64	128
255	512	256	256	64	128	256	128
148	256	128	128	128	>512	256	16
43	0.5	32	2	4	8	128	64
166	<0.5	32	8	2	8	64	64
186	4	128	4	2	4	32	8
233	0.5	16	8	64	128	128	256
149	256	128	32	2	4	128	128
405	4	64	2	2	8	32	8
437	8	128	8	1	4	64	128
400	<0.5	1	<0.5	0.5	2	4	2
268	0.5	16	32	128	256	128	64
401	0.5	32	8	0.5	8	128	16
398	<0.5	8	0.5	2	4	8	4
495	32	128	64	256	512	>512	128
460	1	32	16	2	16	64	32
458	128	128	32	4	1	64	32
430	0.5	4	64	1	8	128	64
568	2	64	4	1	16	32	8
524	1	64	2	0.5	2	16	4
654	128	256	64	2	2	64	32
684	256	256	128	0.5	4	128	64
677	>512	256	128	2	8	128	128

agents, chemical modifications of currently known agents to over come resistance, and the development of potentiators of known antimicrobial represent three areas that have been partially exploited in the past continue to represent fertile fields for additional investigations. A number of investigators are working to develop inhibitors of new bacterial targets and to develop inhibitor of genes relating to virulence or pathogenesis. So the development of antisence nucleotides as antimicrobial agents is theoretically appealing to date, to develop any of these agents for clinical use (Moellering, 1998). The developing resistance in neomycin and lincomycin that was most effective during the late few decades (Givental *et al*., 1983).

Pakistan like other developing countries, there is general increase in drug-resistance especially to all the commonly used antibiotics. Hence there is a need to monitor the prevailing levels of resistance, so that the effective therapy can be given. Moreover, it is due to irrational use of antibiotics in human medicines. This principle is applied in both developing and in developed countries, national situation and to hospital and community setting (Willim and Heymann, 1998).

The aim of this study was to characterize morphologically, biochemically as well as to investigate the *in-vitro* activity of penicillin, ampicillin, cloxacillin, streptomycin, clindamycin, neomycin, lincomycin, and resistance developed in *Staphylococcus aureus*

MATERIAL AND METHOD

Staphylococcus aureus isolates employed in this study were obtained from the Microbiology Laboratory, Pakistan

Institute of Medical Sciences, Islamabad. A total of 50 Gram-positive cocci were selected for identification and sensitivity pattern.

IDENTIFICATION OF ISOLATES

These bacterial isolates were collected from blood, urine and pus samples of patients suffering from various infectious diseases from hospitalized as well as OPD. The blood samples were incubated at 37°C in broth for 3-7 days in Brain Heart Infection Broth/Nutrient Broth as one mL in 150 mL of B.H.I. broth, the turbidity was checked.

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. On the next day if growth is positive, then identification tests were performed. In case of urine samples, were inoculated on Cled media till 48 hours if growth occurs on the Cled agar, there is possibility of pathogenic organisms.

50 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. Out of these 50 isolates 30 were identified *S. aureus*. Identification tests performed include: Gram-staining, β-haemolysis by hot–cold technique, coagulase, catalase test and DNase test to identify the pathogenicity of *S. aureus* (Collin *et al*., 1995).

Determination of minimum inhibitory concentrations (MIC) by agar dilution method:

Stock solutions of different antibiotic injections were prepared according to their labelled potencies. Stock solutions were dispensed in aliquots and stored immediately at -70°C.

Agar dilution method was used to determine the minimum inhibitory concentrations (MIC), or the lowest concentration of antimicrobial agent required to inhibit the microorganism. Serial two-fold dilution of penicillin, ampicillin, cloxacillin, clindamycin, lincomycin, streptomycin were made and tested against *S. aureus*.

Four to five well isolated colonies of various *S .aureus* isolates from a blood agar plate were inoculated in tube containing 5mL of tryptone soya broth (CM129-OXOID) and incubated at 35°C until it achieved or exceeded the turbidity of 0.5 McFarland standards. After incubation, if turbidity was exceeded 0.5 McFarland standard, then adjusted by using sterile saline to give the density equivalent approximately 10^8 cfu/mL. Then it was diluted accordingly to give 10^4 cfu/mL and 10µL of inoculum was transferred on Mueller-Hinton agar plates containing different concentrations of antibiotics with the help of micropipette. Mueller-Hinton agar was used as a growth media with 4% sodium chloride (Connie and Georgre, 2000).

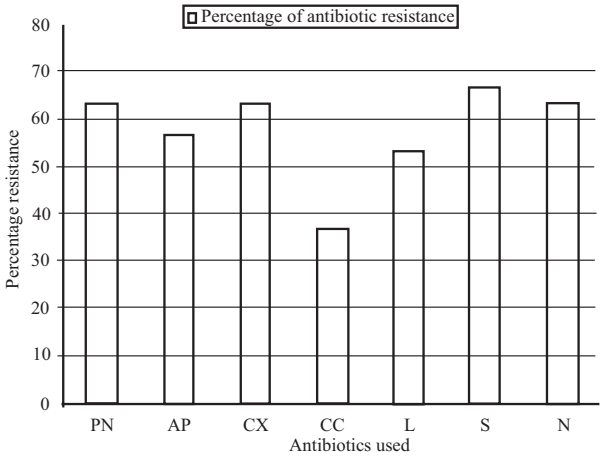

Table 2
Inhibitory range of MICs µg/mL of various antibiotics against 30 isolates of *Staphylococcus aureus*

Antibiotic	Inhibitory Ranges (µg/mL)
Penicillin(PN)	<0.5->512
Ampicillin(AP)	0.5-256
Cloxacillin(CX)	<0.5-256
Streptomycin(S)	4->512
Clindamycin(CC)	0.5-256
Lincomycin(L)	0.5->512
Neomycin(N)	2-256

Abbreviations used

S. aureus : *Staphylococcus aureus*
PN : Penicillin
AP : Ampicillin
CX : Cloxacillin
CC : Clindamycin
L : Lincomycin
S : Streptomycin
N : Neomycin
ICU : Intensive Care Unit
PIMS : Pakistan Institute of Medical Sciences
MIC : Minimum inhibitory concentration
NCCLS : National Committee for Clinical Laboratory Standards

Plates in inverted position were placed in an incubator at 35°C for 18 hours. Control strains were also run with the isolated strains of *S. aureu*s (Collin *et al*., 1995). The MIC is the lowest concentration of the agent that completely

inhibits visible growth was judged by the naked eye. The concentration, in which the petri plate showed no growth from inoculum spot, was considered as the MIC of the specific antimicrobial agent.

RESULTS

Antibiotic sensitivity pattern

Among the 50 Gram-positive cocci, 30 were *S. aureus* from various clinical samples, as determined by their sensitivity pattern; more than 50% were resistant to penicillin. The 57% of the isolates were resistant to ampicillin, 63% isolates were resistant to cloxacillin, 37% were resistant to clindamycin, 53% isolates were resistant to lincomycin, and 67% were resistant to streptomycin and 63% to neomycin. These results show more than 90% isolates were multidrug resistance (tables 1 and 2; fig. 1).

Only two isolates *S. aureus* 400 and *S. aureus* 398 were found to be sensitive to all the antibiotics used for sensitivity (penicillin, ampicillin, cloxacillin, clindamycin streptomycin, lincomycin and neomycin). Streptomycin was found to be most resistant, only 10% isolates were sensitive to this antibiotic. Whereas ampicillin and lincomycin were effective against 43% and 47% isolates respectively. Ampicillin, cloxacillin and neomycin were effective against 37% isolates. Sixty three percent isolates were sensitive to Clindamycin according to the MICs given in table 1.

Minimum inhibitory concentration of antibiotics

The susceptibility of 30 *S. aureus* isolates when tested against seven antibiotics was measured with their MIC values. The susceptibility of each bacterial isolates to these antibiotics tested varied greatly, ranging from < 0.5 to >512 µg/mL (table 2). Penicillin exhibited the broadest rang <0.5 to >512 µg/mL, followed by lincomycin 0.5 to >512 µg/mL. Highest MIC values were exhibited by streptomycin 4 to >512 µg/mL. The MIC ranges of other antibiotics occupied intermediate spectrum, i.e. neomycin 2 to 256 µg/mL, ampicillin 0.5 to 256 µg/mL and cloxacillin 0.5 to 256 µg/mL (tables 1 and 2; fig. 1).

Most of the isolates were inhibited by clindamycin, cloxacillin and neomycin at MIC 64 µg/mL, and the isolates showed inhibition by ampicillin at 256 µg/mL. Only one isolate was inhibited at MIC 4 µg/mL concentration of streptomycin. Seven isolates were inhibited at MIC $\leq$8 µg/mL concentration of neomycin. Streptomycin was the least effective antibiotics against these isolates studied. Isolate nos. *S. aureus* 77, *S. aureus* 400 were found very sensitive to penicillin and cloxacillin, they were inhibited even at MIC 0.5 µg/mL concentration of most antibiotics (sensitive to all these antibiotics used).

Isolates *S. aureus* 77, *S. aureus* 477, *S. aureus* 43, *S. aureus*, 233, *S. aureus* 400, *S. aureus* 268, *S. aureus* 401, *S. aureus* 398, *S. aureus* 430 *S. aureus* 517 and *S. aureus* 166 were inhibited by 0.5 µg/mL concentration of penicillin. *S. aureus* 148 isolate was resistant to lincomycin at MIC 512 µg/mL, while *S. aureus* 495 was also resistant to lincomycin and streptomycin at MIC 512 µg/mL (table1).

NCCLS (1997) gave MICs of these antibiotics as:
Penicillin >0.5 µg/mL, ampicillin >32 µg/mL, cloxacillin>4 µg/mL, clindamycin 2 µg/mL, lincomycin >4 µg/mL, streptomycin 32 µg/mL and neomycin >16 µg/mL.

DISCUSSION

S. aureus is one of the most common causes of nosocomial infection. It had produced a wide variety of major and minor pyogenic infections. The excessive use of antibiotics, patient's non-complaince and easy availability of antibiotics has made the *S. aureus* to develop high degree of resistance (Abu Saud, 1996; Lemaitre *et al*., 1998). The minimum inhibitory concentration (MIC) values of various antibiotics were determined against 30 isolates of *S. aureus* from clinical specimen samples (patients visited PIMS). Minimun inhibitory concentrations of these antibiotics (penicillin, ampicillin, cloxacillin, clindamycin, lincomycin, streptomycin and neomycin) *against S. aureus* were evaluated according to NCCLS interpretive standards. Our study findings were similar with the findings of Odelola *et al*., (1989) who studied penicillin and gentamycin against clinical isolates of *S. aureus*, *E. coli*, and *P. aeruginosa* and observed comparable minimum inhibitory concentrations.
Mahmood *and* co-workers (2001) studied 25% isolates resistant to clindamycin, whereas our study had 37% resistant isolates. In present study streptomycin had a resistance range 4 µg/mL to >512 µg/mL against 67% isolates. Devriese *et al*., (1997) reported similar resistance range 26% to 33% during 1971 to 1980 against streptomycin. He also studied that the lincomycin had increased resistance rate among 10 % of isolates (1971 to 1996), whereas our study was 53%. The high resistance rate was observed in penicillin and 38 to 81% (1971 to 1977) by Devriese and co-workers (1997), 91.3% by Cheong and co-workers (1995). This high percentage of resistant range is comparable with the present study, where >63% isolates were resistant to penicillin and 57% were resistant to ampicillin. It was reported that of *S. aureus* isolates clinical samples when tested against penicillin and ampicillin, found 100% resistant (But *et al*., 2004). Craig, (1998) suggested, when low doses of antibiotics were 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.

This study documents the importance of Gram-positive pathogen, *S. aureus* among the Pakistani people, due to lack

of awareness, patients' non-compliance, easy availability of antibiotics (without prescription) of these drugs has aided in this problem. The solution can be planned by continuous efforts of clinician, microbiologists and community, to promote greater understanding of this problem and to practice preventive measures rather than curative ones such as better hygiene, post-operative care and management.

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