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*Full Length Research Paper*

# Comparative analysis of minimum inhibitory concentration of various brands of cephalosporin against clinical isolates of *Staphylococcus aureus*

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***Staphylococcus aureus*** **poses a serious global threat due to difficulty in treatment of its multi-drug resistant strains. One hundred and fifty isolates were collected from pathology laboratory of Nashtar Medical Hospital, Multan; out of which, 50% isolates were found resistant to erythromycin, 100% to carbenicillin and 97% to ampicillin. Vancomycin was comparatively most effective as compared to other antibiotics. Regarding the cephalosporin groups, cefotaxime (73%), ceftazidime (81%), ceftriaxone 82%, cefaclor and cefoperazone (90%) and cephradine was 50% effective against these isolates. Bacterial resistance of twenty five isolated and identified strains of *S. aureus* were tested against local and multinational brands from 1st, 2nd and 3rd generations' injectables. It was noted that 1st generation brands were more resistant than 2nd and 3rd generation. On the basis of mean value, minimum inhibitory concentration (MIC) of local brands were better than multinational brands but on the basis of statistical analysis by ANOVA, the MIC obtained for local and multinational brands were equally effective against the *S. aureus* isolates.**

**Key words:** *Staphylococcus aureus*, antibiotics, local and multinational brands, cephalosporins, minimum inhibitory concentration.

## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is recognized as an important bacterial pathogen contributing towards hospital acquired infections globally. *S. aureus* causes localized infections spreading into the blood stream. *Staphylococci* inhabit the nasopharynx and skin of most healthy people and prevail in our homes and hospitals, but few belong to virulent strains and they are infectious and frequently become invasive and progressive coagulase-positive *S. aureus. Staphylococci* can cause inflammation in any body site. Coagulase-negative *Staphylococci* (*Staphylococcus epidermis, Staphylococcus saprophyticus*) are less virulent and infect mainly previously damaged tissues and hosts (Frobes et al., 2002). *Staphylococci* can produce disease both through their ability to multiply and spread widely in tissues. *S. aureus* produces a large number of proteins that specially bind to human plasma or the extracellular matrix. Some strains are capable of producing a highly

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heat stable protein or toxin that causes illness in humans (Food and Drug Administration, 2003). *S. aureus* is the most frequently isolated bacterial pathogen in hospital acquired infections and is a common cause of community acquired infections including endocarditis, osteomylitis, septic arthritis, pneumonia and abscesses (Lowy, 1998). The symptoms of *Staphylococcal* food poisoning are nausea, vomiting, itching, abdominal pain and prostration (Food and Drug Administration, 2003). Leukocidin produced by *S. aureus* is exfoliative toxin that yields skin syndrome. The emergence of pathogens with increased resistance to available antibacterial has led to development of modern antibiotics with improved activity for these resistant isolates (Polk, 1999). *Cephalosporin acremonium*, the first source of the cephalosporins was isolated in 1948; crude filtrates from the cultures of this fungus were found to inhibit the *in vitro* growth of *S. aureus,* staphylococcal infections and typhoid fever in human being. Culture fluids of sardinian fungus contain three distinct antibiotics named cephalosporins P, N and C with the isolation of active nucleus of cephalosporin C (7-aminocephalosporanic acid) having greater bactericidal activity (Neiss, 1973). Resistant infections are associated with the increased morbidity, prolonged hospital stays, greater direct and indirect costs, prolonged periods during which the individuals are infectious and greater opportunities for the spread of infection to other individuals (Acar, 1997). In many developing countries, the availability and use of antibiotics are poorly controlled which result in a high rate of resistance particularly to the older antibiotics (Kunin, 1993).

The present study was conducted to investigate the prevalence of antibiotic resistance among the various antibiotics as well as the comparative minimum inhibitory concentration study of six brands of 1st, 2nd and 3rd generation cephalosporins.

## MATERIALS AND METHODS

The study was approved by the Board of Advanced Studies and Research and was conducted at Pathology Laboratory, Nashtar Medical College, Multan during January to December 2010. The sensitivity patterns of *S. aureus* isolated from various clinical samples brought to microbiology laboratory were determined against commonly used antibiotics. This study was accomplished according to the principles of 'good clinical practice'.

### Identification and morphological and biochemical characterization of bacterial strains

Isolated colonies, after purification were initially Gram-stained by using Bergey's manual of determinative bacteriology (Collins et al., 1995). The isolates were biochemically characterized and identified up to species level by performing various biochemical tests.

1) β-Hemolysis.
2) Coagulase test.
3) Catalase test.
4) DNase test (Collins et al., 1995).

### Disc diffusion (Bauer kirby) susceptibility test

The disc diffusion test of each *S. aureus* isolates were performed on Mueller Hinton agar (CM337-OXOID) as growth medium. Antibiotic resistance pattern of the isolated *S. aureus* were studied and the resistance pattern among these isolates against different brands of the cephalosporin groups as well as commonly used antibiotics were determined by disc diffusion method (National Committee for Clinical Laboratory Standards, 1993) to interpret diameter of inhibition zone with specified potencies (Connie and Geogre, 2000; Bauer et al., 1996).

### Determination of minimum inhibitory concentration (MIC) by agar dilution method

Agar dilution method was used to determine the minimum inhibitory concerned (MIC) or the lowest concentration of antimicrobial agent required to inhibit the microorganism. Serial two fold dilution concentrations of 6 brands of cephalosporin group of antibiotics belonging to 1st, 2nd, 3rd generation for test were prepared against *S. aureus* isolates from the clinical specimen (Table 3). The six brands of antibiotics in injection form along with the information of potency, manufacturer/suppliers and expiry dates were obtained from Serve Pharmacy, Lahore, Pakistan. Stock solutions of all antimicrobials were prepared by with the help of the following formula:

$$\text{Weight of powder (mg)} = \frac{\text{volume of solvent (ml)} \times \text{concentration (mg/ml)}}{\text{potency of powder (mg/g)}}$$

Whereas, stock solutions of different brands were prepared according to their labeled potencies/concentrations and instructions to reconstitute. Stock solutions dispensed in aliquots and stored immediately at -70°C. Four to five well isolated colonies of various *S. aureus* isolates from a blood agar plate were inculcated in tube containing 5 ml of Tryptone Soya broth (CM129-OXOID) and incubated at 35°C until it was achieved or the turbidity of 0.5 McFarland standards. If turbidity was exceeded McFarland adjusted standard against 0.5 McFarland standards using sterile saline to give the density equivalent to $10^8$ cfu/ml. Then this diluted 10 µl inoculum was transferred on Mueller Hinton agar plates containing various concentrations of antibiotics with the help of micropipette.

Mueller Hinton agar plates containing antibiotic was prepared and Mueller Hinton agar was cooled to 50°C after autoclaving. Dilution series of antimicrobial agents were added depending upon antimicrobial agent. The containers were mixed thoroughly and agar was poured into already labeled sterile petri plates on a leveled surface.

The plates were allowed to set at room temperature and dried. There should be no moisture on the surface of agar. Plates were marked with concentration. Using micropipette, inoculum was transferred to the series of agar plates, including a control without antimicrobial allowed to dry at room temperature, and the plates were placed in inverted position in incubator at 35°C for 18 h. Controls were also run with the isolated strains of *S. aureus*. The MIC is the lowest concentration of the test antimicrobial agent that completely inhibits visible growth as judged by the naked eye was considered the MIC (Espersen, 1995).

### Statistics

ANOVA based statistics was applied to elaborate significance of

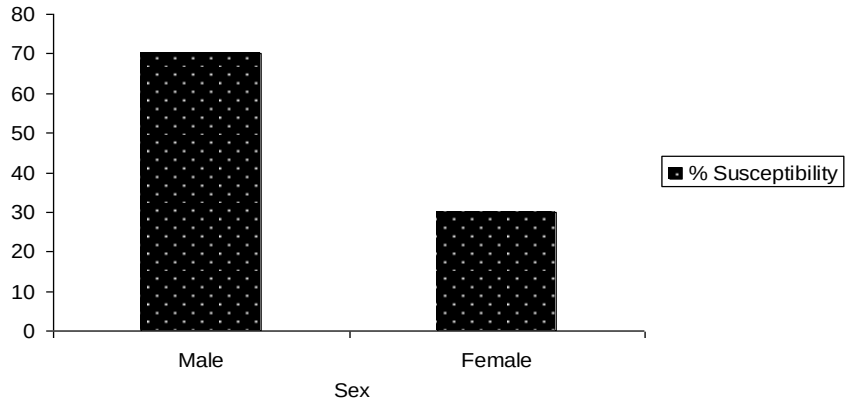


**Figure 1.** Prevalence of *S. aureus* isolates among male and female.

difference among various values. The level of significance was set at 0.05.

## RESULTS

It is a comparative analysis of minimum inhibitory concentration of various brands of cephalosporin against clinical isolates of *S. aureus*. *S. aureus* found in wound exudes; tracheal secretions and catheter tip swab were collected from Pathology Laboratory, Nashtar Hospital, Multan. One hundred and fifty clinical samples were selected for routine screening for antimicrobial assay. These cultures were found to be causative agents, in hospital, out of one hundred and fifty patients, 70% were male and 30% were female (Figure 1). Sensitivity of these *S. aureus* isolates against 25 antimicrobial agents was evaluated by disc diffusion method. Cefaclor, cefoperazone and sparfloxacin were found to be effective (90, 90 and 70%). Amikacin, ciprofloxacin, urixin and meropenem were also effective (100, 90, 100 and 100%, respectively). Only 35 and 11% were resistant to oxacillin and enoxacin. Piperacillin, aztrconam, oflaxacin were also found to be effective; resistance percentage of these *S. aureus* against the antibiotics were 11, 15 and 15%, respectively. The efficacy of ceftazidime, tobramycin, cefotaxime and chloramphenicol were also comparable to each other. As 19% isolates of *S. aureus* were found to be resistant to ceftazidime 20% isolates of *S. aureus* were found to be resistant to tobramycin, 27% were resistant to cefotaxime and 30% were found to be resistant to chloramphenicol. The isolates were 50% resistant to the erythromycin and whereas vancomycin (9%). The 97% isolates showed resistance against trimethoprim, whereas 97% isolates were resistant to ampicillin (Figures 2 and 3). Antibiotic sensitivity with the clinical isolates from indoor and outdoor patients were compared to evaluate the trend of usage of different antibiotic groups such as penicillin, cephalosporin, quinolones, aminoglycosides and other antibiotics. Although cephalosporin was largely prescribed and is used in antimicrobial therapy but ampicillin was found to be the most ineffective against all isolates from ICU, with similar antibiotic resistance pattern which was 34% gentamicin, 50% cephradine, 27% cefotaxime, 18% against ceftriaxone and 19% ceftazidime in the present study. Twenty-five isolates were randomly selected for comparing the efficiency of different brands of cephalosporin group by agar dilution.

The inhibitory activity of two brands of 1st generation (Batosef and Cefatil), two brands of 2nd generation (Ceclor and Cefaclor) and two brands of 3rd generation cephalosporin (Forax and Claforan) were used (Table 1). The six brands were compared as one brand of each antibiotic is local and the other is multinational. Mean values were calculated and it was observed that mean values for the local brand was less than the multinational brand (Table 2). Minimum inhibitory activities of 6 brands of cephalosporins 1st, 2nd and 3rd generations cephalosporins against clinical isolates of *S. aureus* were determined and found comparable values for the local and multinational brands having no major difference (Table 2). This study documents the importance of *S. aureus* as important pathogen among Pakistani population and highlights the fact that there is an alarming development of resistance among clinical isolates. Although, the differences in mean values for local multinational brands by ANOVA, it was observed that all *p*-values were non-significantly ($p>0.05$) different from one another (Table 3).

## DISCUSSION

In this study, comparative analysis of MIC of various brands of cephalosporin against clinical isolates of *S. aureus* was conducted. *S. aureus* from wound exudes; tracheal secretions and catheter tip swab were collected

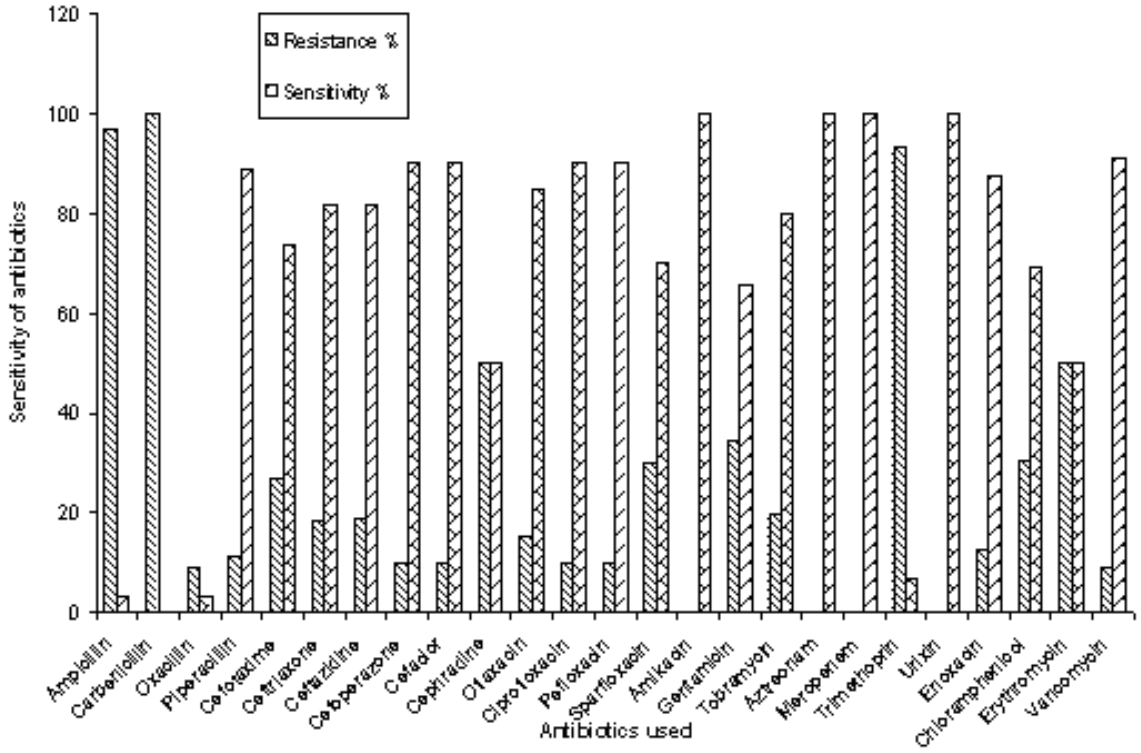


**Figure 2.** Percentage resistance of *S. aureus* against various antibiotics.

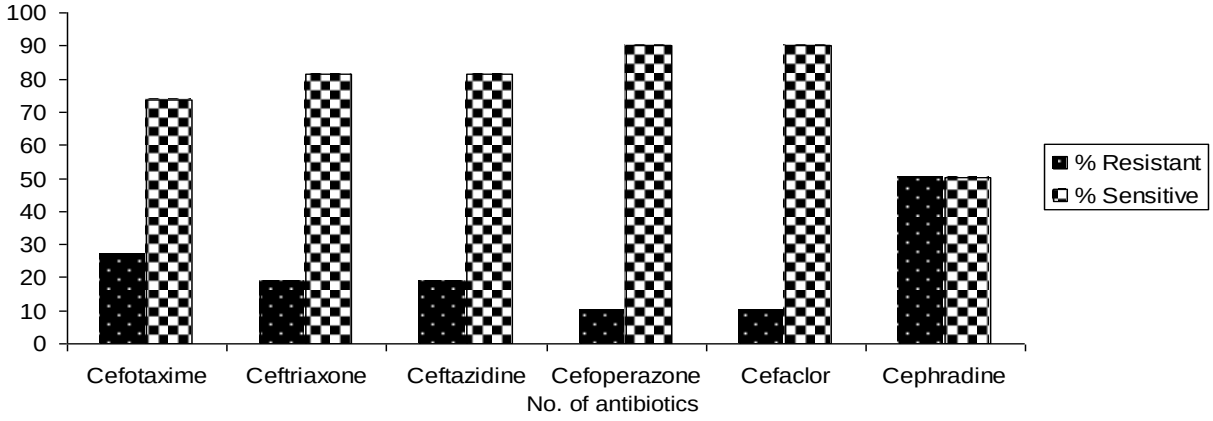


**Figure 3.** Percentage susceptibility of *S. aureus* isolates against cephalosporin group.

from Pathology Laboratory, Nashtar Hospital, Multan. One hundred and fifty clinical samples were selected for routine screening for antimicrobial assay; all these isolates were in line with the work of other researchers (13 to 16). These cultures were found to be causative agents, in hospital, out of one hundred and fifty patients, 70% were male and 30% were female (Figure 1).

Sensitivity of these *S. aureus* isolates against 25 antimicrobial agents was evaluated by disc diffusion method. Cefaclor, cefoperazone and sparfloxacin were found to be effective (90, 90 and 70%). Amikacin, ciprofloxacin, urixin and meropenem were also effective (100, 90, 100 and 100%, respectively). Only 35 and 11% were resistant to oxacillin and enoxacin. Piperacillin, aztrconam, oflaxacin were also found to be effective; resistance percentage of these *S. aureus* against the

**Table 1.** Brand used in study for minimum inhibitory concentration.

| Variable | Brands | Manufacturer |
|---|---|---|
| First generation | Batosef (L) | Swiss pharma |
| | Cefatil (M) | Highnoon |
| Second generation | Ceclor (L) | E-Lilly |
| | Cefaclor (M) | Bosche |
| Third generation | Forax (L) | Emson |
| | Claforan (M) | Aventis |

Note: Brand name of local company (L) and brand name of multinational company (M).

**Table 2.** MICs (mg/ml) of six brands cephalosporin against clinical isolates of *S. aureus.*

| Variable | 1st generation | | 2nd generation | | 3rd generation | |
|---|---|---|---|---|---|---|
| | Batosef | Cefatil | Ceclor | Cafaclor | Forax | Claforan |
| *S.a.*1 | 512 | 256 | 256 | 512 | 256 | 256 |
| *S.a.*2 | 1024 | 512 | 512 | 1024 | 512 | 512 |
| *S.a.*3 | 128 | 256 | 512 | 256 | 256 | 512 |
| *S.a.*4 | 1024 | 512 | 512 | 256 | 1024 | 512 |
| *S.a.*5 | 512 | 512 | 256 | 256 | 512 | 512 |
| *S.a.*6 | 512 | 512 | 256 | 256 | 512 | 512 |
| *S.a.*7 | 64 | 128 | 128 | 128 | 256 | 256 |
| *S.a.*8 | 1024 | 512 | 254 | 512 | 512 | 1024 |
| *S.a.*9 | 8 | 8 | 8 | 16 | 16 | 8 |
| *S.a.*10 | 64 | 32 | 64 | 64 | 32 | 64 |
| *S.a.*11 | 512 | 256 | 256 | 256 | 256 | 256 |
| *S.a.*12 | 512 | 256 | 512 | 256 | 256 | 256 |
| *S.a.*13 | 8 | 16 | 32 | 32 | 16 | 16 |
| *S.a.*14 | 8 | 16 | 8 | 32 | 16 | 8 |
| *S.a.*15 | 256 | 256 | 512 | 512 | 128 | 128 |
| *S.a.*16 | 256 | 256 | 512 | 512 | 1024 | 1024 |
| *S.a.*17 | 512 | 256 | 256 | 256 | 512 | 1024 |
| *S.a.*18 | 512 | 512 | 1024 | 256 | 512 | 512 |
| *S.a.*19 | 16 | 16 | 64 | 64 | 32 | 64 |
| *S.a.*20 | 512 | 512 | 1024 | 512 | 512 | 1024 |
| *S.a.*21 | 512 | 512 | 256 | 256 | 16 | 8 |
| *S.a.*22 | 8 | 8 | 512 | 512 | 512 | 512 |
| *S.a.*23 | 256 | 256 | 256 | 512 | 128 | 128 |
| *S.a.*24 | 8 | 256 | 512 | 128 | 128 | 512 |
| *S.a.*25 | 512 | 256 | 128 | 64 | 16 | 8 |

antibiotics were 11, 15 and 15%, respectively. The efficacy of ceftazidime, tobramycin, cefotaxime and chloramphenicol were also comparable to each other. As 19% isolates of *S. aureus* were found to be resistant to ceftazidime, 20% isolates of *S. aureus* were found to be resistant to tobramycin, 27% were resistant to cefotaxime and 30% were found to be resistant to chloramphenicol. The isolates were 50% resistant to the erythromycin and whereas vancomycin (9%). The 97% isolates showed resistance against trimethoprim, whereas 97% isolates were resistant to ampicillin (Figures 2 and 3). Antibiotic sensitivity with the clinical isolates from indoor and outdoor patients were compared to evaluate the trend of usage of different antibiotic groups such as penicillin, cephalosporin, quinolones, aminoglycosides and other antibiotics. Although cephalosporin was largely

**Table 3.** Statistical analysis of minimum inhibitory concentration of six brands of cephalosporins against *S. aureus* isolates (ANOVA).

| Source of variation | SS | Df | MS | F | P-Value | F- critical |
|---|---|---|---|---|---|---|
| Antibiotics | 28208.41 | 2 | 14104.21 | 0.163907 | 0.848979 | 3.058928 |
| Made | 93520.17 | 1 | 96520.17 | 1.121673 | 0.291332 | 3.966849 |
| Interaction | 253224.9 | 2 | 126612.4 | 1.47138 | 0.23304 | 3.058928 |
| Within | 12391223 | 144 | 86050.16 | | | |
| Total | 12769177 | 149 | | | | |

prescribed and is used in antimicrobial therapy, but ampicillin was found to be the most ineffective against all isolates from ICU with similar antibiotic resistance pattern which was 34% gentamicin, 50% cephradine, 27% cefotaxime, 18% against ceftriaxone and 19% ceftazidime in the present study.

Mahmood et al. (2001) reported similar results as 20% isolates from blood were resistant to ceftriazone, gentamicin, more than 50% resistance was observed in case of penicillin, ampicillin and erythrocin (Mahmood et al., 2001). Twenty-five isolates were randomly selected for comparing the efficiency of different brands of cephalosporin group by agar dilution. The inhibitory activity of two brands of 1st generation (Batosef and Cefatil), two brands of 2nd generation (Ceclor and Cefaclor) and two brands of 3rd generation cephalosporin (Forax and Claforan) were used (Table 1). The six brands were compared as one brand of each antibiotic was local and the other was multinational. Mean values were calculated and it was observed that mean values for the local brand is less than the multinational brand (Table 2).

Among penicillin, ampicillin and other antistaphylococcal agent has also contributed to the emergence of multidrug-resistant strains in the patient of present study. Previous researchers found resistant strains of MRSA with 1st, 2nd, 3rd and 4th generation of cephalosporins (James and Reeves, 1996). 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 than eventually affecting other cells (Khalid et al., 2011). Cephalosporin group included cefaclor, cephradine, cefoperazone, cefotaxime, ceftazidime and ceftriaxone were also tested against *Pseudomonas aeruginosa* isolates. Most of the isolates were resistant to cephradine and half of the isolates were sensitive to cefotaxime. Findings from this study for cephradine and cefaclor get support from the aforementioned report (Khalid et al., 2011). Ciprofloxacin and pefloxacin possess good spectrum of activity against Gram-Positive bacteria, while it retains excellent activity against Gram-negative microorganism. Only 10% of the isolates were resistant to the ciprofloxacin and pefloxacin. In the present study, the development of resistance with quinolones to *S. aureus* had been created by previous antimicrobial chemotherapy of either patient before hospitalization or surgery (Andersen et al., 2001).

In the present study, most of the isolates were sensitive to vancomycin with having 9% were the resistant and 91% of the isolates were sensitive. Vancomycin is a drug of choice for *S. aureus* especially MRSA. Chloramphenicol is very active against Gram-positive bacteria but *S. aureus* is found to be usually resistant to it. In the present study also, some of the *S. aureus* isolates were resistant. All the isolates of *S. aureus* were resistant to carbenicilin in the present work. Aztreonam a monobactam was noted to be effective. Only 14% of the isolates were resistant in the present study against *S. aureus*. The effectiveness of aztreonam was also reported previously (Ahmad et al., 2001). Among carbapenems, the activity of meropenem is greater than the aztreonam in the present study and the result is comparable to various workers (Farzana et al., 2011; Edwards-Jones et al., 1996). The kinetics of inhibitory activity show that all brands of each antibiotic possessed almost similar activity against clinical isolated of *S. aureus* tested. This finding is similar to previous finding (Ejaz et al., 2011) who studied 6 brands of cephalosporins, ampicilin and 4 brands of gentamicin against clinical isolate and observed comparable minimum inhibitory activities (Ahmad et al., 2010).

Minimum inhibitory activities of 6 brands of cephalosporins 1st, 2nd and 3rd generation cephalosporins against clinical isolates of *S. aureus* were determined and found comparable values for the local and multinational brands having no major difference (Table 2). Mean values for first generation cephalosporin for local and multinational brand were 452.16 and 295.68 µg/ml. The average value for the 2nd generation cephalosporin for local brand slightly greater from local to multinational (360.24 and 320.24 µg/ml, respectively). Whereas, 3rd generation cephalosporin were 337.6 and 381.48 µg/ml for local and multinational brands. Ampicillin is semisynthetic penicillin. Resistance of these strains was 96% among isolates against ampicillin. Antibiotic use provides selective pressure favouring resistant bacterial strains. Another factor responsible for development of

antibiotic resistance in bacteria could be due to non-access of health workers for health information (Ahmad et al., 2010). During last two decades, the sensitivity of 2nd generation's cephalosporins, ceftriazone was foundto be effective against *S. aureus,* whereas other workers have reported variable results with this antibiotic (Khalid et al., 2011; Farzana et al., 2011; Ahmad et al., 2008). During last two decades, the sensitivity of 3rd generation cephalosporins, cefoperazone and cefotaxime has dramatically decreased against *P. aeruginosa* in developed countries. This development of resistance by *P. aeruginosa* might be due to wide spread use of these antimicrobials.

The kinetics of inhibitory activity show that all brands of each antibiotic possessed almost similar activity against clinical isolated of *S. aureus* tested. This finding is similar to the finding of previous researchers (Ejaz et al., 2011) who studied 6 brands of cephalosporins, ampicilin and 4 brands of gentamicin against clinical isolate and observed comparable minimum inhibitory activities (Ejaz et al., 2011). Minimum inhibitory activities of 6 brands of cephalosporins 1st, 2nd and 3rd generations cephalosporins against clinical isolates of *S. aureus* were determined and found comparable values for the local and multinational brands having no major difference. Our object of this study was to observe the effect of hospital environment on the emergence of resistant strains of *S. aureus*. This study documents the importance of *S. aureus* as important pathogen among Pakistani population and highlights the fact that there is an alarming development of resistance among clinical isolates. Although the differences in average mean values for local multinational brands by ANOVA, it was observed that all *p*-values were not significantly ($p>0.05$) different from one another (Table 3).

## Conclusion

On the basis of average mean value that minimum inhibitory concentration of local brand were better than multinational brands but on the basis of statistical analysis by ANOVA, the most powerful tool for analyzing, the MIC obtained for each brand against the *S. aureus* isolates, the local and multinational brands were equally effective.

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