# REPORT

# Comparative study on resistant pattern of clinical isolates against Levofloxacin and Cefepime

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**Abstract**: The aim of this study is to evaluate the susceptibility and resistance pattern of clinical isolates causing different types of infections and to compare the efficacy of antibiotics namely Levofloxacin and Cefepime. The in-vitro antibacterial activity and resistance patterns of these two well known antibiotics were studied and compared using disk diffusion method. For this, one hundred clinical isolates comprising of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from different local pathological laboratories and hospitals. *Escherichia coli* (17.95% against cefepime and 30.77% against levofloxacin), *Staphylococcus aureus* (30% against cefepime and 46.66% against levofloxacin) and *Pseudomonas aeruginosa* (23.53% against cefepime and 35.29% against levofloxacin) were found resistant against the studied antibiotics which show that cefepime is more effective than levofloxacin. In case of *Klebsiella pneumoniae,* resistance was 42.85% against cefepime and 35.71% against levofloxacin thereby showing that levofloxacin is more effective than cefepime. Concluded that the clinical isolates collected were susceptible to both the antibiotics but the microbial resistance against these antibiotics is increasing in our population which is alarming. Therefore, it is recommended the physicians may prescribe these antibiotics unless no other substitute is available in clinical practice.

**Keywords**: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, Cefepime and Levofloxacin.

## INTRODUCTION

During the past decades, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes but these bacteria and microbes are remarkably resilient and have developed several ways to resist antibiotics. This problem is due to increasing use, and misuse, of the existing antibiotics in human being and animals. Antibiotic resistance in bacteria may be an inherent trait of the organism due to which bacteria get resistance, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source (Kenneth, 2008). It is widely accepted that the greater the use of antimicrobials, the greater will be the emergence of resistance (Bearden, 2001). The extensive use of antibiotics has resulted in bacteria rapidly developing resistance to these agents (Joaquim, 2003). Bacterial resistance to cephalosporin antibiotic is due to the production of beta-lactamase, a bacterial enzyme that breaks the core beta-lactam ring, leaving the antibiotic unable to bind Pencillin bindng proteins (Harrison *et al*., 2008). Combinations of fluoroquinolones with β-lactams or amikacin show an enhanced activity against *P. aeruginosa* and *Acinetobacter* spp. (Drago *et al*., 2004). Levofloxacin is safe and effective in the treatment of ear infections in children (Arguedas, 2006). The increased rates of antimicrobial resistance commonly found among isolates causing community-acquired pneumonia and hospital-acquired pneumonia indicate that extended-spectrum antimicrobial agents, such as cefepime, would be more appropriate therapeutic agents (Lin *et al*., 2001). Antibiotic treatments for lower respiratory tract infections depend upon to gender, age and the treatment duration (Bertrand *et al*., 1993). Resistance was more common among vancomycin-resistant enterococci and methicillin-resistant Staphylococci (Eliopoulos *et al*., 1996). The bactericidal effect of levofloxacin is concentration-dependent; it is possible to increase the peak concentration by increasing the dose, resulting in even better tissue concentration (and a possible reduction in the development of resistance) (Wolfgang *et al*., 2004). Cefepime had an extended-spectrum against many important Gram-positive and Gram-negative bacteria including those producing cephalosporiases. Cefepime had broader spectrum than other third generation cephalosporins (Surapee *et al*., in 1994). Levofloxacin plus a β-lactam was synergistic more often than levofloxacin combinations and levofloxacin in combination with another active agent against *Ps.*

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*aeruginosa* may prove to be clinically beneficial and superior to combinations using lower doses of levofloxacin (David *et al*., 2003). Levofloxacin is a new fluoroquinolones to which the most common clinical isolates are susceptible. The susceptibility of *Enterococcus* spp. and *S. pneumoniae* to levofloxacin was particularly remarkable. This compound appears to be a promising therapeutic alternative for the treatment of Gram-positive infections (Hans *et al*., 1996).

The objective of the present work was to determine the resistance pattern of hundred clinical isolates comprising of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* against two antibiotics namely levofloxacin and cefepime which were selected for their wider use by the physician.

## MATERIALS AND METHODS

***Collection of clinical isolates***
One hundred clinical isolates were procured from different pathological laboratories of metropolitan city.

***Antimicrobial agents***
Standard discs of cefepime and levofloxacin were procured from market. Cartridges containing discs were stored in refrigerator ($2^oC$ to $8^oC$).

***Preparation of media***
Mueller Hinton Agar and Mueller Hinton Broth were prepared and sterilized according to manufacturer's instructions (Merck).

***Preparation of media plates***
Mueller Hinton Agar plates were prepared for this research task.

***Preparation of Inoculum:***
The inoculation was prepared by touching the top of the colonies of the isolates with sterile wire loop and suspending in a tube containing 4-5 ml of broth and incubated at 37°C for 4-6 hours.

***Inoculation of plates:***
Sterile swab was dipped into inoculum suspension. Excess fluid was removed by pressing and rotating the swab against the side of tube above the level of suspension. The swab was then spell evenly over the surface of the medium in three directions, rotating the plates approximately 60 degree to ensure even distribution. After inoculation, surface of agar was allowed to dry for 5 minutes. McFarland standards were prepared by mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. For example, A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid ($H_2SO_4$). The cell density /concentration was approx 1.5X10^8 CFU/mL while % Transmittance at wavelength of 600 nm was 74.3 and Absorbance was 0.132.

***Placement of antibiotic disc:***
Using sterile forceps, the appropriate antimicrobial discs of cefepime and levofloxacin were placed on the agar surface and slightly pressed down to ensure firm.

***Incubation of Plates:***
Within 30 minutes of applying the discs, plates were incubated at $37^oC$ in incubator for 18-24 hours.

***Measurement of Zone diameter and interpretation of result:***
After 24 hours of incubation, the plates were examined and zone of inhibition was measured (in mm) which were shown in tables 1 to 4.

## RESULTS

In the present study, resistant pattern of hundred (100) clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were studied using cefepime and levofloxacin and the results are presented in Tables 1-4 and graph 1-2. The results revealed that 17.95% clinical isolates of *Escherichia coli*, 30% *Staphylococcus aureus*, 23.53% *Pseudomonas aeruginosa and* 42.85% *Klebsiella pneumoniae* were resistant to cefepime. In case of levofloxacin, the results showed that 30.77% clinical isolates of *Escherichia coli*, 46.66% *Staphylococcus aureus*, 35.29% *Pseudomonas aeruginosa* and 35.71% *Klebsiella pneumoniae* were resistant to levofloxacin. From these figures, it is clear that *Klebsiella pneumoniae* is more resistant to cefepime as compare to levofloxacin but in case of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* it appears that these isolates are more resistant to levofloxacin as compare to cefepime.

## DISCUSSION

During the present study two antimicrobial agents i.e. levofloxacin and cefepime, were used against 100 clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* The antibacterial activity of cefepime is more than levofloxacin. Both antibiotics have antibacterial activity against *Escherichia coli* but this activity is not 100%. Approximately 20% to 40% isolates have developed resistance against these two antibiotics and this is alarming situation, so it is very important that these drugs should be prescribed under condition which is related to *Escherichia coli* unless other alternate is not available. Both antibiotics are active against *Staphylococcus aureus*

**Table 1**: Resistance pattern of Levofloxacin and Cefepime against *Escherichia coli*

| Name of clinical isolates | No. of clinical isolates. | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Levofloxacin 5µg | | | Cefepime 30µg | | |
| | | Resistant (R) ≤13mm | Intermediate Resistant (R) 14-16mm | Sensitive (S) ≥ 17mm | Resistant (R) ≤14mm | Intermediate Resistant (R) 15-17mm | Sensitive (S) ≥ 18mm |
| *Escherichia coli* | 39 | 8 | 4 | 27 | 4 | 3 | 32 |

**Table 2**: Resistance pattern of Levofloxacin and Cefepime against *Staphylococcus aureus*

| Name of clinical isolates | No of clinical isolates. | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Levofloxacin 5µg | | | Cefepime 30µg | | |
| | | Resistant (R) ≤ 15mm | Intermediate Resistant (R) 16-18mm | Sensitive (S) ≥ 19mm | Resistant (R) ≤ 14mm | Intermediate Resistant (R) 15-17mm | Sensitive (S) ≥ 18mm |
| *Staphylococcus aureus* | 30 | 10 | 4 | 16 | 7 | 2 | 21 |

**Table 3**: Resistance pattern of Levofloxacin and Cefepime against *Klebsiella pneumoniae*

| Name of clinical isolates | No of clinical isolates. | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Levofloxacin 5µg | | | Cefepime 30µg | | |
| | | Resistant (R) ≤13mm | Intermediate Resistant (R) 14-16mm | Sensitive (S) ≥ 17mm | Resistant (R) ≤ 14mm | Intermediate Resistant (R) 15-17mm | Sensitive (S) ≥ 18mm |
| *Klebsiella pneumonia* | 14 | 4 | 01 | 9 | 4 | 2 | 8 |

**Table 4**: Resistance pattern of Levofloxacin and Cefepime against *Pseudomonas aeruginosa*

| Name of clinical isolates | No of clinical isolates. | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Levofloxacin 5µg | | | Cefepime 30µg | | |
| | | Resistant (R) ≤ 13mm | Intermediate Resistant (R) 14-16mm | Sensitive (S) ≥ 17mm | Resistant (R) ≤ 14mm | Intermediate Resistant (R) 15-17mm | Sensitive (S) ≥ 18mm |
| *Pseudomonas aeruginosa* | 17 | 3 | 3 | 11 | 3 | 01 | 13 |

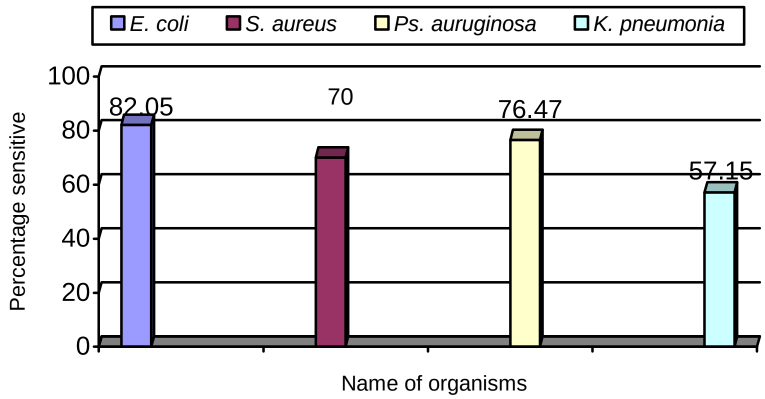
but the antibacterial activity of cefepime is more as compared to levofloxacin. Levofloxacin is more effective than cefepime against *Klebsiella pneumoniae.* Cefepime is more active than levofloxacin against *Pseudomonas aeruginosa.* This study revealed that clinical isolates collected from different pathological laboratories and hospitals of Karachi were susceptible to both antibiotics.

Antibiotic resistance is due to increasing use, and haphazard of existing antibiotics in human therapy (Kenneth, 2008). The extensive use of antibiotics has resulted in bacteria rapidly developing resistance to these agents (Ruiz, 2003).

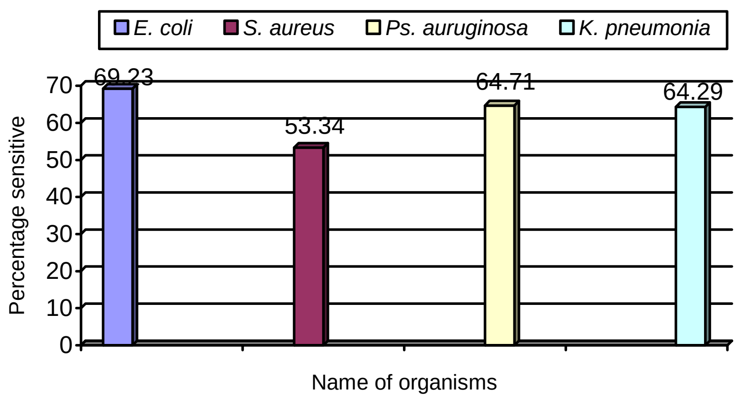
Several workers throughout the world have reported resistance of various organisms against levofloxacin and cefepime. Blondeau *et al* (1999) reported 1466 clinical non-repeat isolates of *Pseudomonas aeruginosa,* 21 of *Acetinobacter spp.* and 31 *Stenotrophomas maltophilia.* Against each group of isolates, cefepime was active against 87%, 86.4% and 15.6%, respectively. This *in vitro* study showed that cefepime may be a useful additional agent in the treatment of infections caused by *Pseudomonas aeruginosa* and *Acinetobacter spp*. During present study the activity of cefepime against *Pseudomonas aeruginosa* was 76.47%.

In another work the activity of several beta-lactam antimicrobial agents, including Piperacillin/Tazobactam and cefepime, against 108 *Escherichia coli* and *Klebsiella pneumoniae* strains resistant to oxyimino cephalosporins. On the basis of the percentage of susceptible, 52 to 64% isolates were susceptible to cefepime. The results of present study against these isolates are 82.05% to 57.14% respectively (Jett *et al*., 1995).

Reported that twelve beta-lactam and non-beta-lactam antibiotics were evaluated against 115 clinical isolates of




**Graph 1**: Percentage of cefepime sensitive bacteria/isolates




**Graph 2**: Percentage of levofloxacin sensitive bacteria/isolates

extended-spectrum beta-lactamase-producing (ESBLs) *Escherichia coli* using a broth microdilution test in accordance with the Clinical Laboratories Standard Institutes (CLSI) guidelines. Susceptibility was 80% with cefepime (Antonio *et al*., 2007). Presently the susceptibility of *Escherichia coli* was 82.05% with cefepime.

Sader *et al*. (1998) evaluated antimicrobial susceptibility of 556 strains from the lower respiratory tract by the SENTRY Antimicrobial Surveillance Program (1997). The isolates were tested against more than 70 drugs by the reference broth microdilution method. The five most frequently isolated species were (n/%): *Pseudomonas aeruginosa* (149/26.8%), *Staphylococcus aureus* (127/22.8%), *Acinetobacter spp.* (66/11.9%), *Klebsiella spp. (56/10.1%), and Enterobacter spp.* (40/7.2%). *Ps. aeruginosa* demonstrated high degree of resistance to a majority of the antimicrobial drugs tested. The lowest resistance rate was observed for cefepime (6.7%). Cefepime was active against 90.0% of the isolates. The present results revealed that 70% *Staphylococcus aureus* were found susceptible to cefepime where as 76.47% of *Pseudomonas aeruginosa* were found 76.47% susceptible to cefepime.

David *et al*. (2003) evaluated the in vitro activity of two concentrations of levofloxacin (500 mg and 750 mg daily dose) against *Ps. aeruginosa.* The percentage susceptibility for levofloxacin was 67%. Levofloxacin 4 μg/mL (750 mg/day) alone reached 99.9% killing and maintains this killing over the time period more often than levofloxacin 2 μg/mL (500 mg/day). It is concluded after this experiment that levofloxacin 750 mg/day in combination with another agent active against *Ps. aeruginosa* may prove to be clinically beneficial and superior to combinations using lower doses of levofloxacin. During the present study the activity of levofloxacin against *Pseudomonas aeruginosa* was 64.71%.

Hans *et al*., (1999) examined the susceptibility of the clinical isolates to levofloxacin, a fluoroquinolone with extended activity against Gram-positive bacteria, and other antibiotics in 12 Swiss clinical microbiology laboratories using the NCCLS disc diffusion technique. A total of 310 Gram-positive and 580 Gram-negative isolates from the respiratory tract (36%), skin/wounds (12%), blood (16%), urine (17%) and other sources (19%) were tested. The percentage of the isolates susceptible to levofloxacin for *Staphylococcus aureus* (95 strains, including 2% MRSA) was 94%, coagulase-negative staphylococci (85) 65%, *Enterobacter* spp. (75) 99%, *Escherichia coli* (111) 97%, *Klebsiella pneumoniae* (45) 98% and *Pseudomonas aeruginosa* (124) 87%. In conclusion, levofloxacin is a new fluoroquinolone to which the most common clinical isolates in Switzerland are susceptible. This compound appears to be a promising therapeutic alternative for the treatment of Gram-positive infections**.** During the present study the percentage of susceptibility *Staphylococcus aureus* to levofloxacin was 53.34%, *Escherichia coli* 60.23%, *Klebsiella pneumoniae* 64.20% and *Pseudomonas aeruginosa* 64.71%.

It may be concluded that levofloxacin and cefepime are two very good antibacterial agents in the field of antimicrobial chemotherapy but different isolates have started developing resistance against these antibiotics so we recommend physicians to prescribe this group unless no other alternative is available.

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