RESEARCH ARTICLE

COMPARISON OF IN VITRO ANTIBACTERIAL ACTIVITY OF LEVOFLOXACIN AND CIPROFLOXACIN

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ABSTRACT

Levofloxacin and ciprofloxacin are widely used antibiotics that belong to the class of fluoroquinolones. The in vitro antibacterial activity of these two antimicrobials were evaluated and compared in the present study by Disk Diffusion (Kirby-Bauer) method against 52 clinical isolates of *Staphylococcus aureus* (n = 36) and *Klebsiella pneumoniae* (n = 16). These clinical isolates were obtained from different pathological laboratories and medical centers of Karachi. Among clinical isolates of *Staphylococcus aureus* the average zone of inhibition for levofloxacin was 16.7 mm while for ciprofloxacin the zone of inhibition was 21 mm. For the clinical isolates of *Klebseilla pneumoniae* the average zone of inhibition of levofloxacin was 16.5 mm while for ciprofloxacin was 16.9 mm. Comparison of susceptibility results indicated that both fluoroquinolones have excellent in vitro antibacterial activity but comparatively ciprofloxacin showed broader spectrum of activity against most of the tested clinical isolates.

Keywords: Fluoroquinolones, antibacterial activity, clinical isolates, disk diffusion method.

1. INTRODUCTION

Quinolone antibiotics were developed in the 1960s. In 1967, nalidixic acid was given license for the treatment of urinary tract infections (UTIs) caused by majority of Gram-negative bacteria with the exception of *Pseudomonas aeruginosa*. However, majority of the Gram-positive organisms are usually resistant to these early Quinolones[1]. Until the development of flumequine, the first monofluoroquinolone in 1976, none of the earlier compounds had offered any significant improvements over nalidixic acid. Flumequine was the first compound to be developed with the fluoro group at position 6. This development provided the indication that the modifications of the basic chemical structure could improve the activity of quinolones against Gram-positive organisms[2].

Having taken two decades to produce significant improvements in the bioavailability and spectrum of these drugs from the earlier compounds, the next phase of development followed very rapidly. Between 1979 and 1982, a number of fluoroquinolones were patented including ciprofloxacin which is still in widespread clinical use today[3,4]. With the development of fleroxacin in 1986, the first trifluorinated quinolone, the class entered the third decade of development and use. Fleroxacin was distinguished from its predecessors by its excellent bioavailability, high concentrations in the plasma and other body fluids[5].

The next significant advancement occurred in the early 1990s, with the synthesis of temafloxacin which has four to eight fold greater activity against *Streptococcus pneumoniae* and also shows good effect against anaerobes such as the *Bacteroides* and *Prevotella species*[6]. By the mid 1990s the development of sparfloxacin, grepafloxacin, levofloxacin and gatifloxacin took place[7]. Optimism increased with the discovery of grepafloxacin, clinafloxacin and moxifloxacin, all of which have significantly further improved activity against Gram-positive species, notably against *Streptococcus pneumoniae*[8].

The present study has been carried out to investigate and compare the antibacterial activity of two most widely used fluoroquinolone antibiotics, levofloxacin

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and ciprofloxacin against *S. aureus* and *K. pneumoniae*. Such study will give an idea regarding the sensitivity and resistance pattern of the said antibiotics against the clinical isolates of *S. aureus* and *K. pneumoniae* in our local population.

2. MATERIALS AND METHODS

2.1. Materials

Mueller-Hinton agar and Mueller-Hinton broth were purchased from Oxoid (UK). All apparatus employed in the study were used after thorough sterilization.

2.2. Procurement and Identification of Clinical Isolates

A total of fifty two clinical isolates of *S. aureus* (n = 36) and *K. pneumoniae* (n = 16) were collected from different pathological laboratories of Karachi. The procured cultures were further identified by routine morphological and cultural methods.

2.3. Determination of Antibacterial Activity

The antibiotic disks of 20 µg concentration (Oxoid) were stored in a refrigerator (2 to 8 °C). The antibacterial activity of the test antibiotics was performed by the Disk diffusion (Kirby-Bauer) method according to the guidelines of Clinical Laboratory Standard Institute (CLSI). The details of the method are as follows:

2.3.1. Preparation of culture medium

Mueller-Hinton agar and broth were prepared according to the manufacturer's instruction. The prepared medium was then autoclaved at a temperature of 121 °C for 15-20 min for complete sterilization before use.

2.3.2. Preparation of susceptibility plates

Susceptibility plates were prepared by pouring around 25-30 ml of Mueller-Hinton agar onto 100 mm diameter Petri plates on a smooth horizontal surface in order to give a uniform depth of approximately 4 mm. The agar medium was then cooled at room temperature for solidification.

2.3.3. Preparation of McFarland turbidity standard

A 0.5 McFarland standard was prepared by adding 0.5 ml of 0.048 M barium chloride to 99.5 ml of 0.36 N sulfuric acid. An aliquot of 4 to 6 ml of barium sulfate turbidity standard was distributed to screw-capped tubes (same size as inoculums tube) which were sealed tightly and stored in the dark at room temperature.

2.3.4. Preparation of inoculum

With the flame sterilized wire loop, culture was transferred to a test tube containing 4 to 5 ml of Mueller-Hinton broth. The broth culture was allowed to incubate for 2 to 6 hours at 37°C until it achieved the turbidity of the McFarland 0.5 standard. The turbidity standard line chart was used for the comparison of standard and inoculum. The number of bacteria in the inoculating suspension was further confirmed by performing Colony Count Method.

2.3.5. Inoculation of susceptibility plates

With the help of sterile cotton swab, inoculum suspension i.e. broth culture was inoculated to Mueller-Hinton agar plates. The culture was streaked three times over the entire agar surface in different directions. Each plate was allowed to dry approximately 15 minutes before placing the antibiotics disks.

2.3.6. Application of the test antibiotic disks to the agar plates

By using flame-sterilized forceps, antibiotic-containing disks were placed on equal distance on the dried agar surface.

2.3.7. Preparation of controls

A negative control of the medium was prepared i.e. without antibiotic disks and inoculum to check its sterility. Similarly, a positive control was prepared without antibiotic disks to confirm that the medium supports the growth of the test bacteria.

2.3.8. Incubation of susceptibility plates

The plates were incubated at 37°C for 16-18 hrs.

2.3.9. Measurement of zones of inhibitions

After incubation period, zones of inhibition appeared around the antibiotic disks. The diameter of each zone of inhibition including the diameter of the disk was measured using calibrated vernier caliper. Zones were measured from the back of the petri plate using a bright source of transmitted light. The size of the zone of inhibition was interpreted for susceptibility and resistance.

Table 1. Comparative zone of inhibition (mm) pattern of levofloxacin and ciprofloxacin against clinical isolates of *Staphylococcus aureus*.

S. No.	DIAMETER OF ZONE OF INHIBITION (mm)	
	Levofloxacin	Ciprofloxacin
1	24	26
2	22	21
3	24	0
4	0	0
5	12	14
6	25	28
7	0	10
8	20	22
9	11	16
10	0	11
11	35	30
12	35	22
13	21	20
14	0	14
15	23	26
16	0	27
17	23	26
18	0	0
19	20	16
20	25	28
21	22	28
22	18	35
23	0	13
24	19	22
25	31	31
26	20	28
27	18	16
28	18	18
29	23	20
30	19	34
31	0	31
32	20	26
33	14	21
34	22	20
35	16	27
36	20	28

Table 2. Comparative zone of inhibition (mm) pattern of levofloxacin and ciprofloxacin against clinical isolates of *Klebsiella pneumonia.*

S. No.	DIAMETER OF ZONE OF INHIBITION (mm)	
	Levofloxacin	Ciprofloxacin
1	17	17
2	29	33
3	27	27
4	23	24
5	0	0
6	0	0
7	8	0
8	30	27
9	29	30
10	0	30
11	13	12
12	12	9
13	29	21
14	29	20
15	18	8
16	0	12

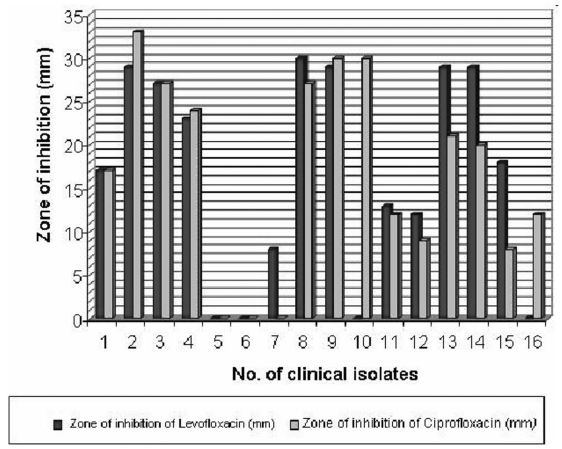

Fig. 1. Comparative zone of inhibitions of levofloxacin and ciprofloxacin against clinical isolates of *Klebsiella pneumonia*.

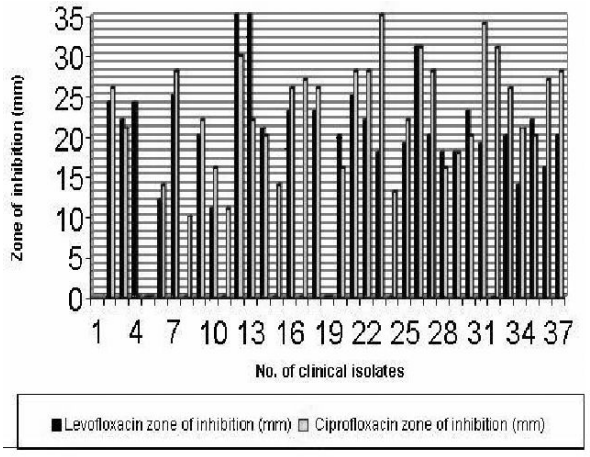

Fig. 2. Comparative zone of inhibitions of levofloxacin and ciprofloxacin against clinical isolates of *Staphylococcus aureus*.

3. RESULTS AND DISCUSSION

Levofloxacin and ciprofloxacin are fluoroquinolones antibiotics. They are rapidly growing class of antimicrobial agents that have proven to be useful in the treatment of many soft tissues infections, UTI, ulcers, skin infections and toxic shock syndrome etc.[9,10]. Comparison of in vitro antibacterial activity of these two antibiotics has been carried out in the present study by Disk Diffusion (Kirby-Bauer) method (Table 1 and 2) against clinical isolates of *Staphylococcus aureus* and *Klebsiella pneumoniae.*

Out of 36 clinical isolates of *Staphylococcus aureus*, 8 isolates were resistant to levofloxacin and 3 isolates were resistant to ciprofloxacin. The average zone of inhibition of levofloxacin was 16.7 mm while the average zone for ciprofloxacin was 21 mm, which showed that ciprofloxacin is more active than levofloxacin against clinical isolates of *Staphylococcus aureus*. The present work is in agreement with the work of Kowalski *et al.*[11] who investigated that fourth generation fluoroquinolones i.e. ciprofloxacin demonstrated increased susceptibility for *Staphylococcus aureus* isolates that were resistant to levofloxacin and ofloxacin. The present study is also in agreement with the work of Matsuzaki *et al*.[12] who reported that ciprofloxacin was more active than levofloxacin against *Staphylococcus aureus* isolates.

Out of 16 clinical isolates of *Klebsiella pneumoniae*, 5 each were found to be resistant to ciprofloxacin and levofloxacin. The average zone of inhibition of levofloxacin was 16.5 mm, while for ciprofloxacin was 16.9 mm, which shows that ciprofloxacin is more effective than levofloxacin against clinical isolates of *Klebsiella pneumoniae*. The result is in agreement with the work of Matsuzaki *et al.*[12] This study revealed that clinical isolates collected from Karachi were susceptible to both antibiotics. It is apparent from the disk diffusion susceptibility study that ciprofloxacin and levofloxacin possesses reasonably good antibacterial activity against all tested clinical isolates.

4. CONCLUSION

On the basis of the present study it can be concluded that levofloxacin and ciprofloxacin are two very good antibacterial agents. However, different clinical isolates have started to develop resistance against these antibiotics, which is alarming. It is expected that the present work will be a milestone in the antibacterial treatment and will bring new ideas in the field of antimicrobial chemotherapy that will enable the physicians, practitioner and pharmacist to select the most appropriate and effective quinolone antibiotics in the treatment of various infectious diseases.

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