# Comparison of the antibacterial activities of different brands of Ciprofloxacin

Comparación de la actividad antibacterial de diferentes marcas de Ciprofloxacina

**Muhammad Shahid NAZIR MUGHAL✉, Muhammad Tahir ASGHAR, Muhammad Atif ZIA and Tariq ISMAIL**

Pharmaceutical Lab, Punjab Institute of Paramedical Studies. 13 Mamdot Block Mustafa Town Lahore Pakistan.
E-mail: shahidbiochemist@yahoo.com ✉ Corresponding author

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## ABSTRACT

The present study was carried out to evaluate and compare the antibacterial susceptibility of Gram-positive and Gram-negative bacteria to Cyrocin (Ciprofloxacin). The following three bacterial strains were used: *Staphyloccocus aureus* [ATCC 25923], *Escherichia coli* [ATCC 25922] and *Pseudomonas aeruginosae* [ATCC 27853]. Standard commercial discs of definite potency are used as reference standard (Ciprofloxacin 5µg [CTO425B - OXOID Ltd. UK]). The test products were 250 mg and 500 mg tablets of the following brands: Cyrocin (Highnoon Laboratories Limited), Ciproxin (Bayer Pharma (Pvt) Ltd. – Pakistan), Mercip (Merck Marker (Pvt.) Ltd., Pakistan) and Axcin (Sandoz - Norvatis Pharma Ltd., Pakistan). The media used were: Nutrient Broth (Cat. No. 1.05443, Merck, Germany) and Mueller Hinton Agar [Oxoid]. The study showed no statistically significant difference in the results of different brands.

**Kew words**: Antibacterial properties, *Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosae*, Ciprofloxacin

## RESUMEN

El presente estudio se realizó para evaluar y comparar la susceptibilidad antibacterial de las bacterias Gram-positiva y Gram-negativa al Cyrocin (Ciprofloxacina). Se usaron las cepas bacetriales *Staphyloccocus aureus* [ATCC 25923], *Escherichia coli* [ATCC 25922] y *Pseudomonas aeruginosae* [ATCC 27853]. Se utilizaron discos comerciales estandars de potencia definida como estandar de referencia (Ciprofloxacin 5µg [CTO425B - OXOID Ltd. UK]). Los productos evaluadoes fueron tabletas de 250 mg y 500 mg de las siguientes marcas: Cyrocin (Highnoon Laboratories Limited), Ciproxin (Bayer Pharma (Pvt) Ltd. – Pakistan), Mercip (Merck Marker (Pvt.) Ltd., Pakistan) y Axcin (Sandoz - Norvatis Pharma Ltd., Pakistan). Los medios usados fueron: Nutrient Broth (Cat. No. 1.05443, Merck, Germany) and Mueller Hinton Agar [Oxoid]. El estudio mostró diferencias estadísticamente no significativas en los resultados de las diferentes marcas,

**Palabras clave**: Propiedades antibacteriales, *Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosae*, Ciprofloxacina

## INTRODUCTION

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agents under suitable conditions to inhibit bacterial growth *in vitro* (Inhibitory effect on micro-organism) (Bauer *et al.* 1966).

For evaluating the safety and effectiveness of antibiotic products, several types of antimicrobial susceptibility (sensitivity) tests are recommended. The choice of the method depends on local needs and resources, however, the disk diffusion test has a long and successful track record; it is still the most common test used for antimicrobial susceptibility testing. In this method, the paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with test organism. A concentration gradient of the antibiotic forms by diffusion from the disc and growth of test organism is inhibited at a distance from the disc that is related among other factors to the susceptibility of the organism.

The modified "Kirby Bauer Method" is the recommended method by National Committee on Clinical Laboratory Services (NCCLS-USA) subcommittee on Antimicrobial Susceptibility testing (Bauer *et al.* 1966). The Bauer Kirby procedure has been standardized to correlate the zone diameter

produced by the fixed amount of antimicrobial agent in the disc with an MIC for the drug–organism combination. The results may be interpreted as resistant, intermediate, moderately susceptible or susceptible. The term intermediate is important. It generally means that the result is inconclusive for that drug-organism combination. The term moderately susceptible is applied to those situations where a drug may be used for infections in a particular body site, e.g. cystitis, because it is highly concentrated in the urine. The interpretive standards for Ciprofloxacin 5 µg disc are given by National committee for clinical laboratory standards is: Resitatant ≤ 15; Intermediate 16-20 y Susceptible ≥ 21.

Ciprofloxacin (Cipro®) was discovered in 1960s by Bayer. Its discovery stemmed from researchers in the 1960s looking for an alternative treatment to malaria. Cipro® was approved in 1987 by the U.S. Food and Drug Administration as a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Since then it has been prescribed to over 500 million patients worldwide. Cipro® has been approved for the treatment of 14 types of infection including respiratory and urinary tract infections, skin, and other gastro-intestinal infections (SIS, 1987). Cipro® is the most widely used fluoroquinolone antibiotic in the world, which testifies to its wide range of uses. It is also the first antibiotic to be approved specifically for an indication associated with the intentional use of a lethal biological weapon (Hilliard *et al.* 1995). Cipro is available in three different forms: Tablets, Oral Suspension (strawberry-flavored liquid to be taken by mouth), and I.V. (which a doctor or nurse injects directly into the bloodstream) (Drusano *et al.* 1986).

Because of its general safety, potency and broad spectrum activity, Ciprofloxacin was initially reserved as a "last-resort" drug for use on difficult and drug-resistant infections. As with any antibiotic, however, increasing time and usage has led to an increase in Ciprofloxacin-resistant infections, mainly in the hospital setting. Also, implicated in the rise of resistant bacteria is the use of lower-cost, less potent fluoroquinolones, and the widespread addition of Ciprofloxacin and other antibiotics to the feed of farm animals, which leads to greater and more rapid weight gain, for reasons which are not clear (Brouwers, 1992). The toxicity of drugs that are metabolised by the cytochrome P450 system is enhanced by concomitant use of some quinolones (Janknegt, 1990). They may also interact with the GABA A receptor and cause neurological symptoms; this is further augmented by certain non-steroidal anti-inflammatory drugs (Krishek and Smart, 2001).

The present study was carried out to evaluate and compare the antibacterial susceptibility of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia-coli* and *Pseudomonas aeruginosae*) bacterial strains to Cyrocin (Ciprofloxacins) 250 mg and 500 mg tablets of Highnoon Laboratories and three other leading brands of the same drug.

## MATERIAL AND METHODS

### Test organisms

The following three bacterial strains were used for the study:

| | |
|---|---|
| *Staphyloccocus aureus* | [ATCC 25923] |
| *Escherichia coli* | [ATCC 25922] |
| *Pseudomonas aeruginosae* | [ATCC 27853] |

### Reference standard

Standard commercial discs of definite potency are used as reference standard (Ciprofloxacin 5µg [CTO425B - OXOID Ltd. UK])

### Test products

The 250 mg and 500 mg tablets of the following brands were tested: Cyrocin (Highnoon Laboratories Limited), Ciproxin (Bayer Pharma (Pvt) Ltd. – Pakistan), Mercip Merck Marker (Pvt.) Ltd., Pakistan) and Axcin (Sandoz - Norvatis Pharma Ltd., Pakistan).

### Media

Nutrient Broth (Cat. No. 1.05443, Merck, Germany) and Mueller Hinton Agar [Oxoid].

### Preparation of Turbidity Standard

The turbidity standard was prepared by pouring 0.6ml of a 1% (10 g $L^{-1}$) of solution of Barium chloride dehydrate into a100ml graduated cylinder and making up the volume to 100ml with 1% (10ml/l) sulfuric acid.

**Preparation of antimicrobial susceptibility test discs**

**Standard discs of Ciprofloxacin** (Andrews, 2001).

Ciprofloxacin sensitivity disc (5µg) of OXOID- UK were used as a Reference Standard.

**Preparation of test disc**

Discs (6mm in diameter) were punched out from 47 mm Petri Pad (Millipore Corporation, USA) and placed in Petri dishes allowing a distance of 2-4 mm between each disc and sterilized in a hot air oven at 160°C for 1 hour.

The average weight of five tablets was taken and the tablets were ground and the powder equivalent to 50 mg was taken in a 100mL volumetric flask. Added 15-20 mL distilled water into the flask and sonicated for few minutes and made up the volume upto the mark. An aliquot of 0.01mL (10µL) was pipetted onto a separate disc incubated at 37°C for 1 hour placed in labeled air tight container and kept in refrigerator at 4°C until use.

**Procedure for inoculation of plates and application of plates (The Modified Kirby Bauer Method)** (Barry *et al.* 1980).

The inoculum is prepared and disc is applied as per following procedure:

**Inoculum *Preparation***

1. To prepare the *inoculum* from culture plate, touch with a loop the tops of each 3.5 colonies of similar appearance of the organism to be tested.

2. To make the *inoculum* from a pure culture, a loopful of confluent growth is similarly suspended in saline.

3. Compare the tube with turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline. Proper adjustment to the turbidity of the *inoculum* is essential to ensure that the resulting lawn growth is confluent or almost confluent.

**Inoculation of plates and application of discs**

1. The plates were inoculated by dipping a sterile swab into the *inoculum*. The excess *inoculum* was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.

2. The swab were streaked all over the surface of the medium three times rotating the plates through an angle of 60° after each application. Finally, the swab was passed around the edge of the agar surface. The agar was left to dry for a few minutes at room temperature with the lid closed. The antibiotic discs were placed on the inoculated plates using a sterile forceps.

3. The plates were placed in an incubator at 35°C within 30 minutes of preparation in a $CO_2$ free atmosphere.

4. After overnight incubation, the diameter of each zone was measured and recorded in 'mm'.

## RESULTS AND DISCUSSION

The study was conducted to compare the antibacterial susceptibility of Highnoon brands of Ciprofloxacin (*i.e.* Cyrocin) 250 mg and 500 mg tablets with the pure Ciprofloxacin (as standard) and three other leading brands of Ciprofloxacin tablets of same strength.

The results of the study in terms of inhibition zone diameters produced by the 5 µg potency discs are given in tables 1 and 2. Also, the photograph of the plates with the zone of inhibition of different brands of Ciprofloxacin tablets against the tested bacterial strains is given in figure 1.

The comparison of the results with the NLCCS Control limits for monitoring inhibitory zone diameters (mm) shows that all the results fall within the acceptance range (NCCLS, 1994). The control limits for monitoring inhibitory zone diameter with 5 µg disc content of Ciprofloxacin for the bacterial strains is given below:

*Escherichia coli* (ATCC#25922): 30-40mm
*Staphylococcus aureus* (ATCC#25923): 22-30mm
*Pseudomonas aeruginosae* (ATCC#27853): 25-33mm

The results for 250 mg tablets were median whereas the results for 500 mg tabs fall within the upper range.

Apparantly, all the results are comparable and are similar than standard. Also, the results of Ciproxin [Bayer] showed the most consistent zones of inhibition against three studied bacterial strains followed by Mercip [Merck], Axcin [Sandoz] and Cyrocin [Highnoon].

Table 1. Antimicrobial susceptibility testing of different brands of Ciprofloxacin 250 mg tablets

| Bacterial Strains | Sample No. | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Standard | Axcin | Ciproxin | Cyrocin | Mercip |
| *Escherichia coli* [ATCC # 25922] | 1 | 34.96 | 35.37 | 35.65 | 35.15 | 34.90 |
| | 2 | 33.71 | 34.90 | 35.15 | 34.85 | 34.40 |
| | 3 | 33.85 | 35.20 | 34.70 | 34.65 | 34.70 |
| | Avg. | 34.17 | 35.16 | 35.17 | 34.88 | 34.67 |
| | STDEV | 0.68 | 0.24 | 0.48 | 0.25 | 0.25 |
| *Staphylococcus Aureus* [ATCC # 25923] | 1 | 24.93 | 26.15 | 26.07 | 25.80 | 25.13 |
| | 2 | 24.71 | 25.50 | 25.45 | 25.30 | 24.80 |
| | 3 | 24.80 | 24.90 | 26.10 | 25.95 | 25.30 |
| | Avg. | 24.81 | 25.52 | 25.87 | 25.68 | 25.08 |
| | STDEV | 0.11 | 0.63 | 0.37 | 0.34 | 0.25 |
| Pseudomonas aeruginosa [ATCC # 27853] | 1 | 27.68 | 28.69 | 29.07 | 28.28 | 28.03 |
| | 2 | 27.20 | 28.10 | 28.10 | 27.70 | 27.40 |
| | 3 | 26.90 | 27.90 | 28.65 | 27.90 | 27.75 |
| | Avg. | 27.26 | 28.23 | 28.61 | 27.96 | 27.73 |
| | STDEV | 0.39 | 0.41 | 0.49 | 0.29 | 0.32 |

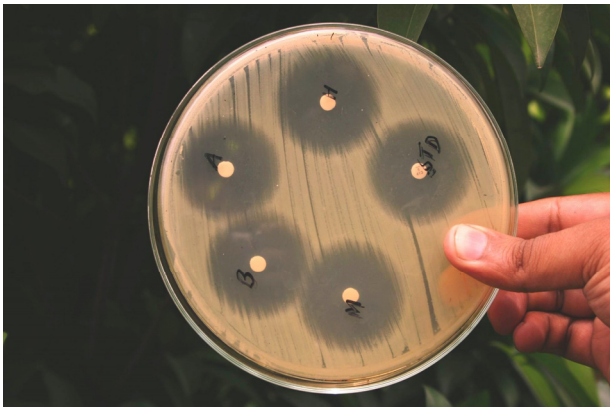
Avg.: Average; STDEV: Standard Deviation

Table 2. Antimicrobial susceptibility testing of different brands of Ciprofloxacin 500 mg tablets.

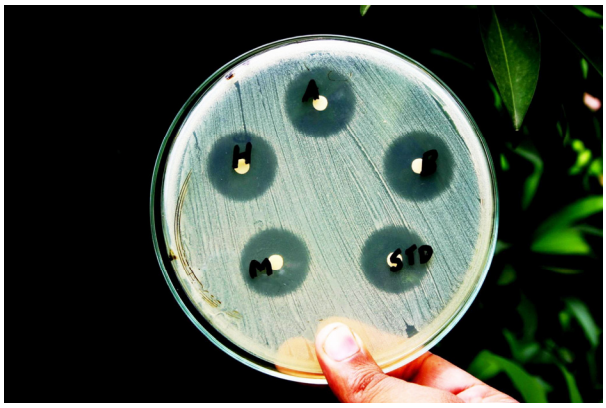
| Bacterial Strains | Sample No. | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Standard | Axcin | Ciproxin | Cyrocin | Mercip |
| *Escherichia coli* [ATCC # 25922] | 1 | 33.72 | 34.00 | 35.00 | 34.20 | 33.97 |
| | 2 | 34.10 | 33.82 | 37.45 | 33.80 | 37.99 |
| | 3 | 33.06 | 36.62 | 34.47 | 36.15 | 34.50 |
| | Avg. | 33.63 | 34.81 | 35.64 | 34.72 | 35.49 |
| | STDEV | 0.53 | 1.57 | 1.59 | 1.26 | 2.18 |
| *Staphylococcus Aureus* [ATCC # 25923] | 1 | 28.00 | 27.50 | 28.77 | 27.00 | 27.98 |
| | 2 | 26.90 | 27.19 | 27.32 | 26.99 | 26.84 |
| | 3 | 27.00 | 26.92 | 29.00 | 27.41 | 27.00 |
| | Avg. | 27.30 | 27.20 | 28.36 | 27.13 | 27.27 |
| | STDEV | 0.61 | 0.29 | 0.91 | 0.24 | 0.62 |
| Pseudomonas aeruginosa [ATCC # 27853] | 1 | 27.95 | 30.02 | 30.03 | 29.27 | 29.60 |
| | 2 | 31.00 | 32.19 | 32.00 | 32.00 | 32.42 |
| | 3 | 28.37 | 31.00 | 32.72 | 34.68 | 32.00 |
| | Avg. | 29.11 | 31.07 | 31.58 | 31.98 | 31.34 |
| | STDEV | 1.65 | 1.09 | 1.39 | 2.71 | 1.52 |

Avg.: Average; STDEV: Standard Deviation

The statistical analysis revealed that there is no significant difference in the results for different brands and statistically the antibacterial activities of all the brands are similar.



*Escherichia coli* [ATCC #. 25922]



*Staphylococcus aureus* [ATCC #. 25923]

*Pseudomonas aeruginosae* [ATCC #. 27853]

Figure 1. Zone of inhibition of different brands of Ciprofloxacin tablets against the tested bacterial strains.

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