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Comparative Study of the Antibacterial Activity of Selected Ciprofloxacin Tablet Brands Available in Iraq

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Abstract

Ensuring the microbiological quality of antibiotics is essential for effective treatment and resistance prevention. In Iraq, several ciprofloxacin tablet brands are commercially available, yet limited data exist on their *in vitro* antibacterial potency. This study compared five ciprofloxacin brands from Switzerland, Germany, Iraq, Jordan, and Turkey. Antibacterial activity was assessed using disk diffusion, broth microdilution for Minimum Inhibitory Concentration (MIC), and time-kill kinetics assays, following CLSI standards. Significant differences in antibacterial activity were observed. Brand A (Switzerland) and brand B (Germany) showed the largest inhibition zones (35.7 mm and 34.3 mm for *Escherichia coli*) and the lowest MICs (0.008 µg/mL for *E. coli*, 0.25 µg/mL for *Pseudomonas aeruginosa*, and 0.125 µg/mL for *Staphylococcus aureus*), matching the reference standard. Brand E (Turkey) had the smallest zones (30.7 mm) and the highest MICs (0.031-1.0 µg/mL). In time-kill assays, brands A and B achieved 3-log bacterial reduction within 4-6 hours, while brands C, D, and E required 6-10 h. Correlation analysis revealed strong relationships between antibacterial performance and price ($r = 0.896$, $p = 0.016$) and tablet hardness ($r = 0.782$, $p = 0.038$), while disintegration time and weight variation showed no significant impact.

Major Findings: Swiss and German ciprofloxacin brands demonstrated superior antibacterial efficacy, reflected in larger inhibition zones, lower MICs, and faster killing rates. These findings emphasise the need for mandatory microbiological quality testing of antibiotics marketed in Iraq.

Keywords: Antibacterial Activity, Ciprofloxacin, Disk Diffusion, Iraq, Minimum Inhibitory Concentration, Pharmaceutical Quality, Time-Kill Kinetics

1. Introduction

Substandard and falsified antibiotics remain a major global health threat, especially in low- and middle-income countries. Poor-quality medications—whether counterfeit, improperly manufactured, or degraded—can result in treatment failure, antimicrobial resistance, increased morbidity and mortality, and loss of trust in healthcare systems[1,2]. According to a WHO report in 2003, approximately 28% of antibiotics were falsified[2]. Countries such as China, India, and Jordan have been identified as frequent sources of poor-quality pharmaceuticals, and Iraq is also a known site of local substandard production. Resistance trends among both Gram-positive and Gram-negative bacteria further compound the dangers of inadequate antibiotic quality[3].

Ciprofloxacin is a widely prescribed second-generation fluoroquinolone antibiotic with broad-spectrum

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bactericidal activity against various Gram-positive and Gram-negative organisms. Its antibacterial effect results from inhibition of DNA gyrase and topoisomerase IV—enzymes essential for bacterial DNA replication, transcription, and repair[4]. Inhibition of these targets leads to cell death. Ciprofloxacin is highly effective against *E. coli*, *P. aeruginosa*, and *Haemophilus influenzae*, and exhibits moderate activity against *S. aureus* and *Streptococcus pneumoniae*[4]. Although pharmacopeial standards define the identity and amount of active pharmaceutical ingredients (APIs), they do not directly assess antibacterial potency, which may be influenced by excipients, manufacturing methods, and bioavailability[5,6].

In Iraq, the pharmaceutical market contains several ciprofloxacin tablet brands varying in origin and cost. However, due to limited regulatory oversight stemming from economic sanctions and political instability, substantial differences in product quality persist[7,8]. While local manufacturers routinely test physical and chemical parameters such as hardness and weight variation, microbiological activity is rarely assessed[8]. This raises concerns over the therapeutic efficacy of some marketed antibiotics.

This lack of microbiological evaluation reflects a critical quality gap. Studies from countries such as Pakistan, Bangladesh, Saudi Arabia, and the UAE have demonstrated wide variability in the potency of generic antibiotics, and in some cases, complete absence of active ingredients[9]. Antibacterial activity, which directly affects clinical efficacy, is rarely considered in routine quality assurance despite its central importance[6,10].

Therefore, this study aimed to evaluate the *in vitro* antibacterial activity of five ciprofloxacin tablet brands marketed in Iraq using disk diffusion, broth microdilution (MIC), and time-kill kinetics methods following CLSI guidelines, and to assess correlations between antibacterial efficacy, tablet price, and physical quality attributes to determine their therapeutic values.

2. Materials and Methods

2.1 Material

2.1.1 Study Design

Research compared antibacterial properties between different brands of ciprofloxacin tablets available throughout the Iraqi pharmaceutical sector using an experimental approach. The research took place at the Microbiology Laboratory of the Technical Institute in Al-Mussaib, which belongs to Al-Furat Al-Awsat Technical University.

2.1.2 Ciprofloxacin Brands

The five ciprofloxacin drug brands that Community pharmacies in Baghdad made available came with 500 mg ciprofloxacin hydrochloride content. The research obtained five ciprofloxacin tablets with 500 mg of ciprofloxacin hydrochloride from multiple manufacturer locations that followed available market brands. The identities of pharmaceutical tablets under institutional protection were identified as A, B, C, D together with E. The details about the five ciprofloxacin drug brands can be found in Table 1.

The experiments utilised USP ciprofloxacin hydrochloride reference standard (Sigma-Aldrich, USA) as a control.

2.1.3 Bacterial Strains

Three clinical bacterial isolates were used in this study: *E. coli* (EC-K1), *P. aeruginosa* (PA-K1), and *S. aureus* (SA-K1). These isolates were obtained from diagnostic samples at Al-Hussein General Hospital, Karbala, Iraq. They were identified and confirmed using standard microbiological and biochemical methods. All procedures involving bacterial handling were performed under Biosafety Level 2 (BSL-2) conditions. The strains were preserved on nutrient agar slants and stored at 4°C, with subculturing every two weeks to ensure viability.

2.2 Method

2.2.1 Preparation of Ciprofloxacin Solutions

The measurement process involved the individual weighing of 20 tablets for each brand to obtain their weight average. The tablets were then crushed to a fine

Table 1. Information about the ciprofloxacin brands included in the study

Brand code	Manufacturing country	Price (IQD)
Brand A	Switzerland	15,000
Brand B	Germany	13,000
Brand C	Iraq	5,000
Brand D	Jordan	4,000
Brand E	Turkey	3,000

Note: IQD = Iraqi Dinar

powder using a mortar and pestle. A quantity of powder equivalent to 50 mg of ciprofloxacin hydrochloride was accurately weighed and transferred to a 100 mL volumetric flask. The powder was dissolved in 0.1 N hydrochloric acid, sonicated for 15 min, and the volume was made up to 100 mL with the same solvent to obtain a stock solution of 500 µg/mL. The solution was filtered through a 0.45 µm membrane filter to remove any undissolved particles. For the reference standard, 50 mg of USP ciprofloxacin hydrochloride was dissolved in 100 mL of 0.1 N hydrochloric acid to obtain a stock solution of 500 µg/mL.

Working solutions of appropriate concentrations were prepared by diluting the stock solutions with sterile distilled water for the various assays.

2.2.2 Disk Diffusion Assay

Experimental procedures followed the protocols provided by Clinical and Laboratory Standards Institute (CLSI)[11]. Bacterial solutions with concentrations of around 1-2 × 10^8 CFU/mL were adjusted to a 0.5 McFarland standard before they were used to inoculate Mueller-Hinton agar plates. Each brand and reference standard received 10 µL of 5 µg/disk ciprofloxacin solution, which was applied to sterile 6 mm filter paper disks. Sterile forceps were used to place the discs, which received 10 µL of ciprofloxacin solution (5 µg/disk) after allowing them to dry at room temperature. The culture incubated at 35 ± 2°C under 18-24 h developed at this temperature range. The measurement of inhibition zones surrounding test discs was conducted using a digital calliper after incubation. The assay measurements were performed three times for each brand against each bacterial strain, and the obtained values were calculated as mean zone diameters.

2.2.3 Minimum Inhibitory Concentration (MIC) Determination

The MIC values for the ciprofloxacin brands were determined using the broth microdilution method[12]. Microtiter plates contained Mueller-Hinton broth to dilute ciprofloxacin solutions from each brand and the reference standard at eight concentrations, ranging from 8 µg/mL to 0.0039 µg/mL. Control wells containing only bacteria and broth (no ciprofloxacin) were included to monitor natural bacterial growth.

To prepare bacterial cultures, Mueller-Hinton broth was used to dilute the bacterial suspension to the 0.5 McFarland standard, which corresponds to approximately 5 × 10^5 CFU/mL. A 100 µL bacterial inoculum was added to each well, resulting in a final volume of 200 µL per well. The plates were incubated at 35 ± 2°C for 18-24 h.

Bacterial growth was assessed by visual inspection of the wells for turbidity (indicating bacterial growth) and Optical Density (OD) measurements at 600 nm using a microplate reader. The MIC was defined as the lowest concentration of ciprofloxacin that resulted in no visible bacterial growth or a significant reduction in OD, indicating bacterial inhibition.

The assay was performed in duplicate for each bacterial strain and ciprofloxacin brand, and the geometric mean MIC values were calculated for each brand.

2.2.4 Time-Kill Kinetics Assay

Bactericidal effects between different ciprofloxacin brands were measured through time-kill kinetics testing according to Gajic *et al.*[12], with minor experimental changes. Laboratory technicians prepared bacterial solutions by using Mueller-Hinton broth for suspending bacteria at approximately 5 × 10^5 CFU/mL. The testing solutions of Ciprofloxacin obtained from brands and the reference standard were added to bacterial suspensions at double and quadruple MIC values discovered from the previous assay. The mixtures were incubated at 35 ± 2°C with shaking at 150 rpm. Aliquots (0.1 mL) were removed at 0, 2, 4, 6, 8, and 24 h, serially diluted in sterile saline, and plated on Mueller-Hinton agar plates. The plates were incubated at 35 ± 2°C for 24 h, and the number of Colony-Forming Units (CFU) was counted. The log10 CFU/mL was calculated and plotted against time to generate time-kill curves.

Bactericidal activity was defined as a ≥3-log10 reduction (99.9% killing) in the number of viable bacteria compared to the initial inoculum. The time required to achieve this level of killing was determined for each brand at each concentration.

2.3 Statistical Analysis

Data from the disk diffusion assays, MIC determination, and time-kill kinetics were analysed using IBM SPSS Statistics software (version 26.0). The results were presented as mean values with Standard Deviations (SD). To compare the antibacterial activity among the ciprofloxacin brands, one-way Analysis of Variance (ANOVA) was conducted, followed by Tukey's

Honestly Significant Difference (HSD) test for pairwise comparisons. Differences between the brands were considered statistically significant if the p-value was less than 0.05.

For the MIC values, the geometric mean was calculated for each bacterial strain and ciprofloxacin brand combination to summarise the central tendency of the results. The geometric mean was chosen due to the skewed distribution of antimicrobial susceptibility data. Additionally, correlation analysis was performed to examine the relationship between antibacterial performance (inhibition zone size, MIC values, time-kill kinetics) and factors such as price and tablet hardness.

3. Results

3.1 Antibacterial Activity of Ciprofloxacin Brands

The antibacterial activity of five ciprofloxacin tablet brands available in Iraq was evaluated against three standard bacterial strains using the disk diffusion method. Table 2 presents the mean zone of inhibition diameters (in millimetres) for each brand against each bacterial strain.

All ciprofloxacin brands (Figure 1) demonstrated antibacterial activity against the tested organisms, with zone diameters exceeding the susceptibility breakpoints established by Clinical and Laboratory Standards Institute (CLSI)[11]: ≥21 mm for *E. coli* and *S. aureus*, and ≥21 mm for *P. aeruginosa*. However, significant differences were observed in the zone diameters between the brands for all three bacterial strains ($p<0.001$).

The assessment showed brand A produced the largest *E. coli* inhibition zone of 35.7 ± 0.6 mm, which matched the reference standard measurement of 36.3 ± 0.6 mm. Among the brands, brand E had the smallest size of zone inhibition (30.7 ± 0.6 mm), with brands B through E showing successively decreasing zones. The post-hoc Tukey's HSD test demonstrated significant differences ($p<0.05$) between brand A and brands C, D, E, yet did not establish any distinctions ($p = 0.142$) between brand A and rademark B.

Table 2. Mean Zone of Inhibition Diameters (mm) of Ciprofloxacin Brands Against Test Organisms

Brand	*E. coli*	*P. aeruginosa*	*S. aureus*
A	35.7 ± 0.6	30.3 ± 0.6	27.7 ± 0.6
B	34.3 ± 1.2	29.7 ± 0.6	26.3 ± 1.2
C	32.7 ± 0.6	27.3 ± 1.2	24.7 ± 0.6
D	31.3 ± 1.2	26.7 ± 0.6	23.3 ± 1.2
E	30.7 ± 0.6	25.3 ± 0.6	22.7 ± 0.6
Control	36.3 ± 0.6	30.7 ± 0.6	28.3 ± 0.6
p-value	<0.001*	<0.001*	<0.001*

Note: Values are presented as mean ± standard deviation (n=3).
*Statistically significant difference ($p<0.05$) by one-way ANOVA.

The inhibitory patterns of *P. aeruginosa* and *S. aureus* growth were similar in the experiment. Brand A demonstrated the largest inhibitory zone size (30.3 ± 0.6 mm and 27.7 ± 0.6 mm) among all test brands during the antibacterial test. The four remaining brands started after brand A, as shown by the zones of inhibition rankings. The antibacterial assay revealed that brand A possessed similar zones compared to brand B but demonstrated essential differences with brands C, D, and E regarding both bacterial testers ($p<0.05$).

3.2 Minimum Inhibitory Concentration (MIC) Results

The MIC values of the five ciprofloxacin brands against the three bacterial strains were determined using the broth microdilution method. Table 3 presents the geometric mean MIC values (in µg/mL) for each brand against each bacterial strain.

All ciprofloxacin brands (Figure 2) demonstrated potent antibacterial activity, with MIC values well below the susceptibility breakpoints established by Clinical and Laboratory Standards Institute (CLSI)[11]: ≤1 µg/mL for *E. coli* and *S. aureus*, and ≤1 µg/mL for *P. aeruginosa*. However, significant differences were observed in the MIC values between the brands for all three bacterial strains ($p<0.05$). For *E. coli*, Brands A and B exhibited the lowest MIC values (0.008 µg/mL), identical to the reference standard. Brands C and D showed slightly higher MIC values (0.016 µg/mL), while Brand E had the highest MIC value (0.031 µg/mL). Mann-Whitney U tests revealed significant differences between Brands A/B and Brand E ($p<0.05$), but not between Brands A/Band Brands C/D ($p = 0.067$).

The reference standard maintained its position as the lowest MIC value (0.25 µg/mL) against *P. aeruginosa* through Brands A and B. An analysis found that Brand E showed the maximum MIC value of 1.0 µg/mL, but Brands C and D demonstrated an MIC measurement of 0.5 µg/mL. Both Brands A/B and Brand E exhibited statistically

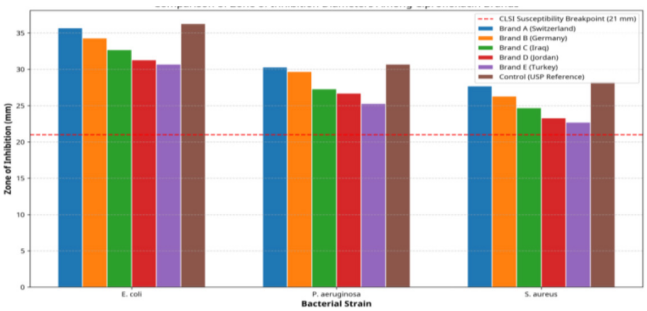


Figure 1. Comparison of zone of inhibition diameters among ciprofloxacin brands.

Table 3. Minimum Inhibitory Concentration (MIC) values (μg/mL) of ciprofloxacin brands against test organisms

Brand	*E. coli*	*P. aeruginosa*	*S. aureus*
A	0.008	0.25	0.125
B	0.008	0.25	0.125
C	0.016	0.5	0.25
D	0.016	0.5	0.25
E	0.031	1.0	0.5
Control	0.008	0.25	0.125
p-value	0.003*	0.002*	0.004*

Note: Values are presented as geometric mean (n=3). *Statistically significant difference (p<0.05).

significant differences in their results (p<0.05), yet no such variances existed between Brands A/B and Brands C/D (p = 0.089).

The lowest minimum inhibitory concentration values of 0.125 μg/mL for *S. aureus* were found in the reference standard and Brands A and B. The MIC value from Brands E reached 0.5 μg/mL, yet Brands C and D showed MIC values at 0.25 μg/mL. Analysis revealed a p<0.05 value between Brands A/B and Brand E, along with a p = 0.076 value between Brands A/B and Brands C/D, indicating their antibacterial activity.

3.3 Time-Kill Kinetics Results

Time-kill kinetics assays were performed to evaluate the bactericidal activity of the five ciprofloxacin brands over time. Figure 3 illustrates the time-kill curves for each brand against *E. coli* at 2× MIC concentration. Similar patterns were observed for the other bacterial strains and concentrations. All ciprofloxacin brands demonstrated concentration-dependent bactericidal activity against the tested organisms. At 2× MIC, Brands A and B achieved a

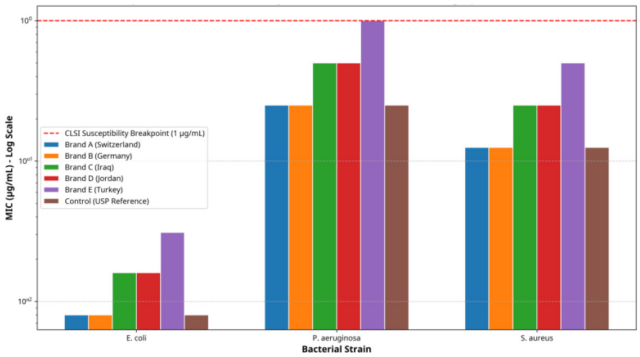


Figure 2. Comparison of Minimum Inhibitory Concentration (MIC) values among ciprofloxacin brands.

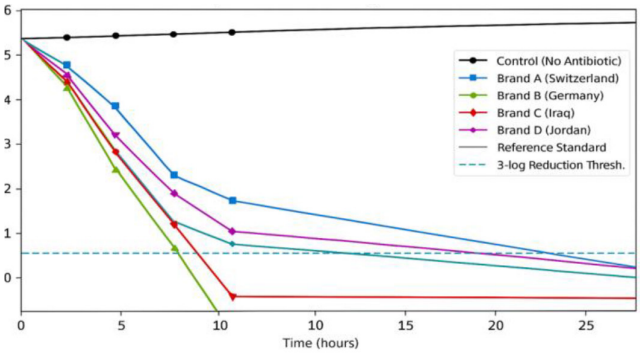


Figure 3. Time-kill curves of ciprofloxacin brands against *E. coli* at 2× MIC.

3-log reduction in viable count (99.9% killing) of *E. coli* within 4 h, comparable to the reference standard. Brands C and D required 6 hours to achieve the same level of killing, while Brand E required 8 h (Figure 3).

For *P. aeruginosa*, Brands A and B achieved a 3-log reduction within 6 h at 2× MIC, while Brands C, D, and E required 8, 10, and 12 h, respectively. For *S. aureus*, Brands A and B achieved a 3-log reduction within 6 h at 2× MIC, Brands C and D within 8 h, and Brand E within 10 h.

At 4× MIC, all brands demonstrated accelerated killing kinetics, with Brands A and B achieving a 3-log reduction of all tested organisms within 4 h, Brands C and D within 6 h, and Brand E within 8 h. Table 4 summarises the time required for each ciprofloxacin brand to achieve a 3-log reduction in viable count at 2× MIC concentration for each bacterial strain.

3.4 Correlation Analysis

Correlation analysis was performed to examine the relationships between antibacterial activity, price, and

Table 4. Time (hours) required to achieve 3-log reduction in viable count at 2× MIC

Brand	*E. coli*	*P. aeruginosa*	*S. aureus*
A	4	6	6
B	4	6	6
C	6	8	8
D	6	10	8
E	8	12	10
Control	4	6	6

Note: Values represent the time (in hours) required for each ciprofloxacin brand to achieve a 3-log reduction (99.9% killing) in viable bacterial count at 2× MIC concentration.

Table 5. Pearson Correlation Coefficients (r) between various parameters

Parameter 1	Parameter 2	rvalue	pvalue
Zone diameter	MIC values	-0.943	<0.001*
Price	Zone diameter	0.896	0.016*
Price	MIC values	-0.874	0.023*
Hardness	Zone diameter	0.782	0.038*
Disintegration time	Zone diameter	-0.651	0.113
Weight variation	Zone diameter	-0.423	0.345

Note: *Statistically significant correlation (p<0.05).

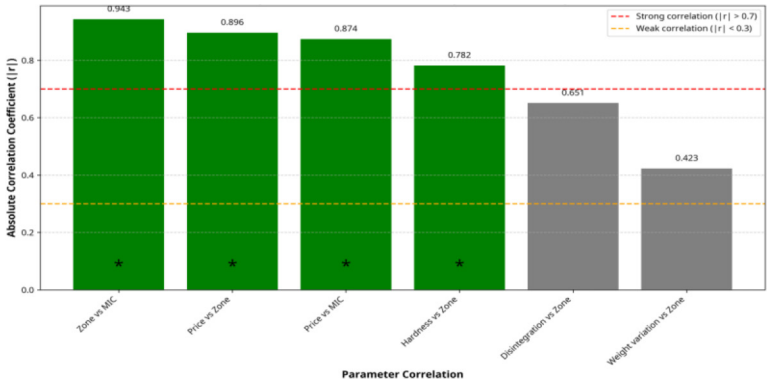


Figure 4. Correlation analysis between antibacterial activity, price, and quality parameters.

previously determined quality parameters. Table 5 and Figure 4 present the Pearson correlation coefficients for these relationships. An inverse relationship existed between antibacterial activity measurements, as indicated by the substantial negative correlation (r = -0.943, p<0.001) between zone diameter and MIC values.

4. Discussion

Better antibacterial performance traits were exhibited by more expensive brands since both zone diameter and MIC showed strong positive correlations with price (r = 0.896, p = 0.016 and r = -0.874, p = 0.023).

The quality characteristic of tablet hardness showed a statistically meaningful positive relationship (r = 0.782) to the inhibition zone dimensions (p = 0.038) between tested brands. The antibacterial activity showed no significant statistical relationship with either disintegration time or weight variation because their p-values exceeded 0.05.

This study evaluated the antibacterial activity of five ciprofloxacin tablet brands available in the Iraqi pharmaceutical market against three bacterial isolates using multiple complementary *in vitro* methods. The findings revealed significant variations in antibacterial potency across the brands, with observed differences correlating positively with price and certain physicochemical quality parameters. These results carry meaningful implications for healthcare professionals, patients, and regulatory authorities concerned with drug efficacy and quality assurance in Iraq.

All ciprofloxacin brands demonstrated antibacterial activity within the susceptibility breakpoints defined by CLSI, confirming that each contained adequate levels of active pharmaceutical ingredient[11]. However, notable variations were observed in inhibition zone diameters, MIC values, and bacterial kill rates, highlighting the variability in product performance. Brands A (Swiss) and B (German) consistently demonstrated superior potency, closely matching the USP reference standard. These findings align with previous studies reporting that ciprofloxacin formulations produced by well-established manufacturers in developed countries tend to exhibit higher pharmaceutical quality and bioefficacy[5,9].

The comparative potency of the brands appeared to follow a trend consistent with their country of manufacture, with locally produced and regionally sourced brands (Iraq,

Jordan, Turkey) showing relatively lower antibacterial activity. Although all brands remained within acceptable MIC limits, the reduced efficacy observed in Brand E suggests potential differences in manufacturing standards, formulation stability, and dissolution profiles[4]. Ciprofloxacin is known to be sensitive to formulation variables, and differences in excipients, granulation methods, and tablet coatings may all contribute to altered bioavailability and *in vitro* performance. Prior research has demonstrated that variability in dissolution rates can significantly influence antibacterial outcomes and therapeutic reliability[1,13].

Time-kill kinetics data further emphasised these disparities, with Brands A and B achieving a 3-log reduction in viable bacterial counts within 4-6 hours—faster than other brands. Rapid bacterial killing is particularly critical in clinical settings, as it reduces infection persistence, minimises treatment duration, and lowers the likelihood of resistance emergence[12]. Since all bacterial isolates were held constant, the observed variability reinforces that the product formulation, not the organism, was responsible for potency differences.

A positive correlation was observed between product price and antibacterial efficacy, although causality cannot be definitively established from this study. This finding aligns with previous work indicating that higher-priced ciprofloxacin brands in Iraq often reflect superior manufacturing practices and formulation quality[8]. Additionally, tablet hardness showed a meaningful positive correlation with antibacterial activity, suggesting a potential link between physical robustness and stability of the active ingredient. This observation is supported by reports that firmer tablets may preserve ingredient integrity and enhance dissolution stability[13]. In contrast, disintegration time and weight variation did not significantly correlate with antibacterial potency, underscoring that not all physical quality parameters equally predict therapeutic outcomes[6].

The findings of this study also align with global concerns about antibiotic quality variation, particularly in low- and middle-income countries. The World Health Organisation has highlighted that substandard and falsified medical products are more prevalent in regions with weak regulatory systems[2,7]. Thus, post-marketing surveillance studies such as this are essential for ensuring antibiotic efficacy and preventing the emergence of antimicrobial resistance.

Overall, the results support the need for routine microbiological evaluation of antibiotic products in pharmaceutical markets where regulatory oversight is inconsistent. Strengthening quality control standards and improving post-marketing surveillance are essential to ensure clinical efficacy, maintain public trust, and reduce the risk of antimicrobial resistance development.

5. Conclusion

Antibacterial properties of Iraq market-accessible ciprofloxacin tablet brands were studied through disk diffusion tests together with broth microdilution testing and time-kill kinetic analysis against *E. coli*, *P. aeruginosa*, and *S. aureus*. The manufacturer-listed activity standards were achieved, but the brands showed considerable variation in their antibacterial effects. All antibacterial tests demonstrated that Brand A, Switzerland and Brand B, Germany, surpassed the antibacterial performance of Iraqi Brand C, Jordanian Brand D, and Turkish Brand E. Studies demonstrated that antibacterial activism increases as brands increase their price points, thus making higher-priced items more powerful against bacteria. The antibacterial performance of tablets increased with higher tablet hardness values, which implies a potential relation between formulation quality levels and therapeutic outcomes. Research moving forward should analyse a broader selection of brands, clinical strains and realistic environmental conditions, such as shelf life and economic considerations, to develop better antibiotic management programs in Iraq and comparable regions.

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7. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors prepared this manuscript without the utilisation of generative AI or any AI-assisted technology. The authors developed the entire content without AI or generative AI tools.

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