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RESEARCH ARTICLE

IN VITRO ANTIMICROBIAL ACTIVITY EVALUATION FOR DIFFERENT PHARMACEUTICAL DOSAGE FORMS OF CIPROFLOXACIN IN ADEN-YEMEN

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Abstract

Ciprofloxacin (CIP) is classified as a second-generation fluoroquinolone structurally similar to nalidixic acid. It is a widely used antibiotic to treat different types of bacterial infections. The present study was carried out to evaluate the antimicrobial activity of three different dosage forms of CIP [tablets (Tab coded I, II, III), CIP infusion (Infusion coded I, II, III) and CIP eye drop (Eyedrop coded I, II, III)]. Three most commonly prescribed and dispensed brands for each dosage form were selected. All studied brands were within their shelf life. All brands examined by spectroscopy and the quantity of the active ingredients was with the permitted limits of British pharmacopeia (95-105%). The disk diffusion method was used to evaluate the antimicrobial activity of CIP against *E. coli* and *Staphylococcus Aureus*. The highest inhibition zone was at low concentration against *E. coli*, by Tab-II, Tab-I, and Tab-III tablets respectively. While in the case of infusion, the Infusion-III showed the highest inhibition zone, followed by Infusion-I and Infusion-II. In the case of *Staphylococcus Aureus*, all Tab I, II, and III have similar potency. At low concentration, Infusion II, III indicated similar while Infusion I had lower potency. However, all brands had slightly higher potency over the standard. All brands of eye drops showed nearly similar potencies against *Staphylococcus Aureus* with a slight superiority of Eyedrop-I over Eyedrop-II then Eyedrop-III in the highest concentration. All the brands of eye drops showed antimicrobial activity slightly lower than standard. Post-marketing surveillance is an essential issue to distinguish poor-quality medicines. The current study revealed that the marketed CIP pharmaceutical dosage forms showed reasonable antimicrobial activity except for the eyedrops dosage forms which showed slightly lower inhibition zone in comparison to standard.

Keywords: Ciprofloxacin, disk diffusion method, *E. coli*, and *Staphylococcus Aureus*.

1. Introduction

Ciprofloxacin (CIP) is classified as a second-generation fluoroquinolone structurally similar to nalidixic acid. It is effective against gram-positive and negative bacteria, commonly used in clinical and hospital cases **[1]**. In 1987 it was approved by the FDA to be the first oral broad-spectrum antibiotic **[2]** and included in the WHO essential drugs list **[3]**. It can be used orally and intravenously for several diseases such as; urinary tract infection, gastrointestinal and skin infection.

There are several brands of CIP tablets available within the drug delivery system worldwide as well as in Aden-Yemen. In Yemen, several medicines in the markets are either imported or locally manufactured. Various pharmaceutical dosage forms of CIP are available in Aden markets for both local or systemic application. The quality of the marketed drugs in Yemen is in doubt because the markets are submerged with the counterfeit and falsified medicines. According to a study carried in Yemen, about 80% of medicines enter into the country via illegal routes and about 40% were fake or of low quality **[4]**. This finding is a normal consequence of unstrict control and monitoring of drug manufacturing, importation, marketing, distribution, prescription, and consumption. Substandard and counterfeit medicines are the main cause of morbidity, mortality and the issue are more serious in case of antibiotics because these drugs may lead to developing resistance bacteria and treatment failure **[5, 6]**. According to the WHO report, about 10% of drugs were falsified worldwide, around 50% of these

were antimicrobial medication **[7]**. The falsified antimicrobials, either have no active pharmaceutical ingredients, poor quality, low quantity, or are with wrong ingredients **[8,9]**.

Bacterial resistance to the CIP was reported in the early nineties and is incessantly growing ever since **[10-12]**. CIP resistance spread globally and differs significantly among countries with the highest prevalence described in developing countries **[13]**. According to a study carried in Aden-Yemen to evaluate the antimicrobial resistance profiles for clinical specimens, the total antimicrobial resistance to CIP was 25% distributed as; *Klebsiella species* 41.66 %, *E. coli* 27.18%, *Pseudomonas species* 35.71 %, *Staphylococcus Aureus* 20.0% and *Enterobacter species* 20.0% **[14]**. The relatively high microbial resistance to CIP may be due to several reasons such as; overuse, self-medication, dispensing without physician prescription, and low antibiotic efficiency of the commercially available CIP in the Aden markets. Using substandard or falsified dosage forms leads to treatment failure and increasing bacterial resistance **[15,16]**. Low socioeconomic status of the Yemeni citizen forced them to select the low-priced drugs which may be of low quality. The over-prescription of antibiotics which exceeds the WHO recommendation value is another serious problem in Aden according to the study carried out in 2016 **[17]**. An important issue is the degradation of medicines due to improper storage conditions due to the continuous electricity shortage in this city. For all these reasons, the present study was carried out to evaluate the antimicrobial activity of three different dosage forms of CIP (tablets, infusion, and eye drops). Three most commonly prescribed and dispensed brands for each dosage form were selected. In-vitro disk diffusion method was used to evaluate the antimicrobial activity of CIP against *E. coli* and *Staphylococcus Aureus* using the Bauer-Kirby disk diffusion method **[18]**.

The literature review inducted the presence of similar comparative antimicrobial activity studies for different brands of CIP in many countries. A study conducted in Pakistan for four brands of CIP (250 and 500 mg) tablets, revealed that there were no significant differences among the studied brands **[19]**. An alternative study was performed in Tanzania to assess the antimicrobial effects of nine brands of CIP Tablets, the finding of this study indicated that a great variation in the antimicrobial activities among the verified brands, some of the brands showed poor antimicrobial quality **[20]**. In Nigeria, five brands of CIP tablets were evaluated and the result indicated slight variation in the activities of different brands, however, all of the brands were within the acceptable limit **[21]**. In Yemen, only one study related to the quality control of the CIP marketed in Sana'a pharmacies was conducted. Evaluation of several quality control tests for different brands of tablets was performed except the antimicrobial susceptibility test. All brands have an acceptable quality that met the official pharmacopeias **[22]**. In the current study, antimicrobial activity for the selected CIP was performed because it can estimate both potency and bioactivity of antibiotics, in contrast to the quantitative methods which only quantify the percentage of the active ingredients.

The post-marketing quality surveillance of the antimicrobial agents is an essential aspect to weed out the poor-quality medicines from the local markets. It should be routinely performed since the antimicrobial activities of antibiotics is inherently related to quality assurance, from production to supply and storage in the distribution chain, until the drug is consumed by the patient.

2. Experimental Section

2.1 *Materials and methods*

Test organisms:

The bacterial strains used for this study were:

- *Staphyloccocus aureus* [ATCC 25923].
- *Escherichia coli* [ATCC 25922].

Reference standard:

The standard of CIP-HCl was provided from the Modern pharma -Yemen as a gift.

Test products:

Three pharmaceutical dosage forms that were used (tablets, infusion, and eye drops), three brands for each dosage form. Tablet of 500 mg named (Tab I, II, III), infusion 2 mg mL-1 superscript named (Infusion I, II, III), and eye drop of 0.3% CIP named (Eyedrop I, II, III). All of the dosage forms were dissolved in water and diluted to get 0.25, 0.5, and 1 µg .5 μL^{-1} of CIP.

Media:

Mueller Hinton Agar (TM MEDIA-TITAN BIOTECH.LTD. India).

Preparation of Turbidity Standard (MacFarland Solution):

To prepare turbidity standard, exactly 0.6 ml of a 1% (10 g L^{-1}) of the Barium chloride dehydrate solution was poured into a100 ml graduated cylinder, and the volume made up to 100 mL with 1% sulfuric acid.

Preparation of Test Disc:

Discs (5mm in diameter) were punched out from 9 cm qualitative filter paper and placed in the Petri dish and

sterilized in a hot air oven at 120°C for 1 hour. Then amount equivalent to 1, 0.5, 0.25 µg. 5 μL^{-1} of CIP of standard (CIP-HCl) dissolved in water was pipetted onto a separate disc, the same procedure was carried out for all dosage forms.

Inoculum Preparation:

To prepare the inoculum from culture plate, touch with a loop the tops of each colony and dissolve it in sterile water or saline solution. Compare the tube with turbidity standard (i.e. 0.5 MacFarland 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 (approximately 1 x 10^7 CFU mL^{-1} of bacterial growth) lawn growth. The bacteria inoculated on the Muller Hinton agar (M.H.A).

Inoculation of plates and application of discs:

The plates were inoculated by dipping a sterile swab into the inoculum. The swab was streaked all over the surface of the medium three times rotating the plates through an angle of 60oC Superscript 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 with the lid closed. The antibiotic discs were placed onto the surface of the inoculated plates using sterile forceps, all steps were carried out under laminar flow. After overnight incubation, the diameter of each zone was measured and recorded in 'mm' **[19, 21]**.

Statistical data analysis:

Each concentration was repeated three times for standard and different pharmaceutical dosage forms, and the data were expressed as means. The difference in the inhibition zone for the three brands of each dosage form and standard as well as among different dosage forms was evaluated by using one-way ANOVA. Statistical analysis of data was carried out by using Graph Pad Prism v 6.0 b software.

3. Results

Resistance to antimicrobial agents is a worldwide problem. This problem is exacerbated in developing countries due to the presence of substandard medicines and other problems mentioned above. Three concentrations of CIP were studied because the responses of bacteria are concentration-dependent. At high concentrations, most of the antibiotics have antibacterial activities of liable cells while low concentrations have a more discriminative inhibitory effect **[23]**. The results of the study in terms of inhibition zone diameters produced by the different potency of discs for all brands and standards are given in Table 1. Also, the photograph of the plates with the inhibition zone of different brands of CIP-HCl against the tested bacterial strains is given in Figure 1.

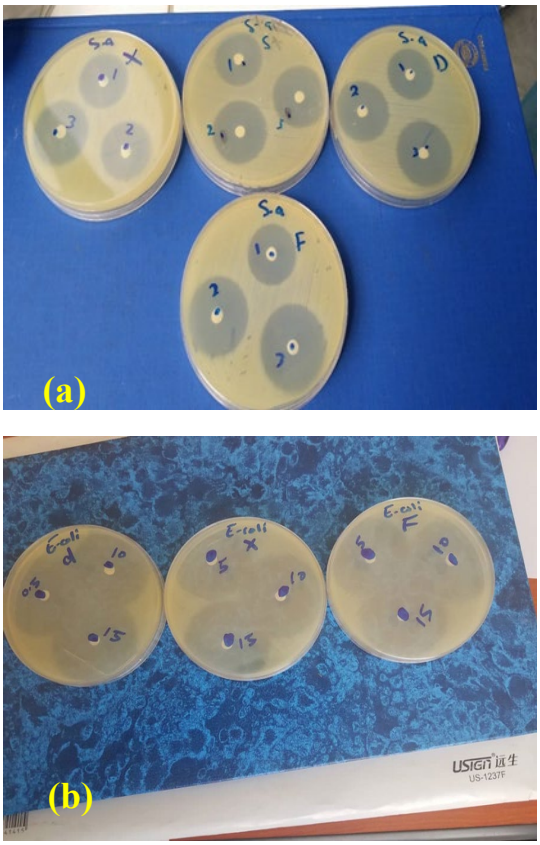

Figure 1: Zone Inhibition **(a)** CIP-HCl Standard and Tablet, Infusion and Eye Drop against Staphylococcus Aureus, **(b)** CIP-HCl Standard, and Tablet, Infusion against Escherichia coli.

4. Discussion

All brands examined by spectroscopy and the quantity of the active ingredients was with the permitted limits of British pharmacopeia (95-105%). The comparison of the results with the HiMedia company charts for inhibition zone (quality control limits for antibiotics) Control limits for monitoring inhibitory zone diameters (mm) shows that all the results fall within the acceptance range **[24]**.

4.1. CIP Effects against E-coli

The comparison of different brands of tablets and infusion results in the case of *E-coli* strain was comparable and similar to the standard expected at a lower concentration. At high and middle concentration for all brands of tablets and standard, there was no significant difference (p=0.5957, 0.2011 respectively), while it was a significant difference between the different brands of tablets (p=0.0008) and also between them and standard (p=0.0001) at low concentration. Also, all brands of infusions and standard at high and middle concentration showed no significant difference (p= 0.7936, 0.8272 respectively), while it was no significant differences

Table 1 Inhibition Zone of different brands of CIP against studied bacterial strains.

Bacterial Strain	Concentration µg/Disc	Zone of Inhibition (mm)± SD			
		Tablets (n=3)			
		Std	Tab-I	Tab-II	Tab-III
Escherichia coli [ATCC # 25922]	1.25	29.33 ± 0.57	34.66 ± 0.57	36.66 ± 0.57	32.00± 1.00
	2.5	38.00 ± 1.00	39.33 ± 0.57	38.33 ± 0.57	38.66 ± 0.57
	5	40.33 ± 0.57	41.00 ± 1.00	40.33 ± 0.57	41.00± 1.00
Staphylococcus Aureus [ATCC # 25923]	1.25	28.00 ± 0.57	27.33± 1.00	27.00± 0.57	27.33± 1.15
	2.5	30.33±0.57	30.33± 0.57	27.33± 1.00	30.33± 0.57
	5	31.33± 0.00	33.00± 1.15	32.00± 1.00	33.00± 1.00

Bacterial Strain	µg/Disc	Infusion (n=3)			
		Std	Infusion-I	Infusion-II	Infusion-III
Escherichia coli [ATCC # 25922]	1.25	29.33 ± 0.57	35.33± 2.08	34.00 ± 1.73	36.00 ± 1.00
	2.5	38.00 ± 1.00	38.33± 0.57	38.33 ± 0.57	38.66 ± 1.15
	5	40.33 ± 0.57	40.56± 0.98	41.26 ± 1.41	40.90 ± 1.00
Staphylococcus Aureus [ATCC # 25923]	1.25	28.00 ± 0.57	27.33 ± 0.57	28.66 ± 0.57	28.00 ± 0.57
	2.5	30.33 ±0.57	29.33 ± 0.57	30.00 ± 1.00	30.33 ± 0.57
	5	31.33 ± 0.00	32.33 ± 0.57	31.66 ± 0.57	33.33 ± 0.00
Bacterial Strain	**µg/Disc**	**Eye Drop (n=3)**			
		Std	Eyedrop-I	Eyedrop-II	Eyedrop-III
Staphylococcus Aureus [ATCC # 25923]	1.25	28.00 ± 0.57	26.66 ± 0.57	25.00 ± 0.00	25.66 ± 0.57
	2.5	30.33 ±0.57	28.33 ± 0.57	28.33 ± 0.57	28.00 ± 0.00
	5	31.33 ± 0.00	30.00 ± 0.00	29.33 ± 0.57	28.66 ± 0.57

between the different brands of infusions (p= 0.3861) at low concentration, however, there was a significant difference between them and standard (p=0.0022). The highest inhibition zone was in Tab-II, followed by Tab-I then Tab-III tablets. While in infusion, the Infusion-III showed the highest inhibition zone, followed by Infusion-I then Infusion-II.

4.2. CIP Effects against Staphylococcus aureus

At high and low concentration for all brands of tablets and standard, there was no significant difference (p= 0.1433, 0.4823 respectively), whereas at the middle concentration there was a difference between the different brands of tablets (p=0.0022) and also between them and standard(p=0.0007). All Tab I, II, and III have almost similar potency. The infusion, revealed no significant difference at the high and middle concentration for all brands of infusions and standard (p= 0.0126, 0.3300 respectively), whereas at the low concentration there was a difference between the different brands of infusions (p= 0.0370) and also between them and standard (p= 0.0553). Infusion II, III indicated similar activity while Infusion I had slightly lower potency.

In the case of Eye drops, there was a significant difference between the all brands and standard at the high, middle and low concentrations (p= 0.0011, 0.0016, 0.0001 respectively), however, there was no significant difference between the different brands of eye drops at the middle concentration (p= 0.6297). All brands showed nearly similar potencies with a slight superiority of Eyedrop-I over Eyedrop-II then Eyedrop-III in the highest concentration. All the brands showed antimicrobial activity slightly lower than standard.

The results were similar to the previous studies carried out in different developing countries as mentioned above for evaluation of the antimicrobial activity of the marketed CIP tablet dosage forms **[19, 21]**, except the result from Tanzania which revealed a low-quality of some marketed brands **[20]**. Of note, the current study also evaluated the infusion and eye drops which were have not to be assessed in the previous literature.

5. Conclusion

Antimicrobial sensitivity against marketed antibiotics drugs is dynamic and alters with the development of resistance in microorganisms. The key role of delivering safe and efficient drugs is drug quality control. It is essential to have strict regulation for illegal smuggling of substandard and falsified drugs to the local market, in addition, there must be periodic inspection campaigns to detect the counterfeit, and fake medicines in the community pharmacies. The post-marketing surveillance is an essential issue to distinguish poor-quality medicines. The current study revealed that the marketed CIP pharmaceutical dosage forms showed reasonable antimicrobial activity except for the eyedrops dosage forms which showed slightly lower inhibition zone in comparison to standard. There was a slight difference between the studied brands which may be related to the differences in the manufactures production procedures. The poor-quality antibiotics are not only the developing countries' health-related issues but rather have global dimensions due to widespread of resistant bacteria globally.

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مقالة بحثية

تقييم النشاط الحيوي المضاد للميكروبات لأشكال الجرعات الدوائية المختلفة من سيبروفلوكساسين في عدن ـ اليمن

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استلم في: 04 يونيو 2020 / قبل في: 21 يونيو 2020 / نشر في: 30 يونيو 2020

الملخص

يصنف السيبروفلوكساسين من الجيل الثاني ضمن مجموعة الفلوروكينولون, يشبه هيكلياً حمض الناليدكسيك (Nalidixic acid), و هو مضاد حيوي يستخدم على نطاق واسع لعلاج أنواع مختلفة من الالتهابات البكتيرية. أجريت الدراسة الحالية لتقييم النشاط المضاد للميكروبات لثلاثة أشكال صيدلانية مختلفة من CIP [أقراص (ترميز III، II، Tab I)، محاليل وريدية CIP (ترميز III، II، Infusion I) وقطرة العين CIP (ترميز III، II، Eye drop I)]، حيث تم اختيار ثلاث علامات تجارية أكثر شيوعاً واستخداماً. كانت جميع العلامات التجارية المدروسة ضمن فترة صلاحيتها. تم فحص جميع العلامات التجارية بواسطة طريقة التحليل الطيفي وكانت كمية المادة الفعالة ضمن الحدود المسموح بها للدستور الادوية البريطاني (95-105 ٪). استخدامت طريقة انتشار القرص لمقارنة النشاط المضاد للميكروبات للعلامات التجارية المختلفة ضد سلالتين من البكتيريا: المكورات العنقودية الذهبية ستافيلوكوكس اوريس (Staphylococcus aureus) والعصيات القولونية (اي كولايEscherichia coli). كانت أعلى منطقة تثبيط عند تركيز منخفض ضد *E. coli*، بواسطة أقراص Tab-II وTab-I وTab-III على التوالي. بينما في حالة المحاليل الوريدية، أظهر Infusion-III أعلى منطقة تثبيط، يليه Infusion-I وInfusion-II. في حالة المكورات العنقودية الذهبية، فإن جميع الاقراص I,II,III كانت لها قوة مماثلة. عند التركيز المنخفض، اعطى المحلول الوريدي II,III, نتائج متشابهة بينما كان المحلول الوريدي I ذو فاعلية أقل. ومع ذلك، كان لجميع العلامات التجارية قوة أعلى قليلاً من المعيار. أظهرت جميع العلامات التجارية لقطرات العين فعاليات متشابهة تقريبًا ضد المكورات العنقودية الذهبية مع تفوق طفيف لـ Eyedrop-I على Eyedrop-II ثم Eyedrop-III في أعلى تركيز. أظهرت جميع العلامات التجارية لقطرات العين نشاطًا مضادًا للميكروبات أقل بقليل من المعيار.

الكلمات الرئيسية: سيبروفلوكساسين، طريقة انتشار القرص، العصيات القولونية، المكورات العنقودية الذهبية.