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ORIGINAL ARTICLE

Study Of Antibacterial and Physicochemical Properties of Local and International Brands of Moxifloxacin Used in Clinical Patient Care in Pakistan

Fozia Israr[1], Fouzia Hassan[2], Huma Ali[1]*, Huma Sharif[1], Syed Muhammad Farid Hasan[2], Sabahat Jabeen[2], Zobia Israr[3]

[1]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan.
[2]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
[3]Department of Economis, University of Karachi, Karachi, Pakistan.

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****Address of Correspondence Author:***

huma.ali@jsmu.edu.pk

ABSTRACT

Objective: Moxifloxacin antibiotic belongs to fourth-generation fluoroquinolone class and used to treat various Gram-positive and Gram-negative bacterial. The present work designed to evaluate the pharmaceutical quality attributes and antibacterial activity of five different local and international brands of moxifloxacin 400mg tablets (B1-B5) available in Pakistan with different price ranges.

Methodology: Different physicochemical parameters including weight, thickness variation, disintegration time, chemical assay and in vitro dissolution evaluation were performed on above mentioned brands of moxifloxacin. Disk diffusion method (Bauer-Kirby susceptibility test) were also used to evaluate the in-vitro antibacterial activity of these brands on three standard bacterial culture of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* by micro dilution minimum inhibitory concentration (MIC) assay technique and compared with their respective reference standard (A).

Results: All the brands was found to be similar according to compendial quality standards tested for physicochemical evaluation with more than 90% drug release in 0.1N HCl (pH 1.2). All brands exhibited good in vitro activity (zone of inhibition: 26 – 38 mm) against all the standard cultures in terms of clinical effectiveness despite of little variation in price.

Conclusion: It was concluded that a good pharmaceutical association was observed amongst all the local and international brands of moxifloaxcin and each product were also observed within a particular microbial limits exhibits satisfactory effectiveness required for the treatment of particular infectious disease conditions. This study also help medical practitioner to prescribe alternative antibiotic product to the patients in case of unavailability of the brand and for those who are unable to afford the costly treatment.

Keywords: *Moxifloxacin, Different brands, Cost effective, Pharmaceutical quality attributes, Antibacterial effectiveness*

INTRODUCTION

Selection of one product among others numerous drug with good compliance of efficacy relative to its price is called cost effective evaluation in which analyze the cost of the product and consequences of other alternatives resources of achieving an objective of requirement [1]. The emergence of resistance due to irrational use of broad spectrum antibiotics is a main reason of life threatening infections especially in those countries which are in a developing stage like Pakistan [2]. Development of National surveillance programs for antibiotic resistance, developing prescription strategies and emphasizing persevering with clinical and public training are the critical equipment to enhance health care system [3].

Moxifloxacin belongs to fourth generation synthetic fluoroquinolone chemotherapeutic agent and effective against various types of microbial diseases recognized as serious and life threatening bacterial infections caused by Gram-positive and Gram-negative bacteria which includes chronic bronchitis, acute bacterial sinusitis, community acquired pneumonia and complicated Intra-abdominal infections [4, 5]. Quality estimation studies of pharmaceutical generics/brands may be useful in demonstration of the counterfeit products [6]. Pharmaceutical formulations having similar generic moieties may greatly diverge in quality perspectives. Furthermore, these products are available as alternative choice the same therapeutic ingredient at variable cost in particular community and retail setups [7].

The ability of an antibiotic or different other antimicrobial agents to inhibit the in vitro growth of microbes under suitable environmental conditions is termed as antimicrobial susceptibility analysis [8]. Different types of antimicrobial susceptibility (sensitivity) techniques are recommended for safety and efficacy determination of these antibiotic products, depending upon the types of resources and accessibility for evaluation. The utmost frequent technique to determine the susceptibility evaluation of antimicrobial agents is a disk diffusion method due its protective and most effective history profile [9].

Present work is designed to evaluate the qualitative characteristics including numerous physico – chemical tests along with in vitro dissolution profile determination of dissimilar international and local brands of moxifloxacin 400mg tablets (B1-B5) available in Pakistani drug market with different price range. Furthermore, antibacterial susceptibility of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosae*) bacterial strains was also evaluated by disk diffusion method (Bauer-Kirby susceptibility test) using micro dilution technique of moxifloxacin international and local brands.

MATERIALS AND METHODS

Materials

Moxifloxacin (Reference standard: A) was obtained from Helix Pharma (Pvt.) Ltd. All moxifloxacin 400 mg brands (B1 to B5) were bought from local and international manufacturer available in Pakistani drug marketplace. Hydrochloric acid and methanol (Merck, Darmstadt, Germany) of analytical grade. *Staphyloccocus aureus* [ATCC 6538], *Escherichia coli* [ATCC 25422], *Pseudomonas aeruginosae* [ATCC 9027] bacterial strains used for the test. Nutrient Broth (Merck, Germany), Mueller Hinton Agar (Oxoid, England), Mac-Farland 0.5 turbidity standard (Thermo Fischer Scientific). Standard disks of Moxifloxacin 5µg (Oxide Ltd. Hampshire, England).

Methods

Pharmaceutical quality evaluation of moxifloxacin tablet brands

Physicochemical estimation of tablets

Different pharmaceutical parameters of five selected brands of moxifloxacin 400 mg (B1-B5) tablets were determined according to pharmacopeial and non-pharmacopeial approaches like weight variation (Analytical balance: Sartorius, Germany), thickness variation (Vernier caliper: CD-6, CSX, Mitutoyo, Japan) and disintegration test (Erweka, ZT2, Heusenstamm Germany) [10].

Assay Method

Uniformity of dosage form were determined by simple, accurate, cost effective and reproducible spectro-photometeric method to perform assay of test formulations (B1-B5) [11]. For assay performance weigh and powder 20 tablets to a fine powder and transfer in to 200 ml volumetric flask containing the equivalent quantity of 100 mg moxifloxacin. Add 100 ml of 0.1N HCl and mix for 30 minutes on magnetic stirrer. Make up the volume with 0.1N HCl and then filter through filter paper. Final dilution of solution with concentration of 5 µg/ml prepared by transferring 2 ml

of filtered aliquot in to 200ml volumetric flask and make up volume with 0.1N HCl. UV/V double beam spectrophotometer (1800, Shimadzu, Japan) used to measure the drug absorbance at 294nm to evaluate the content uniformity of formulations.

In vitro dissolution studies

Moxifloxacin drug releases patterns were determined by placing tablets in a 900 ml of dissolution medium of 0.1N HCl with pH 1.2 (37±0.5°C) using USP type II dissolution paddle apparatus (Erweka DT, Heusenstamm, Germany) at 100 rpm rotation speed. Approximately 10 ml aliquot of medium withdrawn at different time intervals like 5, 10, 15, 20, 25, 30, 45, 60, 90 and at 120 minutes compensated with equal quantity of fresh dissolution medium to keep total volume. The absorbance of moxifloxacin were obtained by suitably dilute the removed filtered test sample solutions with dissolution medium and results were note down at 294 nm λ max using UV/V double beam spectrophotometer (1800, Shimadzu, Japan) taken dissolution medium as blank sample [12].

Antibacterial activity of moxifloxacin brands

Preparation of antibiotic disks

Serial dilution method (MIC assay) used to prepare 0.1% (1mg/ml) concentration of moxifloxacin disks of the moxifloxacin 400mg tablets (B1-B5) and reference standard of moxifloxacin (A). First prepare the stock solutions and then it is further diluted for making the final concentration of 0.1% (1 mg/ml). The disks were already circularly cut with the help of punch machine. The final solution was poured on disks and the disks were soaked for 24 hrs in petri plate. After that the disks were refrigerated and used against the microbial plates [13].

Bacterial cultures and procedure

In vitro antibacterial performance of moxifloxacin brands were determined by using standard culture of Gram positive *Staphylococcus aureus*, Gram negative *Pseudomonas aeruginosa* and Gram negative *E.coli.* These standard cultures were collected from various hospital and laboratories in Karachi. Clinical isolates of standard culture will be identified on the basis of morphological, cultural and biochemical reactions.

Subcultures had been suspended overnight in sterile Nutrient broth (Oxoid, U.K) and the very last turbidity adjusted to compare with McFarland standard 0.5 [13]. Mueller Hinton's sensitivity agar (Oxoid) was placed on petri plates to make lawn of each bacterial suspension with the help of sterile cotton swabs [14]. Prepared discs of 0.1% of every product have been located at suitable distances on the identical Mueller Hinton's agar petri dish streaked one after the other with each culture. The batch of all plates were incubated for overnight at 37°C for 24 hours, and CLSI (*Clinical & Laboratory Standards* Institute) guidelines and Kirby-Bauer disc diffusion method were used as reference standard to determine inhibition zone diameters carefully measured with the help of vernier caliper [15, 16]. All assessments had been run concurrently to make sure uniformity of conditions, along with control reference standard of commercially available moxifloxacin 5 ug discs (Oxoid) (Table **1**).

RESULTS

The safety and efficacy of pharmaceutical formulation with different price cost significantly be contingent on its quality features, formulation properties and clinical effectiveness. Physico- chemically comparable brands ought to be equal with respect to purity, quality, strength and bioavailability [10]. In the present study at first step, pharmaceutical quality estimation was performed on different brands of moxifloxacin (B1-B5) purchased from commercial market of Karachi, Pakistan with different prices. Results of physico-chemical parameters evaluation for weight, thickness variations and disintegration time are shown in table **2**. All of the obtained brands were assayed by validated spectrophotometric test procedure [11] and observed with satisfactory results (Table **2**). Dissolution medium of 0.1N HCl having pH 1.2 were used to performed the multiple point dissolution studies of different brands of moxifloxacin tablets as per USP guidelines showed 70% of drug release within 15 minutes (Figure **1**). This work was also used to evaluate the cost value of different products of moxifloxacin (B1-B5) tablets against the sensitivity of standard culture of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. All brands exhibited good in vitro antibacterial activity (26 – 38 mm) against all the standard cultures in terms of clinical effectiveness despite of little variation in price (Table **3** and Figure **2, 3, 4**).

Table 1. Susceptibility (Zone Diameter Breakpoints, nearest whole mm) of microbial culture to moxifloxacin (CLSI guidelines).

Microbial cultures	Disk content	Zone diameter break points nearest whole mm		
		S	I	R
Staphylococcus aureus	5µg	≥24	21-23	≤20
Pseudomonas aeruginosa	5µg	≥ 25	18-24	≤ 17
Escherichia coli	5µg	≥21	18-20	≤17

Table 2. Pharmaceutical properties of different brands of moxifloxacin tablet.

Product code	Weight	Thickness	Disintegration time	Assay	Dissolution test
	(mg) (n=20)	(cm) (n=20)	(min) (n=6)	(%) (n=20)	(%) (n=6)
Pharmacopoeial limits (USP 36)	**±5%**	**±5%**	**within 30 minutes**	**95-105%**	**NLT 80%**
B1	749.56 ± 0.36	0.85 ± 0.38	12	101.71±1.05	100.5 ± 0.28
B2	706.68 ± 0.31	0.75 ± 0.54	16	100.56±0.70	99.97 ± 0.48
B3	726.83 ± 0.54	0.80 ± 0.35	17	96.97±0.66	99.23 ± 0.31
B4	716.82 ± 0.75	0.65 ± 0.48	15	97.56±1.20	99.89 ± 0.18
B5	770.30 ± 0.58	0.81 ± 0.51	13	99.97±0.44	98.32 ± 0.45

Table 3. Comparative prices and average zones of inhibition of reference standard and different brands of moxifloxacin against standard culture of microorganisms.

S. No.	Ref std. and different brands of moxifloxacin	M. R. Price (PKRS)	Zone of inhibition (mm) at concentration of 0.1% (1mg/ml)		
			E. coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
			ATCC No. 25422	ATCC No. 6538	ATCC No. 9027
1	Reference standard (A)	-	26	27	35
2	B1	375	26	28	34
3	B2	395	34	34	38
4	B3	790	31	37	35
5	B4	350	32	30	36
6	B5	345	30	31	35

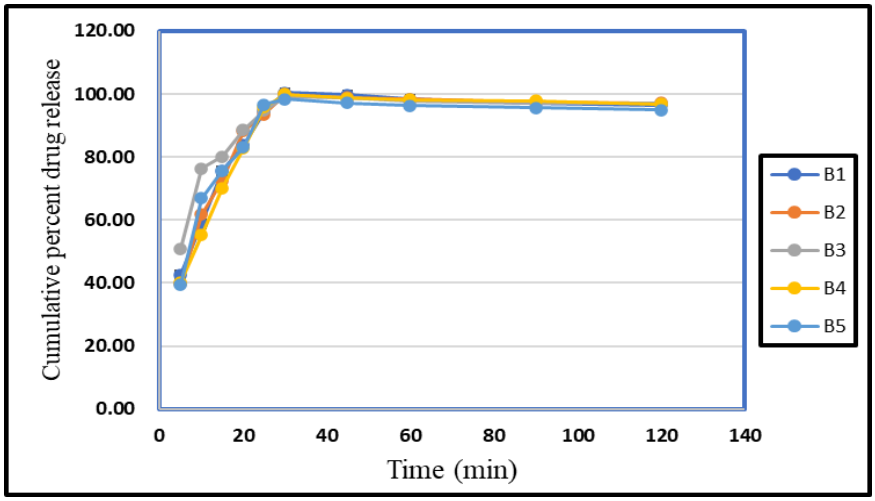

Figure 1. In vitro dissolution profile of moxifloxacin brands formulations in 0.1N HCl of pH 1.2. (N=6).

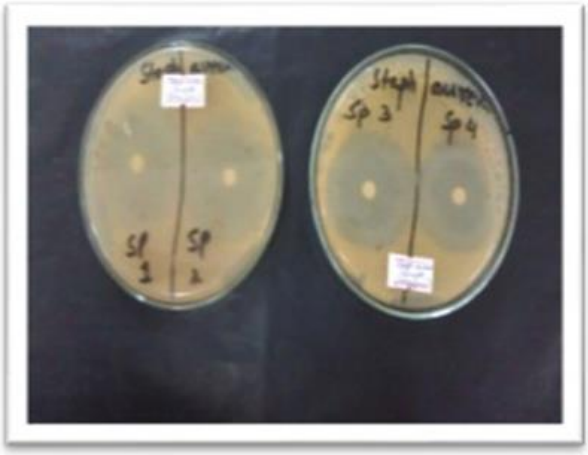

Figure 2(a): Zone of Inhbition of Ref. standard (A) and brand (B1) of moxifloxacin against *Staphylococcus aureus.*

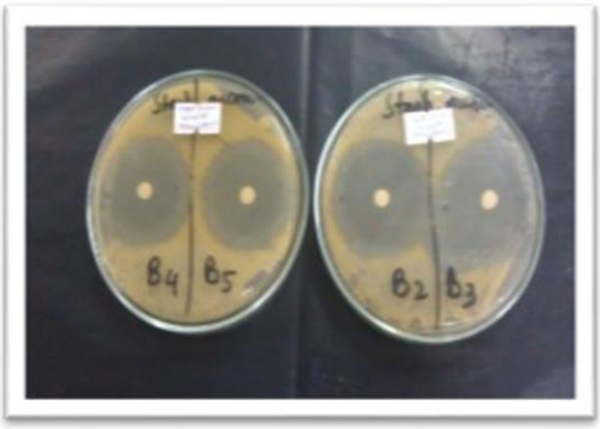

Figure 2(b): Zone of Inhbition of different brands (B2-B5) of moxifloxacin against *Staphylococcus aureus.*

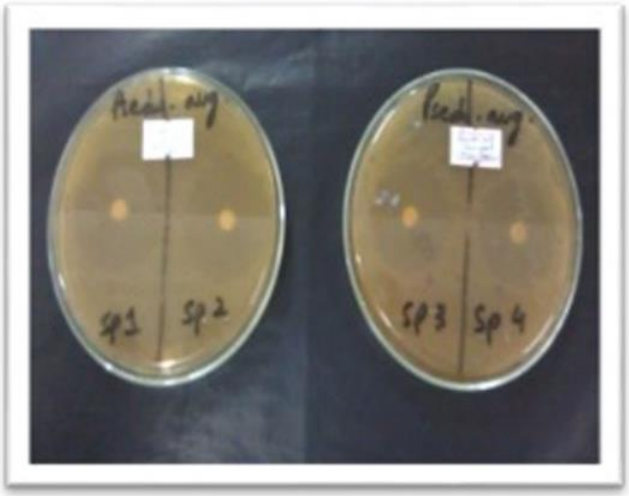

Figure 2(c): Zone of Inhbition of Ref. standard (A) and brand (B1) of moxifloxacin against *Pseudomonas aeruginosa*

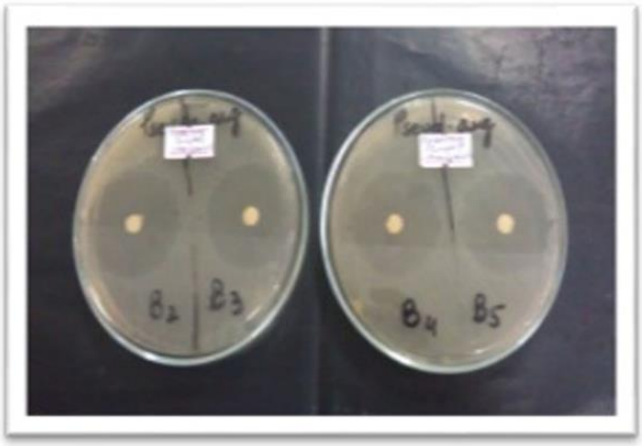

Figure 2(d). Zone of Inhbition of different brands (B2-B5) of moxifloxacin against *Pseudomonas aeruginosa*

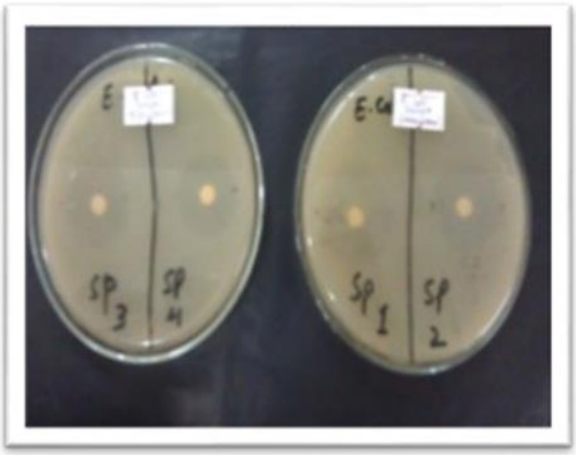

Figure 2(e). Zone of Inhbition of Ref. standard (A) and brand (B1) of moxifloxacin against *Escherichia coli*

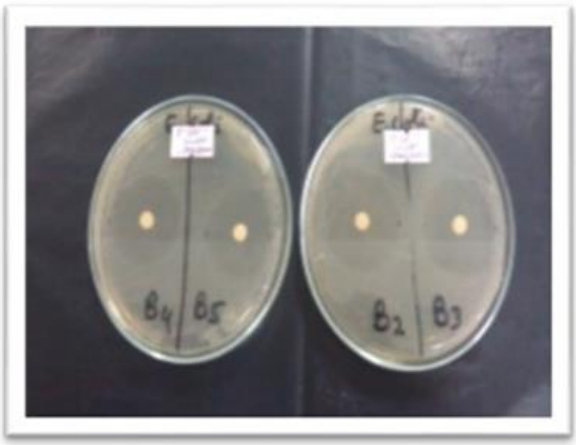

Figure 2(f). Zone of Inhbition of different brands (B2-B5) of moxifloxacin against *Escherichia coli*

DISCUSSION

Moxifloxacin is synthetic fluoroquinolone chemotherapeutic agent belongs to fourth generation class and found to be not affected by penicillin and cephalosporin resistance and it is 32-fold more effective than ciprofloxacin [17]. The present work designed to evaluate the feature characteristics of different products of moxifloxacin tablets obtainable in national marketplace to determine the effectiveness

of pharmaceutical products with different price range. Different manufacturers of such antibiotics provide easy and affordable solution to many health issues with proper availability of cost effective medicine. Although, in many deprived countries the proper cure and treatment for infectious diseases is still not available [18].

Pharmaceutical quality evaluation of moxifloxacin tablet brands

The safety and efficacy of pharmaceutical products substantially relies upon formulation qualities and manufacturing strategies. Physico- chemically comparable brands ought to be equal with respect to purity, quality, strength and bioavailability [10]. In the present study at first step, pharmaceutical quality estimation was performed on different brands of moxifloxacin (B1-B5) purchased from commercial market of Karachi, Pakistan with different prices. Results of physico-chemical findings were observed to be in satisfactory values with average weight and thickness variations of B1-B5 found in order of 706.8±0.31 mg to 770.30±0.58 mg and 0.65±0.48 cm to 0.85±0.38 cm, as shown in table 2. In previous studies different researchers evaluate the quality attributes of diverse antibiotic brands available in Pakistani drug market [19, 20].

According to USP, prescribed period of time is important for tablet disintegration in which tablet break up into a primary powder particles [10]. All the tested antibiotic brands showed disintegration time 12 to 17 minutes and compliance with the USP criteria. The spectrophotometric assay method results revealed that drug content between 96.97%±0.66 to 101.71%±1.05 is acceptable (Table **2**).

Dissolution profile of the drug is a vital component of overall quality control program and also representing in-vivo bioavailability of drug [21]. In the present work 0.1 N HCl dissolution medium with pH 1.2 used to evaluate the drug release profile at multiple time intervals. From figure 1, it can be seen that all products released significant amount of drug (more than 70%) within fifteen minutes time period. More than 90% of drug release were observed during complete dissolution period of 2 hours with maximum drug release of moxifloxacin (B1-B5) at 30 minutes were within the range of 98.32%±0.45 to 100.50%±0.28 according to USP [10], a prescribed test procedure (acceptable NLT 80%).

Antibacterial activity of moxifloxacin brands

The present work was also planned to analyze the different products of moxifloxacin (B1-B5) tablets which are available in Karachi with different costs against the sensitivity of standard culture of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. Antibacterial effectiveness of 34 competitive ofloxacin brands were also evaluated in one of research study having 31 Pakistani manufacturer present in local market place. The activity of these brands were investigated on three standard Quinolone-sensitive ATCC bacterial cultures of *Escherichia coli*, *Proteus vulgaris* and *Staphylococcus aureus* [22]. In another work resistance pattern of ciprofloxacin were investigated against different pathogens by disc diffusion method [23].

In the current study, in-vitro antibacterial activity of diverse products of moxifloxacin were performed by disc diffusion method (Bauer-Kirby susceptibility test) and compared with respective reference standard (A) as well as with each other. Results of the study illustrated that all the brands of moxifloxacin have found to be more effective against standard culture of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* when compare with reference standard of moxifloxacin (Table 3 and Figures 2 (a-f)). The results revealed that among the different brands of moxifloxacin, B2 is found to be most effective against all the microbial culture i.e. *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* in order of 34, 34 and 38 mm zone of inhibition. Brands B3 has shown high activity against *Staphylococcus aureus* (37 mm) and B1 gives good activity against *Pseudomonas aeruginosa* (34 mm), while B4 and B5 has also given the similar pattern of activity against *Escherichia coli* (32 mm and 30 mm), *Pseudomonas aeruginosa* (30 mm and 31 mm) and *Staphylococcus aureus* (36 mm and 35 mm).

Previously a study performed to revealed significant efficiency of antibacterial ceftriaxone moxifloxacin combination showed an immunomodulatory outcome with effective solution against bacterial biofilm infections produced by methicillin resistant *Staphylococcus aureus* [24].

Comparison of in-vitro antibacterial activity of different brands revealed that the moxifloxacin is found to be more sensitive against *Pseudomonas aeruginosa* and *Staphylococcus aureus* as compare to *Escherichia*

coli. The similar pattern were observed by all the antibiotic products however the cost of the different manufacturers are varies but no one is under the standard measures prescribed as per the CLSI guidelines for susceptibility evaluation. Individual in-vitro antibacterial activity of different brands of moxifloxacin showed that B5 have low cost as compare to price range with other brands with parallel however little higher pattern of activity as compare to different products of this class.

In another work sensitivity of clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi, Proteus mirabilis*, *Staphylococcus aureus* and *Klebsiella pneumonia* were evaluated on different products of ceftriaxone brands available in Pakistani drug market with differing costs and concluded that the in-vitro interest of variable products is equal notwithstanding of dissimilarity in costs that is an vital element in the overall price of treatment [25]. Different brands of same antibiotic may be with no trouble utilized for therapy of infectious ailments having cost effective features if other parameters such as pharmacological and chemical investigation are completely full filled.

It is likewise interpreted that the in-vitro effectiveness of all manufacturers of moxifloxacin is sort of identical in spite of variant in their cost. Hence, the treatment of infectious disease for the recovery of non-affording patient can be done with any product of antibiotic with low price range.

CONCLUSION

Our study confirms the same pharmaceutical attributes of different local and international brands of moxifloxacin with quality control limits. The study also revealed that these brands fourth generation fluoroquinolone moxifloxacin showed good in vitro bactericidal activity against clinically relevant pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* Therefore outcome of such studies may be useful in selection of the therapeutic alternative moxifloxacin antibiotic for the treatment of acute exacerbations of chronic bronchitis, community-acquired pneumonia, acute bacterial sinusitis and uncomplicated skin and skin structure infectious disease conditions. It is also helpful for the physician to prescribe another antibiotic brand with lesser price for the treatment of the patients who are incapable to meet the expense of costly therapy and the supportive tool for the drug legislation authorities, manufacturers and regulating bodies to constantly scrutinize the provision of quality products.

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