In vitro antibacterial susceptibility of different brands of oral levofloxacin 250 mg tablet against *Staphylococcus aureus* and *Escherichia coli*

Raheela Bano[1] and Adeel Arsalan[2*]

[1]Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Toll Plaza, Super Highway, Gadap Road, Karachi, Pakistan
[2]College of Pharmacy, Ziauddin University, 4/B, Shahrah-e-Ghalib, Clifton, Karachi, Pakistan

Abstract: Antibiotics are not only used in morbidity but also help in prevention of infection. The irrational use of broad spectrum antibiotics is now increasing the resistance against pathogens. This present study has been carried out to evaluate the *in-vitro* antibacterial effect of levofloxacin against clinical isolates. According to Clinical and Laboratory Standards Institute (CLSI) guidelines, minimum inhibitory concentrations 90% (MIC_{90}) of the levofloxacin tested were evaluated by an agar dilution method. Total 63 clinical isolates *Staphylococcus aureus* (n=34) and *Escherichia coli* (n=29) were collected from different hospitals at Karachi and were evaluated MIC_{90} of eleven different brands of levofloxacin tablet (250 mg). Levofloxacin (Reference) was tested against E.coli standard (American Type Culture Collection) (ATCC=25922) with (MIC_{90}; 0.03µg/ml) and compared with different eleven brands of levofloxacin tablets 250mg (MIC_{90}; 0.5µg/ml -16.0µg/ml). Levofloxacin (Reference) sensitivity against *S. aureus* standard (ATCC=25923) is (MIC_{90}; 0.12µg/ml) and similarly when it was compared with same levofloxacin tablets (MIC_{90}; 0.5-16.0µg/ml). It has been concluded by the present study, a large number of strains of bacteria have shown better bactericidal action of different brands of levofloxacin and nearly all commercialized drugs were appropriate for therapeutic use.

Keywords: Levofloxacin, generics, *in vitro,* antibacterial activity, susceptibility.

INTRODUCTION

Microorganisms are ubiquitous found from shallow of seas to air. These may be pathogenic or beneficial for human being (Arsalan *et al*., 2013a, 2013b, 2013c, 2013d). Antibiotics are not only used in morbidity but also help in prevention of infection (Arsalan *et al*., 2014a, 2014b). The irrational use of broad spectrum antibiotics has now increased the resistance against pathogens (Arsalan *et al.*, 2010, 2015, 2017). Antibiotics are the most often given drug in modern medicine to treat and/or prevent disease due to its bactericidal or bacteriostatic effect (Arsalan *et al*., 2016). Quinolones have been probably the fastest growing group of antibiotics with broader and wider spectrum of anti-bacterial activity (Bhanot *et al.*, 2001; Ahmad *et al*., 2016). Among quinolones, levofloxacin third generation has been possessed wide range of bactericidal effect against Gram-negative and positive and atypical pathogens. Levofloxacin has shown its lethal activity by inhibiting enzymes topoisomerase IV and DNA gyrase against virulent and resistant organisms like *Pseudomonas aeruginosa,* methicillin-resistant *Staphylococcus aureus, Mycobacterium tuberculosis,* penicillin-resistant *Streptococcus pneumoniae, Escherichia coli* (Zhanel *et al.*, 2002; Stein and Goldstein, 2006). Therapeutically, it is used for urinary tract infections (UTIs); respiratory tract infections (RTIs); biliary tract infections (BTIs); sinusitis; chronic bronchitis; pneumonia and uncomplicated mild to moderate infection of skin and skin structure (Blondeau 1999; File 2004; Prajapati *et al.*, 2008). Orally levofloxacin has been well absorbed, because tissue and fluid concentrations often exceed the serum drug concentration; peak plasma concentration has been usually attained within one to two hours (Zhanel and Noreddin, 2001).

Among Gram-positive *S. aureus* infections have been a major cause of morbidity and mortality worldwide (Bishop and Howden, 2007). *S. aureus* may cause severe infections like endocarditis, pneumonia, osteomyelitis, septicemia (Gagliardi *et al.,* 1998, Mader *et al.,* 2000). Since last two decades, the resistance of *S. aureus* to various antibiotics has been raised. The incidence has been accompanied by a rise in antibiotic-resistant strains particularly, methicillin-resistant *Staphylococcus aureus* (MRSA) and more recently, vancomycin-resistant *Staphylococcus aureus* (VRSA) (Stevenson *et al.*, 2005). *Escherichia coli* is a Gram-negative bacilli and one of the most frequent clinical isolated pathogen has been involved in infections like cholangitis, pneumonia, meningitis, urinary tract infections (UTI), gastro-intestinal infections (GII) and diarrhea (Bano *et al*., 2011). The resistance of *E. coli* against levofloxacin has been observed by several workers (Tenover 2006; Reinert *et al.*, 2007; Jang *et al.*, 2011).

**Corresponding author:* e-mail: drarsalanadeel@gmail.com

MATERIALS AND METHODS

Standard (STD) levofloxacin drug was kindly gifted from M/s *Sanofi-Aventis (Pakistan) Limited*. Twelve different marketed Levofloxacin were purchased from local pharmacies of Karachi, Pakistan. STD Gram-negative (American Type Culture Collection; ATCC) (*E. coli*, ATCC® 25922) and Gram positive (*S. aureus*, ATCC® 25923) have been kindly gifted by M/s. Brookes Pharmaceutical (Private) Limited. The clinical isolates were obtained from January 2013 to August 2013 from different hospitals at Karachi, Pakistan. Mueller-Hilton broth (Merck Germany) and freshly boiled distilled water was always used to prepare different dilution (Wiegand *et al*., 2008).

Instrumentation

The determination of MICs of levofloxacin drug and their dosage form a Shimadzu UV-1601 spectrophotometer was used for quantitative calculation.

Broth dilution method

A complete protocol has been found in the clinical and laboratory standards institute (CLSI), for broth dilution methods by reducing the quantity of the anti-bacterial agent to be evaluated, generally prepared in sequential two fold dilutions in broth tubes (Andrews, 2001).

Preparation of MacFarland standard

Sulphuric acid 1% is prepared and 1.175% aqueous solution of barium chloride is added in it. Now with constant slow agitation, add the designated amounts of the two solutions to the tubes make a total of 10 ml per tube. The suspended barium sulfate precipitate corresponds approximately to homogenous *E. coli* cell densities per ml throughout the range of standard (Wiegand *et al*., 2008).

Preparation of inoculum

These suspensions were prepared by using the top of the colonies of the standard and isolated microorganisms. STD *E. coli* (ATCC® 25922) and STD *S. aureus* (ATCC® 25923) and clinical isolates of *E. coli* and *S. aureus* were incubated in test tubes at 37°C for 2-8 hours until the turbidity exceeds that of 0.5 McFarland standards (Wiegand *et al*., 2008).

Preparation of antibiotic stock solutions

Weighed accurately for a required amount of standard antibiotic powder (standard powder of levofloxacin and its different brands). Prepare stock solution using the formula (Wiegand *et al*., 2008).

$$W = \frac{V \times C \times 1000}{P}$$

Where V is volume in ml required,
W is weight of the antimicrobial to be dissolved in V,
C is final concentration of solution and
P is potency of the antibiotic base.

Preparation of antibiotic dilution range

Generally prepared a series of varying concentrations two fold serial dilutions (0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16 µg/ml). Equal volume of inoculum has been added in test tubes (Wiegand *et al*., 2008).

Procedure

Prepared Muellur-Hilton broth, arranged sufficient sterile test tubes for levofloxacin to cover the range of antibiotic dilutions. 9ml of broth and 1ml of each antimicrobial agent's dilution was transferred in separate broth tubes. Now inoculum was added to each anti-microbial containing tube in the dilution series. The tubes were incubated at 37°C for 12 to 18 hours. Examined the tubes with visually and with spectrophotometer (546 nm) for the existence or absence of microbial growth and compared the result with the growth in the control tube.

STATISTICAL ANALYSIS

The data were analyzed by one way ANOVA (by Graphpad software, Quick calcs online calculator for scientists).

RESULTS

In our study, total 63 most common clinical isolates *Staphylococcus aureus* (n=34) and *Escherichia coli* (n=29) were collected from different hospitals at Karachi, Pakistan as shown in table 1 and MIC_{90} were evaluated against eleven different brands of levofloxacin tablet (250 mg) has been shown in table 2 and table 3. The overall resistance pattern of Gram-negative *E. coli* and Gram-positive *S. aureus* clinical isolates has been illustrated in table 4 and fig. 1.

Levofloxacin (Reference) was tested against *E.coli* standard (ATCC = 25922) with (MIC_{90}; 0.03µg/ml) and compared with different eleven brands of levofloxacin tablets 250mg (MIC_{90}; 0.5µg/ml -16.0µg/ml). Levofloxacin (Reference) sensitivity against *S. aureus* standard (ATCC=25923) is (MIC_{90}; 0.12µg/ml) and similarly when it was compared with same levofloxacin tablets (MIC_{90}; 0.5- 16.0µg/ml).

DISCUSSION

In-vitro analysis of antibacterial activity has been conducted commonly, as consequences of the antimicrobial susceptibility have been done to observe antibiotic efficacy inside the body (Hannan *et al*., 2008). For a long period of time, resistance of bacteria to antibiotics has been appeared as one of the major troubles encountered by health associated professionals (Arsalan *et al.,* 2014). Levofloxacin is a third generation quinolone, exhibits fine bactericidal activity against Gram-positive and Gram-negative. Because of its wide spectra even

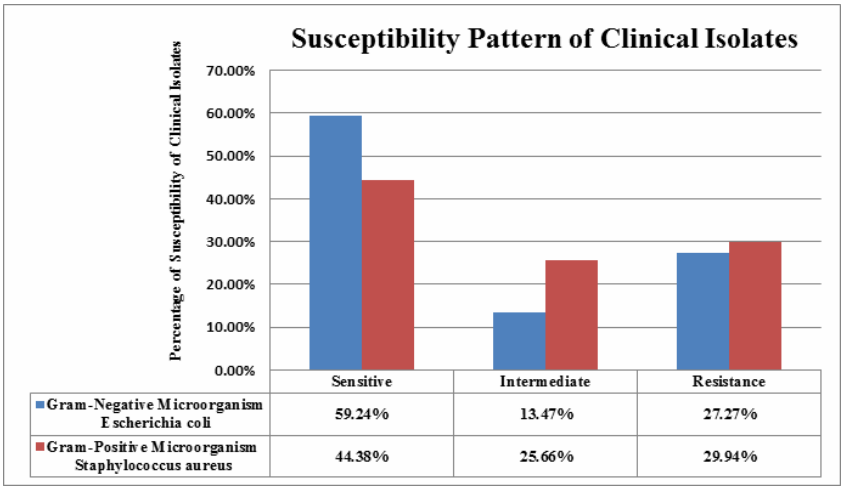

Fig. 1: Susceptibility Pattern of Clinical Isolates against Marketed Levofloxacin 250 mg tablets

Table 1: Summary of Clinical Isolates

Clinical Isolates	Source of Isolates	Number of Isolates
Staphylococcus aureus	Surgical, burn and accidental wound pus, pneumonia, blood sample	41
Escherichia coli	Stool and urine, blood sample	37

Table 2: Comparison of standard levofloxacin and different brands of levofloxacin against standard (STD) *Escherichia coli* & different clinical isolates of *Escherichia coli*

MICROBES n=29	LEVOFLOXACIN (µg/ml)											
	STD LF	LF 01	LF 02	LF 03	LF 04	LF 05	LF 06	LF 07	LF 08	LF 09	LF 10	LF 11
STD *E. coli* ATCC 25922	0.03	0.06	0.06	0.12	1.0	0.5	0.5	0.03	0.06	0.25	0.25	0.12
Ec 01	0.25	0.5	0.5	1	0.5	1	1	4	1	1	16	1
Ec 02	0.25	4	1	1	16	16	0.5	1	1	16	16	1
Ec 03	0.5	1	4	1	1	16	16	1	1	1	16	1
Ec 04	0.25	0.5	0.5	1	16	1	1	1	0.5	0.5	1	16
Ec 05	0.06	4	1	1	0.5	16	16	1	1	1	1	16
Ec 06	0.5	0.5	1	16	1	8	16	16	1	16	1	2
Ec 07	0.5	0.5	4	1	16	16	1	2	16	4	1	1
Ec 08	0.25	1	1	4	16	1	4	1	0.5	16	1	1
Ec 09	0.12	1	1	16	1	1	1	1	8	1	16	1
Ec 10	0.12	16	0.5	0.5	8	16	4	1	1	1	1	1
Ec 11	0.25	1	1	0.5	1	16	1	4	16	16	4	16
Ec 12	0.25	0.5	1	16	16	4	1	16	4	1	1	1
Ec 13	0.12	1	0.5	1	1	16	1	1	1	16	8	1
Ec 14	0.06	16	4	1	0.5	8	0.5	1	16	4	0.5	1
Ec 15	1	16	16	1	16	16	16	16	2	16	1	16
Ec 16	0.5	16	0.5	1	8	16	16	1	16	0.5	16	1
Ec 17	0.06	1	1	4	16	1	1	16	0.5	1	4	1
Ec 18	0.06	4	16	1	1	0.5	16	0.5	0.5	4	0.5	1
Ec 19	0.12	0.5	1	16	1	1	4	16	1	1	16	8
Ec 20	0.5	1	4	16	16	16	1	0.5	16	1	1	1
Ec 21	0.12	16	1	4	16	1	1	4	1	1	1	16
Ec 22	0.25	4	1	1	0.5	1	16	16	1	16	1	1
Ec 23	0.12	1	1	4	16	4	1	16	16	1	4	0.5
Ec 24	0.06	1	16	1	1	1	16	4	16	1	1	16
Ec 25	0.5	1	1	16	16	16	16	0.5	1	1	0.5	1
Ec 26	0.5	1	1	16	16	1	16	1	0.5	16	4	4
Ec 27	0.06	0.5	1	1	16	1	1	1	1	16	1	1
Ec 28	0.12	1	4	1	1	4	1	16	1	0.5	1	4
Ec 29	0.5	4	1	0.5	1	16	16	1	1	1	16	1

Table 3: Comparison of standard levofloxacin and different brands of levofloxacin against standard (STD) *Staphylococcus aureus* and different clinical isolates of *Staphylococcus aureus*

MICROBES n=34	LEVOFLOXACIN (µg/ml)											
	STD LF	LF 01	LF 02	LF 03	LF 04	LF 05	LF 06	LF 07	LF 08	LF 09	LF 10	LF 11
STD. *S. aureus* ATCC 25923	0.12	0.12	0.5	0.12	0.12	0.5	0.25	0.25	0.12	0.5	0.12	0.25
Sa 01	0.12	0.5	8	1	4	8	1	8	8	2	1	0.5
Sa 02	0.25	0.5	4	0.5	4	8	1	0.5	2	1	8	8
Sa 03	0.25	4	1	2	0.5	8	4	1	0.5	2	1	4
Sa 04	0.12	2	0.5	0.5	1	0.5	8	4	0.5	0.5	2	0.5
Sa 05	0.5	8	8	0.5	0.5	8	0.5	0.5	0.5	8	8	8
Sa 06	0.12	0.5	0.5	1	0.5	2	8	0.5	2	1	1	8
Sa 07	0.12	0.5	0.5	4	0.5	8	2	1	0.5	2	0.5	0.5
Sa 08	0.25	1	4	8	1	0.5	0.5	0.5	0.5	0.5	8	0.5
Sa 09	0.5	1	0.2	0.5	0.5	8	8	0.5	8	2	0.5	0.5
Sa 10	0.5	0.5	8	1	0.5	8	8	0.5	0.5	8	0.5	8
Sa 11	0.25	0.5	0.5	8	0.5	8	0.5	8	0.5	1	0.5	0.5
Sa 12	0.12	0.5	1	8	0.5	8	0.5	8	1	8	0.5	0.5
Sa 13	0.5	8	0.2	0.5	0.5	1	0.5	1	2	0.2	0.5	8
Sa 14	0.25	0.5	8	1	8	8	1	0.5	1	4	8	0.5
Sa 15	0.12	0.5	8	0.5	1	8	0.5	2	0.5	0.5	8	1
Sa 16	0.25	4	0.5	8	0.5	8	8	8	0.5	0.5	0.5	0.5
Sa 17	0.12	1	8	1	8	2	0.5	0.5	2	0.5	8	0.5
Sa 18	0.12	0.5	2	8	0.5	0.5	0.5	8	0.5	8	0.5	0.5
Sa 19	0.5	8	8	0.5	8	4	8	8	2	8	0.5	2
Sa 20	0.25	0.5	0.5	8	0.5	0.5	0.5	1	0.5	0.5	0.5	4
Sa 21	0.12	0.5	8	0.5	0.5	1	0.5	0.5	0.5	8	0.5	8
Sa 21	0.25	8	0.5	8	0.5	0.5	2	0.5	1	0.5	1	0.5
Sa 23	0.5	0.5	1	0.5	0.5	8	8	8	8	0.5	8	0.5
Sa 24	0.12	0.5	0.2	0.5	2	8	0.5	1	8	0.5	0.5	2
Sa 25	0.25	4	0.5	0.5	1	2	0.5	0.5	0.5	8	8	0.5
Sa 26	0.25	8	0.5	8	0.5	8	0.5	2	0.5	8	0.5	8
Sa 27	0.12	0.5	0.5	1	8	8	0.5	0.5	8	0.5	8	2
Sa 28	0.12	0.5	8	0.25	0.5	0.5	8	8	1	0.5	0.5	0.5
Sa 29	0.12	0.5	8	0.5	1	8	0.5	0.5	0.5	8	0.5	8
Sa 30	0.25	1	2	0.5	8	0.5	8	0.5	0.5	0.5	8	0.5
Sa 31	0.12	8	8	0.5	2	0.5	0.5	1	0.5	0.5	1	8
Sa 32	0.25	0.5	0.5	8	0.5	0.5	2	8	0.5	2	8	8
Sa 33	0.12	0.5	8	0.5	0.5	1	8	8	0.5	8	0.5	8
Sa 34	0.25	8	8	0.5	0.5	8	0.5	8	8	8	0.5	2

Table 4: Resistance Pattern of *Escherichia coli* and *Staphylococcus aureus* against Eleven Brands of Marketed Levofloxacin

Resistance Pattern	Gram-Negative Microorganism	Gram-Positive Microorganism
	Escherichia coli	*Staphylococcus aureus*
Sensitive	59.24%	44.38%
Intermediate	13.47%	25.66%
Resistance	27.27%	29.94%

against resistant pathogens, it has been recommended in severe infections like community acquired pneumonia (CAP) (Mandell *et al.*, 2007), nosocomial pneumonia (Welte and Pletz, 2010), inhalation anthrax (Li *et al.*, 2010), treatment of pulmonary infections due to *Pseudomonas aeruginosa* and other bacteria in patients

with cystic fibrosis (Mesaros *et al.*, 2007). Due to irrational use of Levofloxacin and change in serotype and source of microbes, there is a change in resistance pattern of clinical isolates as shown in various studies (Bucaneve *et al*., 2005; MacDougall *et al.*, 2005; Lockhart *et al.*, 2007). According to CLSI (2011), for *E. coli* $MIC_{90} \leq$ 2µg/ml possessed susceptible, 4 to 8µg/ml and ≥8µg/ml has intermediate and resistant concentration, respectively, in broth dilution method of levofloxacin, while ≤1µg/ml has susceptibility, 2 to 4µg/ml and ≥4µg/ml have intermediate and resistant concentration of levofloxacin against *S. aureus*.

Antimicrobial assay of different marketed levofloxacin by broth dilution method was evaluated against standard *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) and their clinical isolates. The broth dilution tests have been allowed the determination of the MIC_{90} of an antimicrobial agent (Basset *et al.*, 2011). The broth dilution test has been specified that the MIC_{90} of standard levofloxacin against standard *E.coli* (MIC_{90} =0.03µg/ml) indicated by Drago *et al.* (2002). The present studies has shown that resistance against levofloxacin has acquired by *E. coli* and its clinical isolates, significant variations have been found in MIC_{90} of different marketed levofloxacin (MIC_{90} =0.5µg/ml-16.0µg/ml). Soriano and co-workers (2005) have been found MICs of standard levofloxacin (MIC_{90} =0.12µg/ml) against standard *S. aureus*. The present study has prevailed that different brands of 250mg levofloxacin tablets has shown resistance acquired by *S. aureus* and significant variations has been found with (MIC_{90} = 0.5µg/ml-8.0µg/ml) against different clinical isolates of *S. aureus*.

The resistance depend upon the source of infections; serotype; genes of microbes; environmental factors; immunity due to socioeconomic conditions (Goossens *et al.*, 2005; Erb *et al.*, 2007; Shakya *et al.*, 2013). In our study, clinical isolates of *E. coli* and *S. aureus* have possessed 27.5% supported by Noviello *et al*. (2006) and Nasiri *et al*. (2013), in contrast to present study Woo *et al.* (2011) found 37.94% and Zemkova *et al.*, 2007 reported 11%.

Marangon and co-workers (2004) reported 25.5% resistance of *S. aureus* against levofloxacin, similarly 23% resistance has been reported by Shafiq *et al.* (2012). These studies have been supported our study in which 29.94% has been a positive sign of resistance while a fire alarming situation for health associated professionals is that only 44.38% sensitivity of *S. aureus* against levofloxacin has been noticed. In the past half a century, there has been a constant increase in the use of antibiotics for the treatment of bacterial infections. The continuous use and often abuse of the drug have led to an increased resistance to levofloxacin.

CONCLUSION

The prime object of all pharmaceutical industries is to provide quality medicines with efficacy and safety profile. It has been concluded from the present study that there were no significant variations found in MIC_{90} values of different brands of levofloxacin which has been indicated that the levofloxacin tablets manufactured either by multinational or local pharmaceutical industries has been produced approximate similar results. The approximate similarity in results of MIC_{90} of levofloxacin 250 mg tablets of pharmaceutical industries in Pakistan is mainly due to fulfill the requirements of drug regulatory authorities.

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