ENHANCED SUSCEPTIBILITY OF ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PYOGENES TO FLUOROQUINOLONES IN THE PRESENCE OF PROCHLORPERAZINE

Uzma Saleem[1], Muhammad Hidayat Rasool[1], Bashir Ahmad[2], Waqas Sadiq[2], Saeed Mahmood[3], Muhammad Saleem[1], Alia Erum[4]

1. College of Pharmacy, Govt. College University, Faisalabad, Pakistan
2. University College of Pharmacy, University of the Punjab, Lahore, Pakistan
3. Lahore General Hospital, Lahore, Pakistan
4. Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan.

ABSTRACT

Combating the problem of microbial resistance is one of the major challenges the medical sciences facing today. Inhibition of resistance mechanism in bacteria seems much better approach than developing new antibiotics only. The present study was aimed to investigate the effect of different concentrations of prochlorperazine in increasing the effectiveness of fluoroquinolones against *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes*. The bacteria were isolated from indigenous sources and identified through cultural characteristics, Grams' staining and biochemical tests. The master suspensions were subjected to viable count and inoculated at the rate of 10^6 CFU/ml of the media for antimicrobial sensitivity testing. The four fluoroquinolones i.e. ciprofloxacin, levofloxacin, pefloxacin and norfloxacin were first applied to bacterial cultures alone and then in combination with four different concentrations (16µg/ml, 32µg/ml, 64µg/ml and 128µg/ml) of prochlorperazine and inhibition of growth was recorded in terms of diameter of zones of inhibition. A linear relationship was found between the increase in concentration of prochlorperazine and diameter of zones of inhibition against all the fluoroquinolones. The diameter of zones of inhibition were significantly ($p<0.05$) greater with 128µg/ml of prochlorperazine in combination with all the four fluoroquinolones against *Staphylococcus aureus* and *Streptococcus pyogenes*. In contrast *E. coli* showed significant susceptibility ($p<0.05$) only to ciprofloxacin and norfloxacin in the presence of prochlorperazine. It was concluded that prochlorperazine is capable of increasing the susceptibility of *Staphylococcus aureus*, *Streptococcus pyogenes* and *E coli* when used in combination with different fluoroquinolones. This approach of hindering the resistance mechanism of bacteria with non-antibiotics can shift many of the resistant strains of bacteria to susceptible.

Keywords: Enhanced susceptibility; prochlorperazine; fluoroquinolones; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus pyogenes*

***Address for correspondence**:* Dr Muhammad Hidayat Rasool, College of Pharmacy, Govt. College University, Faisalabad, Pakistan. Phone: +92-41-9201036; +92-301-7102378,
Email: *drmhrasooluaf@hotmail.com*

INTRODUCTION

Excessive and irrational use of antibiotics had resulted in the emergence of many resistant strains of bacteria, which are not eradicated even with broad spectrum antibiotics. Many drugs have been reported to bear ant-microbial activity and the ability to combat the resistant mechanisms of bacteria when used in combination with antibiotics. The trend of combination therapy with more than one antibiotic for the treatment of diseases is worsening worldwide but combination of antibiotic drugs with non-antibiotics such as the neuroleptics and their isomers appears promising. These non-antibiotics are classed as therapeutic agents not originally designed for antibiotic or chemotherapeutic purposes but subsequently exhibited such properties [8].

Fluoroquinolones are synthetic chemical agents and among the most commonly prescribed antimicrobials because of their broad-spectrum antimicrobial activity. Excessive clinical use of fluoroquinolones has led to high rates of resistance to these agents among pathogenic microbes [4].The most common mechanism of resistance to fluoroquinolones among pathogenic microbes is the mutation of chromosomal genes encoding DNA gyrase or topoisomerase IV. Changes in the expression of efflux pumps and porin proteins are also common fluoroquinolones resistance mechanisms in bacteria [5].

Escherichia coli are the causative agent in approximately 70-90% cases of uncomplicated urinary tract infections (UTIs) [7]. Fluoroquinolones are the most widely used antibiotics for the treatment of UTIs in most countries of Asia. Broad-spectrum quinolones such as ciprofloxacin and norfloxacin are highly effective against gram-negative bacteria and eradicate bacteriuria in more than 90% cases of UTIs and traveler's diarrhea. About 68% phenotypes among *E. coli* and *Klebsiella pneumoniae* from Indian subcontinent are resistant to fluoroquinolones.

During the past few years, sporadic reports have suggested that phenothiazines and chemically related compounds employed today for the management of psychosis and emesis exhibit additional properties such as antimicrobial activity against a wide array of microorganisms. Antimicrobial activity of phenothiazines has generated particular interest since the molecular active sites of these compounds may contribute to modern concepts of pharmacology such as sterioisomeric relationships and specificity of targets.

The antimicrobial activity of phenothiazines might eventually have a place in the armamentarium of antimicrobials especially with regard to the management of infectious diseases caused by highly resistant microorganisms where the possibility of adequate treatment is problematic. Prochlorperazine is a drug that belongs to the phenothiazines class of antipsychotic agents that are used for the treatment of nausea and vertigo. It is also typical antipsychotic drug and highly potent neuroleptic, 10-20 time more potent than chlorpromazine. It was therefore of interest to investigate the effect of prochlorperazine one of the most commonly used phenothiazine, some of which are known to inhibit efflux pump and chemically related compounds on highly antibiotic resistant human pathogens [8].

Keeping in view the importance of the subject, the present study was designed to investigate the effects of different concentrations of prochlorperazine on the susceptibility of *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes* against fluoroquinolones.

MATERIALS AND METHODS

1. Isolation and characterization of microorganisms:

The selected species of bacteria were isolated from ear swab, throat swab, urine and faecal samples of clinically positive patients from Mayo Hospital Lahore, Pakistan. The samples were collected under all the aseptic conditions and inoculated to Nutrient agar, MacKonkey's agar, Blood agar and Staphylococcus medium 110. The inoculated plates were incubated at 37^{O}C for 3-4 days and observed daily for the presence of growth. The cultural characteristics on different media including colony color, colony margins

and haemolysis were observed for the primary identification. Grams' staining was used for the confirmation of bacterial isolates [9]. Biochemical testing of *Escherichia coli* was performed by using API 20E Kits (BioMerieux) as described by Edelmann et. al. [3]. Manual testing was performed for the biochemical characterization of *Staphylococcus aureus* and *Streptococcus pyogenes* using various sugar fermentation tests and the tests for enzymes.

2. Source of drugs:

Prochlorperazine powder was procured from Getz Pharmaceuticals Pvt. Ltd., Pakistan. A 100 mg of this powder was accurately weighed, dissolved in 500 ml of phosphate buffer saline (PBS) to prepare the stock solution and sterilized using syringe filters of Minisart (Sartorius). Different fluoroquinolones antibiotic discs including ciprofloxacin (5µg), levofloxacin (5µg), pefloxacin (5µg) and norfloxacin (10µg) were also purchased from Oxoid, UK.

3. Standardization of bacterial suspensions:

Master suspensions of isolated bacterial species were prepared separately in Nutrient broth to standardize the inoculums for anti microbial susceptibility testing. The viable bacteria per ml were counted using pour plate method as described by Tortora et. al. [9]. The results of colony forming unit per ml (CFU/ml) of broth were used to calculate the quantity of broth containing standard amount of inoculum for each bacterial species.

4. Antimicrobial susceptibility testing:

Susceptibility of standard concentrations of isolated bacterial species against four different fluoroquinolones was determined through Kirby Bauer disc diffusion method [2]. Briefly, Mueller Hinton agar (Oxoid) medium was prepared and autoclaved at $121^{O}C$ under 15 $lb/inch^2$ pressures for 20 minutes. The cooled but still molten medium was inoculated and mixed separately with standard inoculum of each bacterial species. Medium was poured in sterilized and labeled Petri plates which were divided in to two groups i.e. control group and test group. In control group only inoculated medium was added but in test group inoculated medium was mixed with prochlorperazine solution in four different concentrations i.e. 64µg (1X), 128µg (2X), 192µg (3X) and 256µg (4X) per ml of the media [6]. Antibiotic discs (Oxoid) of ciprofloxacin (5µg), levofloxacin (5µg), pefloxacin (5µg) and norfloxacin (10µg) were dispensed equidistantly on to the surface of inoculated medium and plates were incubated at $37^{O}C$ for 24 hours. The diameters of zones of inhibition were measured in mm and compared. Each test was performed in replicates of five. Data were analyzed statistically using analysis of variance (ANOVA) followed by Turkeys' test on SPSS version 13.0.

RESULTS AND DISCUSSION

Development of new antibacterial drugs requires a huge amount of resources and time while during the last ten years frequency of emergence of resistant strains of bacteria has increased manifolds. Potential candidates, which already have proven to have a restrictive effect on the resistance mechanisms like efflux pumps in bacteria can be combined with the broad-spectrum antibiotics to increase their effectiveness. In the present study, different concentrations of prochlorperazine were used with same intentions in combination with fluoroquinolones against *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes*.

Three different bacterial species i.e. *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes* were isolated from faecal samples, ear swabs and throat swabs of clinically positive patients, respectively. *Escherichia coli* produced small pin point blood red colonies on MacKonkey's agar but showed no haemolysis on blood agar. *Staphylococcus aureus* colonies were smooth with entire edges and orange in color on Staphylococcus medium 110 and showed partial haemolysis on blood agar. The colonies were

irregular with dull surfaces and cream colored on nutrient agar in case of *Streptococcus pyogenes* and showed complete haemolysis on blood agar. On Grams' staining, *Escherichia coli* were G-ve coccobacilli whereas both, *Staphylococcus aureus* and *Streptococcus pyogenes* were G+ve cocci. The *Staphylococcus aureus* were arranged in clusters but *Streptococcus pyogenes* were in the form of long chains. The results are in agreement with those described by Tortora et. al. [9].

Biochemically, *Staphylococcus aureus* was nitrate reduction test, catalase test, coagulase test, glucose, lactose and manitol fermentation tests positive whereas it showed negative results for Voges Proskauer test, citrate utilization test and xylose fermentation test. For *Streptococcus pyogenes*, arginine test, pyrrolydonyl arylamidase test, bacitracin sensitivity test, lactose and trehalose fermentation tests were positive whereas Voges Proskauer test, hippurate test, insulin test, catalase test, ribose, sorbitol, raffinose fermentation tests showed negative results. The results of asculin test and manitol fermentation test were variable. The results of all the 20 biochemical tests performed for *Escherichia coli* using API 20E kit were positive. These results are in line with those described by Awan and Rahman [1].

Pour plate method was used to count viable number of bacteria. The viable count of *Staphylococcus aureus* and *Streptococcus pyogenes* was $4.11x10^{10}$ CFU/ml of the master suspension, whereas each 0.024µl of this suspension contained 10^6CFU/ml, which was used as standard inoculum for antimicrobial sensitivity testing. The viable count of *Escherichia coli* was $3.56x10^7$ CFU/ml and 28µl of master suspension was added per ml of the media to have10^6CFU/ml for antimicrobial sensitivity testing. The same method for viable counting of bacteria has been described by other scientists [1, 9]. The results of antimicrobial sensitivity testing against fluoroquinolones without and with different concentrations of prochlorperazine for *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes* are shown in Table 1, Table 2 and Table 3, respectively.

Table 1: Diameter of zones of inhibition of fluoroquinolones with increasing concentrations of prochlorperazine against *Escherichia coli*

Antibiotics used	Diameter of zones of inhibition (mm) Mean ± SD				
	Negative control	Concentrations of prochlorperazine (µg/ml)			
		16	32	64	128
Ciprofloxacin (5µg)	28.2±0.84	35.6±0.55	35.4±0.55	39.6±0.55	42.4±0.89*
Levofloxacin (5µg)	24.6±0.55	28.4±0.55	27.4±0.55	32.4±0.55	38.2±0.84
Norfloxacin (10µg)	27.0±0.71	35.6±0.55	36.8±0.84	42.2±0.84	48.0±0.71*
Pefloxacin (5µg)	18.6±0.89	23.6±0.55	24.8±0.45	29.6±0.55	31.8±0.45

Each value represents Mean ± SD of five replicates (n=5)
* indicates p value <0.05

In case of *Escherichia coli*, diameter of zones of inhibition in negative control group were 28.2 ± 0.84, 24.6 ± 0.55, 27 ± 0.71 and 18.6 ± 0.89 against ciprofloxacin, levofloxacin, norfloxacin and pefloxacin, respectively. The diameters increased with the increase in concentration of prochlorperazine. The diameter

of zones of inhibition were significantly ($p<0.05$) greater against ciprofloxacin (42.4 ± 0.89) and norfloxacin (48 ± 0.71) when combined with 128µg/ml of prochlorperazine as compared to negative control group (Table 1). Prochlorperazine has the ability to inhibit the efflux pump on the cell membranes of G+ve and G-ve bacteria. P-glycoproteins are the most likely targets of prochlorperazine and specific blockers for P-glycoproteins can be used to confirm these results [8]. In the present study increase in susceptibility of *Escherichia coli* against levofloxacin and pefloxacin was found to be in significant even with the addition of the highest concentration (128µg/ml) of prochlorperazine, suggesting that both of these drugs are pumped out through some transporters other than P-glycoproteins due to difference in chemical structures than the other two drugs used.

Table 2: Diameter of zones of inhibition of fluoroquinolones with increasing concentrations of prochlorperazine against *Staphylococcus aureus*

Antibiotics used	Diameter of zones of inhibition (mm) Mean ± SD				
	Negative control	Concentrations of prochlorperazine (µg/ml)			
		16	32	64	128
Ciprofloxacin (5µg)	13.4±0.89	20.8±0.84	37.6±1.14	45.2±0.84	51.8±0.45*
Levofloxacin (5µg)	17.6±0.55	21.6±1.14	39.0±0.71	45.8±0.84	54.2±1.30*
Norfloxacin (10µg)	10.0±0.71	13.8±0.45	34.8±0.45	42.0±0.71	47.6±0.55*
Pefloxacin (5µg)	10.4±0.89	24.0±0.84	32.0±1.30	38.4±0.45	44.8±0.84*

Each value represents Mean ± SD of five replicates (n=5)
* indicates p value <0.05

The diameters of zones of inhibition for*, Staphylococcus aureus* were 13.4 ± 0.89, 17.6 ± 0.55, 10 ± 0.71 and 10.4 ± 0.89 against ciprofloxacin, levofloxacin, norfloxacin and pefloxacin, respectively in negative control group. There was a linear relationship in increase of diameters with increase in concentration of prochlorperazine. The diameter of zones of inhibition were significantly greater ($p<0.05$) against all four fluoroquinolones when combined with 128µg/ml of prochlorperazine (Table 2). *Streptococcus pyogenes* also showed a linear increase in susceptibility against fluoroquinolones with the addition of different concentrations of prochlorperazine. The diameter of zones of inhibition were 22.8 ± 0.84, 22.4 ± 0.55, 20.2 ± 0.84 and 20.4 ± 0.89 against ciprofloxacin, levofloxacin, norfloxacin and pefloxacin, respectively in negative control group. The diameters were significantly greater ($p<0.05$) against all the four fluoroquinolones when used in combination with the highest concentration (128µg/ml) of prochlorperazine as compared to the negative control group (Table 3).

Table 3: Diameter of zones of inhibition of fluoroquinolones with increasing concentrations of prochlorperazine against *Streptococcus pyogenes*

Antibiotics used	Diameter of zones of inhibition (mm) Mean ± SD				
	Negative control	Concentrations of prochlorperazine (µg/ml)			
		16	32	64	128
Ciprofloxacin (5µg)	22.8±0.84	24.4±0.89	37.6±0.55	44.8±1.64	53.6±0.55*
Levofloxacin (5µg)	22.4±0.55	26.2±0.45	50.2±0.84	61.6±0.55	72.6±0.89*
Norfloxacin (10µg)	20.2±0.84	23.4±0.55	36.4±0.55	41.8±0.84	51.6±0.89*
Pefloxacin (5µg)	20.4±0.89	21.8±1.92	37.2±0.84	51.6±0.55	74.6±0.54*

Each value represents Mean ± SD of five replicates (n=5)
* indicates p value <0.05

Overall results showed that most significant increase in susceptibility of bacterial isolates against fluoroquinolones with prochlorperazine was shown by *Streptococcus pyogenes* followed by *Staphylococcus aureus* and *Escherichia coli*, respectively. These results are in line with those described by Kristiansen et. al. [8] who reported that phenothiazine derivatives like thioridazine, prochlorperazine and chlorpromazine demonstrated resistance reversal effects for methicillin-resistant *Staphylococcus aureus* tested with oxacillin and erythromycin-resistant *Streptococcus pyogenes* tested with erythromycin at a concentration of 812mg/L.

Based on these results it was concluded that non-antibiotics such as prochlorperazine can be combined with certain fluoroquinolones to combat the problem of resistance against G+ve and G-ve bacteria. Moreover, the linear relationship between the concentration of prochlorperazine and increase in the effectiveness of fluoroquinolones in terms of increase in diameter of zones of inhibition is also a significant finding of this study. This approach has exciting prospects and may lead to new concepts of infection containment while shedding light on resistance and efflux mechanisms. Further investigations are required to confirm the nature of efflux transporters and to determine *in vivo* dose of prochlorperazine to inhibit them.

REFERENCES

1. Awan, J.A.; Rahman, S.U. (2002). Microbiology Manual. Unitech Communication, Pakistan.

2. Doern, G.V.; Scot, D.R.; Rashad, A.L.; Kim, K.S. (1981). Evaluation of a direct blood culture disc diffusion antimicrobial susceptibility test. *Antimicrobial Agents Chemother*. 20, 696-698.

3. Edelmann, A.; Pietzker, T.; Wellinghausen, N. (2007). Comparison of direct disc diffusion and standard microtiter broth and dilution susceptibility testing of blood culture isolates. *J. Med. Micro*. 56, 202-207.

4. Engberg, J.; Aarestrup, F.M..; Taylor, D.E.; Smidt, P.G.; Nachamkin, I. (2001). Quinolone and macrolide resistance in *Compylobacter jejuni* and *Compylobacter coli*: resistance mechanisms and trends in human isolates. *Emerg. Infect. Dis.* 7, 24-34.

5. Hooper, D.C. (2001). Principles and practice of infectious diseases. Churchill Livingstone, New York.

6. Jabra, M.A.; Meiller, T.F.; James, C.E.; Shirtliff, M.E. (2006). Effect of farnesol on *Staphylococcus aureus* biofilm formation and antimicrobial susceptibility. *Antimicrobial Agents Chemother*. 50, 1463-1469.

7. Kahlmeter, G. (2000). The ecosens: a prospective, multinational, multicentre epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens. *J. Antimicrobial Chemother*. 46, 15-22.

8. Kristiansen, J.E..; Hendricks, O.; Delvin, T. (2007). Reversal of resistance in microorganisms by the help of non-antibiotics. *J. Antimicrobial Chemother*. 59, 1271-1279.

9. Tortora, G.J.; Funke, B.R.; Case, C.L. (2002). Microbiology: An introduction. Addison Wesley Longman, Int. California.