INT. J. BIOL. BIOTECH., 6 (3): 191-195, 3009.

RESISTANCE PATTERN OF GRAM NEGATIVE UROPATHOGENS ISOLATED FROM COMMUNITY ACQUIRED URINARY TRACT INFECTIONS AGAINST QUINOLONES

Sabahat Saeed[1] and Perween Tariq[2]

[1] *Department of Microbiology, Jinnah University for Women, Karachi, Pakistan.*
[2] *Department of Microbiology, University of Karachi, Karachi Pakistan.*

ABSTRACT

The fluoroquinolones are synthetic compounds with greater potency and a broader antimicrobial activity than their quinolone precursor, nalidixic acid, which is used only as urinary antiseptic. Susceptibility tests of 345 strains, belonging to 9 species of Gram negative uropathogens against quinolones, were carried out by disc diffusion method. This comprised *Escherichia coli* (270 strains), *Klebsiella pneumoniae* (51), *K. ozaenae* (03), *Proteus mirabilis* (05), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (01), *S. paratyphi A* (02), *S. paratyphi B* (01) and *Serratia marcescens* (02). The highest rate of resistance was recorded against ciprofloxacin and nalidixic acid i.e. each 6.1% (21/345) followed by ofloxacin (4.3%, 15/345) and norfloxacin (0.3%, 1/345). Continued surveillance is required to prevent the future spread of these resistancet strains.

Key words: Nalidixic acid, Ciprofloxacin, Ofloxacin, Norfloxacin, *Escherichia. Coli*, Uropathogens.

INTRODUCTION

The fluoroquinolones has become increasingly popular class of antibiotics and is bactericidal against a variety of bacteria including both Gram positive and Gram negative. Examples of fluoroquinolones include ciprofloxacin, ofloxacin, levofloxacin and norfloxacin (Scholar and Pratt, 2000). Their mode of action is inhibition of bacterial DNA synthesis (Drlica and Zhao, 1997; Nester *et al.,* 2004). Fluoroquinolones have also been reported to affect cytokine production *in vitro* (Ogino *et al.,* 2009). The fluoroquinolones are superior to nalidixic acid in their activity against bacterial urinary pathogens, therefore, are used for the treatment of uncomplicated urinary tract infections (UTIs). These are the drug of choice for the treatment of UTIs caused by organisms resistant to beta-lactams. These are also used to treat complicated UTIs caused by multi-drug resistant Gram negative bacteria (Scholar and Pratt, 2000).

The antibiotics of this group used in the present study were ciprofloxacin, ofloxacin and norfloxacin. Ciprofloxacin is a broad spectrum antibiotic. This drug is also compatible with breast feeding. The major side effect of using ciprofloxacin is gastrointestinal irritation (Scholar and Pratt, 2000). It is also known to cause swelling of joints and cartilage and cause tendon rupture and chronic pain. It is used for the treatment of UTIs, lower respiratory tract infections, septicemias and Legionellosis. It is contraindicated in children, during pregnancy and in patients with epilepsy (Hilliard *et al.,* 1995).

Ofloxacin achieves good concentration in genitourinary tract tissues and fluids. It demonstrates consistent efficacy, achieving bacterial response rates of 80% in uncomplicated and 70% in complicated UTIs. The most commonly reported adverse effects with ofloxacin are gastrointestinal, neurological and dermatological disturbances. It was associated with lower incidence of photosensitivity and tendonitis and higher incidence of some neurological events than some other fluoroquinolones. Ofloxacin is also used to treat community acquired pneumonia, gastroenteritis, traveler's diarrhea, veneral diseases and osteomyelitis (Scholar and Pratt, 2000).

Norfloxacin, a broad spectrum antibiotic of fluoroquinolones group, is usually prescribed for the treatment of UTIs (Wajeeha *et al.,* 2006). It is also sometimes used to treat stomach infections (Goldstein, 1987). The use of norfloxacin is limited to infections of the gastrointestinal tract. It plays a key role in the prophylaxis of spontaneous bacterial peritonitis in the cirrhotic patients. However, there are few reports of probable norfloxacin-induced liver toxicity. Although the incidence of hepatic toxicity is low and norfloxacin has been used successfully in existing impairment (Lucena *et al.,* 1998).

The quinolones are the family of broad spectrum antibiotics. One of the attractive features of the quinolones is the ability to kill bacteria rapidly, an ability that differs widely among the various derivatives. The intracellular targets of quinolones are two DNA topoisomerases; gyrase and topoisomerase IV. Gyrase tends to be the primary target in Gram negative bacteria, while topoisomerase IV is preferentially inhibited by most quinolones in Gram positive organisms (Drlica *et al.,* 2009). The nalidixic acid is the member of this group. It is more effective against

Gram negative bacteria than Gram positive. The strains of *E. coli, Klebsiella, Enterobacter, Proteus, Providencia, Pasturella, Salmonella* and *Shigella* are susceptible to it (Scholar and Pratt, 2000).

MATERIALS AND METHODS

Bacterial isolates:

A total of 345 Gram negative uropathogens isolated from community acquired UTIs in females were used to determine their resistance/susceptiblity pattern against fluoroquinolones (ciprofloxacin, ofloxacin and norfloxacin) and quinolones (nalidixic acid). The microbial strains comprised *Escherichia coli* (270 strains), *Klebsiella pneumoniae* (51), *K. ozaenae* (03), *Proteus mirabilis* (05), *Pseudomonas aeruginosa* (10), *Salmonella typhi* (01), *S. paratyphi A* (02), *S. paratyphi B* (01) and *Serratia marcescens* (02).

Determination of antimicrobial susceptibility:

Antimicrobial susceptibility testing was performed using disc diffusion method as described by National Committee for Clinical Laboratory Standards (Presently called as Clinical Laboratory Standars institute) (Cheesbrough, 2000).

Media:

Mueller Hinton Agar (MHA) (Merck) was employed for determination of antimicrobial susceptibility test and Mueller Hinton Broth (MHB) (Merck) was used for preparation of inoculum.

Antibiotic discs:

Different antibiotic discs of ciprofloxacin, ofloxacin, norfloxacin and nalidixic acid were used for microbial susceptibility / resistance (Table 1).

Preparation of turbidity standard:

McFarland Nephlometer Standard tuber number 0.5 was used to standardize the turbidity of test inoculum (Sonnenwirth and Jerett, 1980; Baron *et al.*, 1994).

Preparation of inoculum:

A loopful of pure culture of organisms was transferred to 5ml MHB. The broth was incubated at 35-37^{o}C for 18-24 hours. After incubation, the turbidity of the culture was compared with 0.5 McFarland Nephlometer Standard to get approximate cell density 150 x 10^{6} CFU/ml.

Inoculation of medium:

A sterile cotton swab was immersed into the standardized inoculum suspension. Then it was streaked evenly on the surface of MHA plate.

Disc placement:

Antibiotic susceptibility discs were placed on the surface of inoculated MHA plates by using a sterile forcep.

Incubation:

Plates were incubated at 35-37^{o}C for 18-24 hours.

Interpretation:

Inhibition zone diameters (including diameter of disc) were measured with a ruler. The susceptibility or resistance was interpreted on the basis of criteria mentioned in Table 1.

RESULTS AND DISCUSSION

Because of the increasing resistance to fluoroquinolones, the use of fluoroquinolones as first line therapy for the treatment of uncomplicated UTI is discouraged except in patients who can not tolerate sulfonamides or trimethoprim or who have a high frequency of antibiotic resistance because of recent antibiotic treatment or who reside in area in which significant resistance to trimethoprim-sulfamethoxazole has been noted (Orenstein and Wong, 1999; Nester *et al.*, 2004). The antibiotics used for the study were ciprofloxacin, ofloxacin, norfloxacin and nalidixic acid. Results of the resistance rates of Gram negative uropathogens are shown in Table 2.

Among tested fluoroquinolones, norfloxacin was found most effective antibiotic. Only 0.4% (1/270) isolates of *E. coli* were found resistant to this antibiotic. As far as ciprofloxacin is concerned, 20% (2/10) isolates of *P. aeruginosa*, 20% (1/5) of *P. mirabilis*, 5.9% (3/51) of *K. pneumoniae* and 5.6% (15/270) isolates of *E. coli* were resistant to this antibiotic. Ofloxacin was also found effective, such that 40% (2/5) *P. mirabilis*, 30% (3/10) of *P. aeruginosa*, 3.3% (9/270) of *E. coli*, and 2% (1/51) isolates of *K. pneumoniae* were resistant to ofloxacin. In case of nalidixic acid, 30% (3/10) isolates of *P. aeruginosa*, 20% (1/5) of *P. mirabilis*, 5.6% (15/270) of *E. coli* and 3.9% (2/51) isolates of *K. pneumoniae* were found resistant.

Ciprofloxacin is the most frequently prescribed fluoroquinolones to UTIs because of its availability in oral and intravenous formulations (Warren *et al.,* 1997). In the present study, 5.6% isolates of *E. coli* were resistant to ciprofloxacin. These results are in fair correlation with some previous studies who reported low resistance rates of *E. coli* to ciprofloxacin, such as, 1.03% (Perfetto *et al.,* 2004), 4.5% (Stratchounski and Rafalski, 2006), 8.7% (Kahan, 2006), 9.3% (Bean *et al.,* 2008) and 9.5% (Karlowsky *et al.,* 2003). However, many studies worldwide have also reported a sharp increase in ciprofloxacin resistant *E. coli* isolates from UTIs. For example in Bangladesh the resistance to ciprofloxacin was recorded as 26% (Gupta *et al.,* 2001) and 11.5% (Bhowmick and Rashid, 2004), in Spain 23.9% (Andreu and Planell, 2008) and 22.7% (Garcia *et al.,* 2007), in Korea 23.4% (Kim *et al.,* 2008) and in Pakistan 10% (Memon, 2007). In another study carried out by Kumar and Dass (2004), 38% *E. coli* isolated from hospitalized patients were resistant to ciprofloxacin while 32% *E. coli* were from out patients. In this connection Kawlowskey *et al.,* (2002) demonstrated an increased resistance to ciprofloxacin from 1995 (0.7%) to 2001 (2.5%).

In the present study, the resistant rates of *E. coli* were 3.3% and 0.4% to ofloxacin and norfloxacin respectively. These resistant rates were low as compared to other studies. For instance, Sotto *et al.,* (2007) and Meomon (2007) recorded 5% resistance to ofloxacin, and Goettsch *et al.,* (2000) noted incresea in resistance to norfloxacin in *E. coli* from 1.3% in 1989 to 5.8% in 1998.

Table 1: Criteria for the interpretation of antibiotic susceptibility/resistance.

Antibiotics	Disc code (µg)	Potency	Inhibition zone diameters (mm)		
		Resistant	Intermediate		Sensitive
Ciprofloxacin	CP	05	≤ 15	16-20	≥ 21
Ofloxacin	OF	05	≤ 12	13-15	≥ 16
Norfloxacin	NR	10	≤ 12	13-16	≥ 17
Nalidixic acid	NA	30	≤ 13	14-18	≥ 19

Table 2: Antimicrobial resistance patterns of uropathogens.

Organisms	No. of isolates	Percentage of isolates resistant to			
		CP	OF	NR	NA
E. coli	270	5.6 (15)	3.3 (9)	0.4 (1)	5.6 (15)
K. pneumoniae	51	5.9 (3)	2.0 (1)	0 (0)	3.9 (2)
K. ozaenae	03	0 (0)	0 (0)	0 (0)	0 (0)
P. mirabilis	05	20 (1)	40 (2)	0 (0)	20 (1)
P. aeruginosa	10	20 (2)	30 (3)	0 (0)	30 (3)
S. typhi	01	0 (0)	0 (0)	0 (0)	0 (0)
S. paratyphi A	02	0 (0)	0 (0)	0 (0)	0 (0)
S. paratyphi B	01	0 (0)	0 (0)	0 (0)	0 (0)
S. marcescens	02	0 (0)	0 (0)	0 (0)	0 (0)
Total	345	6.1 (21)	4.3 (15)	0.3 (1)	6.1 (21)

Figures in parenthesis are number of isolates.
CP = Ciprofloxacin, OF = Ofloxacin, NR = Norfloxacin, NA = Nalidixic acid

In the present study, the prevalence of nalidixic acid resistant *E. coli* was 5.6%. This level of resistance is quite low than the resistance reported in a previous study i.e. 40% (Bhowmick and Rashid, 2004).

As far as *Klebsiella* species are concerned, Low level of resistance against fluoroquinolones viz., ofloxacin, ciprofloxacin and norfloxacin; was observed in the present study. All isolates of *K. ozaenae* were found susceptible to fluoroquinolones while only 2.2% and 5.9% isolates of *K. pneumoniae* were resistant to ofloxacin, and ciprofloxacin respectively. In contrary to the present findings, in a previous study (Nwanze *et al*., 2007) 53% and 6% isolates of *K. pneumoniae* were found to be resistant to ciprofloxacin and ofloxacin, respectively. Similarly

Hasan *et al.* (2007) reported 62.8% resistance to ciprofloxacin and norfloxacin against *Klebsiella* species. Furthermore, relatively high level of resistance was recorded by Magalit *et al.* (2004). The rate of resistance against *K. pneumoniae* isolated from community acquired UTIs was 75% to both ofloxacin and ciprofloxacin, while, it was 80% and 100% to ofloxacin and ciprofloxacin, respectively.

The antibiotic resistance pattern observed in the present study revealed that resistance rates of *K. pneumoniae* to nalidixic acid was 3.9% while all isolates of *K. ozaenae* were sensitive to it. These results are contradictory to a study carried out by Nwanze *et al.* (2007) who reported that 94% isolates of *K. pneumoniae* were nalidixic acid-resistant.

Trends of increase of antibiotic resistance in uropathogens and dissemination of their resistant strains have necessitated continued monitoring of antibiotic resistance in uropathogens.

REFERENCES

Andreu, A. and I. Planells (2008). Community acquired lower urinary tract infections and antimicrobial resistance of *Escherichia coli*: a national surveillance study. *Med Clin (Barc).,* 130(13): 481-486.

Baron, E.J., L.R. Peterson and S.M. Finegold (1994). Baily & Scott's Diagnostic Microbiology, 9th edition. The C.V. Mosby Company. Pp. 333-351.

Bean, D.C., D. Krahe and D.W. Wareham (2008). Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates, London 2005-2006. *Annals of Clinical Microbiology and Antimicrobials,* 7: 13-16.

Bhowmick, B.K. and H. Rashid (2004). Prevalence and antibiotic susceptibility of *E. coli* isolated from urinary tract infection in Bangladesh. *Pakistan Journal of Biological Sciences,* 7(5): 717-720.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 1. pp. 370-387.

Drlica, K. and X. Zhao (1997). DNA gyrase, a topoisomerase IV and the 4-quinolones. *Microbiol Mol Biol Rev.,* 61: 377-380.

Drlica, K., M. Malik, R.J. Kerns and X. Zhao (2009). Quinolone-mediated bacterial death. *Antimicrobial Agents and Chemotherapy,* 52(2): 385-392.

Garcia, G.M.I., M.J.L. Munoz and G.J.A. Rodriguez (2007). *In vitro* susceptibility of community acquired urinary tract pathogens to commonly used antimicrobial agents in Spain: comparative multicenter study (20002-2004). *J Chemother.,* 19(3): 263-270.

Goettsch, W., W.V. Pelt, N. Nagelkerkr, M.G.R. Hendrix, A.G.M. Buiting, P.l. Petit, L.J.M. Sabbe, A.J.A. Griethuysen and A.J.F. Neeling (2000). Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in Netherlands. *Journal of Antimicrobial Chemotherapy,* 46: 223-228.

Goldstein, A (1987). Norfloxacin, a fluoroquinolones antibiotic agent. Classification, mechanism of action and *in vivo* activity. *Am J Med.,* 82(6B): 3-17.

Gupta, K., T.M. Hooton and W.E. Stamm (2001). Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Annals of Internal Medicine,* 135(1): 41-50.

Hasan, A.S., D. Nair, J. Kaur, G. Baweja, M. Deb and P. Aggarwal (2007). Resistance patterns of urinary isolates in a tertiary Indian Hospital. *J Ayub Med Coll. Abbotabad,* 19(1): 39-41.

Hilliard, J.J., H.M. Krause, J.I. Bernstein, J.A. Fernandez, V. Nguyen, K.A. Ohemeng and J.F. Berrett (1995). A comparison of active site binding of 4-quinolones and novel flavone gyrase inhibitors to DNA gyrase. *Adv Exp Med Biol.,* 39: 59-69.

Kahan, N.R. (2006). Emperic treatment of urinary tract infection with fluoroquinolones in older women in Israel: Another treatment option? *The Annals of Pharmacotherapy,* 40(12): 2223-2227.

Karlowsky, J.A., C. Thornsberry, M.E. Jones and D.F. Sahm (2003). Susceptibility of antimicrobial-resistant urinary *Escherichia coli* isolates to fluoroquinolones and nitrofurantoin. *Clin Infect Dis.,* 36 (2); 183-187.

Kawlowskey, J., L.J. Kelly, C. Thornsberry, M.E. Jones and D.F. Sahm (2002). Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Antimicrobial Agents and Chemotherapy,* 46(8): 2540-2545.

Kim, M.E., U.S. Ha and Y.H. Cho (2008). Prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in female outpatients in South Korea: A multicenter study in 2006. *Int J Antimicrob.,* 31(1): 15-18.

Kumar, K.A. and S.M. Dass (2004). Antimicrobial resistance patterns of Gram negative bacteria isolated from urine cultures at a general hospital. *Saudi J Kidney Dis. Transpl.,* 15(2): 135-139.

Lucena, M.I., R.J. Andrade, H.S. Martinez, J.M.P. Serrano and A.G. Outes (1998). Norfloxacin-induced cholestatic jaundice. *American Journal of Gastroenterology,* 93: 2309-2311.

Magalit, S.L., M.T.S. Gler and T.E. Tupasi (2004). Increasing antimicrobial resistance of community acquired and nosocomial uropathogens in Makati Medical Center. *Phil J Microbiol Infect Dis.*, 33(4): 143-148.

Memon, B.A. (2007). Predominant and common cause of urinary tract infections in Sukkur city. *Rawal Medical Journal*, 32(2): 99-101.

Nester, E.W., D.G. Anderson, C.E. Roberts, N.N. Pearsall and M.T. Nester (2004). Microbiology: A human perspective, International edition, McGraw Hill. Pp. 633-664, 495-516.

Nwanze, P.I., L.M.Nawaza, S. Oranusi, M.U. Dimkpa, M.U. Okwu, VB.B. Babatunde, T.A. Anake, W. Jatto and C.E. Asagwara (2007). Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. *Scientific Research & Essay*, 2(4): 112-126.

Ogino, H., M. Fujii, M. Ono, K. Maezawa, S. Hori and J. Kizu (2009). *In vivo* and *in vitro* effects of fluoroquinolones on lipopolysaccharide-induced pro-inflammatory cytokine production. *J. Infect. Chemother.*, 15(3): 168-172.

Orenstein, R. and E.S. Wong (1999). Urinary tract infections in adults. http://www.aafp.org/afp/990301ap/12251.html.

Perfetto, E. M., K. Keating, S. Merchant and B.R. Nichols (2004). Acute uncomplicated urinary tract infection and *E. coli* resistance: Implications for first-line empirical antibiotic therapy. *J Manag Care Pharm.*, 10(1): 17-25.

Scholar, E.M., and W.B. Pratt (2000). The Antimicrobial Drugs. Second edition. Oxford University Press. Pp. 242-279.

Sonnenwirth, A.C. (1980). Bacteriology. In: Sonnenwirth A.C. and Jarrett L. (eds): Gradwohl's Clinical Laboratory Methods and Diagnosis. 8th edition. The C.V. Mosby Company. Pp. 480-500.

Sotto, A., C.M.D. Boever, P. Fabbro-Peray, A. Gouby, D. Sirot and J. Jourdan (2007). Risk factors for antibiotic resistant *Eschericia coli* isolated from hospitalized patients with urinary tract infections: a prospective study. *J. Clin. Microbiol.*, 39(2): 438-444.

Stratchounski, L.S. and V.V. Rafalski (2006). Antimicrobial susceptibility of pathogens isolated from adult patients with uncomplicated community-acquired urinary tract infections in the Russian Federation: two multicenter studies, UTIAP-1 and UTIAP-2, *International Journal of Antimicrobial Agents*, 28(1): 4-9.

Wajeeha, F., H. Khan and I. Javed (2006). Bioavailability and pharmacokinetics of norfloxacin after intramuscular administration in goats. *Pakistan Vet. J.*, 26(1): 14-16.

Warren, J.W., E. Abrutyn, J. R. Hebel, J.R. Johnson, A.J. Schaeffer and W.E. Stamm (1997). Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clin Infect Dis.*, 29: 745-758.

(*Accepted for publication May 2009*)