REPORT

Emergence of resistance to fluoroquinolones among gram positive and gram negative clinical isolates

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Abstract: Fluoroquinolones are broad-spectrum antibiotics that are considered as first line drugs to treat infectious diseases. In order to find out useful fluoroquinolones, the antibiotic resistance of fluoroquinolones, namely, ofloxacin (OFL), ciprofloxacin (CIP), norfloxacin (NRF), enoxacin (ENX), pefloxacin (PFL) and levofloxacin (LVF) was investigated against ninety five clinical isolates that includes *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis*. *In vitro* activity of these isolates was carried out by agar dilution method. All *Staphylococcus aureus* were sensitive to OFL at 2 µg/ml. About 6% isolates of *Klebsiella pneumoniae* were found to be resistance to LVF and ENX, 6% to CIP, OFL and PFL and none of the isolates were resistant to LVF and ENX. Percentage resistance of *P. aeruginosa* was found to be 4.35% to CIP, 7% to OFL and 2.2% to NRF, whereas 8.69% to ENX, 0% to PFL and 17.4% to LVF, respectively. The present study provides the data about the emergence of resistance to fluoroquinolones among gram positive and gram negative bacteria and strongly recommends the rational and appropriate use of these antibiotics.

Keywords: Fluoroquinolones, antibiotic resistance, *Staphylococcus aureus*; *Pseudomonas aeruginosa*, agar dilution method.

INTRODUCTION

Bacterial resistance to antimicrobial agents is a serious problem in the treatment of Infectious diseases. One of the most effective ways to control antibiotic resistance is the development of surveillance programs. Fluoroquinolones belong to broad spectrum antibiotics which are used in the management of many infectious diseases. These broad spectrum antibiotics are easily administered and have excellent gastrointestinal absorption, tissue penetration and lack of unwanted side effect (Bonomo *et al.,* 1977). These are classified into first, second and third generation (Albrech *et al.,* 1997, Koga *et al.,* 1980 and Wise *et al.,* 1986) and includes norfloxacin, ciprofloxacin, ofloxacin, pefloxacin, enoxacin etc. These quinolones are analogues of the earlier developed agent, nalidixic acid. They are more potent and broad antibacterial spectrum than nalidixic acid. The fluoroquinolones exert their effect by binding to the enzymes DNA gyrase and topoisomerase IV, which are involved in DNA replication (Luttinger *et al.,* 1995). The lethal effect of quinolone occurs when an intermediary complex of drug and enzymes blocks its replication, and *gyrA* and *parC* genes encode two key target enzymes. Fluoroquinolones resistance may result from chromosomal mutations coding for modification in target subunits (primary gyrase A but also gyrase B) of bacterial topoisomerase (Gootz *et al.,* 1996, Bryan *et al.,* 1989, Wolfson *et al.,* 1989) ciprofloxacin has rapidly become one of the most frequently prescribed oral antibiotics. We have observed widespread inappropriate use of ciprofloxacin and describe several patterns of misuse.(Thomas and Richard 1990).

Ciprofloxacin is more active than norfloxacin against *P. aureginosa,* enterococci and pneumococci. Values of MIC90 range from 0.5 to 6 µg/ml. Ciprofloxacin, ofloxacin, pefloxacin and sparfloxacin also have good activity against *Staphylococci.* MIC90 range from 0.1-1µg/ml. Norfloxacin, ciprofloxacin and ofloxacin (second generation quinolones) are given for 5 days in the treatment of patients with shigellosis. Ciprofloxacin and ofloxacin treatment cures most patients with enteric fever caused by *S. typhi*. Levofloxacin has superior activity against gram positive organisms. The quinolones are being used as part of multiple-drug regimen for the treatment of multiple-drug-resistant tuberculosis (William *et al.,* 2006). The antimicrobial surveillance program should continue to monitor the antimicrobial activity of these newer agents throughout the world, to identify emerging resistant strains and to facilitate possible

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intervention strategies as these newer compounds are used in the clinical setting.

MATERIALS AND METHODS

Collection of clinical isolates

Gram positive and Gram negative clinical isolates were collected from different pathological laboratories and hospital in Karachi. These strains were inoculated in slants containing Muller Hinton Agar (Darmstadt, Germany) at 37°C and stored at temperature between 2-4°C (Frobes *et al.,* 1998).

Preparation of stock solution

Reference Standardized powder of different newer quinolones has collected from different pharmaceutical industries, i.e. Ciprofloxacin (Bayer AG Pakistan (Pvt.) Ltd.), Levofloxacin (Aventis Pharma (Pvt.) Ltd.), Pefloxacin (Sami Pharma (Pvt.) Ltd.), Enoxacin (Abbott Laboratories (Pvt.) Ltd.), Ofloxacin (Hoechst Marion Roussel Pakistan (Pvt.) Ltd.), Norfloxacin (Novartis Pharma (Pak.) Ltd.). For preparing antibiotics stock solution, standardized (lyophilized) powder materials were completely dissolved in sterilized double distilled water or other suitable solvent. The stock solution was then used for making dilution to give the required concentration of each drug and stored in a highly sealed containers at 20°C (Lorian; 1999).

Preparation of media

Muller Hinton Agar and Muller Hinton Broth were prepared in screw capped flask, test tubes. After autoclaving the medium was cooled at 45-50°C in water bath (Bertina; 1987)

Agar dilution susceptibility test

In order to perform agar dilution susceptibility test, the antibiotic was incorporated into a liquefied agar medium (40-45°C), which was then mixed, poured into Petri dishes and allow to solidify. A series of Petri plates were prepared with increasing concentration of drug and with the aid of a multiple inoculums replicator containing 11 wire loops, one for the standard and 10 for the clinical isolates (Steers *et al.,* 1959). The inoculated plates were allowed to stand undisturbed until the spot of inoculum absorbed completely. These plates were then inverted and incubated at 37°C for 16 to 24hrs (Lorian, 1991). The incubated plates were examined for the presence or absence of growth. The lowest concentration of each antimicrobial agent that inhibited growth was considered the MIC. In order to evaluate MIC results, NCCL (National Committee for Clinical Laboratories Standards) recommendations were applied. The Acquisition of resistance was defined as increase in MIC of at least 4-fold (Hamzehpour *et al.,* 1994).

RESULTS

To determine bacterial resistance to fluoroquinolones, clinical isolates were collected from different hospitals of Karachi. Drug susceptibility testing was performed. Six fluoroquinolones were tested against 95 clinical isolates including 27 Gram-positive and 68 Gram-negative bacteria. MIC_{90} and percentage of susceptible and resistant isolates among 95 clinical isolates are summarized in table and fig.

DISCUSSION

Bacterial resistance to antimicrobial agents is a serious problem in the treatment of Infectious diseases. To observe the emergence of resistance, six fluoroquinolones were tested against 95 clinical isolates including 27 Gram-positive and 68 Gram-negative bacteria. Clinical isolates of *Staphylococcus aureus* (n=27) showed highly variable pattern of antibiotic sensitivity. All of the isolates were sensitive to OFL at 2 µg/ml was observed. The present studies showed that against *S.aureus*, OFL is superior to other antibiotics tested. Almost similar findings were also reported (George *et al.,* 1995 and Goldblatt *et al.,* 1998). Fluoroquinolones resistance of *Pseudominas aeruginosa* (n= 46) was found to be 4.35% to CIP, 7% to OFL and 2.2% to NRF, whereas 8.69% to ENX, 0% to PFL and 17.4% to LVF, respectively. Aerobic gram negative bacilli are the most susceptible bacteria to CIP (William and Petri; 2006). The result in the present study showed that *P. aeruginosa* is moderately susceptible to CIP. Another researcher also reported similar increase in resistance to CIP among *P.aeruginosa* (Walker; 1999). Fluoroquinolones are concentration dependent antibiotics and when bacteria are exposed to sub lethal concentration, resistance can be induced (Regis; 2003). Norfloxacin, ofloxacin and ciprofloxacin are the fluoroquinolones with mainly gram-negative coverage used for UTIs. Ciprofloxacin is most notably used for its coverage against *Pseudomonas aeruginosa.* Its use is limited in some case due to ciprofloxacin resistance strains (Karlowsky; 2003 and Smith *et al.,* 2005).

During the present study, all (n=17) isolates of *Klebsiella pneumoniae* were found to be sensitive to LVF and ENX whereas, 6% to CIP, OFL and PFL. Increased resistance to NRF was observed (12%). Oni *et al.* in 2001 reported the increase in resistance among *Klebsiella spp.* to CIP. Five isolates of *Proteus mirabilis* analyzed during this study showed 0% resistance to CIP, OFL, NRF, PEF and ENX. 40% resistance to LVF among *Proteus mirabilis* was observed. Percentage sensitivity to different fluoroquinolones of *P.mirabilis* as exhibited in this study may partly be attributed to the fact that the total number of isolates was too small. Ongoing surveillance of Enterobacteriaceae will be particularly important to monitor changes in fluoroquinolone susceptibility as well

Table: Standard MIC, MIC_{90} and percentage of resistant isolates among 95 bacterial isolates.

Bacterial isolates	Fluoroquinolones*	Standard MIC^{ø}	MIC_{90}	Resistance
		µg/ml	µg/ml	%
Staphylococcus aureus (n=27)	CIP	0.5	>8	49
	OFL	0.5	1	0
	NRF	1	>16	33.3
	ENX	2	>32	22.3
	PFL	0.5	>8	52
	LVF	0.5	>8	63
Klebsiella pneumoniae (n=17)	CIP	0.5	2	6
	OFL	1	4	6
	NRF	0.25	>4	12
	ENX	0.5	0.25	0
	PFL	0.5	0.25	5.88
	LVF	0.25	0.125	0
*Pseudomonas aeruginosa (*n=46)	CIP	4	8	4.35
	OFL	2	4	7
	NRF	2	8	2.2
	ENX	8	64	8.69
	PFL	8	4	0
	LVF	8	>64	17.4
Proteus mirabilis(n=5)	CIP	0.0625	<0.03125	0
	OFL	0.5	<2	0
	NRF	0.25	<0.5	0
	ENX	0.25	0.125	0
	PFL	2	1	0
	LVF	0.25	>4	40

*Ofloxacin (OFL), ciprofloxacin (CIP), norfloxacin (NRF), enoxacin (ENX), pefloxacin (PFL) and levofloxacin (LVF). ^{ø}Standard MIC(minimum inhibitory concentration)

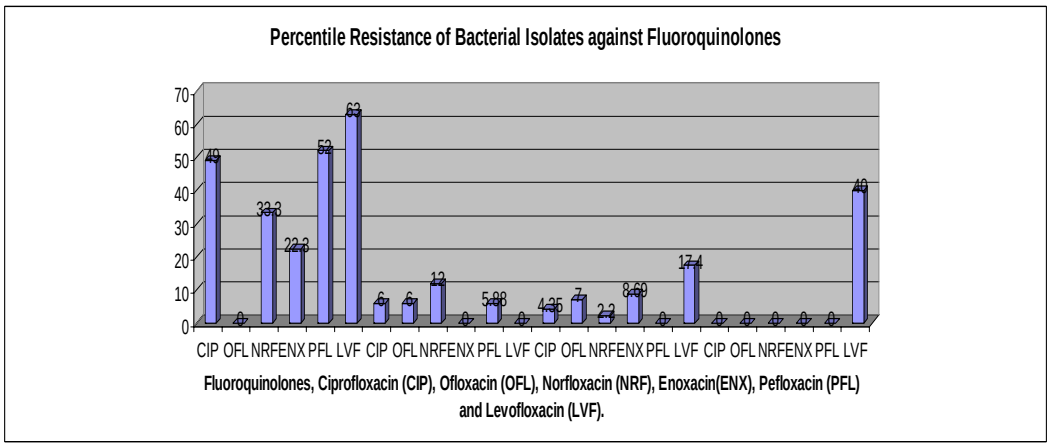

Fig: Percentage resistance against fluoroquinolones among 95 bacterial isolates

as changes in the prevalence of isolates resistance to multiple classes of antimicrobial agents (Jones and Pfaller; 2000). Table and graph show the MIC90 of all fluoroquinolones against different groups of organisms. The MIC90 of OFL against gram-positive bacteria was lower than that of CIP and NRF. The bactericidal activity of CIP and NRF against Gram negative isolates is comparable but MIC90 of NRF is higher than MIC90 of CIP and OFL against Gram positive as well as Gram negative bacteria (Tripathi; 2008). Increase in MIC90 of

ENX, PEF and LVF against *S.aureus* and *P. aeruginosa* was observed. MIC90 of LVF against all bacterial isolates was increased except *Klebsiella pneumonia.* Monotherapy with fluoro-quinolones is a better choice because of quicker clinical response and less toxicity. Heavy dispensing of fluoroquinolones could eventually lead to resistance among pathogens to these drugs (Al Gamdi, 2001 and Knox *et al.,* 2003). Ofloxacin has shown among six fluoroquinolones used in our study, promising activity (above 95% inhibition) against clinical isolates at concentrations lower than the peak serum levels. The present study provides the data about the emergence of resistance to fluoroquinolones among Gram positive and Gram negative bacteria from South Asia. Similar results were also reported from different parts of the World (Panahi *et al.,* 2008).

Emergence of bacterial resistance has observed in the study. Antibiotic surveillance program should be conducted to reduce the risk of development of resistance. The study strongly recommends the adherence to the antibiotic policy and regular susceptibility testing to overcome the problem associated with antimicrobial resistance.

REFERENCES

Albrecht R (1997). Development of antibacterial agents of nalidixic acid type. *Prog. Res.*, **21**: 9-104.

Bertina B. WentWorth (2005). *In:* Diagnostic Procedure for Bacterial Infection. 7th edition, American Public Health Association, Inc. Washington, DC, pp. 471-489, 638-640, 824.

Bryan LE, Bedard J, Wong S and Chamberland S (1989). Quinolone antimicrobial agents: Mechanisms of action and resistance development. *Clin. Invest. MED*, **12**: 14-19.

Frobes BA, Sahm DF and Weissfeld AS (Eds.) (1998). Overview of conventional methods for identification, Chapter 13. *In*: Bailey and Scott's Diagnostics Microbiology, 10th edition, The CV Mosby Company, St. Louis, pp.167-181.

George O Akpede, Olumuyiwa Adeyemib and Jose P Ambec (1995). Trends in the susceptibility to antimicrobial drugs of common pathogens in childhood septicaemia in Nigeria: experience at the University of Maiduguri Teaching Hospital, Nigeria, 1991-1994. *Int. J. Antimicrobial. Agent*, **6**(2): 91-97.

Goldblatt EL, Dohar J, Nozza RJ, Nielsen RW, Goldberg T, Sidman JD and Seidlin M (1998). Topical ofloxacin versus systemic amoxicillin/clavulanate in purulent otorrhea in children with tympanostomy tubes. *Int. J. Pediatr. Otorhinolaryngol*., **46**(1-2): 91-101.

Gootz TD and Brighty KE (1996). Fluoroquinoloe antibacterials: SAR, mechanism of action, resistance and clinical aspects. *Med. Res. Rev.,* **16**: 433-486.

Hamzehpour MM, Pechere JC, plesiat P and Kohler P (1994). Oprk and OprM define two genetically distinct multi drug efflux systems in Pseudomonas aeruginosa. *Antimicrob. Agents Cherm*, **39**(11): 2392-2396.

Jones RN and Pfaller MA (2000). *In vitro* activity of newer fluoroquinolones for respiratory tract infections and emerging patterns. of antimicrobial resistance: data from the SENTRY antimicrobial surveillance program. *Clin. Infect. Dis*., **31**(2): S16-S23.

Karlowsky JA (2003). Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* & *Acinetobacter bavmannii* from hospitalized patients in USA, 1998-2001. *Antimicrob. Agent Chemother.,* **47**(5): 1681-8.

Knox K, Lawsony W, Deanyz B and Holmesx A (2003). Mutidisciplinary antimicrobial management and the role of infectious disease pharmacist, A UK perspective. *JHI*, **53**: 85-90.

Koga H, Itoh A, Murayama S, Suzue S and Irikura T (1980) Structure activity relationships of antibacterial 6,7 and 7-8- disubstituted 1- alkyl -1,4 dihydro-4- oxo-quinolone-3-carboxylic acids. *J. Med. Chem.* **23**: 1358-1363.

Lorian Victor (1991). Antibiotics and laboratory Medicine, Williams and Wilkins, Baltimore, Maryland 21202, USA, 3rd edition, pp.1-16, 53-105, 599-644, 795.

Luttinger A (1995). The twisted "life" of DNA in the cell: bacterial topoisomerases. *Mol. Microbiol.,* **15**: 601-606.

McFarland J (1907). The nephelometar: Instrument for estimating the number of bacteria in suspension used for calculating the opsonic index and for vaccines. *J. Am. Med. Assoc*, **49**: 1176.

Mastour S and Al Gamdi (2001)**.** Empirical treatment of uncomplicated urinary tract infection by community pharmacist in the Eastern province of Saudi Arabia. *Saud. Med. J.,* **22**(12): 1105-1108.

National Committee for Clinical Laboratory Standards (1997). Approved standard M7-A4. Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. 4th ed. NCCLS, Wayne.

Oni AA, Bakare RA, Arowojolu AO, Kehinde Toki RA and Fasina NA (2001). Comparative in *vitro* activities of commonly available quinolones and other antibiotics on bacterial isolates in Ibadan, Nigeria. *Afr. J. Med. Med. Sci*., **30**(1-2): 35-37.

Regis P Kowalski, Lisa M Karenchak and Eric G Romanowski (2003). Infectious disease: Changing antibiotic susceptibility. *Ophthalmol. Clin. N. Am* .**16**: 1-9.

Robert A Bonomo, John Avcott and Robert A Salata (1997). Oral antibiotics in ninety's new drugs new challenges in primary care. *FBS*, **2**(15): 63-71.

Smitha S, Lalitha P, Prajna VN and Srinivasan M (2005). Susceptibility trends of *Pseudomonas species* from corneal ulcers. *Indian J. Med. Microbiol.,* **23**: 168-171.

Steers E, Foltz EL and Graves BS (1959). An inoculating apparatus for routine testing of bacterial Susceptibility to Antibiotics. *Antibit. Chemother.*, **9**: 307-311.

Thomas R Frieden and Richard J Mangi (1990). Inappropriate Use of Oral Ciprofloxacin. *JAMA.* **264**(11): 1438-1440.

Tripathi TD (2008). *In*: Essentials of Medical Pharmacology, 6th edition, Jaypee's Brothers (Pvt.) Ltd., New Dehli, India, pp.647-651.

Walker RC (1999). The fluoroquinolones. *Mayo. Clin. Proc*., **74**(10): 1030-1037.

William A and Petri Jr (2006). *In*: Goodman Gilman's, The Pharmacological Basis of Therapeutics (Laurence L Brunton, John S Lazo and Keith L Parker, eds.), 10th edition, McGraw Hill, New York, pp.1119-1122.

Wise R, Lister D, McNulty CAM, Griggs D and Andrews JM (1986). The comparative pharmacokinetics of five quinolone. *J. Antimicrob. Chemother*. **18**(Suppl. D): 71-81.

Wolfson JS and Hooper DC (1989). Bacterial resistance to quinolones: Mechanisms and clinical importance. *Rev. Infect. Dis.*, **11**(5): S960-S968.

Yunes Panahi, Mojtaba Mojtahedzadeh, Fatemeh Beiraghdar, Marzeyeh Pazooki and Yashar Moharamzad (2008). Prevalence of microorganisms causing septicemia and determination of antimicrobial resistance in intensive care unit. *IJPR*, **7**(4): 305-309.