Zagazig J. Pharm. Sci., June 1996,
Vol. 5, No. 1, pp. 91-97

ISSN 1110-5089

COMPARATIVE IN-VITRO STUDY OF THE EFFICACY OF NEW QUINOLONES VERSUS NALIDIXIC ACID AND NITROFURANTION AGAINST BACTERIAL UROPATHOGENS

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ABSTRACT

Bacteriological examination of 161 patients with urinary tract infections enrolled in the study, revealed that *Pseudomonas aeruginosa* was the commonest organism isolated from urine samples in all patients (36%) followed by *Staphylococcus aureus* (21%), *Klebsiella pneumoniae* (16.8%), *Escherichia coli* (12.4%). Other isolates included *Streptococus pneumoniae* 4.3%), *Proteus mirabilis* (3.7%), *Staphylococus epidermidis* (1.9%), *Streptococcus faecalis* (2.5%) and *Enterobacter aerogenes* (1.2%). Antimicrobial susceptibility patterns highlightened the spectra and efficiencies of the tested antimicrobials. About 86, 80, 74, 24 and 15% of the isolates were sensitive to ciprofloxacin, norfloxacin, pefloxacin, nalidixic acid and nitrofurantoin, respectively. There was an increased emergence of antimicrobial resistant organisms against many agents (multidrug resistance). Nine, two and six isolates of *Ps. aeruginosa, E. coli* and *K. pneumoniae* were resistant to 5,4 and 2 drugs respectively. Meanwhile, 5,2 and one isolates of *S. aureus, St. species and S. epidermidis* were resistant to 3,2 and 5 drugs, respectively. Because of the superior efficacy, broader spectra, proper pharmacokinetics and lower side effects, this study recommends the necessity for use the recent quinolone antibacterials such as ciprofloxacin, norfloxacin or pefloxacin in the treatment of urinary tract infections.

INTRODUCTION

Infection of the urinary tract is one of the most common bacterial diseases. It is notoriously resistant to treatment and can produce serious complications. The proper management and prevention of these infections require knowledge of the type of the bacteria involved and their susceptibility to antibacterial agents.

Nalidixic acid, a pyridone carboxylic acid with its older related derivatives have been available for years (since 1963), primarily for the treatment of urinary tract infections caused by Gram negative enteric bacilli. Recently, structurally related derivatives have been developed; included are ciprofloxacin[1] norfloxacin[2] and pefloxacin[3]. These agents have been shown to have antibacterial activity not only against gram negative bacilli[4] but also gram positive bacteria[1,5,7].

The unpredictability and breadth of drug resistance of many isolates and the development of newer broad spectrum agents such as Quinolones, B-lactamase stable cephalosporins and many others, make in-vitro susceptibility testing a major component of rational therapy.

The purpose of this study was to evaluate the in-vitro susceptibility of uropathogens to three quinolones (ciproflxacin, norfloxacin and pefloxacin) by means of an agar diffusion method and to compare with those of other urinary antibacterial agents such as nalidixic acid (an older quinolone) and nitrofurantoin (synthetic nitrofuran).

MATERIALS AND METHODS

Bacteria:

The organisms studied were all clinical isolates from urine of different patients suffering from urinary tract infections referred to the urology clinic, Hosh Issa central hospital, Beherah, Egypt (1955-1996).

All urine samples were collected in sterile universal bottles, each specimen was cultured and only specimens yielded a pure heavy growth ($\geq 10^5$ CFU/ML) were included in the study. Bacterial isolates were identified conventionally (Cruickshank, 1975)[5].

Antibacterial susceptibility testing :

The in-vitro activity of antibacterials was determined by an agar diffusion method[8] and also by a

Antibacterial & Disc content	Zone diameter (mm)					
	WHO (1977)			Shungu et al (1983)		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Quinolone (10µg)	≤ 15	16 - 22	≥23	≤12	13 - 16	≥17
Nalidixic acid (40µg)	≤13	14 - 18	≥ 19	≤ 13	14 - 18	≥19
Nitrofurantoin (300 µg)	≤ 14	15 - 16	≥ 17	≤ 14	15 - 16	≥17

standardised microdilution test (NCCLS, 1988) with commercially prepared discs (Oxoid) and Mueller Hinton broth with inoculum of approximately 5×10^5 CFU/ml and ciprofloxacin powder (against ciprofloxacin-resistant strains only) provided by Miles Inc., Pharmaceutical division. The antibacterial agent disc contents were: ciprofloxacin (10 µg) norfloxacin (10 µg), pefloxacin (10 µg), nalidixic acid (30 µg) and nitrofurantoin (300 µg).

Zone sizes were determined according to standard recommendation. The interpretative criteria for susceptibility of urinary tract bacterial isolates to the mentioned disc contents of antimicrobials, according to WHO (1977) and Shungu et al. (1983)[10] are as follows:

RESULTS

A total of 161 strains isoalted from specimens of urine from patients submitted to the urology clinic, was studied. They comprised *Ps. aeruginosa* (85 strains) *S. aureus* (34 strains), *K. pneumoniae* (27 strains), *E. coli* (20 strains), *St. pneumoniae* (7 strains), *P. mirabilis* (6 strains), *S. epidermidis* (3 strains), *St. faecalis* (4 strains) and *Ent. aerogenes* (2 strains) as shown in Table (1).

Table (1): Bacterial isolates of urinary tract infection in 161 patients.

Isolate	No.	%
Ps. aeruginosa	58	36
S. aureus	34	21
K. pneumoniae	27	16.8
E. coli	20	12.4
St. pneumoniae	7	4.3
P. mirabilis	6	3.7
S. epidermidis	3	1.9
St. faecalis	4	2.5
Ent. aerogenes	2	1.2
Total	161	100

Table (2) compares the activity of the antibacterial agents against urinary isolates according to WHO (1977)[9]. Ciprofloxacin was the most active compound tested with all of the strains highly suseptible. The suseptibility patterns were intermediate in about 56% and about 34% of the isoaltes with the new quinolones and classic old antiuropathogens, respectively.

About 35, 27, 24, 18 and 15% of the isolates were sensitive to ciprofloxacin, norfloxacin, nalidixic acid, pefloxacin and nitrofurantoin, respectively. The highest frequency of resistance was exhibited with nitrofurantoin (55%) followed by nalidixic acid (38%) and pefloxacin (26%) but with ciprofloxacin and norfloxacin, the isolates exhibited only 9 and 15% resistance, respectively.

Table (3) illustrates the multiple resistance patterns exhibited by nalidixic acid and nitrofurantoin-resistant isoaltes. Among the 25 isolates of the *Ps. aeruginosa*-nalidixic acid resistant, 22 were nitrofurantoin-resistant 14 were norfloxacin and pefloxacin resistant and only nine were ciprofloxacin-resistant. Therefore, 9 of the these isolates were multiple resistant to all 5 tested antibacterials and 14 isolates were resistant to more than three of them.

Among the 12 isolates of *E. coli*- nalidixic acid resistant, only 2 were norfloxacin and nitrofurantoin resistant; 6 were pefloxacin-resistant and none was resistant to ciprofloxacin.

The able also, dipicts that the 6 isolates of *K. pneumoniae* were nalidixic acid and nitrofurantoin-resistant but sensitive to the new quinolone antibacterials. Among the 21 nitrofurantoin resistant (Nalidixic acid-sensitive) *Ps. aeruginosa* isolates, only 2 were pefloxacin-resistant. Two isolates of *E. coli* and 9 isolates of *K. pneumoniae* were only nitrofurantoin-resistant.

Table (3) also, illustrates the multiple resistance of Gram positive bacterial isolates against new quinolone antibacterials and nitrofurantoin but not nalidixic acid.

Regarding *S. aureus*, 5 isolates were resistant to all tested quinolone antibacterials but sensitive to nitrofurantoin, while other 5 isolates were resistant only to pefloxacin. Four isolates were resistant to nitrofurantoin and pefloxacin while another 3 were resistant only to nitrofurantoin. The resistance exhibited to one isolate of *S. epidermidis* was against all tested antibacterials.

Concerning the 11 isolates of nitrofurantoin-resistant streptococci, only for were pefloxacin-resistant, too. The presumptive criteria of activity according to Shungu et al. (1983)[10] highlightened the results as shown in tables (5&6).

The sensitivity patterns took the rank of ciprofloxacin (85.7%), norfloxacin (78.9%), perfloxacin (73.9%), nalidixic acid (24%), and nitrofurantoin (15%).

* National Commitee for Clinical Laboratory Standards; (NCCL, 1988). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A; Villanova, Pennsylvania: National committee of clinical laboratory standards, (1988).

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Table (2): Comparative activity of three new quinolones, nalidixic acid and nitrofurantoin against the isolated uropathogens (WHO, 1977).

Isolate & NO	Susceptibility Patterns (No. Of Isolates)														
	Ciprofloxacin			Norfloxacin			Pefloxacin			Nitrofurantion			Nalidixic Acid *		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ps. aeruginosa (58)	19	30	9	11	33	14	9	33	16	9	5	43	14	19	25
S. aureus (34)	11	18	5	11	18	5	9	11	14	5	22	7			
K. pneumoniae (27)	12	15	-	9	18	-	6	21	-	3	9	15	6	15	6
E. coli (20)	8	12	-	8	10	2	2	12	6	8	8	4	2	6	12
St. pneumoniae (7)	-	7	-	-	7	-	-	5	2	-	-	7			
P. mirabilis (6)	4	2	-	2	4	-	2	4	-	-	-	6	4	2	-
S. epidermidis (3)	2	-	1	2	-	1	2	-	1	2	-	1			
St. faecalis (4)	-	4	-	-	4	-	-	2	2	-	-	4			
Ent. aerogenes (2)	-	2	-	-	-	2	-	2	-	-	-	2	-	2	-
Total (161)	56	90	15	43	94	24	30	90	41	24	48	89	26	44	43
%	34.8	65	9.3	26.7	58.4	15	18.6	56	25.5	15	29.8	55.3	23	39	38

Table (3): Resistance patterns of nalidixic acid-resistant isolates of gram negative and gram positive bacteria.

Organism	Patterns of resistance to				
	Nalidixic acid	Ciprofloxacin	Norfloxacin	Pefloxacin	Nitrofurantion
Ps. eruginosa	25	9	14	14	22
	-	-	-	2	2
	-	-	-	-	19
E.Coli	12	-	2	6	2
	-	-	-	-	2
K.pneumoniae	6	-	-	-	6
	-	-	-	-	9
Total	43	9	16	22	62
S.aureus	NOT TESTED	5	5	5	-
		-	-	-	3
		-	-	4	4
		-	-	5	-
S. epidermidis		1	1	1	1
St. pneumoniae		-	-	2	2
St. faecalis		-	-	2	2
		-	-	-	2
Total		6	6	19	19

On the contrary, the resistance patterns took the reverse profile. As shown in Table (4), the 25 ciprofloxacin-resistant isolates discovered by disc agar diffusion method were also, exhibited resistant patterns by broth dilution method (MIC ranged from 3.5-6 ug/ml) whereas the cutoff value of ciprofloxacin sensitivity is ≤ 2 ug/ml as reported by NCCLS (1988)*

Table (4): Susceptibility testing of ciprofloxacin-resistant isolates by micro-dilution (MIC) and agar diffusion (zone diameter).

Ciproflaxacin-resistant isolate	No.	Mean zone diameter	Mean MIC (µg/ml)
Ps. aeruginosa	9	12.5	4.8
S. aureus	15	10.0	4.3
S. epidermidis	1	00.0	6.0

Table (5&6) depict susceptibility patterns and the percentage patterns of susceptibility of each microorganisms to each antibacterial. Complete sensitivity was exhibited by *K. pneumoniae, P. mirabilis* and *St. faecalis* to ciprofloxacin and norfloxacin; *E. coli* to ciprofloxacin and *St. pneumoniae* and *Ent. aerougenes* to ciprofloxacin and pefloxacin. Meawhile, complete resistance was exhibited by *St. pneumoniae, P. mirabilis, St. faecalis* and *Ent. aerogenes* to nitrofurantoin.

DISCUSSION

New quinolone antibacterials exhibited many differences compared with the older quinolone derivatives, such as nalidixic acid, pipemidic acid or oxolinic acid. They are considerably more active against gram negative bacteria and active also, against genera resistant to the older drugs, such as gram positive cocci.

In the present, study ciprofloxacin demonstrated a very promising broad spectrum of activity against various gram negative and gram positive organisms including multiply-resistant hospital strains. Comparative in-vitro studies, previously have shown ciprofloxacin to be superior to cefaclor, ceflazidime, cefotaxime aztreonam, imipenem, gentamicin, cefuroxime, amikacin, mezlocillin, nalidixic acid and norfloxacin[1]. The present study confirms such studies and shows that 100% of the gram negative isolates as well as more than 70% of *Ps. aeroginosa* isolates were sensitive to ciprofloxacin. In addition, more than 80% of most gram positive bacteria isolates were also, sensitive to such antibacterial. However, 85.7% of the overall isolates were ciprofloxacin-sensitive.

Even if it is assumed that the mode of action of ciprofloxacin is similar to that of nalidixic acid[11], it is difficult to speculate on its enhanced activity which may will be attributable to increased permeability and drug transport across bacterial cells or possibily because it is not affected by nal B or nal D mutations.

In a similar study carried out by Garcia-Rodriguez et al.[12]) in Spain, the susceptibility patterns showed higher percentage of sensitives than these illustrated in this study. In that study 99,96 and 92% comparing 85,15 and 35% in this study of *E. coli* uropathogens were sensitive to norfloxacin, nalidixic acid and nitrofurantoin. In addition, the present study showed that 100% and 70% of *E. coli* isolates were sensitive to ciprofloxacin and pefloxacin, respectively.

Correspondingly, the % sensitvities of *K. pneumoniae* uropathogens in this study, 100, 22.2 and 11% were sensitive to norfloxacin, nalidixic acid and nitrofurantoin respectively, which are incompatible with those in Spain study where 84 and 76% were sensitive to nalidixic acid and nitrofurantoin. In agreement with the present study, 100% of *K. pneumoniae* isolates were norfloxacin-sensitive. The present study also, showed that 100 and 85.2% of *K. pneumoniae* isolates were sensitve to ciprofloxacin and pefloxacin, respectively.

As regards, *Ps. aeruginosa* isolates in this study showed 75.5, 55.3, 24.1 and 10.3% sensitivity to norfloxacin or ciprofloxacin; pefloxain; nalidixic acid and nitrofurantoin, respectively. These findings are comparable to some extant with those of Garcia-Rodriguez et al.[12] that reported 94% of *Ps. aeruginosa* isolates were norfloxacin-sensitive and none was sensitive to nalidixic acid or nitrofurantoin.

The findings in this study demonstrated that 100 and 66.7% of *Proteus species* isolates were sensitive to ciprofloxacin or norfloxacin and nalidixic acid or pefloxacin, while none was sensitive to nitrofurantoin. These are not comparable to those reported by Garcia-Rodiguez et al.[12] where 100, 86 and 7% of Proteus isolates were sensitive to norfloxacin, nalidixic acid and nitrofurantoin, respectively.

Garcia-Rodriguez[12] reported 100, 90 and 86% of *Enterobacter species* were sensitive to norfloxacin, nalidixic acid and nitrofurantoin, respectively. The two strains of *Enterobacter aerogenes* isolated in this study were not sensitive to any of them. By far, they were sensitive to ciprofloxacin and pefloxacin.

In the present study, the activity of the tested antibacterials against gram positive uropathogens, was also, illustrated where 100% sensitivity was exhibited by streptococci to ciprofloxacin, norfloxacin and pefloxacin, while more than 75% sensitivity was exhibited by stahylococci to ciprofloxacin and norfloxain.

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Table (5): Activity patterns of the tested antibacterials against the isolated uropathogens according to Shungu et al (1983).

Isolate & NO	Susceptibility Patterns (No. Of Isolates)														
	Ciprofloxacin			Norfloxacin			Pefloxacin			Nitrofurantion			Nalidixic Acid *		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ps. aeruginosa (58)	42	8	8	42	14	2	32	14	12	6	8	44	14	20	24
S. aureus (34)	28	-	6	25	3	6	18	13	3	6	22	6	-	-	-
K. pneumoniae (27)	27	-	-	27	-	-	24	3	-	3	9	15	6	15	6
E. coli (20)	20	-	-	17	3	-	14	3	3	7	7	6	3	6	11
St. pneumoniae (7)	7	-	-	4	3	-	7	-	-	-	-	7	-	-	-
P. mirabilis (6)	6	-	-	6	-	-	4	2	-	-	-	6	4	2	-
S. epidermidis (3)	2	-	1	2	-	1	2	-	1	2	-	1	-	-	-
St. faecalis (4)	4	-	-	4	-	-	2	2	-	-	-	4	-	-	-
Ent. aerogenes (2)	2	-	-	-	2	-	2	-	-	-	-	2	-	2	-
Total (161)	138	8	15	127	25	9	105	37	19	24	46	91	27	45	41
%	85.7	5	9.3	78.9	15.5	5.6	73.9	14.3	11.8	14.9	28.6	56.5	23.9	39.8	36.3

S; Sensitive, I: Intermediate, R: resistant

Table (6): Percentage susceptibility pattern of uropathogens to antibacterials.

Isolate & No.	Pattern	% Susceptibility patterns				
		Ciprofloxacin	Norfloxacin	Pefloxacin	Nitrofurantoin	Nalidixic Acid
Ps.aeruginosa(58)	S	72.4	72.5	55.3	10.3	24.1
	I	13.8	24.1	24.1	13.8	34.5
	R	13.8	3.4	20.6	75.9	41.4
S. aureus (34)	S	82.4	73.6	53	17.6	
	I	0	8.8	38.2	64.8	
	R	17.6	17.6	8 8	17.6	
K.pneumoniae(27)	S	100	100	85.2	11.1	22.5
	I	0	0	14.8	33.3	55.6
	R	0	0	0	55.6	22.2
E. coli (20)	S	100	85	70	35	15
	I	0	15	15	35	30
	R	0	0	15	30	55
St. pneumoniae (7)	S	100	57.2	100	0	
	I	0	42.8	0	0	
	R	0	0	0	100	
P. mirabilis (6)	S	100	100	66.7	0	66.7
	I	0	0	33.3	0	33.3
	R	0	0	0	100	0
S. epidermidis (3)	S	66.7	66.7	66.7	66.7	
	I	0	0	0	0	
	R	33.3	33.3	33.3	33.3	
St. faecalis (4)	S	100	100	50	0	
	I	0	0	50	0	
	R	0	0	0	100	
Ent. aerogenes (2)	S	100	100	100	0	0
	I	0	0	0	0	100
	R	0	0	0	100	0

S : Sensitive , I : Intermediate , R : resistant

The high prevalence of resistance to the commonly used antibacterials such as nitrofurantoin and nalidixic acid has caused considerable alarm. The factors favouring antibacterial resistance are though to be previous use of an antibacterial by the individual or widespread use of antimicrobials in the community, a thing that can cause a shift in the species resistance pattern of organisms prevalent in a community.

Such a sitation is very common in developing countries, mainly because of failure to restrict the use of antibacterials in hospitals and control their sale and use in the community.

Taking in consideration the resistance patterns exhibited by *Ps. aeruginosa* isolates, this study included 3.4, 41.4 and 75.9% of isolates were resistant to norfloxacin, nalidixic acid and nitrofurantoin, respectivly, whereas in Spain study 85 and 100% were resistant to nalidixic acid and nitrofurantoin while none was resistant to norfloxacin. In agreement with Spain study, this study showed none of *E. coli* nor *K. pneumoniae* isolates was resistant to norfloxacin. While 3 and 4% of *E. coli* isolates and 8 and 18% of *Klebsiella species* isolates were nalidixic acid and nitrofurantoin-resistant, respectively in Spain study, this study showed 55 and 30% of *E. coli* and 22.2 and 55.6% of *K. pneumoniae* wre nalidixic acid and nitrofurantoin-resistant, respectively.

Resistance to ciprofloxacin develops in gram positive organisms according to amultisteps pattern (stepwise mannar). First step mutants usually showed a fourfold to eightfold decrease in susceptibility. Contrary to the situation in gram negative organisms, resistant mutants did not show reduced growth[13]. A point should be noted that selection of resistant mutants could occur during treatment of systemic gram positive infections with orally administered ciprofloxacin.

In general, this study is on shares with others[4,14,15] to assist the fact that new quinolone antibacterials such as ciprofloxacin, norfloxacin and pefloxacin are active against a wide variety of aerobic gram positive and gram negative bacteria. Furthermore, there was little cross-resistance between those agents and other. Nalidixic acid resistant strains remained susceptible to such new quinolones but were significantly less susceptible than nalidixic acid-susceptible strains.

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دراسة معملية لمقارنة كفاءة مضادات البكتريا الكينولونية مع مثيلاتها القديمة مثل حمض الناليدكسيك والنيتروفيورانتيوين ضد المسببات البكتيرية لأمراض المسالك البولية

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أوضحت الدراسة البكتريولوجية لـ ١٦١ مريض بالتهابات المسالك البولية أن بكتريا الزودوموناس هى المسبب الأكثر تكراراً بين ٣٦٪ من المرضى ثم البكتريا العنقودية ٢٣٪ والكليبسيلا ١٦ر٨٪ والإيشير يشيا ١٢ر٤٪، البروتياس ٣ر٧٪، الإنتروباكتر ١ر٢٪ أما البكتريا السبحية فقد عزلت من ٦ر٨٪.

هذا وقد أوضحت الدراسة مدى كفاءة مضادات البكتريا التى تم إختبارها، فقد ثبتت فاعلية السيبروفلوكساسين مع ٨٦٪ من العزلات، أما النورفلوكساسين والبفلوكساسين فثبتت مع ٨٠، ٧٤٪ على الترتيب، أما المركبات قديمة الاستخدام مثل حمض النليدكسيك، فقد أثبتت فاعليته فى ٢٤٪ من العزلات السالبة لصبغة جرام فقط. وكذا مركب النيتروفيورانتوين فى ١٥٪ من العزلات.

وقد أثبتت الدراسة أيضاً أن هناك تزايد مستمر فى انتشار سلالات بكتيرية عديمة التأثر بعديد من المضادات البكتيرية. فقد وجد أن ٩، ٢، ٦ عزلات من الزودوموناس والإيشيريشيا والكليبسيلا كانت عديمة التأثر بـ ٥، ٤، ٢ مضاد بكتيرى على الترتيب. كذلك ٥، ٢ عزلة من البكتريا العنقودية والسبحية كانت عديمة التأثر بـ ٣، ٢ مضاد بكتيرى على الترتيب. والأكثر من ذلك أن سلالة من الميكروب العنقودى الانتهازى كانت عديمة التأثر بجميع المضادات البكتيرية المستخدمة.

ونظراً للكفاءة العالية ومدى التأثير الواسع وتحقيق تركيزات عالية فى البول وكذلك محدودية الآثار الجانبية لتلك المركبات المستحدثة، فتوصى الدراسة بضرورة استخدام مركبات الكينولون الجديدة المضادة للبكتريا مثل السيبروفلوكساسين والنورفلوكساسين والبفلوكساسين لعلاج مرضى المسالك البولية.