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Susceptibility Pattern of Gram Negative Urine Pathogens with Exclusion of *Escherichia coli* to Quinolone/ Fluoroquinolone Antibiotics

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Abstract
Quinolone/Fluoroquinolones group of antibiotics are frequently used for the treatment of urinary tract infection (UTI). Because of high frequency of Gram negative bacterial infection in UTI and emerging resistant urinary pathogen in spite of lower prevalence with exclusion of *Escherichia coli*, the susceptibility pattern was subjected in this study. The study was conducted at National Public Health Laboratory (NPHL) Teku, Kathmandu with an objective to study the antibiotic Quinolone/Fluoroquinolones susceptibility pattern.Out of 550 urine samples, 100 (18.18%) bacteria were isolated from NPHL. Also, 69 isolates from Blue-Cross Hospital and 83 isolates from Medicare Hospital were collected. Altogether 252 urine isolates, showing significant growth in MacConkey Agar and blood agar were identified by standard microbiological techniques. Among 62 Gram negative isolates other than *Escherichia coli* subjected for the study; *Proteus mirabilis* (27.42%), *Proteus vulgaris* (11.29%), *Klebsiella oxytoca* (12.90%), *Klebsiella pneumoniae* (17.75%), *Citrobacter freundii* (8.06%), *Enteribacter cloaecae* (6.45%), *Pseudomonas aeruginosa* (12.90%), *Acinetobacter* spp. (3.23%). Altogether eight types of antibiotics belonging to quinolone/fluoroquinolone group were used in this study. Among them, norfloxacin was found most sensitive and nalidixic acid was most resistant against Gram negative isolates.

Key words: AST, Gram negative, Quinolone/Fluroquinolone, urinary tract infection

Introduction
Urinary tract infection (UTI) simply means the presence of bacteria undergoing multiplication in human urine within the urinary drainage system (Leigh 1990). From a microbiological perspective, UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney or prostate. In most instances, growth of more than 10^5 organisms per milliliter from a properly collected midstream "clean-catch" urine sample indicates infection (Stamm 2003). There are wide varieties of Gram positive and Gram negative organisms that cause the UTI. Among Gram negative *E. coli* is the most common cause of UTI.Such as *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., and *Staphylococcus aureus* are generally associated with hospital acquired infection followed by catherization and gynecological surgery. Infections due to *Proteus* spp. are associated with renal stones. *Staphylococcus saprophyticus* infection is usually found in sexually active women (Forbes *et al.* 2002).

There has been a recent dramatic growth in information on the use of fluoroquinolone antimicrobial agents for the oral treatment of bacterial infections of the genitourinary tract. The fluoroquinolones include norfloxacin, ciprofloxacin, ofloxacin, non fluorinated agent nalidixic acid. The low risk of nephrotoxicity of antibiotics in patients with complicated urinary tract infection may be especially advantageous (Fluit *et al.* 2004).

The quinolones also referred to as fluoroquinolones are a family of synthetic broad-spectrum antibiotics. The first generation of the quinolone begins with the introduction of nalidixic acid in 1962 for the treatment of urinary tract infections in humans (Denyer *et al.* 2007). Quinolones or fluoroquinolones, some of the most frequently prescribed antimicrobial agents worldwide target the bacterial type II Topoisomerase, Gyrase and Topoisomerase IV. Fluoroquinolones increase the longetivity of the normally short-lived cleaved DNA topoisomerase intermediates. DNA tracking machinery somehow is affected by these intermediates, resulting in multiple subsequent effects, such as chromosome fragmentation, the inhibition of DNA synthesis and death (Lauren 2009).

The fluoroquinolones resistance is acquired by changes in outer-membrane permeability for gram negatives organisms. Efflux, however, does contribute to resistance mainly low level for both Gram positive and Gram negative bacteria (Denyer *et al.* 2007).

Methodology

This study was conducted at the National Public Health Laboratory, Kathmandu, Teku from Sep, 2009-Mar, 2010. Cross-sectional study was performed.

Sample collection and transport

Each patient was given a sterile dry, wide-naked leak proof container and requested for 10-20 ml mid-stream urine sample. He/She was instructed properly for collecting mid stream urine sample before providing the container. The clinical urine samples were collected from Medicare Hospital and Blue-Cross Nursing home by sub-culturing on nutrient agar (NA) slants.

Culture of sample

The urine samples were cultured into the MacConkey agar and blood agar medium by semi-quantitative culture technique using standard loop to detect the presence of significant bacteriuria (Cheesbrough 2000).

Identification of isolates

Standard protocols provided by (Cheesbrough 2000) and *(*Collee 1996) were followed for identification of bacteria isolated from urine specimens.

Antibiotic susceptibility testing (AST)

According to protocols of (Vandepitte *et al.* 2003) and (Sapkota *et al.* 2014), the tests were performed.

Selected antibiotics for AST

Antibiotics used for UTI from quinolone/ fluoroquinolone group were Nalidixic acid (NA), Moxifloxacin (MFX), Levofloxacin (LEV), Gatifloxacin(GAT), Ofloxacin (OF), Ciprofloxacin (CIP), Norfloxacin (NOR) and Enrofloxacin (ENF).

Data analysis

The data were analyzed by statistical package for social science (SPSS) software version 16.0 and One-way ANOVA.

Results and Discussion

During the study period, a total of 550 patients suspected of urinary tract infection visiting National public Health Laboratory (NPHL), were included for urine culture. One hundred isolates from NPHL, 83 isolates from Medicare Hospital and (69) isolates from Blue-Cross Nursing home were the sources of the selected isolates used for antibiotic sensitivity testing**.**

The total Gram negative isolates were 62 excluding *E.coli*. Most isolates were *Proteus mirabils* (27.42 %) and least were *Acinetobacter* spp. (3.23%).

Table 1. Total bacterial isolates from urine culture

Bacterial isolates of UTI		
Gram negative isolates (61.51 %)	No. of isolates	Percentage (%)
Proteus vulgaris	7	11.29
Proteus mirabils	17	27.42
Klebsiella oxytoca	8	12.90
Kelbsiella pneumoniae	11	17.75
Citrobacter freundii	5	8.06
Enterobacter cloaecae	4	6.45
Pseudomonas aerogionosa	8	12.90
Acinetobacter spp.	2	3.23
Total Gram negative isolates	62	100

Table 2. Quinolone\Fluoroquinolone sensitivity pattern of *P. vulgaris*

Proteus vulgaris (n=7)							
Groups of antibiotics	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\Fluoroquinolone	NA	5	71.43	0	0	2	28.57
	MFX	3	42.86	0	0	4	57.14
	ENF	5	71.43	0	0	2	28.57
	LEV	5	71.43	0	0	2	28.57
	GAT	3	42.86	0	0	4	57.14
	OFX	3	42.86	2	28.57	2	28.57
	CIP	5	71.43	0	0	2	28.57
	NOR	2	28.57	0	0	5	71.43

In Quinolone/Fluoroquinolones group of antibiotics Nalidixic acid, Enrofloxacin, Levofloxacin and Ciprofloxacin showed similar resistance pattern i.e., 71.43%. Norfloxacin (71.43%) was found to be more sensitive towards the isolates in this study.

In Quinolone\Fluoroquinolones group, Nalidixic acid was found most resistant (76.47%) whereas Norfloxacin was found most sensitive (82.35%) and Gatifloxacin and Ciprofloxacin were sensitive (76.47%) towards isolates.

Table 3. Quinolone\Fluoroquinolone sensitivity pattern of *P. mirabilis*

Proteus mirabilis (n=17)							
Antibiotics groups Quinolone\Fluoroquinolone	Antibiotics	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
	NA	13	76.47	3	17.65	1	5.88
	MFX	6	35.29	0	0.00	11	64.71
	ENF	4	23.53	1	5.88	12	70.59
	LEV	4	23.53	0	0.00	13	76.47
	GAT	2	11.76	2	11.76	13	76.47
	OFX	5	29.41	2	11.76	10	58.82
	CIP	2	11.76	3	17.65	12	70.59
	NOR	2	11.76	1	5.88	14	82.35

Table 4. Quinolone\Fluoroquinolone sensitivity pattern of *K. oxytoca*

Klebsiella oxytoca (n=8)							
Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\Fluoroquinolones	NA	3	37.50	0	0.00	5	62.50
	MFX	4	50.00	0	0.00	4	50.00
	ENF	3	37.50	0	0.00	5	62.50
	LEV	3	37.50	0	0.00	5	62.50
	GAT	3	37.50	0	0.00	5	62.50
	OFX	3	37.50	0	0.00	5	62.50
	CIP	3	37.50	0	0.00	5	62.50
	NOR	2	25.00	0	0.00	6	75.00

Among Quinolone\fluoroquinolone, the isolates were sensitive towards Norfloxacin where as resiatance towards Nalidixic acid, Moxifloxacin, Enrofloxacin, Levofloxacin, Gatiflixacin, Ofloxacin, Ciprofloxacin.

Table 5. Quinolone\Fluoroquinolone sensitivity pattern of *K. pneumonia*

Klebsiella pneumoniae (n=11)

Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\Fluoroquinolones	NA	8	72.73	0	0.00	3	27.27
	MFX	6	54.55	2	18.18	3	27.27
	ENF	6	54.55	0	0.00	5	45.45
	LEV	6	54.55	0	0.00	5	45.45
	GAT	6	54.55	1	9.09	4	36.36
	OFX	6	54.55	1	9.09	4	36.36
	CIP	7	63.64	0	0.00	4	36.36
	NOR	2	18.18	0	0.00	9	81.82

In Quinolone/Fluoroquinolones; the isolates were most resistant towards Nalidixic acid (72.73%) followed by Ciprofloxacin 63.64% and 81.82% sensitive for Norfloxacin.

Among the Quinolone\fluoroquinolone; isolates were most sensitive towards Norfloxacin (100%) followed by Moxifloxacin, Enrofloxacin, Levofloxacin, Gatifloxacin and Ciprofloxacin (80%).

Table 6. Quinolone\Fluoroquinolone sensitivity pattern of *C. freundii*

Citrobacter freundii (n=9)

Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\Fluoroquinolones	NA	4	80	0	0	1	20
	MFX	1	20	0	0	4	80
	ENF	1	20	0	0	4	80
	LEV	1	20	0	0	4	80
	GAT	1	20	0	0	4	80
	OFX	1	20	1	20	3	60
	CIP	1	20	0	0	4	80
	NOR	0	0	0	0	5	100

Table 7. Quinolone\Fluoroquinolone sensitivity pattern of *E. cloaecae* isolates

Enterbacter cloaccae (n=4)

Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\Fluoroquinolones	NA	2	50	0	0	2	50
	MFX	1	25	1	25	2	50
	ENF	1	25	0	0	3	75
	LEV	2	50	0	0	2	50
	GAI	1	25	0	0	3	75
	OFX	1	25	2	50	1	25
	CIP	1	25	0	0	3	75
	NOR	0	0	0	0	4	100

Isolates were 100% sensitive towards Norfloxacin, while towards Enrofloxacin, Gatifloxacin and Ciprofloxacin they were 75 % sensitive.

In quinolone/fluoroquinolone group of antibiotics, 6 isolates were resistant to Nalidixic acid (75%). Moreover,Moxifloxacin and Ofloxacin showed 50% resistivity, while towards Gatifloxacin, Levofloxacin and Norfloxacin were least resistant.

Table 8. Quinolone\Fluoroquinolone sensitivity pattern of *P. aeruginosa* isolates

Pseudomonas aeruginosa (n=8)							
Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\fluoroquinolone	NA	6	75.00	0	0	2	25.00
	MFX	4	50.00	1	12.5	3	37.50
	ENF	3	37.50	0	0	5	62.50
	LEV	2	25.00	0	0	6	75.00
	GAT	2	25.00	0	0	6	75.00
	OFX	4	50.00	0	0	4	50.00
	CIP	3	37.50	0	0	5	62.50
	NOR	2	25.00	0	0	6	75.00

Table 9.Quinolone\Fluoroquinolone sensitivity pattern of *Acinetobacter* spp. isolates

Acinetobacter spp. (n=2) Antibiotics group	Antibiotic	Resistant		Intermediate		Sensitive	
		Isolates	%	Isolates	%	Isolates	%
Quinolone\fluoroquinolone	NA	2	100	1	50	0	0
	MFX	1	50	0	0	0	0
	ENF	1	50	0	0	1	50
	LEV	1	50	0	0	1	50
	GAT	1	50	0	0	1	50
	OFX	1	50	0	0	1	50
	CIP	1	50	0	0	1	50
	NOR	0	0	1	50	1	50

In *Acinetobacter* spp.; 100% isolates showed resistance to Nalidixic acid and 0% towards Norfloxacin in Quinolone/fluoroquinolones.

In the present study, among 252 bacterial isolates; 155 were Gram negative rods. In a similar study by (Shrestha 2005) reported higher growth rate of gram negative rods.

Among 62 Gram negative isolates subjected for study, *P. mirabilis* (27.42%), *P. vulgaris* (11.29%), *K. oxytoca* (12.90%), *K. pneumoniae* (17.75%), *C. freundii* (8.06%), *E. cloaecae* (6.45%), *P. aeruginosa* (12.90%) and *Acinetobacter* spp. (3.23%). Similar results were reported by several authors (Gautam *et al.*1997, Manandhar *et al.* 2005, Chhetri *et al.* 2001, Jha & Bapat 2005 Dhakal 1999, Farrell *et al.* 2003).

In a study done by Fluit *et al.* (2004) in Europe found, *Klebsiella* spp. 7%, *Proteus* spp. 7%, *P. aeruginosa* 7%, and *Enterobacter* spp. 5%. In a study done by (Obi *et al.* 1996) in Africa among 10 species of bacteria, the distribution of gram negative and gram positive bacteria were 88.5% and 9.7% respectively in UTI positive samples. *Proteus mirabilis* is the commonest organism isolated. In *Proteus mirabilis* high sensitivity i.e. 82.35%, 70.59% and 76.47% for norfloxacin, ciprofloxacin and gatifloxacin .In fluoroquinolone group of antibiotics *Proteus vulgaris* was found to be most sensitive for norfloxacin i.e.

71.43%. *Klebsiella pneumonia* is one of the isolate which was used for sensitivity pattern of variety of quinolone/fluoroquinolone antibiotics and found to be sensitive to norfloxacin (81.82%), while enrofloxacin and levofloxacin both were found to be 45.45% sensitive towards these isolates. Nalidixic acid shows 72.73% resistant for the isolate. *Klebsiella oxytoca* were found 37.5% resistanct to nalidixic acid, moxifloxacin, enrofloxacin, levofloxacin, gatifloxacin, ofloxacillin and ciprofloxacin while norfloxacin (75%) was found to be effective one among them. The *Pseudomonas aeruginosa* were also isolated. Similar result was obtained by (Kosakai *et al.* 1990). The study performed by different person at different places found that *P. aeruginosa* affect the urinary bladder and is considered as primary pathogens in compromised host (Manandhar *et al.* 2005) and uncomplicated urinary tract infection (Gautam *et al.* 1997) *Pseudomonas aerugenosa* was 75% resistant to Nalidixic acid, 75% sensitive Norfloxacin, Gatifloxacin and Levofloxacin. *Enterobacter cloaecae* was not found resistant to norfloxacin and 50% of the isolates were found to be resisatant for nalidixic acid, moxifloxacin and levofloxacin. For *Citrobacter freundii* norfloxacin was 100% sensitive while ths same isolates were subjected for the sensitivity pattern towards moxifloxacin, enrofloxacin, levofloxacin, gatifloxacin, ofloxacin, ciprofloxacin and found 20% resistant nalidixic acid shows 80% resistant to the given isolates.

Among fluoroquinolones, norfloxacin was found to be 50% sensitive, 50% intermediate and 0% resistant for the isolates.In quinolone group; nalidixic acid was 100% resistant. In the present study, norfloxacin was seen to be effective while nalidixic acid was more resistant antibiotic, similar study performed by (Barrette *et al.* 1999) in Britain found that 98.9% of all isolates were sensitive to norfloxacin and to ciprofloxacin. Norfloxacin was most sensitive one in this study which was also similar in (Maigaord *et al.* 1978). Overall fluoroquinolone resistance was near 23.0%, but this rate varied significantly according to sex, age, type of urinary infection and geographic region (Andreu *et al.* 2005).

Among 62 Gram negative isolates other than *E.coli* subjected for study, *P. mirabilis* (27.42%), *P. vulgaris* (11.29%), *K. oxytoca* (12.90%), *K. pneumoniae* (17.75%), *C. freundii* (8.06%), *E. cloaecae* (6.45%), *P. aeruginosa* (12.90%), *Acinetobacter* spp. (3.23%). Altogether eight types of antibiotics belonging with quinolone/fluoroquinolone group (nalidixicacid, ciprofloxacin, ofloxacin, norfloxacin, gatifloxacin, levofloxacin, enrofloxacin, moxifloxacin) were used in this study. Of them, norfloxacin was found most sensitive and nalidixic acid was most resistant against Gram negative isolates.This indicates that norfloxacin when recommended for individuals of corresponding UTI would be the better antibiotic for treatment.

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