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Antibiotic resistance pattern of *Escherichia coli* causing urinary tract infection with an emphasis on fluoroquinolone resistance

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ABSTRACT

INTRODUCTION: **Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the community and the hospital settings. UTIs are more common in females compared to males and are mostly caused by Escherichia coli accounting for more than 70% of uncomplicated cases both in outpatients and inpatients. With increasing antibiotic resistance, management of urinary tract infection has become complicated with limited therapeutic options.**

OBJECTIVES: **The present study was undertaken to detect the current antibiotic resistance pattern of Escherichia coli with a special reference to fluoroquinolone resistance.**

MATERIALS AND METHODS: **A total of 1248 urine samples collected between November 2011 to May 2013 were cultured and pathogens identified by conventional methods. Antibiotic susceptibility pattern determined was by Kirby-Bauer method and the minimum inhibitory concentration (MIC) of fluoroquinolones was determined by microbroth dilution method as per CLSI guidelines.**

RESULTS: **Among 311culture positive urine samples, 203 were Escherichia coli. High resistance rate to Ampicillin (81.3%), Co-trimoxazole (83.3%) and low resistance rate to Nitrofurantoin (17%) were noted for Escherichia coli. Among the 203 Escherichia coli, 141 and 121 isolates showed a MIC of ≥ 4 µg/ml for Ciprofloxacin and ≥ 8 µg/ml for Levofloxacin respectively. The resistance rate to other antibiotics and the MIC of Ciprofloxacin and Levofloxacin increased as the patient's age increases.**

CONCLUSION: **The increased resistance to fluoroquinolones in *Escherichia coli* could be due to its inappropriate usage. It is imperative to rationalize the use of fluoroquinolones in order to prevent the dissemination of resistant strains in the population.**

Keywords: Antibiotic resistance, Microbroth dilution, Minimum Inhibitory Concentration, Urinary Tract Infection.

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INTRODUCTION

Urinary Tract Infections are one of the most common bacterial infections, both in the community and in hospital settings.[1] Urinary Tract Infections are caused mostly by Escherichia coli accounting for more than 70 % of uncomplicated cases both in outpatients and inpatients.[2] Urinary Tract Infections are more common in females compared to males due to anatomic and physical factors.[3] Urinary Tract Infections are usually treated with broad-spectrum cephalosporins, fluoroquinolones and aminoglycosides.[4] The minimum inhibitory concentration of fluoroquinolones has increased and is significantly increasing with age of the patient due to frequent exposure to fluoroquinolones and to prolonged antimicrobial therapy, especially in the elderly patients.With the increasing trend of antibiotic-resistance in Escherichia coli, the management of urinary tract infections is likely to become complicated with limited therapeutic options.[5] So there is a need for a constant surveillance of resistance rates among Escherichia coli isolates to ensure appropriate recommendations for treatment of urinary tract infections.

Objectives of the Study

The cross sectional study was undertaken to detect the current antibiotic resistance pattern of Escherichia coli with a special reference to fluoroquinolone resistance.

MATERIALS AND METHODS

1248 urine samples received from inpatients and outpatients of Vinayaka Missions Kirupananda Variyar Medical College and Hospitals, Salem from November 2011 to May 2013 were included in the study.

Sample Collection Method

Freshly voided midstream urine specimens and catheter samples were collected under strict aseptic precautions in a sterile wide mouthed container. Samples were transported immediately to the laboratory.

a) Culture and Identification

Each urine sample was inoculated on MacConkey agar and blood agar. The culture plates were incubated at 37°C for 24 hours and observed for growth.[6] The plates showing significant growth as per Kass count were processed for further identification.[7] Escherichia coli isolated was identified by microscopy, colony morphology and biochemical tests.[5]

b) Antimicrobial Susceptibility Testing of Escherichia coli

The antibiotic susceptibility testing was performed using Kirby-Bauer's disc diffusion technique as described by Clinical and Laboratory Standards Institute guidelines.[8] The antimicrobial agents used were: Ampicillin10µg (AMP), Amikacin30µg (AK), Cotrimoxazole25µg (COT), Nalidixic acid30µg (NA), Nitrofurantoin 300µg (NIT), Norfloxacin10µg (NOR), Ciprofloxacin5µg (CIP), Levofloxacin5µg (LE), Cefotaxime30µg (CTX), Cefepime30µg (CPM), Imipenem10µg (IPM) and Piperacillin/Tazobactum100/10µg (PIT).

c) Determination of minimum inhibitory concentration (MIC) of Ciprofloxacin and Levofloxacin by micro broth dilution method

Ciprofloxacin hydrochloride monohydrate was obtained from Hi-media (Mumbai, India) and Levofloxacin was obtained from Sigma-Aldrich. Dilutions of antibiotics for MIC testing were prepared as per CLSI guidelines.[8] The antibiotics were used immediately after reconstitution.

Figure 1: Antibiogram of Gram Negative Bacilli

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The different concentrations of the drug analyzed were 0.125 to 512 µg/ml. ATCC Escherichia coli 25922 were inoculated on each plate as the growth control. The growth control was read first followed by the MIC of the test strains. The breakpoints of resistance for ciprofloxacin were ≥ 4 µg/ml and for levofloxacin were ≥ 8 µg/ml.[8]

Statistical Analysis

Statistical analysis using chi-square test was performed to analyze antimicrobial susceptibility pattern and minimum inhibitory concentration of Ciprofloxacin and Levofloxacin in Escherichia coli isolates. P value < 0.05 is considered statistically significant

RESULTS

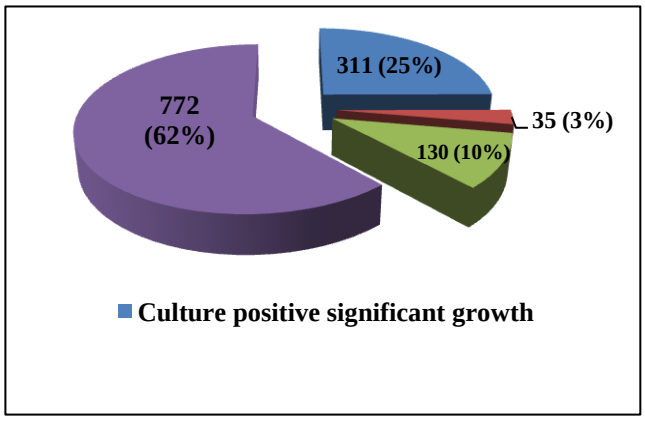

Figure 2: Distribution of growth pattern in processed urine samples

Out of the 1248 urine samples received from patients having clinically suspected urinary tract infections attending VMKVMCH, Salem subjected to culture, 311 (25 %) showed significant growth, 772 (62 %) were negative for culture, 103 (10 %) showed contamination and 35 (3 %) showed insignificant growth. **[Fig.2]**

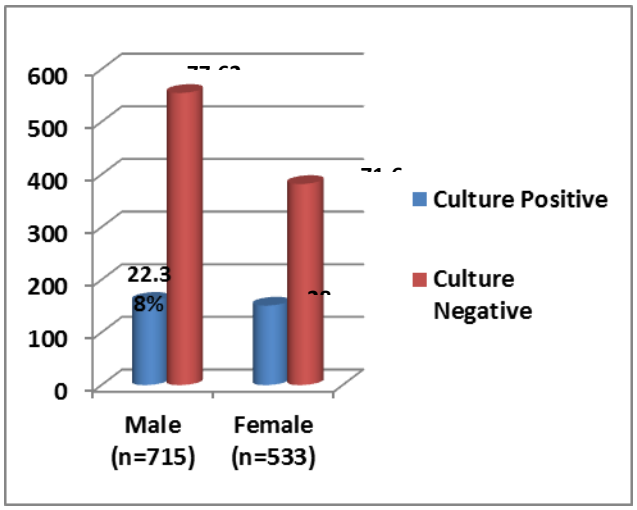

Figure 3: Percentage distribution of gender-wise processed urine samples

Among the 1248 urine samples received for culture, 715 (57.3 %) urine samples were from male patients and 533 (42.7 %) urine samples were from female patients respectively. Of the 715 urine samples received from male patients, 160 (22.38 %) showed significant growth in male patients and of the 533 urine samples received from female patients, 151 (28.33 %) showed significant growth in female patients. **[Fig.3]**

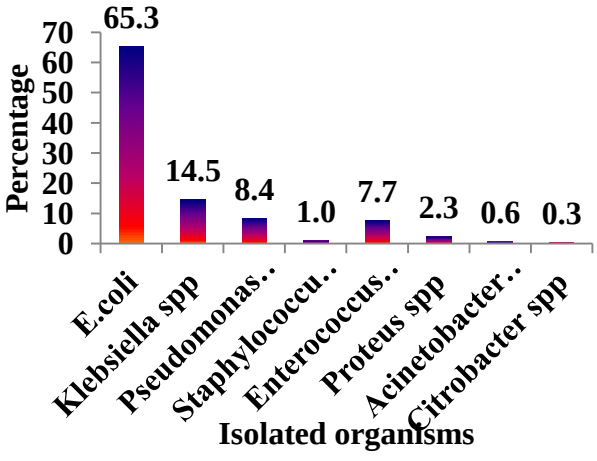

Figure 4: Isolated organisms among culture positive urine samples

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Among the 311 culture positive urine samples, 203 (65.3 %) were *Escherichia coli*, 45 (14.5 %) were Klebsiella species, 26 (8.4 %) were Pseudomonas species, 24 (7.7 %) were Enterococcus species, 7 (2.3 %) were Proteus species, 3 (1.0 %) were Staphylococcus aureus, 2 (0.6 %) were Acinetobacter species and 1 (0.3 %) was Citrobacter species. **[Fig.4]**

Table 1: Antibiotic Resistance pattern of Escherichia coli (% resistance)

Organism Isolated	AMP	AK	COT	NA	NIT	NX	CIP	LE	CTX	CPM	IPM	PIT
Escherichia coli	81.3	23.2	83.3	73.4	17	73.4	68.9	59.6	68.5	65	0	0

Escherichia coli exhibited more than 70 % resistance to ampicillin, cotrimoxazole, nalidixic acid and norfloxacin. It showed more than 60 % resistance to ciprofloxacin, cefotaxime and cefepime and 59 % resistance to levofloxacin. The resistance rate of *Escherichia coli* was 23.2 % to amikacin and 17 % to nitrofurantoin.

Table 2: Distribution of Ciprofloxacin and Levofloxacin resistant Escherichia coli as per their Minimum Inhibitory Concentration

ANTIBIOTIC	Minimum Inhibitory Concentration (µg/ml)							Total
	4	8	16	32	64	128	256	
Ciprofloxacin	5	6	32	28	52	15	3	**141**
Levofloxacin	-	28	28	46	18	1	-	**121**

112 Escherichia coli isolates showed ciprofloxacin MIC ranging from 16 µg/ml – 64 µg/ml, 15 isolates showed MIC of 128 µg/ml and 3 isolates showed MIC of 256 µg/ml. 102 Escherichia coli isolates showed levofloxacin MIC ranging from 8 µg/ml – 32 µg/ml, 18 isolates showed MIC of 64 µg/ml and 1 isolate showed MIC of 128 µg/ml. **[Table 2]**

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Table 3: Distribution of the Ciprofloxacin resistant Escherichia coli as a function of Age and MIC group among In/Out patients

Organism Isolated	In/Out Patient	MIC Group	Age(yrs)							Total	P
			0 – 5	6 - 10	11 – 18	19 - 25	26 - 40	41 - 60	> 60		
Escherichia coli	In Patient	4 to 16	4	1	2	3	4	5	0	19	< 0.001**
		32 to 64	0	0	0	0	3	23	16	42	
		≥ 128	0	0	0	0	0	2	10	12	
		Total	4	1	2	3	7	30	26	73	
	Out Patient	4 to 16	2	0	4	4	9	4	1	24	< 0.001**
		32 to 64	0	0	0	2	8	22	6	38	
		≥ 128	0	0	0	0	0	1	5	6	
		Total	2	0	4	6	17	27	12	68	

**- significant

Among Escherichia coli which were resistant to ciprofloxacin, the above table clearly shows that as the age of the patient increases, the ciprofloxacin MIC level increases statistically for both inpatient and outpatient groups. ($p < 0.05$).

Table 4: Distribution of the Levofloxacin resistant Escherichia coli as a function of Age and MIC group among In/Outpatients

Organism Isolated	In/Out Patient	MIC Group	Age(yrs)							Total	*P*
			0 – 5	6 – 10	11 - 18	19 – 25	26 – 40	41 - 60	> 60		
Escherichia coli	In Patient	8 to 16	3	1	2	1	4	7	2	20	< 0.001 **
		32 to 64	0	0	0	0	1	16	23	40	
		≥ 128	0	0	0	0	0	0	1	1	
		Total	3	1	2	1	5	23	26	61	
	Out Patient	8 to 16	2	0	3	4	13	13	1	36	< 0.001 **
		32 to 64	0	0	0	1	1	12	10	24	
		≥ 128	0	0	0	0	0	0	0	0	
		Total	2	0	3	5	14	25	11	60	

**- significant

The levofloxacin resistant Escherichia coli, in the above table clearly shows that with increasing age, the levofloxacin MIC level also increases statistically for both inpatient and outpatient groups ($p < 0.05$). **[Table 4]**

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Table 5: Percentage of resistance to antibiotics among Ciprofloxacin resistant Escherichia coli

CIP MIC Group	AMP (n=124) (87.9%)	AK (n=33) (23.4%)	COT (n=120) (85.1%)	NA (n=116) (82.3%)	NIT (n=33) (23.4%)	NX (n=140) (99.3%)	LE (n=140) (99.3%)	CTX (n=109) (77.3%)	CPM (n=104) (73.8%)
4 to 16 (n=43)	83.7	16.3	79	74.4	7	100	100	79	74.4
32 to 64 (n=80)	91.3	28.8	86.3	86.3	28.8	98.8	98.8	75	71.3
≥ 128 (n=18)	83.3	16.7	94.4	83.3	38.9	100	100	83.3	83.3

When Escherichia coli isolates were placed into three groups based upon ciprofloxacin MIC (4 to 16, 32 to 64, and ≥ 128 µg/ml), increasing rates of resistance to other antibiotics were observed. **[Table 5]**

Table 6: Percentage of resistance to antibiotics among Levofloxacin resistant *Escherichia coli*

LE MIC Group	AMP (n=108) (89.3%)	AK (n=31) (25.6%)	COT (n=105) (86.8%)	NA (n=101) (83.5%)	NIT (n=31) (25.6%)	NX (n=121) (100%)	CIP (n=121) (100%)	CTX (n=96) (79.3%)	CPM (n=91) (75.2%)
8 to 16 (n=56)	85.7	23.2	85.7	76.8	23.2	100	100	80.4	73.2
32 to 64 (n=64)	92.2	28.1	87.5	89.1	28.1	100	100	78.1	76.6
≥ 128 (n=1)	100	0	100	100	0	100	100	100	100

When *Escherichia coli* isolates were placed into three groups based upon levofloxacin MIC (8 to 16, 32 to 64, and ≥ 128 µg/ml), increasing rates of resistance to other antibiotics were observed. **[Table 6]**

DISCUSSION

Due to the increasing use of antibiotics, urinary pathogens have shown a slow but steady increase in resistance to several antibiotics. The distribution of species and their susceptibility to antibiotics vary with time and place.[4]

Out of the 1248 urine samples processed, 25 % showed significant growth and this data was comparable to the study done by Mandal et al [5] which showed 26 % of significant growth. Studies have reported that women have a higher prevalence of urinary tract infection than men, mainly due to anatomic and physical factors.[3]Similarly, our study also showed that the urinary pathogens were isolated predominantly from women (28.3 %) than in men (22.4 %). Escherichia coli (65.3 %) was the most predominant species isolated in our study population which was similar to Akram et al [3] and Aypak et al [9].The second commonest organism isolated in our study was Klebsiella species (47%) which was comparable to studies of Akram et al [3] and Rajesh et al[10]. Pseudomonas species was the third common organism isolated from the urinary samples in our study which was comparable to Tankhiwale et al. [11].The other organisms causing urinary tract infection were Enterococcus species, Proteus species, Staphylococcus aureus, Acinetobacter species and Citrobacter species which were also reported in earlier studies.[5,12]

Escherichia coli demonstrated high level of resistance

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to most of the antimicrobial agents except amikacin (23.2 %) and nitrofurantoin (17 %) which was comparable with the results of Sonavane et al [13]. Resistance to cotrimoxazole (83.3 %) and nalidixic acid (73.4 %) in our study was similar to findings of Gupta et al [14]. Resistance of Escherichia coli to cefotaxime in our study was 68.5 % and this finding was at par with the observation made by Ullah et al [4]. The resistance rate of Escherichia coli to ciprofloxacin (68.9 %) in our study was comparable to the results of the study done by Ullah et al [4] (62.1 %) and Levofloxacin resistance (58.6 %) was comparable to Hyuk et al [15]. Escherichia coli isolates showed ciprofloxacin MIC ranging from 16 µg/ml – 64 µg/ml similar to Mandel et al [4] and levofloxacin MIC range was from 8 µg/ml – 32 µg/ml.

With the increasing patient's age, ciprofloxacin and levofloxacin resistance increased significantly in our study ($p < 0.05$). Based on the age of the patient the ciprofloxacin and levofloxacin resistance were more common in the age group of 41 – 60 years with similar distribution among inpatient and outpatient. For inpatients and outpatients aged < 40 yrs, the MIC range of ciprofloxacin was 4 – 16 µg/ml and levofloxacin was 8 – 16 µg/ml and in those aged 41 – 60 years the MIC range for ciprofloxacin was 32 – 64 µg/ml and for levofloxacin it was 8 – 16 µg/ml. The male patients in the age group of > 60 years had MIC of ≥ 128 µg/ml for ciprofloxacin which was comparable with the results of Mandal et al [5]. This increased MIC in the elderly could be due to repeated exposure to fluoroquinolones due to recurrent infections and also may require prolonged antimicrobial therapy, especially in the males as they do not readily penetrate the prostate and so MIC of ≥ 128 µg/ml were more common in males and these results were similar to study by Karlowsky et al [16].

Our study showed that when Escherichia coli isolates were placed into three groups based on MIC of ciprofloxacin (4 to 16, 32 to 64, and ≥ 128 µg/ml) it was observed that the resistance rate of ampicillin, cotrimoxazole, nalidixic acid, norfloxacin, levofloxacin, cefotaxime and cefepime were increased and these were comparable with studies by Karlowsky et al[16]. When Escherichia coli isolates were placed into three groups based upon levofloxacin MIC (8 to 16, 32 to 64, and ≥ 128 µg/ml), increasing rates of resistance to ampicillin, cotrimoxazole, nalidixic acid, norfloxacin, ciprofloxacin, cefotaxime and cefepime were increased. Thus, these findings clearly indicates that MIC of Escherichia coli to ciprofloxacin and levofloxacin has increased and also the resistance to other antibiotics has increased as the MIC increases.

CONCLUSION

Escherichia coli was the common urinary pathogen isolated, which showed increased resistance to commonly used antibiotics. It also demonstrated that the MIC of ciprofloxacin and levofloxacin has increased and as the MIC increases an increase in resistance to other antibiotics was noted. So the treatment of Urinary Tract Infections by antimicrobial agents should be strongly guided by in-vitro susceptibility testing to avoid further spread of antimicrobial resistance in both, in-patients and out-patients and development of multi-drug resistance.

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