SOHAG MEDICAL JOURNAL
Vol. 22 No.1 Jan 2018

Prevalence of Fluoroquinolone-Resistant Clinical Isolates of Escherichia Coli in Urinary Tract Infection

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Abstract

Introduction: The commonest bacterial agent involved in causation of UTIs is Escherichia coli. The emergence of FQ resistant uropathogenic E. coli is of great concern.

Aim of the work: to study resistance towards urinary E. coli with various generations of fluoroquinolones.

Patients & Methods:: our study was carried out in the Clinical Pathology Department, Sohag University Hospital during the period from June 2016 to May 2017. Our study included 140 participants. Isolates from the specimens were obtained and identified using; Gram staining, colony characteristics on different culture medias. VITEC 2 Compact 15 identification kits were be used to confirm the identification of the isolates

Results: E.coli was isolated from 100 patients (71%) of all patients complaining of UTI with positive urinary culture (study or case group). By studying prevalence of Antibiotic resistance of E.coli isolates reveals that fluoroquinolones show sensitivities of 42-46%. Also Nitrofurantoin has the highest sensitivity of 87%. This is followed by meropenem (67%). Ampicillin shows sensitivity in only 6% of cases. Regarding drug sensitivity in out & inpatients, we find that all generations of fluoroquinolones show highly significant resistance ratios among inpatients compared to outpatients. Meropenem show resistance more in inpatients than outpatients, with significant difference, Ampicillin and Nitrofuratoin show non-significant difference.

Conclusion: our study show an increased fluoroquinolone resistance among uropathogenic E. coli isolates mainly in hospital admitted patients.

Keywords: Urinary tract infection (UTI), Escherichia coli and fluoroquinolone resistant E.coli.

Introduction

UTIs are among the most common infectious diseases encountered in clinical practice all over the world[(1)]. UTI is a bacterial infection that affects any part of urinary tract[(2)]. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic Escherichia coli (UPEC)[(3).] Also, it is the principal pathogen both in the community as well as in the hospital(4). E.coli has been indicated as the most frequent uropathogen involved in the community-acquired UTI due to the fact of belonging to the normal flora of the human intestine and therefore easily colonizing the urinary tract[(5)]. Virulence factors of E. coli that have been potentially implicated as important to establish UTIs can be divided into two groups: (i) virulence factors associated with the surface of bacterial cell and (ii) virulence factors, which are secreted and exported to the site of action[(6)]. UTIs in hospital and community setting are initially treated empirically based on frequency of pathogens, local antimicrobial resistance rates and illness severity. Treatment of UTI constitutes a great portion of prescription of antibiotics[(7).] Urinary pathogens have shown a changed pattern of susceptibility to antibiotics,

resulting in an increase in resistance to commonly used antibiotics[8]. Fluoroquinolones are preferred as initial agents for empiric therapy because of high bactericidal and clinical cure rates as well as low rates of resistance among uropathogens[9]. The emergence of fluoroquinolone resistant uropathogenic E. coli is of great concern because these pathogens account for 20% of all hospital acquired infections[10-12]. Despite prescribing guidelines now recommending reserving fluoroquinolone use, resistance continues to rise and is a major problem encountered in the clinical setting. The percentage of E. coli isolates in the UK resistant to fluoroquinolones rose from 6% to 20% from 2001 to 2006 and remained at about 17% for the rest of the decade[13]. Bacteria can become resistant to quinolones by mutations in the target molecules, that is, the topoisomerases II and IV, or by active efflux. Earlier observations of plasmid-mediated resistance have been confirmed but quinolone resistance determinants seem essentially chromosome encoded in both mechanisms[14].

Aim of the work:

After notifying the role of fluoroquinolones in UTIs caused by E. coli, our study was done to study resistance towards urinary E. coli with various generations of fluoroquinolones and also to assess sensitivity pattern of other drugs in place of fluoroquinolones resistant E. coli urinary tract infections with an objective to define appropriate intervention strategies to be applied in patient care and management.

Patients and Methods:

Patients: our study was carried out in the Clinical Pathology Department, Sohag University Hospital during the period from June 2016 to May 2017. Our study included 140 participants, 96 female and 44 male, aged from 12-60 years. Of them, 100 were cases group (positive isolation for E coli) and 40 were negative isolation for E coli but positive isolation for others organisms and considered as control group.

Our study was approved by the Research and Ethical Committee at Faculty of medicine, Sohag University. All subjects were informed about the aim of this study and gave written consents.

Inclusion criteria: patients that were suspected to have urinary tract infection.

Exclusion criteria: patients had taken antibiotic treatment within 3 days prior to initial visit.

Methods: all participants were subjected to:

- Full history taking: including age, sex, socioeconomic status, previous history of UTIs, previous history of antibiotic use, any anatomic abnormalities, hospitalization etc.
- Clinical examination.
- Laboratory investigations.

1. Complete blood count (CBC): The test was performed on Cell Dyn 3700, automated cell counter, abbott diagnostics (USA).
2. Serum Creatinine: The test was done by Roche/Hitachi cobas c311 system.
3. Urea: The test was done by Roche/Hitachi cobas c311 system.
4. Urine analysis.
5. Urine culture.

Isolates from the specimens were obtained and identified according to Bergey's manual of Bacteriology, using; Gram staining, colony characteristics on culture media as nutrient agar, MacConkey's agar and blood agar. VITEC 2 Compact 15 identification kits were used to confirm the identification of the isolates.

Statistical analysis:

- Statistical package for social sciences (IBM-SPSS), version 17

was used for statistical data analysis.

- Data expressed as mean, standard deviation (SD), number and percentage. Mean and standard deviation were used as descriptive value for quantitative data, while number and percentage were used to describe qualitative data.
- Student t test was used to compare the means between two groups, and one-way analysis of variance (ANOVA) test was used to compare means of more than two groups.
- Pearson Chi square was used to compare percentages of qualitative data, and Fisher's Exact test was used for non parametric data.
- Pearson correlation test was used to compare two quantitative variables. The value of (r) is explained in the following figures:
  r <0.2 è negligible correlation
  r 0.2-0.4 è weak correlation
  r 0.4-0.7 è moderate correlation
  r 0.7-1 è strong correlation
  r positive è positive correlation
  r negative è negative correlation
- For all these tests, the level of significance (P-value) can be explained as:
  1 No significance P > 0.05
  2 Significance P < 0.05
  3 High significance P < 0.001.

Results

Our study included 140 participants, 96 female and 44 male, aged from 12-60 years. Of them, 100 were cases group (positive isolation for E coli) and 40 were negative isolation for E coli but positive isolation for others organisms and considered as control group (figure 1).

By comparison between demographic data of case and control group, it was found that the mean age of our cases was 23±13 years, and the mean age of control group was 25±13 years.

In both case & control groups 42% were adult (>20 years), and 58% were Young adults (12-20 years), the comparison is non-significant (P value≥0.05). Regarding sex of our study population 31% of cases were males, 69% were females, also similar percentage was for control group, the comparison is non-significant (P value≥0.05). 46% of our cases were from urban areas, 54% from rural areas, but half of control group were from urban and other half from rural area, the comparison is also non-significant (P value≥0.05). (table 1).

Prevalence of antibiotic resistance in our study group show that ampicillin was resistant in 94% of cases, sensitive in only 6%, but Meropenem was sensitive in 67% of cases, and resistant in 33%. On the other hand, Ciprofloxacin (1st generation Fluoroquinolone) showed a sensitivity of 46%, levofloxacin (2nd generation Fluoroquinolone) showed 45% sensitivity. Moreover, Gatifloxacin and Moxifloxacin (3rd and 4th generation Fluoroquinolone) showed only sensitivity of 42%. We also found that Nitrofurantoin was resistant in 13% of our cases.

By Comparison between 1st&4th generation Fluoroquinolones resistance in case group we found that the majority of cases (88%) were either sensitive to both ciprofloxacin and moxifloxacin (38%) or resistant to both (50%). Only 8 cases were sensitive to ciprofloxacin but resistant to moxifloxacin, and only 4 cases showed the reverse sensitivity to both drugs, so the comparison is non-significant (P value ≥0.05) in such few number of cases (Table 2).

Also, when we compared between ciprofloxacin and demographic data, we found that ciprofloxacin showed resistance more adults than young adult, males more than

females and rural more than urban. However, these differences were small and do not show significant differences (P value ≥0.05) (figure 2).

When we compared between Moxifloxacin and demographic data, we found that moxifloxacin showed resistance more adults than young adults, males more than females and rural more than urban. However, these differences were small and do not show significant differences (p value≥0.05) (figure 3).

In our study, by comparison between outpatients & inpatients regarding drug sensitivity in case group which is divided to 66% outpatients, and 34% inpatients, Regarding drug sensitivity in out& inpatients, we found that 100% of inpatients were resistant to ampicillin, but with non significant difference compared to outpatients (P value ≥0.05). Meropenem showed resistance more in inpatients than outpatients, with significant difference (P value=0.032). Moreover, All Fluoroquinolones generations showed highly significant resistance ration among inpatients compared to outpatients (P value =0.001). Nitrofuratoin showed non significant difference, may be due to the limited number of resistant cases (only 13 cases) (P value ≥0.05) (Table 3).

Table 1: Comparison of clinical data between Group A and Group B.

Variable		Group		Chi square or T test	P value
		A	B		
Age	Mean±SD	23.96±13.4	25.5±13.03	0.620	0.536(NS)
Age Group	Adult (>20 years)	42(42%)	17(42.5%)	0.003	0.957(NS)
	Young adults (12-20 years)	58(58%)	23(57.5%)		
Sex	Male	31(31%)	13(32.5%)	0.030	0.863(NS)
	Female	69(69%)	27(67.5%)		
Residence	Urban	46(46%)	20(50%)	0.183	0.668(NS)
	Rural	54(54%)	20(50%)		

NS : Non significant (P value >0.05) **S:** Significant (P value <0.05) **HS:** Highly significant (P value <0.001).

Table 2: Comparison between 1st&4th generation Fluoroquinolones resistance in case group

			Moxifloxacin		Total
			R	S	
Ciprofloxacin	R	Count	50	4	54
		%	50.0%	4.0%	54.0%
	S	Count	8	38	46
		%	8.0%	38.0%	46.0%
Total		Count	58	42	100
		%	58.0%	42.0%	100.0%

McNemar Chi square = 57.667, p value 0.388

NS : Non significant (P value >0.05) **S:** Significant (P value <0.05) **HS:** Highly significant (P value <0.001).

Table 3: Comparison between outpatients & inpatients regarding drug sensitivity in case group

Variable		Outpatients	Inpatients	Chi square	P value
Ampicillin	Resistant	60(90.9%)	34(100%)	3.288	0.076(NS)
	Sensitive	6(9.1%)	0		
Meropenem	Resistant	17(25.8%)	16(47.1%)	4.605	0.032(S)
	Sensitive	49(74.2%)	18(52.9%)		
Ciprofloxacin	Resistant	26(39.4%)	28(82.4%)	16.672	<0.001(HS)
	Sensitive	40(60.6%)	6(17.6%)		
Levofloxacin	Resistant	27(40.9%)	28(82.4%)	15.573	<0.001(HS)
	Sensitive	39(59.1%)	6(17.6%)		
Gatifloxacin	Resistant	29(43.9%)	29(85.3%)	15.754	<0.001(HS)
	Sensitive	37(56.1%)	5(14.7%)		
Moxifloxacin	Resistant	29(43.9%)	29(85.3%)	15.754	<0.001(HS)
	Sensitive	37(56.1%)	5(14.7%)		
Nitrofuratoin	Resistant	6(9.1%)	7(20.6%)	2.623	0.105(NS)
	Sensitive	60(90.9%)	27(79.4%)		

NS : Non significant (P value >0.05) S: Significant (P value <0.05) HS: Highly significant (P value <0.001).

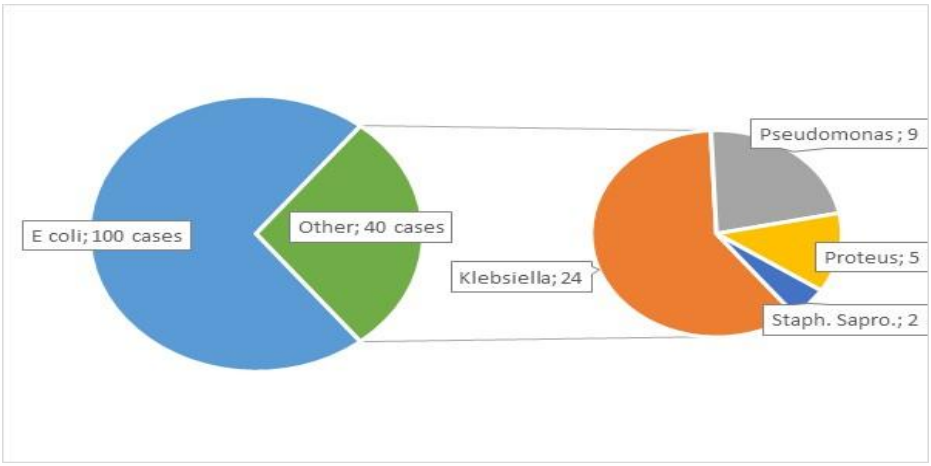

Figure 1: prevalence of isolated organisms "cases and control groups".

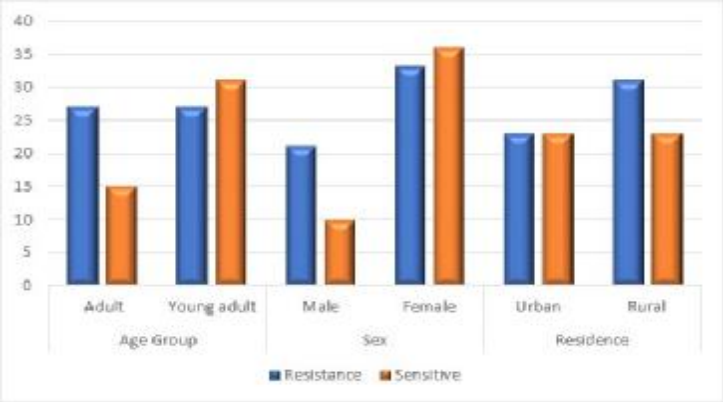

Figure 2: Comparison between Ciprofloxacin and demographic data.

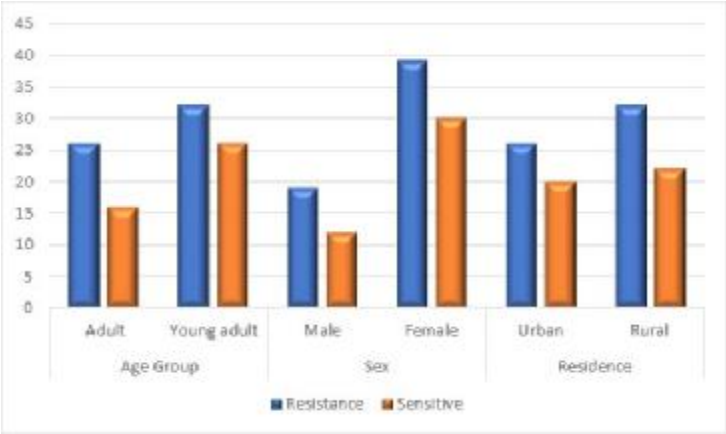

Figure 3: Comparison between Moxifloxacin and demographic data.

Discussion

UTIs are one of the commonly encountered diseases in developing Countries with an estimated annual global incidence of at least 250 million[15]. *E. coli* is the major aetiological agent in causing UTI, which accounts for up to 90% of cases with other pathogens including *Enterococci, Staphylococcus saprophyticus, Klebsiella* spp., Proteus mirabilis and *Pseudomonas*[16]. In UTI cases, antibiotic treatment is often started empirically, before the results of urine culture are available and therapy is based on information obtained from the antimicrobial resistance pattern of the urinary pathogens. Antibiotic resistance is a worldwide problem threatening our ability to treat infections. Treatment failure because of antibiotic resistance inside and outside hospitals results in increasing mortality, morbidity and economic costs[17]. Regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy[18]. Empirical first-line treatment of uncomplicated UTI should preferably be with an antibiotic to which resistance is low and which has a low capacity for co-selection of resistance and a low impact on the normal intestinal flora[19].

Our study group divided into 66% outpatients, and 34% inpatients. In both case & control groups around 42% were Adult (>20 years), and 58% were Young adults (12-20 years).

Regarding sex of our study population 31% of cases were males, 69% were females, also similar percentage was for control group. 46% of our cases were from urban areas, 54% from rural areas, but half of

control group were from urban and other half from rural area.

Regarding drug sensitivity, we found that nitrofurantoin had the highest sensitivity of 87%. This was followed by meropenem (67%). Fluoroquinolone showed sensitivities of 42-46%; being higher among 1st generation (ciprofloxacin; 46%); and lower among the 3rd and 4th generations (gatifloxacin and moxifloxacin; 42%) which may be due to fourth generation drug abuse more than first generation. Ampicillin showed sensitivity in only 6% of cases.

Data of ***Niranjan and Malini. (2014)[20], Saha et al. (2014)[21]*** showed that resistance to fluoroquinolones of 70% and 74.4% was documented from their studies done in Kolkata and Puducherry respectively, also ***Somashekara et al. (2014)***[8] revealed increasing resistance to fluoroquinolones between 74.2% and 86%, lowest resistance is seen to nitrofurantoin in study of ***Mehta et al. (2005)[22]*** as there was a decreasing trend of resistance seen over the three successive years decreasing from 36% (2012) to 18 % (2014) which was close to our results. In Spain, published data indicate a high frequency of resistance to ampicillin, co-trimoxazole and the quinolones among E. coli isolates from outpatient urine samples[23, 24], which seems to indicate that these antimicrobial agents should not be used. ***Hasan et al. (2007)***[25] reported that resistance by *E.coli* to FQ group antibiotics like ciprofloxacin and norfloxacin was 79% and 71% respectively, ***Forbes et al. (2002)***[26] reported that the increasing resistance to third-generation Fluoroquinolones was associated with the presence of ESBLs in their study, as 46.3% of *E.coli* resistant to FQ were ESBL producers. The comparison of ciprofloxacin resistance patterns of uropathogenic *E.coli* in various studies from India and other parts of the world has shown a range from 6 % to 75%[20]. In study of ***Boyd et al. (2008)***[27], 46% isolates of *E.coli* were ciprofloxacin resistant.

Comparing the results obtained from isolates from uncomplicated UTI with those obtained in 1997–98[28], an increase in resistance to quinolones was observed. Increasing FQ resistance among urinary E. coli has also been documented in studies in other countries[29]. Indeed, in a study in the USA, ciprofloxacin was the only agent studied that demonstrated a consistent stepwise increase in resistance from 1995 (0.7%) to 2001 (2.5%)[30]. When we compared between ciprofloxacin and demographic data, we found that ciprofloxacin was resistant in 64% of adults and 47% of young adults, also it was resistant in 68% of males and 48% of females. Regarding residence it was resistant in 50% of urban population and 57% of rural population; all with non significant differences. This was inconsistent with ***Boyd et al. (2008)***[27] who also have reported that fluoroquinolone resistance has increased with time, and patient age. According to Spanish national surveillance study female: male *E. coli* UTI infections are 19%: 28.9%[31]. Regarding drug sensitivity in out & inpatients, we found that 100% of inpatients were resistant to ampicillin, but with non-significant difference compared to outpatients. Meropenem showed resistance more in inpatients than outpatients, with significant difference (p=0.032). Moreover, all generations of FQ showed highly significant resistance ratios among inpatients compared to outpatients. Nitrofuratoin showed non significant difference, may be due to the limited number of resistant cases (only 13 cases). This was similar to results of study of ***Boyd et al. (2008)[27]*** as they found higher rate of resistance is noted

in hospitalized patients than out patients. This may be due to decreased immune system with super added hospital acquired infections and with indwelling catheters, frequent use of FQ and with complicated infections. In study of ***Huang and Stafford. (2002)***[32] laboratory data indicated that 2061 strains of E. coli were isolated from outpatient urine samples during 2002, of which 58.4% were resistant to ampicillin, 19.0%to norfloxacin, 19.2%to ciprofloxacin, 2.3% to fosfomycin, and 1.4% to nitrofurantoin.

Conclusion:

Our study show an increased FQ resistance among uropathogenic E. coli isolates mainly in hospital admitted patients. E.coli was isolated from 100 patients (71%) of all patients complaining of UTI with positive urinary culture (study or case group). By studying prevalence of Antibiotic resistance of E.coli isolates reveals that Fluoroquinolones show sensitivities of 42-46%; being higher among 1st generation (ciprofloxacin; 46%); and lower among the 3rd and 4th generations (gatifloxacin and moxifloxacin; 42%). Also Nitrofurantoin has the highest sensitivity of 87%. This is followed by meropenem (67%). Ampicillin shows sensitivity in only 6% of cases.

In conclusion, our study show an increased FQ resistance among uropathogenic E. coli isolates mainly in hospital admitted patients.

Recommendation: as following

- Close attention to monitor FQ susceptibility patterns and the association of multidrug resistance with FQ resistance in isolates of E. coli and other bacteria causing urinary tract infections and other infections.
- The increased prescription of FQ as first-line therapy for common infections such as cystitis will facilitate the emergence of resistance to this class of compounds and promote the emergence of multidrug-resistant strains and, therefore, should be discouraged as it will undermine the efficacy of FQ to treat more-serious infections.
- FQ- sparing agents should be given higher priority than FQ in the treatment of cystitis.
- Continued surveillance of urinary tract isolates of E. coli and other pathogens is important, and appropriate clinical use of FQ is imperative as they become more widely prescribed.
- Large-scale studies are recommended to reflect the resistance in fourth generation of FQ more than first generation. Also, Further research into the molecular basis of FQ resistance could lead to new therapeutic strategies for FQ-resistant E. coli.

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