Characterization of Quinolone Resistance Genes in Uropathogenic *Escherichia coli* Isolates, in Egypt

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Abstract

**Background:** The resistance of *E. coli* isolates to fluoroquinolone is a significant challenge to antibiotic treatment and infection control policies. A significant increase of ciprofloxacin-resistant *E. coli* has occurred during last years, indicating the need for further analysis.

**Objective:** The current study was aimed to detect quinolone resistance in multi-drug resistant *E. coli* isolates that were recovered from patients with urinary tract infection in the Urology and Nephrology center of El-Mansoura University, Egypt.

**Methods:** The clinical uropathogenic specimens collected were studied. Identification and antimicrobial susceptibility testing were done using VITEK 2. Minimum inhibitory concentrations (MICs) of different quinolones were determined for fluoroquinolone resistant (FQ-resistant) isolates. Also, they were screened for the presence of the plasmid mediated quinolone resistance (PMQR) qnrA, qnrB and qnrS genes by polymerase chain reaction (PCR) techniques.

**Results:** In the present study, 100 (32.7%) *E. coli* of 305 uropathogenic specimens were tested. The patients involved in this study included both genders, females (76%) were the most affected group of patients as compared to males (24%). The quinolones resistance rate was 30% of the isolated *E. coli* samples. The detected genes for the quinolone's resistance samples were: 14 (46.6%) qnrB, 13 (43.3%) qnrS and 2 (6.6%) qnrA.

**Conclusion:** This study identified quinolone resistance (qnr) gene in uropathogenic *E. coli* in EGYPT. These findings suggest a possible resistance to quinolone are of high interest for better management of patients and control of antimicrobial resistance in Egypt.

**Keywords:** Uropathogenic *E. coli* Infections, Quinolone-Resistant *E. coli*, Quinolone-Resistance Genes, Fluroquinolone, PMQR.

1. Introduction:

Among the wide range of uropathogens related to the development of urinary tract infections (UTIs), uropathogenic *Escherichia coli* (UPEC) strains are considered as the main causative agents. UPEC account for the preponderance of both community- and hospital-acquired UTIs (Maria et al., 2017). Several risk factors including renal diseases increase the risk of UTI; however, the treatment of infection more often does not require antimicrobial therapy. Over the recent years, antibiotic therapy of
Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span. Urinary tract infections (UTIs) are very common in the general population, with an estimated overall incidence rate of 17.5 per 1000 person per year (Al-Hasan et al., 2010). Urinary tract infection (UTI) is the second most frequent community-acquired adult infection and the main cause for nosocomial infection. Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases and Gram-negative bacilli cause the overwhelming majority of UTIs (Martínez et al., 2007).

One of the major concerns about antibiotics is related to their inappropriate and widespread use, which induces to a resistance selection in the infecting strains. The dissemination of these resistant organisms occurs, particularly, when basic measures of infection control are not respected. Increasing of antimicrobial resistance is a major issue confronting organized health care today (Kiffer et al., 2007). In order to apply an appropriate therapeutic strategy in each region, it is necessary to have data of the most common pathogens and their antimicrobial resistance pattern (Khameneh, 2009).

Quinolones are a family of synthetic antimicrobial agents with a broad antibacterial activity commonly used as a suitable therapy in patients with UTI. The unnecessary and inappropriate prescription of these agents have directed to appearance of E. coli isolates with multidrug resistance. The severe hospital acquired infections due to these resistant E. coli isolates have been increased (King et al., 2012).

Quinolone’s family has been classified into four generations based on their antimicrobial activity. The most well-known quinolone antibiotics are nalidixic acid, ciprofloxacin, and levofloxacin as members of the first, second, and third generations, respectively. Quinolones prevent bacterial DNA synthesis through inhibiting DNA gyrase and topoisomerase IV enzymes leading to cell death. The emergence of plasmid-mediated quinolone resistance (PMQR) has been reported and indicating that quinolone resistance can also be acquired through horizontal gene transfer. Several PMQR determinants have been identified: (QnrA, QnrB, QnrS, QnrC, and QnrD) (Cattoir et al., 2007). Moreover, PMQR genes have often been found to co-exist on the same plasmid with genes encoding ESBLs and to be co-transferred to recipients. This condition may provide a selective advantage for the development of quinolone resistance which could result in therapeutic failure (Briales et al., 2012).

The aims of this study were to investigate microorganisms isolated from patients with UTI and evaluate their in vitro susceptibility patterns to commonly used antimicrobial agents. In addition, our results can provide proper updates to the clinician and the hospital management about current antibiotic sensitivity pattern and help them updating antibiotic usage guidelines and policy

2. Materials and methods:
2.1. Bacterial isolates and clinical data:
From May 2019 to June 2020, a total of 305 consecutive uropathogenic specimens were recovered as part of standard care of an equal number of individual patients admitted to Urology and Nephrology Center, Mansoura University, Egypt. A detailed history of patient including age, sex, socioeconomic status, previous history of urinary tract infections, previous history of antibiotic use, any anatomic abnormalities, hospitalization etc. were recorded in the prescribed proforma. Patients who were excluded from the study were fluoroquinolone sensitive E. coli isolates, pregnant, lactating or premenopausal women, patients having nosocomial UTI, patients who had taken antibiotic treatment within 3 days prior to initial visit, patients having genital urinary tract disease or abnormalities, patients having gastrointestinal symptoms. Identification of bacterial isolates was performed by the Vitek-2 system (bioMérieux, Marcy l’Étoile, France). Among these clinical samples one hundred (32.7%) isolates were detected as of E. coli strains

2.2. Determination of antimicrobial susceptibility pattern of isolates:
The antimicrobial susceptibility testing to some antimicrobials was carried out by the Vitek-2 system (bioMérieux, Marcy l’Étoile, France). The other antimicrobials which not included in the pattern of Gram negative isolates by Vitek-2 system were determined by Kirby-Bauer disc diffusion technique according to the clinical laboratory standard institute (CLSI, 2018). Antibiotics were purchased from BioRad (Marnes-
Il-Coko and included amikacin (AKN, 30 μg), gentamicin (G 15 μg), amoxicillin / clavulanic acid (AMC 20/10), ciprofloxacin (CIP, 5 μg), ofloxacin (OFL 5 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), cefepime (FEP, 30 μg), imipenem (IPM, 10 μg), aztreonam (ATM, 30 μg), trimethoprim sulfamethoxazole (SXT 1.25/23.75 μg).

2.3. Determination of MICs to different quinolones:
All bacterial isolates were subjected to antimicrobial susceptibility testing by the broth microdilution method using cation modified Mueller-Hinton broth in 96 well microtiter plates according to the clinical laboratory standard institute (CLSI, 2018). Twelve different dilutions of each quinolone were tested by the two-fold dilution method (concentrations tested ranged from 1024 μg/ml to 0.5 μg/ml). Seven quinolone antibiotics, representing the four generations of quinolones, were tested, which included nalidixic acid (NAL), representing the first generation; ciprofloxacin (CIP), norfloxacin (NOR), and ofloxacin (OFL), representing the second generation; levofloxacin (LEV), representing the third generation, and gemifloxacin (GEM) and moxifloxacin (MOX), representing the fourth generation (all from Sigma-Aldrich, USA). The used quality control strains were E. coli ATCC 25922. Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2018). Since the CLSI guidelines do not specify a breakpoint for gemifloxacin, its breakpoint was based on the values proposed by the British Society for Antimicrobial Chemotherapy (≤0.5 mg/L for susceptible, and ≥1 mg/L for resistant). Also, moxifloxacin data were interpreted based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) e-coff and modal MIC, which, for E. coli, are 4 μg/ml and 1 μg/ml, respectively (Le TM et al., 2009).

2.4. Extraction of bacterial DNA
Rapid DNA extraction for QNR genes was performed according to Robiesek et al., (2006), using a boiling technique. Shortly, strains from Tryptic Casein Soy Broth (TCS) broth were reactivated on TCS agar for 18–24 hrs. and two or three isolated colonies were inoculated in Luria Bertani (LB, 2 mL). After 18–24 hrs. of overnight culture, LB broth samples were centrifuged at 10000 rpm/min for 10 min and the pellet suspended in 500 μL of phosphate buffer (100 mM, pH 7) to cell-wall weakening. The mix was heated at 100 °C for 15 min in a water bath to release bacterial nucleic acid. DNA was then precipitated in 250 μL of absolute ethanol, washed twice in 1000 μL of ethanol 75%, dried and re-suspended in 100 μL of sterile water.

2.5. Detection of Qnr encoding genes:
Polymerase chain reaction (PCR) assays were performed for the detection of qnr resistance genes including qnrA, qnrB, and qnrS. The primers used to detect qnr genes were selected from previously described by Doma et al., (2020). PCR was done in a total volume of 25 μL containing 3 μL DNA template, 2.5 μL PCR buffer (1X), 1 μL deoxyribonucleotide triphosphates solution (dNTPs, 200 μM), 1.5 μL MgCl2 (1.5 mM), 0.25 μL Taq DNA polymerase (1 Unit), and 1 μL each specific primers (1 μM). PCR amplifications for the studied genes were carried out on a T100™ thermal cycler (Bio-Rad, Hercules, CA, USA). The cycling conditions were set up as follows: 95°C for 5 mins (step 1), 95°C for 1min (step 2), annealing for 45 sec (step 3), 72°C for 1 min (step 4), and 72°C for 5 mins (step 5); steps 2–4 were repeated for 30 cycles. The amplifications were separated on 1.5% agarose gel prepared in 1X TAE Tris/Acetate/EDTA buffer and visualized using ultraviolet light after staining with safe stain.

2.6. Statistical analysis
Statistical analysis was performed using Epi Info Version 7.1.1.14 software. Fisher’s exact test was used for comparison and the difference was statistically significant when p < 0.05.

3. Results
3.1. Isolation and identification of isolates
In the current study, one hundred E. coli isolates (32.7%) out of 305 specimens were obtained from patients suffering from urinary tract infections. These E. coli isolates were identified by the Vitek-2 system (bioMérieux, Marcy l’Étoile, France). The patients involved in this study included both genders, females (76 samples, 76%) were the most affected group of patients (p < 0.05) as compared to males (24 samples, 24%) and the mean age of affected patients was 52 years (range: 3–87). No differences among the prevalence of E. coli isolated from male outpatients and inpatients infections were found. While E. coli isolated from female patients were mainly from outpatients UTIs. Maximum number of cases was found in female in the age group of 31 - 50 years (31 samples, 31%, p = 0.0335, Table 2).
Table 1: List of primers sequence and PCR condition of QNR genes used.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′–3′)</th>
<th>PCR condition</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnrA</td>
<td>F-ATTTCTCACGCCAGGATTTG R-GATCGGCAAAGGTAGGTCA</td>
<td>95 °C 95 °C 51.2 °C 72 °C 72 °C</td>
<td>3 min 30 s 1 min 2 min 5 min</td>
</tr>
<tr>
<td>qnrB</td>
<td>F-ATCGTGAAAGCCAGAAAGG R-CGATGCCTGGTAGTTGTCC</td>
<td>94 °C 94 °C 53 °C 72 °C 72 °C</td>
<td>5 min 45 s 45 s 5 min 5 min</td>
</tr>
<tr>
<td>qnrS</td>
<td>F-ACGACATTGTCAACTGCAAA R-TAAATTGACCCTGTAGGG</td>
<td>94 °C 94 °C 53 °C 72 °C 72 °C</td>
<td>5 min 45 s 45 s 5 min 5 min</td>
</tr>
</tbody>
</table>

Table 2: Characteristics of the patients with urinary tract infections

<table>
<thead>
<tr>
<th>Age group</th>
<th>All patients, n = 100 (%)</th>
<th>Male, n = 28 (%)</th>
<th>Female, n = 72 (%)</th>
<th>Inpatient, n = 39 (%)</th>
<th>Outpatient, n = 61 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>15 (15%)</td>
<td>6 (6%)</td>
<td>9 (9%)</td>
<td>5 (12.8%)</td>
<td>10 (16.4%)</td>
</tr>
<tr>
<td>11–30</td>
<td>17 (17%)</td>
<td>7 (7%)</td>
<td>10 (10%)</td>
<td>6 (15.3%)</td>
<td>11 (18%)</td>
</tr>
<tr>
<td>31–50</td>
<td>39 (39%)</td>
<td>8 (8%)</td>
<td><strong>31 (31%)</strong></td>
<td>12 (30.7%)</td>
<td>27 (44.3%)</td>
</tr>
<tr>
<td>51–70</td>
<td>13 (13%)</td>
<td>3 (3%)</td>
<td>10 (10%)</td>
<td>5 (12.8%)</td>
<td>8 (13.1%)</td>
</tr>
<tr>
<td>71–80</td>
<td>9 (9%)</td>
<td>4 (4%)</td>
<td>5 (5%)</td>
<td>6 (15.3%)</td>
<td>3 (4.9%)</td>
</tr>
<tr>
<td>&gt; 81</td>
<td>7 (7%)</td>
<td>3 (3%)</td>
<td>4 (4%)</td>
<td>5 (12.8%)</td>
<td>2 (3.3%)</td>
</tr>
</tbody>
</table>

Abbreviation: n = Number of E. coli isolates

3.2. Antimicrobial susceptibility pattern of isolates:

The rates of susceptibility to the selected antimicrobial agents against E. coli isolates are summarized in Table 3. High frequencies of resistance were observed toward trimethoprim–sulfamethoxazole (92%), ampicillin (91%), and aztreonam (72%). Co-resistance between cephalosporins and beta-lactams was frequently observed as the resistance of piperacillin (61%), amoxycillin / clavulanic acid (30%), cefotaxime (68%), ceftriaxone (56%), ceftazidime (62%), cefepime (48%) and Cefuroxime (46%). The resistance against fluoroquinolone in Figure (1) showed, norfloxacin (30%), ciprofloxacin (28%), ofloxacin (27%) and levofloxacin (20%) of the tested isolates. Meropenem and imipenem were the most effective antimicrobials as they showed percentage of resistance 2% and 3% of the isolates, respectively.

Overall, inpatient isolates were slightly more resistant than outpatient isolates (48.5% vs 41.9%, respectively).

The susceptibility of FQ-resistant clinical isolates of E. coli was determined and the calculated MIC50 and MIC90 of different quinolones are shown in Table 4. Resistant and intermediate strains were classified together as resistant. Gemifloxacin showed better activity than nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, and moxifloxacin, with a MIC90 of 8 μg/mL of gemifloxacin versus a MIC90 equal to or higher than 16 μg/mL of the remaining quinolones. Overall, 30% of the analyzed clinical isolates were resistant to gemifloxacin, whereas 66.6% were resistant to ciprofloxacin and norfloxacin, and 56.6%, 46.6%, and 40% were resistant to ofloxacin, levofloxacin, and moxifloxacin, respectively (Table 4).
Table 3: Resistance percentages of E. coli to 20 antibiotics of eight classes.

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Antibiotics (mg/disc)</th>
<th>Resistance of E. coli * (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Isolates (n = 100)</td>
<td>Inpatients n= 39</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin 30</td>
<td>21 (21 %)</td>
</tr>
<tr>
<td></td>
<td>Gentamicin 10</td>
<td>26 (26 %)</td>
</tr>
<tr>
<td>B-lactams</td>
<td>Amoxicillin / clavulanic acid 30/12.5</td>
<td>30 (30 %)</td>
</tr>
<tr>
<td></td>
<td>Ampicillin 10</td>
<td>91 (91 %)</td>
</tr>
<tr>
<td></td>
<td>Piperacillin 100</td>
<td>61 (61 %)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Cefotaxime 30</td>
<td>68 (68 %)</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone 30</td>
<td>56 (56 %)</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime 30</td>
<td>62 (62 %)</td>
</tr>
<tr>
<td></td>
<td>Cefepime 30</td>
<td>48 (48 %)</td>
</tr>
<tr>
<td></td>
<td>Cefuroxime 30</td>
<td>46 (46%)</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Imipenem 10</td>
<td>3 (3 %)</td>
</tr>
<tr>
<td></td>
<td>Meropenem 10</td>
<td>2 (2 %)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Nalidixic acid</td>
<td>54 (54 %)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin 5</td>
<td>28 (28 %)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin 10</td>
<td>30 (30 %)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin 5</td>
<td>27 (27 %)</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin 5</td>
<td>20 (20 %)</td>
</tr>
<tr>
<td>Monobactam</td>
<td>Aztreonam 30</td>
<td>72 (72 %)</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>Trimethoprim–sulfamethoxazole 25</td>
<td>92 (92 %)</td>
</tr>
<tr>
<td>Synthetic drug</td>
<td>Nitrofurantoin 300</td>
<td>56 (56 %)</td>
</tr>
</tbody>
</table>

* Data presented are means of triplicate values; n = Total number of E. coli isolates

Figure 1: The percentage of resistance of E. coli isolates against different antimicrobials.
<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC range μg/ml</th>
<th>MIC50 μg/ml</th>
<th>MIC90 μg/ml</th>
<th>No. of Resistant Isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>8 – 2048</td>
<td>1024</td>
<td>&gt;1024</td>
<td>30 (100 %)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5 – 256</td>
<td>16</td>
<td>64</td>
<td>20 (66.6 %)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>2 – 1024</td>
<td>64</td>
<td>256</td>
<td>20 (66.6 %)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1 – 512</td>
<td>32</td>
<td>128</td>
<td>17 (56.6 %)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.5 – 128</td>
<td>8</td>
<td>32</td>
<td>14 (46.6 %)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤ 0.5 – 32</td>
<td>8</td>
<td>16</td>
<td>12 (40 %)</td>
</tr>
<tr>
<td>Gemifloxacin</td>
<td>≤ 0.5 – 32</td>
<td>4</td>
<td>8</td>
<td>9 (30 %)</td>
</tr>
</tbody>
</table>

MIC50/90: minimum inhibitory concentration for 50% and 90% of the isolates, respectively.

3.3. Distribution of plasmid-mediated quinolone resistance genes:

Overall PMQR genes in FQ-resistant *E. coli* isolates were found in both inpatients and outpatients’ infections as well as in male and female genders. The molecular analysis revealed that the 30 FQ-resistant *E. coli* strains were harbouring at least one qnr gene: 14 (46.6%) qnrB, 13 (43.3%) qnrS and 3 (10 %) qnrA. The concomitant presence of two or three qnr genes was detected (Table 5). The distribution of qnr-positive isolates revealed that there was no significant association between qnr genes and higher antibiotic resistance. However, qnrB gene was found to be relatively higher than qnrS gene among antibiotic-resistant isolates (Table 5).

4. Discussion:

Urinary tract infections are the most common bacterial infections in women and accounts for significant morbidity and increases health care costs (Iravani, 1991). UTI is an old problem that continues to present new challenges due to change in the aetiology of UTI and in the antimicrobial susceptibility of urinary pathogens over the years. Factors such as the changing inpatient population and extensive use and abuse of antimicrobial agents could contribute to changes in the microbial profile of urinary tract isolates. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infection UTI (Warren et al., 1999 and Iroha et al., 2009).

In the current study, one hundred *E. coli* isolates (32.7%) out of 305 specimens were obtained from patients suffered from urinary tract infections. This result was in accordance with results reported by Fam et al., 2011 (42%) [18], Amer et al., 2018 (45%) and Masoud et al., 2021 (47%) in Egypt.

Even our results reveal higher prevalence of urinary tract infections in female patients (72%) than in males (28%). In contrast, the prevalence rates were higher in isolates from male than in those from female patients reported by Linhares et al., 2013. As they explain, it is possible that male infections may be more difficult to eradicate because of the higher rates of antibiotic resistance.

There are significant geographic differences in the susceptibility of commonly used antimicrobials against uropathogens. So, accurate knowledge on local epidemiology and antimicrobial resistance pattern of organisms causing UTI is essential to design effective therapy. Annual determination of bacterial sensitivity pattern in a particular area as a guideline is also recommended (Bidell et al., 2016).

In this study, more than 90% of the isolates showed resistance to trimethoprim–sulfamethoxazole (TMP–SXT) that is recommended as a first choice for UTI treatment (Ali et al., 2016). Previous studies reported similar results (Paniagua-Contreras et al., 2018). Also, 91% of the *E. coli* isolates, showed resistance to ampicillin, 46% to 68% for cephalosporins, while, 56 % showed resistance to nitrofurantoin, 26 % were resistant to gentamicin, and 21% to amikacin, whereas, only 2% and 3% exhibited resistance to meropenem and imipenem, respectively.

Although fluoroquinolones are among the most effective drugs in the treatment of UTI (Kurutepe et al., 2005), diverse studies have reported increasing resistance to fluoroquinolones due to an increase in quinolone prescriptions. Usually, the prevalence of fluoroquinolone resistance is related to the intensity of antibiotic use (Cizman et al., 2001). In this study, the FQ-resistance rate among UPEC was found to be 46.8%. This is quite within the range and this comes consistent with the
previous published rate in Egypt (41.3%) (Samia et al., 2014). These high rates could be explained by the wide and inappropriate use of quinolones as empirical treatment in UTIs. On the other hand, an obviously lower rate (5.3%) was reported by Sotto et al in France in E. coli isolates collected from hospitalized patients. Similar findings were previously reported by El-Mahdy et al., 2017, in Egypt, which may reflect the implementation of a similar antibiotic policy in managing UTI in different Egyptian hospitals.

Our result comes also similar to the findings of Majlesi et al., 2018, who reported that FQ-resistant Enterobacteriaceae isolates showed multidrug resistance to other antimicrobial agents like amoxicillin-clavulanic acid, cefoxitin, cefazidime, cefotaxime, cefepime, aztreonam, tetracyclines, rifampicin, and trimethoprim-sulfamethoxazole, but remain susceptible to carbapenem antibiotics.

On the global level, the rate varies from 35% to 61.9% in different geographical areas (Tandogdu et al., 2014). A higher rate was reported in Iran (61.9%) (Shenagari et al., 2018) and an extremely higher rate was documented in Pakistan (84.2%) (Muhammad et al., 2011). 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% (Mandal et al., 2012).

The frequency of qnrA, qnrB and qnrS genes in FQ-resistant isolates was 10%, 46.6%, and 43.3%, respectively. These findings reveal that, some qnr genes-negative isolates were also resistant to fluoroquinolones which means that, other qnr genes or resistance mechanisms, such as mutations in the target enzyme (e.g., DNA gyrase and topoisomerase IV) and/or activation of efflux pumps, may be involved. Abd El-Salam and his colleges, (2018) in Egypt reported about the same results as QnrS and qnrB were the detected genes in 77.8% and 16.7% of the isolates respectively in Gram negative bacilli clinical isolates which collected from patients attending Misr children hospital, EGYPT.

Globally, in Greece, 10% of CIP-resistant E. coli clinical isolates were qnr-positive (Vasilaki et al., 2008). Also, In Iran, of 116 FQ-resistant isolates, 14 (12.1%) and 9 (7.8%) were positive for qnrA and qnrB, respectively (Firoozeh et al., 2014). On the other hand, the frequency of qnr genes in the present study was higher than that found in China and Japan, where the rates were only 7.5% and 6.5%, respectively (Ode et al., 2009).

In our study, 46% isolates of E. coli were ciprofloxacin resistant. The emergence of resistance for fluoroquinolones is multifactorial (Hooton 2003, Boyd et al., 2008 and Tabasi et al., 2015). The emergence of resistance was predicted on molecular grounds, because single mutation which raises the minimum inhibitory concentration (MIC) of ciprofloxacin by 4 to 16 folds leading to resistance. In addition, the presence of a PMQR determinant increases resistance to fluoroquinolones four to eight times (Ibrahim et al., 2012). This could explain that the majority of FQ-resistant isolates carrying qnr genes had high MIC values (MICs > 32 μg/mL) in our study. However, we noticed a slight decrease in resistance for increasing generations of fluoroquinolones (Table 4). The susceptibility test (MICs) by using 2nd, 3rd, and 4th generation of fluoroquinolones under NCCLS guidelines quinolones for the FQ-resistant E. coli isolates revealed that the 2nd generation fluoroquinolone drugs was showing 56% - 66%. 46% for the 3rd generation and 30% for the 4th generation drugs. These observations were similar to previous papers of susceptibility analysis of isolates to fluoroquinolone drugs (McQuiston et al., 2013, and Kim and Hooper, 2014).

Over all, the present study, showed that the rate of quinolones resistance exceeded 40%, which discourages the empirical use of quinolones in our region since the risk of treatment failure increases when resistance rates exceed 10% to 20% (Carson and Naber, 2004). However, the evidence is insufficient to make a recommendation against using quinolones since no alternative oral antimicrobial options are available for the treatment of pyelonephritis. So, quinolones remain an important treatment option for empirical therapy of complicated urinary tract infections (cUTIs), particularly after stratification of patients based on predicted risk of antimicrobial resistance (Shah et al., 2017).

5. Conclusion:

The incidence of high rates of antibiotic resistance from urinary tract infections related bacteria becomes a public health concern worldwide. We found a high level of quinolones resistance among E. coli strains isolated from patients with UTI in El-Mansoura University Hospitals, EGYPT. Rational use of antimicrobial policy in the hospitals as well as stopping the unnecessary prescription and non-prescription sales in retail pharmacies can be performed as strategies to prevent in order to prevent treatment failure and ensure the best treatment to UTI patients. This study highlights the
importance of antimicrobial resistance of virulent *E. coli* and may help choosing more suitable treatments of UTI patients from urinary tract infection in Egypt. Continuous surveillance of multidrug resistant organisms and patterns of drug resistance are needed in order to prevent treatment failure and reduce selective pressure. These findings may help choosing more suitable treatments of UTI patients.

6. References:


Boyd LB, Atmar RL, Randall GL, Hamill RJ, Steffen D, Zechiedrich L. Increased fluoroquinolone resistance with time in Escherichia coli from >17,000 patients at a large county hospital as a function of culture site, age, sex and location. BMC Infectious Diseases 2008, 8:4.


El-Mahdy RH, Saleh MA, and Aboelnour A. GyrA mutations in nosocomial ciprofloxacin-resistant

### Table 5: Distributions of PMQR genes among FQ-resistant *E. coli* isolates.

<table>
<thead>
<tr>
<th>PMQR genes</th>
<th>Total isolates, n = 30 (%)</th>
<th>Male, n = 8 (%)</th>
<th>Female, n = 22 (%)</th>
<th>Inpatient Infections, n = 11 (%)</th>
<th>Outpatient Infections, n = 19 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnrA</td>
<td>3 (10 %)</td>
<td>1 (12.5 %)</td>
<td>2 (9.1 %)</td>
<td>1 (9.1 %)</td>
<td>2 (10.5 %)</td>
</tr>
<tr>
<td>qnrB</td>
<td>14 (46.6 %)</td>
<td>5 (62.5 %)</td>
<td>9 (40.9 %)</td>
<td>6 (54.5 %)</td>
<td>8 (42.2 %)</td>
</tr>
<tr>
<td>qnrS</td>
<td>13 (43.3 %)</td>
<td>2 (37.5 %)</td>
<td>11 (45.5 %)</td>
<td>4 (36.4 %)</td>
<td>9 (47.3 %)</td>
</tr>
</tbody>
</table>

Abbreviations: PMQR: plasmid mediated quinolone resistance (qnrA, qnrB and qnrS), n = total number of the isolates.


