Impact of co-existence of PMQR genes and QRDR mutations on fluoroquinolones resistance in Enterobacteriaceae strains isolated from community and hospital acquired UTIs

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Abstract

Background: Fluoroquinolones are commonly recommended as treatment for urinary tract infections (UTIs). The development of resistance to these agents, particularly in gram-negative microorganisms complicates treatment of infections caused by these organisms. This study aimed to investigate antimicrobial resistance of different Enterobacteriaceae species isolated from hospital- acquired and community-acquired UTIs against fluoroquinolones and correlate its levels with the existing genetic mechanisms of resistance.

Methods: A total of 440 Enterobacteriaceae isolates recovered from UTIs were tested for antimicrobial susceptibility. Plasmid-mediated quinolone resistance (PMQR) genes and mutations in the quinolone resistance-determining regions (QRDRs) of gyrA and parC genes were examined in quinolone-resistant strains.

Results: About (32.5%) of isolates were resistant to quinolones and (20.5%) were resistant to fluoroquinolones. All isolates with high and intermediate resistance phenotypes harbored one or more PMQR genes. QnrB was the most frequent gene (62.9%) of resistant isolates. Co-carriage of 2 PMQR genes was detected in isolates (46.9%) with high resistance to ciprofloxacin (CIP) (MICs > 128 μg/mL), while co-carriage of 3 PMQR genes was detected in (6.3%) of resistant isolates (MICs > 512 μg/mL). Carriage of one gene only was detected in intermediate resistance isolates (MICs of CIP = 1.5–2 μg/mL). Neither qnrA nor qnrC genes were detected. The mutation at code 83 of gyrA was the most frequent followed by Ser80-Ile in parC gene, while Asp-87 Asn mutation of gyrA gene was the least, where it was detected only in high resistant E. coli isolates (MIC ≥128 μg/mL). A double mutation in gyrA (Lys154Arg and Ser171Ala) was observed in high FQs resistant isolates (MIC of CIP < 128 μg/mL).

Conclusion: FQs resistance is caused by interact between PMQR genes and mutations in both gyrA and parC genes while a mutation in one gene only can explain quinolone resistance. Accumulation of PMQR genes and QRDR mutations confers high resistance to FQs.

Keywords: Enterobacteriaceae, Fluoroquinolones, Plasmid-mediated quinolone resistance, Quinolone resistance-determining regions (QRDRs)

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Background
Urinary tract infections (UTIs) are common infectious diseases in both hospital-acquired UTI (HAUTI) and community-acquired UTI (CA-UTI) [1, 2]. UTI can be diagnosed by at least one of the following clinical symptoms or signs; temperature ≥38°C, suprapubic pain, costovertebral angle pain, urinary urgency, dysuria or frequency. A quantitative urine culture with bacterial counts ≥10^5 colony forming units per mL (CFU/mL) is essential for the diagnosis of UTI [3]. UTIs may be caused by gram-negative, gram-positive bacteria or by fungi. *E. coli* is the most common cause followed by *Klebsiella* and other *Enterobacteriaceae* in both CA-UTI [4] and hospital-acquired UTI (HAUTI) [5]. Fluoroquinolones (FQs) have been considered a highly effective treatment of UTIs. However, the development of resistance to these agents especially by gram-negative micro-organisms complicates the treatment of infections caused by these organisms [6]. FQs resistance is mainly caused by spontaneous mutations in the quinolone resistance determining regions (QRDR) of *gyr A* and *par C* genes, either *gyr A* or *par C*, or both genes [7]. However, in the past few decades, plasmid-mediated quinolone resistance (PMQR) has been increasingly reported in *Enterobacteriaceae* species all over the world [8]. The co-existence of mutations in QRDR and PMQR genes carriage can occur together in *Enterobacteriaceae* species. Moreover, the presence of PMQR determinants may promote QRDR mutations increasing the FQs resistance rates [9]. Some studies in Egypt have previously investigated the FQs resistance [10, 11], but the study of contribution of various mechanisms of resistance in different *Enterobacteriaceae* species in immunocompetent patients was not addressed. Accordingly, the aim of the current study was to investigate antimicrobial resistance of different *Enterobacteriaceae* species isolated from CA-UTI and HAUTI against FQs and correlate its levels with the existing genetic mechanisms of quinolones (Qs) resistance.

Methods
Study design
This is a cross-sectional study was conducted in Minia university hospitals, Egypt, from July 2016 to March 2017. A total of 705 patients with suggested UTI (presented with one or more of UTI symptoms; (Fever ≥38°C, dysuria, increased frequency and suprapubic pain) were included in the study. Urine samples with positive pyuria and urine cultures with a colony count for a single bacterial species ≥10^5 CFU/mL were only included. The study populations were adults (> 18 years). About 32.2% of participants were males and 67.8% were females. The study included 418 outpatients (attended at outpatient’s clinics seeking the treatment) and 286 inpatients (developed their clinical symptoms after 48 h of admission). This study was carried out according to the principles of the declaration of Helsinki. The study was approved by the Medical Ethics Committee of Minia university Hospital, Egypt. As the study used anonymous clinical data, the patients were not required to give informed consent for the study (code: 45 A at 2/5/2016).

Bacterial strains
Urine specimens were collected from symptomatic patients in sterile screw capped containers that were transported within 2 h of collection to bacteriology laboratory in an ice box and processed at once. Well-mixed uncentrifuged urine specimens were streaked by semi-quantitative streaking method onto UTI chrome agar (CHROMagar™ Orientation, paris, France) and by calibrated loop technique on MacConkey and blood agars [12]. After overnight incubation at 37°C, isolated uropathogens were further identified according to their phenotypic criteria based on gram staining, cultural characters and biochemical testing including indole, urease, citrate and sugar fermentation tests [12]. Confirmed *Enterobacteriaceae* strains were kept in tryptcase soy broth with stabilized 15% glycerol at −20°C. A total of 440 non-repetitive *Enterobacteriaceae* isolates were recovered from urine samples.

Antimicrobial susceptibility testing
According to CLSI guidelines [13], disk diffusion method was used to determine antimicrobial susceptibility of the *Enterobacteriaceae* isolates to different antibiotics; amoxicillin/clavulanic acid (AMC) 30 μg, ceftriaxone (CRO) 30 μg, ceftazidime (CAZ) 30 μg, imipenem (IPM) 10 μg, amikacin (AK) 30 μg, sulphamethoxazole/ trimethoprim (SXT) 25 μg, and nitrofurantoin (F) 300 μg. Also 4 different antibiotics disc for quinolone (Q) and fluoroquinolones (FQs) resistance were used; nalidixic acid (NA) 30 μg, ciprofloxacin (CIP) 5 μg, norfloxacine (NOR) 10 μg and ofloxacin (OFX) 5 μg (Thermo Scientific™ Oxoid, UK). Minimum inhibitory concentration (MIC) of ciprofloxacin (CIP) was determined by two methods; MIC strips (E test) which graded from 0.002 to 32 μg/mL and agar dilution method. MIC strips (E test) (Lioflichem s.r.l, Italy) were placed on surfaces of inoculated Mueller-Hinton agar plates as explained previously [12]. MIC determination by agar dilution method was performed according to CLSI guidelines (CLSI, 2015 and CLSI*, 2015), where ten concentrations (4–2048 μg/mL) of CIP were prepared (each in a single agar plate) [14, 15]. The results of disk diffusion assay as well as MIC were interpreted according to CLSI guidelines [13].

DNA extraction and PCR amplification
DNA was extracted using GeneJET genomic DNA purification kit (Thermoscientific, USA) according to the
manufacturer’s instructions. PMQR genes; \textit{qnrA}, \textit{qnrB} and \textit{qnrS} were tested by a multiplex PCR reaction using specific primers (Table 1). PCR was performed in a 25 μL reaction mixture containing 5 μL of purified DNA (approximately 500 ng/μL), 12.5 μL of Platinum® multiplex PCR master Mix (Applied Biosystems™, USA), 0.8 μL (8 pmol) of each primer and 2.7 μL of nuclease free water. Single PCR reactions were used for amplification of each of PMQR genes (\textit{qnrC} and \textit{qepA}) and QRDRs of \textit{gyrA} and \textit{parC} genes using specific primers (Table 1). Each single PCR reaction was performed in a 25 μL reaction mixture containing 300 ng/μL of DNA, 12.5 μL of AmpliTaq Gold® 360 master mix (Applied Biosystems™, USA), 1 μL (10 pmol) of each primer and 7.5 μL of nuclease free water. The primers sequences, annealing temperature, and size of amplified fragments for the studied genes are shown in Table 1 [16–20]. PCR products were resolved on 1% agarose gel with ethidium bromide dye and the gel was visualized under a UV transilluminator (Biometra Goettingen, Germany).

**Table 1** PCR primers used in the current study

| Primer name | Primer sequence (5’ to 3’) | PCR product size (bp) | Annealing Temperature | Ref |
|-------------|---------------------------|-----------------------|-----------------------|-----|
| qnrA m-F    | AGAGGATTCTCACGCGCAGG     | 580                   | 54 °C                 | [16]|
| qnrA m-R    | TGGCGGACAGATCTTGCAC      | 264                   | 54 °C                 | [16]|
| qnrB m-F    | GGMATHGAAATTCGCCACTG     | 428                   | 54 °C                 | [16]|
| qnrB m-R    | TTTGCYGYYCGCCAGTCGAA     | 300                   | 55 °C                 | [19]|
| qnrS m-F    | GCAAGTTCATTGAACAGGGT     | 447                   | 50 °C                 | [17]|
| qnrS m-R    | TCCTACATTGACGCAAGTG      | 502                   | 54 °C                 | [18]|
| qnrC-F      | GGCTTGTACATTATTGATATC    | 343                   | 55 °C                 | [20]|
| qnrC-R      | TCCCTATCCATGAGTTCTTCT    | 300                   | 55 °C                 | [19]|
| qepA-GF     | ACATCTTGGCTTCCTTGTGCG    | 502                   | 55 °C                 | [20]|
| qepA-GR     | AACTGTTGAGCGCGCAGAGT     | 300                   | 55 °C                 | [20]|
| parC-F      | ATG TAC GTG ATC ATG GAC CG | 343                   | 55 °C                 | [20]|
| parC-R      | ATG TCG GAC TAC GAC GAG GT | 343                   | 55 °C                 | [20]|

Statistical analysis
Statistical analysis of demographic, clinical and laboratory data of study subjects was performed using SPSS for windows version 19.0 (IBM, USA).

Results
Prevalence of \textit{Enterobacteriaceae} strains among UTIs
Four hundred and forty isolates belonging to \textit{Enterobacteriaceae} family were recovered from UTI patients with a percentage of (440/705, 62.4%). Two hundred and nine (209/286, 73.1%) were from inpatients and (231/418, 55.1%) were from outpatients. \textit{E. coli} was the most frequent pathogen (281/440 (63.9%), followed by \textit{Klebsiella pneumoniae} (81/440, 18.4%), \textit{Citrobacter} spp. (47/440, 10.7%), \textit{Proteus} spp. (20/440, 4.5%) and lastly \textit{Enterobacter} spp. (11/440, 2.5%), (Table 2).

Antimicrobial susceptibility profiles of \textit{Enterobacteriaceae} isolates
Of 440 \textit{Enterobacteriaceae} clinical isolates tested for antimicrobial susceptibility, the highest rates of resistance were observed against SXT (253/440, 57.5%), CRO (217/440, 49.3%) and AMC (159/440, 36.1%). while the highest susceptibility rate was found to IMP (440/440, 100%). Percentages of resistant isolates to tested antimicrobial agents are summarized in Table 3.

Quinolone (Q) and fluoroquinolones (FQs) susceptibility
A total of (143/440, 32.5%) \textit{Enterobacteriaceae} isolates were resistant to one or more of the tested (Q and FQs). Out of them (90/440, 20.5%) were resistant to FQs. These isolates could be categorized into three phenotypes; high resistance phenotype which included 67 isolates that were highly resistant to all tested Q and FQs.
with MIC of CIP > 32 μg/mL, intermediate resistance phenotype which included 23 isolates with intermediate resistance to all tested Q and FQ with MIC of CIP = 1–2 μg/mL. FQs susceptible phenotype which included 53 isolates that were resistant to NA only (Q) and susceptible to all tested FQs with MIC of CIP ≤ 1 μg/mL.

Prevalence of PMQRs

Of the 143 Q resistant isolates, 90 isolates (62.9%) harbored at least one PMQR gene (54 E. coli, 17 Citrobacter, 9 K. pneumoniae and 10 Proteus). The most frequent PMQR gene was qnrB, which was detected in (90/143, 62.9%) of Q and FQs resistant isolates and in (90/143, 100%) of FQs resistant isolates. QnrS gene was detected in (67/143, 46.9%) of Q and FQs resistant isolates and in (67/143, 74.4%) of FQs resistant isolates (Additional file 1: Figure S1). Neither qnrA nor qnrC were detected in the studied isolates. qepA gene was detected in (9/143, 6.3%) of Q and FQs resistant isolates and in (9/143, 10%) of FQs resistant isolates. (Additional file 2: Figure S2) (Table 4).

QRDR mutations in gyrA and parC genes

Q and FQs resistant isolates were studied by PCR and subsequent sequencing of QRDR of their gyrA and parC genes. Mutation at codon 83 of gyrA was detected in (102/143, 71.3%) of Q resistant isolates; 51 of them belong to high resistance phenotype, 20 in intermediate phenotype and 31 belong to the FQs susceptible phenotype (resistant to NA only) (Figs. 1 and 2). Mutation at codon 87 (Asp87Asn) of gyrA was detected in high resistance phenotype only (6 isolates) (Fig. 3). Three types of amino acid changes resulted from mutation at codon 80 of gyrA protein; change from serine to leucine in E. coli, from serine to tyrosine which was detected in E. coli and Klebsiella and from serine to isoleucine in Citrobacter strains. Mutation at position 80 of parC gene was detected in (78/143, 54.5%) of isolates (Fig. 4); 57 of them belong to high resistance phenotype, 2 isolates (intermediate resistance) and 19 isolates (FQs susceptible phenotype). Isolates’ identification, phenotypes, PMQR genes distribution and detected mutations are summarized in (Table 4).

Correlation between phenotyping and genotyping of resistant isolates

All isolates with high resistance phenotype (67) harbor QnrB and qnrS genes. QepA gene was detected in only 9 isolates with high resistance phenotype. Isolates with intermediate resistance phenotype (23) carry qnrB gene only. None of PMQR genes was found in isolates with FQs susceptible isolates (resistant to NA only). Detection of PMQR genes (qnrB and qnrS) was strongly correlated with FQs resistance levels in resistant isolates with a correlation coefficient equal to 0.8 (r = 0.8) and 0.9 (r = 0.8) respectively which was highly significant (p value; 0.0001) for both genes as well as qepA gene with a correlation coefficient equal to 0.25 (r = 0.25) which was considered fair but significant (p value; 0.006). Detection of specific mutations in gyrA and parC genes was not correlated with susceptibility pattern to tested Q and FQs as these mutations were found in all phenotypes (Table 4).

Discussion

UTIs are common bacterial infections in hospital settings and community [21]. In the current study, 440 Enterobacteriaceae isolates were isolated from UTIs with a percentage of (440/705, 62.4%). The isolation rates from inpatients and

Table 2

| Organism     | Total | Frequency | P value |
|--------------|-------|-----------|---------|
|              | N %   | N %       |         |
|              |       | Outpatients | Inpatients |
| E.coli       | 281   | 147 63.9%  | 134 64.1% | 0.0001 |
| K. pneumoniae| 81    | 147 84.0%  | 41 19.6%  |         |
| Citrobacter spp. | 47    | 17 10.7%  | 30 14.4%  |         |
| Enterobacter spp. | 11    | 9 2.5%    | 2 1.0%    |         |
| Proteus spp.  | 20    | 18 4.5%   | 2 1.0%    |         |
| Total        | 440   | 231 100%  | 209 100%  |         |

UTIs Urinary tract infections

| Antibiotic organism | AMC | CTZ | CRO | IMP | AK | SXT | NA | CIP | NOR | OFX | F |
|---------------------|-----|-----|-----|-----|----|-----|----|-----|-----|-----|---|
| E.coli              | 99  | 35.2| 97  | 34.5| 159| 56.6| 0  | 63  | 22.4| 182| 64.8| 104|
| K. pneumoniae       | 17  | 21.0| 13  | 16.0| 16 | 19.8| 0  | 9   | 11.1| 29 | 35.8| 11 |
| Citrobacter spp.    | 31  | 66.0| 20  | 42.6| 30 | 63.8| 0  | 10  | 21.3| 22 | 46.8| 17 |
| Enterobacter spp.   | 9   | 1   | 9.1 | 1   | 9.1| 1   | 0  | 1   | 9.1 | 2  | 18.2| 1  |
| Proteus spp.        | 11  | 55.0| 11  | 55.0| 11 | 55.0| 0  | 1   | 5.0 | 18 | 90.0| 10 |
| Total               | 159 | 36.1| 142 | 32.3| 217| 49.3| 0  | 84  | 19.1| 253| 57.5| 143|
outpatients were (73.1%) and (55.1%) respectively. A higher frequency of isolation (86%) was recorded previously in Asia-Pacific region [22]. The frequency of isolation from outpatients agrees with a previous report in Ethiopia (57.75%) [23], and disagrees with another report in Korea (89%) [4].

_E. coli_ was the most frequent followed by _K. pneumoniae_, _Citrobacter_ spp., _Proteus_ spp. and lastly _Enterobacter_ spp. These findings agree with several previous studies [24, 25]. In spite of similarity with these reports, _Citrobacter_ spp. isolation rate in the current study remains the highest. The highest antimicrobial resistance rates were recorded in the present study against SXT with a percentage of (57.5%) followed by CRO (49.3%), however, none of the isolates were resistant to imipenem. These findings agree with several previous studies [4, 5]. High resistance to CRO may be caused by extensive use in the locality. NA has been used for treatment of UTIs for more than five decades [26] so the resistance to NA is expected to be higher than to FQs. In the current study, NA has a resistance rate of (143/440, 32.5%), however higher rates were reported previously in several studies [23, 25, 27]. The fact that NA is not an empiric treatment of UTI in Egypt may be the cause of this difference. FQs have an overall resistance rate of (90/440, 20.5%).

### Table 4: Distribution of PMQR and QRDRs among different FQs resistance phenotypes of _Enterobacteriaceae_ isolated from UTIs

| Species            | Phenotype          | Resistant to Quinolones | PMQR genes | MIC of CIP (μg/mL) | Number | gyrA alterations | ParC alterations |
|--------------------|--------------------|-------------------------|------------|--------------------|--------|------------------|------------------|
| _E.coli_           | FQ Susceptible     | NA only                 | No genes detected | 0.5 | 31 | Ser83Leu | No mutation |
|                    |                    |                         |            | 0.25 | 19 | Ile155Phe | Ser80lle |
|                    | Intermediate       | NA, CIP, NOR, OFX       | _qnrB_ | 1.5 | 7 | His80Met Gly81Ala Ser83Leu | Met118Trp Arg119Val |
|                    | resistance         |                         |            | 2 | 13 | Ser83Leu | No mutation |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS_ | 128 | 28 | Ser83Tyr Lys154Arg Ser171Ala Ile174Thr Ala175Val | Ser80lle |
| _K. pneumoniae_    | FQ Susceptible     | NA only                 | No genes detected | 0.5 | 2 | Lys154Arg Ala171Ser | No mutation |
|                    |                    |                         |            | 2 | 2 | No mutation | Ser80lle |
|                    | Intermediate       | NA, CIP, NOR, OFX       | _qnrB_ | 2 | 2 | No mutation | Ser80lle |
|                    | resistance         |                         |            | 128 | 3 | Ser83Tyr Lys154Arg Ala171Ser | Ser80lle Met118lle |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS_ | 512 | 4 | Ser83Tyr Ala175Arg Val176Leu | Ser80lle Met118lle |
| _Citrobacter_ spp. | Intermediate       | NA, CIP, NOR, OFX       | _qnrB_ | 1.5 | 1 | ND | ND |
|                    | resistance         |                         |            | 0.125 | 1 | ND | ND |
| _Enterobacter_ spp.| FQ susceptible     | NA only                 | No genes detected | 0.125 | 1 | ND | ND |
| _Proteus_ spp.     | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS_ | 125–512 | 8 | ND | ND |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS, qepA_ | 2 | 2 | No mutation | Ser80lle |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS, qepA_ | 512 | 1 | ND | ND |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS, qepA_ | 256 | 15 | Ser83Ile Lys154Arg Ser171Ala | Ser80lle |
|                    | High resistance    | NA, CIP, NOR, OFX       | _qnrB, qnrS, qepA_ | 512 | 4 | Ser83Ile Lys154Arg Ser171Ala | Ser80lle |

PMQR Plasmid-mediated quinolone resistance, QRDR Quinolone resistance determining regions, CIP Ciprofloxacin, ND Not determined

Query 4  PHGDLAVYDTVRMAQPSFLRMLVDQGNFSGIDGSAAMRYTEIRLAKIAMELAD 183
Subject 79 PHGDLAVYDTVRMAQPSFLRMLVDQGNFSGIDGSAAMRYTEIRLAKIAMELAD

Query 184  EKETVDFVDNYDGETEKPDPMTKIPNLVNGSSGIA*GMA 306
Subject 139 EKETVDFVDNYDGETEKPDPMTKIPNLVNGSSGIAVMA

**Fig. 1** Alteration in _gyr A_ (codon 83). Nucleotide sequence of a _gyr A_ region of the _E. coli_ FQs-susceptible, WP_074153749.1 DNA gyrase subunit A ( _E. coli_). Alteration in (codon 83)
Korea (24.8%) [4], while a higher resistance rate (54.9%) was recorded in Asian countries [28]. With the analysis of PMQR genes (qnr genes and qepA gene), the most frequent gene was qnrB, which was detected in (62.9%) of Q and FQs resistant isolates, and in (100%) of FQs resistant isolates while qnrS gene was detected in (46.9%) of Q and FQs resistant isolates and in (74.4%) of FQs resistant isolates. In other studies on Egyptian population, qnrB was the most prevalent qnr gene among K. pneumoniae isolates as noted by El-Badawy et al., 2017 which agree with our results [29], while others reported that qnrS was the most prevalent gene among gram negative bacilli isolated from different clinical settings [10, 11].

QnrB and qnrS genes detection rate in our study was higher than that reported in several previous studies [27, 30]. Neither qnrA nor qnrC were detected at all, but qepA gene was detected in (10%) of FQs resistant isolates. In the same context with us, several studies also could not detect qnrA gene among Enterobacteriaceae isolates [27, 30, 31]. However, Szabó et al., 2018 could detect qnrA in their isolates but could not detect neither qnrC nor qepA genes [32]. The difference between our results and others may be caused by geographical distribution of qnr genes, type of clinical isolates or the used methods of detection. With considering the analysis of the QRDRs of gyrA and parC genes, this study reported that the mutation at position 83 of gyrA was the most frequent in Q and FQs resistant strains. Three types of amino acid changes resulted from this mutation. Change from serine to leucine in E. coli strains which was also reported by many reports [33, 34] and change from serine to tyrosine which was detected in K. pneumoniae and E. coli and was also reported previously [35]. The third change (from serine to isoleucine) was detected in Citrobacter strains and was reported in several studies [36, 37].

A double concomitant mutation in gyrA (Lys154Arg and Ser171Ala) was observed in high FQs resistant Citrobacter spp. isolates with MIC of CIP ≥ 256 μg/mL and E. coli isolates with MIC of CIP ≥128 μg/mL. These mutations were reported previously in few studies [35, 38]. Asp-87 Asn mutation of gyrA gene was the least frequent mutation, where it was detected only in high resistant E. coli isolates with MIC ≥128 μg/mL. Therefor the mutation at position 87 of gyrA seemed to contribute to high resistance to FQs, while the mutation at position 83 could contribute to both Q and FQs resistance. This suggestion agrees with other results [34, 39]. Ser80Ile mutation of parC gene was frequent in both Q and FQs resistant strains that agrees with other studies [40, 41]. Single mutation in either gene occurred only in isolates with MIC of CIP ≤ 2 μg/mL, so the presence of double mutation in gyrA and parC genes seemed to be associated with high levels of resistance to FQs. These findings agree with other reports suggested that high levels of FQs resistance appeared to happen as a result of gradual accumulation of QRDR mutations [38, 42]. All isolates with high and intermediate resistance phenotypes harbored one or more PMQR gene, interestingly, isolates with FQs susceptible phenotype (resistant to NA only) harbored none of the tested PMQR genes. This agrees with Rodríguez-Martínez et al., 2016, who stated that resistance to NA only is not enough to suggest presence of PMQR genes [43], while Szabó and his colleagues could found PMQR genes among susceptible or low-level resistance to ciprofloxacin with (MIC = 0.06–1 mg/L) isolates [32]. Our study also agrees with Piekarska et al., 2015, who stated that combination of...
both PMQR genes and mutations in QRDRs of gyrA and parC contributes to high resistance to FQs [9].

**Conclusion**

In the current study *Enterobacteriaceae* remain the most common cause of UTIs. The resistance rate of Q is (32.5%), while the resistance to Q and FQs is (20.5%) among *Enterobacteriaceae* isolates. At least one of PMQR gene was detected in FQs resistant isolates. The most frequent gene was *qnrB*, which was detected in (62.9%) of Q resistant isolates followed by *qnrS* gene which was detected with a percentage of (46.9%). The co-existence of 2 PMQR genes in the same isolate was observed in (46.9%) of resistant isolates, while co-existence of 3 PMQR genes was reported in (6.3%). The presence of at least two PMQR genes together with simultaneous QRDR mutations in each of gyrA and parC genes can describe the mechanism of resistance in high resistance phenotype (highly resistant to all tested Q and FQs), while presence of at least one PMQR gene together with one QRDR mutation at either genes could be the cause of resistance in isolates with intermediate resistance phenotype (intermediate resistance to all tested Q and FQs). Presence of mutation in only one QRDR regions of gyrA or parC genes could be the cause of resistance to NA only. To our knowledge, the current study is the largest study that reported molecular epidemiology of quinolones resistance in different *Enterobacteriaceae* species in the study area and could suggest a phenotypic algorithm to describe genetic mechanisms of quinolone resistance.

### Abbreviations

CA-UTI: Community-acquired urinary tract infection; CIP: Ciprofloxacin; CRO: Ceftriaxone; FQs: Fluoroquinolones; HAUTI: Healthcare-associated urinary tract infection; MIC: Minimum inhibitory concentration; PMQR: Plasmid-mediated quinolone resistance; QRDRs: Quinolone resistance-determining regions; Q: Quinolone; UTI: Urinary tract infection

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### Authors’ contributions

WKMD, MSM and RMK participated in the study design, DNK carried out most of the data collection, DNK and RMK participated in laboratory work, data analysis and interpretation and drafting the manuscript. All authors have read and approved publication of the final manuscript.

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None.

### Availability of data and materials

All data generated or analyzed during this study are included in this article [and its supplementary information files (Additional file 1: Figure S1 and Additional file 2: Figure S2)].

### Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Minia university Hospital, Egypt. As the study used anonymous clinical data, the patients were not required to give informed consent for the study (code: 45 A at 2/5/2016).

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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