International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Determination of *Gyr A* and *Par C* Mutations and prevalence of Plasmid-Mediated Quinolone Resistance Genes in *Escherichia Coli and Klebsiella Pneumoniae* isolated from patients with urinary tract infection in Egypt

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Abstract

The widespread use of antibiotics and disinfectants has hastened the emergence of bacterial resistance. Bacterial resistance has become a global health issue, particularly in developing countries, as a result of the abuse of antimicrobial drugs, which has increased the risk of nosocomial infections. A total of ninety-nine clinical specimens were collected from cases with urinary tract infection. Standard strains obtained from Central laboratories of the Ministry of Health and Health Insurance. Plasmid DNA extracted from quinolone resistant producers were screened for the presence of *qnr* gene families (including *qnrA*, *qnrS*, *qnrB*) encoding for resistance to quinolones *Klebsiella pneumoniae* and *E.coli* clinical isolates using specific primers by PCR amplification technique. Multi-drug resistant isolates were counted for 81 out of 99 isolates (81.8% of initial number of isolates) and all ESBLs producers of *K. pneumoniae* and *E.coli* isolates (41.4%) were multi-drug resistant. *QnrB* gene was the most predominant gene with percentage of 46% and 28% among *K.pneumoniae* and *E.coli* isolates; respectively. On contrast, the least detected gene was *qnrS* that was found in 11.5% of *K. pneumoniae* isolates and 0 % of *E.coli* isolates. The presence of PMQR genes is prevalent in Enterobacteriaceae isolates, with qnrB being the most common. As a result, detecting PMQR determinants among Enterobacteriaceae ESBL producers is critical for optimal empirical therapy and infection management.

Keywords: Escherichia coli, Klebsiella pneumoniae, gyrA, parC, PMQR genes

Full length article **Corresponding Author*, e-mail: ahazem954@gmail.com

1. Introduction

Among *Enterobacteriaceae* family member, *E. coli, Klebsiella pneumoniae and Proteus spp* had a potential medical importance [1]. These bacteria are etiological factor for a many diseases including infections of urinary tract, gastroenteritis, pneumonia, septicemia, and meningitis. If not treated effectively, certain of these disorders have a significant mortality rate. As a result, it is critical to combat them using extremely potent antibiotics [2]. The most used antimicrobial agents are beta-lactams such as penicillin's, cephalosporins and other non-beta lactams such as quinolones [3]. The widespread use of antimicrobials and disinfectants has accelerated the evolution of bacterial resistance. This bacterial resistance has evolved into a global health issue, particularly in developing countries [4]. The antimicrobial susceptibility patterns of the βlactams producing *Enterobacteriaceae* have been changed over the years particularly in *Klebsiella pneumoniae* and *Escherichia coli* isolates [5]. Beta-lactams and fluoroquinolones are the most frequently prescribed antimicrobials worldwide, and considered the second-line agents for empirical treatment of such infections. Cephalosporins are wide-spectrum antibiotics used to treat microorganisms caused diseases [6]. The widespread usage of these antibiotics in hospitals and the community has led to a serious problem with resistance, which has increased death and morbidity rates, reduced treatment options, and raised the expense of healthcare [7,8]. Among the family *Enterobacteriaceae*, production of extended–spectrum beta- lactamase (ESBLs) has emerged an important mechanism of resistance to β-lactam drugs, but these ESBLs can be inhibited by clavulanic acid and sulbactam [9]. The goal of this work is to analyze the existence of PMQR genes (qnrA, qnrB, and qnrS) as well as genetic modifications in the gyrA and parC genes encoding quinolone resistance indicators in plasmids extracted in this investigation. The drug target was tested in a variety of *E. coli* FQR isolates.

2. Materials and methods

2.1. Sample collection

A total of ninety-nine clinical specimens were collected cases admitted royal lab in Cairo during the period from Dec 2021 to Mar 2022. These specimens were taken from urine. Standard *E. coli ATCC (25922) and K. pneumonia ATCC (13883)* strain obtained from Central laboratories of the Ministry of Health and Health Insurance. All specimens were collected aseptically in sterile tubes or bottles and tightly closed to avoid contamination during transport and were subjected immediately for culture on selective and non-selective media for primary isolation and purification. Traditional biochemical tests on different culture media were used to identify the isolates to species level, including: Methyl red indicator, Voges-Proskauer's test, Citrate utilization test, reaction on TSI agar, motility test, Oxidation / fermentation (O/F) test, and VITEK 2 automated systems [10], and urease test [11].

2.2. Plasmid extraction

Promega ™ Plasmid Miniprep kit (Promega Research, Epigenic Company, Madison, USA) was used. The plasmids of quinolone resistant isolates were electrophoresed and visualized according to Sambrook and Russell [12]. A weight of 1 g agarose was dissolved in 100 mL TAE buffer (1% agarose gel) and put in microwaves oven for few minutes to dissolve agarose in buffer completely, the 5 µL ethidium bromide (Et-Br) solution was added to agarose after being slightly cool for staining of DNA. The agarose gel was poured in running chamber with the suitable comb and left to solidify. The comb was removed after flooding the gel with 1X TAE buffer. The extracted plasmid (15 µL) was mixed with 2 µL of 6X loading buffer and each sample was loaded carefully in a separate slot using micropipette. DNA marker was loaded at first slot. The separation of plasmids was performed by electrophoresis at 4V/cm according to their molecular weight. After the bromophenol blue bands had almost reached the end of the gel length, the electrophoresis power was turned off. For documentation, a UV trans-illuminator was used for viewing the gel.

2.3. DNA molecular weight markers

On an agarose gel, a double-stranded DNA in the size range of 100 plus bp to 3000 bp was measured using a Thermo Scientific GeneRuler 100bp DNA ladder. A total of 12 DNA pieces were in the DNA ladder. Also, 1 kb DNA ladder ranging from (250bp – 10000 bp) all were supplied with free vial of 6X gel loading dye and obtained from biotech serve (genedirex) (Taoyuan, Taiwan).

2.4. Polymerase chain reaction (PCR)

GRS Taq (2X) Mstermix supplied in reaction buffer, dNTPs and 4 mM $MgCl_2$ was obtained from (grisp) (portogal). The primers used in this study are listed in table (1). They were purchased from Promega (USA). According to White *et al.* [13] Each PCR mixture contains 30 L of nuclease-free water, 6 L of template DNA, 2.0 L of forward primer, 2.0 L of reverse primer, and 15 L of PCR master mix. DNA (single PCR) is heated at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 45 seconds, 60 °C for 45 seconds, and 72 °C for 1 minute for all genes, followed by 10 minutes of 72 °C heating. For plasmid (multiplex PCR), heat the mixture for 10 minutes at 95°C, then run 35 cycles of all genes at 95°C for 1 minute, 54°C for 1 minute, and 72°C for 1 minute. Finally, heat the mixture for 10 minutes at 72°C. The PCR products were divided on a 1.5% agarose gel, dyed with Et-Br, seen under a UV light source, and captured on camera. The size was determined using a DNA size marker. The BIO-RAD T 100 - thermal cycler equipment (Hercules, California, United States) was used for the amplification.

3. Results

A total of ninety-nine *Klebsiella pneumoniae* and *E. coli* isolates were collected from 100 clinical specimens of cases with urinary tract infection from royal lab in Cairo during the period from Dec 2021 to Mar 2022 (Table 2). The isolates were identified and confirmed based on their biochemical characters as shown in Table (3). The data presented in Figure (1) and (2) showed the susceptibility of *K.pneumonia* to the different antimicrobial. The data revealed that from the 50 *K.pneumonia* isolates 25 isolates were quinolone resistant. The data presented in Figure (3,4) showed the susceptibility of *E. coli* to the different antimicrobial. The data revealed that from the 49 *E. coli* isolates 28 isolates were quinolone resistant. Table (4) summarizes the detection of mutation in Gyr A and Par C in *E. coli* and *K. pneumonia*, In addition, the detection of mutation in plasmid mediate quinolone resistant in *E. coli* and *K. pneumonia* were mentioned in Table (5).

4. Discussion

In this study, all isolates were collected and handled according to the accepted laboratory procedures. Fifty of *Klebsiella pneumoniae* isolates and forty-nine of *Escherichia coli* isolates were recognized based on morphology, culture characteristics and biochemical tests according to Harley *et al.* [14] and Atlas [15]. Screening for antimicrobial resistance producing isolates to the different antimicrobials was carried out with the broth micro-dilution method regarding CLSI [16]. Susceptibility of *Klebsiella pneumoniae* isolates revealed that, most of isolates exhibit high resistance to ampicillin with percentage (100%), cefazolin with percentage (88%), cefuroxime (86%), ceftazidime (86%). The isolates revealed moderate resistance to Piperacillin-tazobactam (52%), cefoxitin (60%), levofloxacin (50%). Intermediate resistance rates were found with Imipenem and nitrofurantoin with percentages of 44% and 34%; respectively. Susceptibility of *K. pneumoniae* isolates agreed with that reported by Du *et al.* [17]. On the other hand, the susceptibility of *K. pneumonia* isolates revealed high rate of resistance when compared to that reported by Kim *et al.* [18] as they found that Klebsiella pneumoniae had resistance rates to 3rd and 4th generation cephalosporins of 36-41% and 33-41%, respectively. For cefoxitin, the resistance rates were 12–17%, for fluoroquinolone, 34%–35%, and for trimethoprim–sulfamethoxazole, it was between 21% and 30%. This study showed moderate resistance rate towards sulphamethoxazole (68.8%) and fluoro-quinolone (53%) which came in agreement with that reported by Shariff *et al.* [19]. Susceptibility of *E. coli* isolates revealed that, most of isolates exhibit high resistance to Ampicillin (90%), Cefazolin (80%). moderate resistance to Trimethoprim-sulfamethoxazole (62%), levofloxacin with percentage (58%), Ceftazidime with percentage (48%) and Ampicillin sulbactam (44%).

Table 1: Primers used in this study that targeting five families of quinolone resistant genes.		
Primer	**Sequence 5`-3`**	**Product size (bp)**
gyrA F	AAATCTGCTCGTGTCGTTGG-3	**349bp**
GyrA R	GCCATACCTACAGCAATACC-3	
ParC F	AAGCCCGTACAGCGCCGTATT-3′	**327bp**
ParC R	AAAGTTATCTTGCCATTCGCT-3′	
ParC F	AAACCTGTTCAGCGCCGCATT	**327 bp**
ParC -R	AAAGTTGTCTTGCCATTCACT	
Qnr A F	AGAGGATTTCTCACGCCAGG	**580bp**
Qnr A -R	TGCCAGGCACAGATCTTGAC	
Qnr B -F	GATCGTGAAAGCCAGAAAGG	**476bp**
Qnr B -R	ATGAGCAACGATGCCTGGTA	
Qnr S -F	GCAAGTTCATTGAACAGGGT	**428bp**
Qnr S -R	TCTAAACCGTCGAGTTCGGCG	

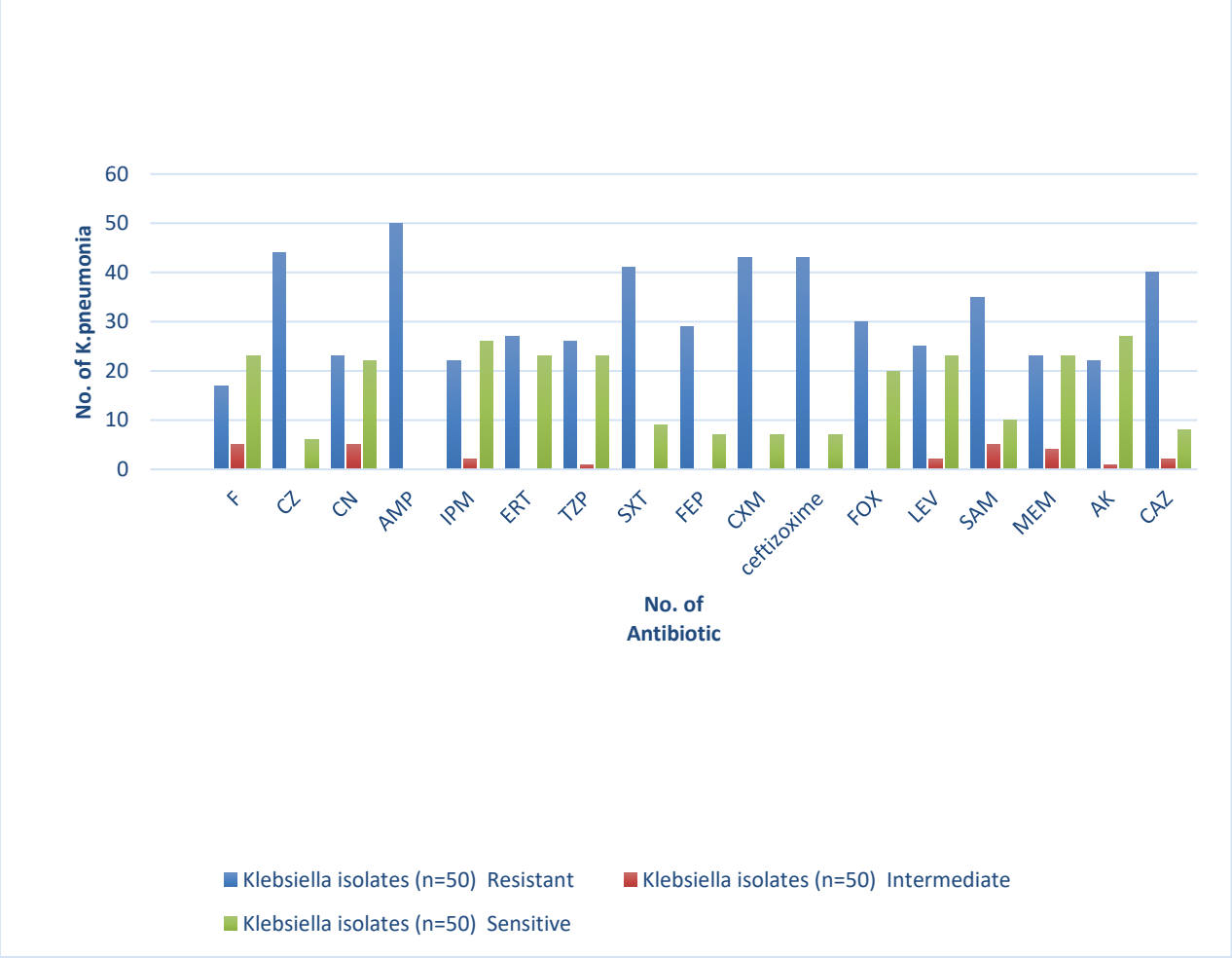

Figure 1: Susceptibility pattern of Klebsiella isolates towards different antibiotic.

S: sensitive, I: intermediate, R: resistant, ATM: Aztreonam, AML: Amoxicillin, CRO: Ceftriaxone, CTX: Cefotaxime, CAZ: Ceftazidime, FOX: Cefoxitin, CXM: Cefuroxime, CN: Gentamycin, CIPRO: Ciprofloxacin, LEVO: Levofloxacin, NOR: Norfloxacin, OFX: Ofloxacin, C: Chloramphenicol, SXT: Sulphamethoxazole/Trimethoprim, and DO: Doxycycline.

Table 2: Distribution of *K. pneumoniae* and *E. coli* isolates according to the clinical sources.

Specimen type	No. of specimen	*Klebsiella pneumoniae*		*Escherichia coli*	
		No. of isolates	Percentage from total (%)	No. of isolates	Percentage e from total (%)
Urine	99	50	49.5	49	48.5

Table 3: Identification of clinical isolates

	K. pneumoniae	*E. coli.*
Indole	-	+
Methyl red	-	+
Voguesproskauer	+	-
Citrate utilization	+	-
TSI	**A/A+CO_2**	**A/A+CO_2**
Motility	-	+
Urease	+	-
Oxidation/ferme ntation test	**O+ / F+**	**O+ / F+**
Eosin methylene blue agar	**Pink mucoid colonies**	**Black colonies with metallic sheen**
TSI: Triple sugar iron agar A/A: Acid slant/Acid butt		

Table 4: Distribution of mutation in Gyr A and Par C in *E. coli* and *K. pneumonia*

E. coli		*K. pneumonia*	
Gyr A	**Par C**	**Gyr A**	**Par C**
9,10,11,17,22,24,25,26,27,29,31,32,33,	**9,10,12,17,20,22,24,25,26,27,29,31,32,33,34,35,36,37,38,39,40,43,44,45,46**	**3,5,8,14,17,18,20,21,23,25,26,30,31,35,40,41,44**	**16,21,22,25,30,31,40,44,47,48**
Total 13	**Total 25**	**Total 17**	**Total 10**

Table 5: Detection of mutation in plasmid mediate quinolone resistant in *E. coli* and *K. pneumonia*

Type of organism	***E. coli***		***K. pneumonia***	
PMQR gene	**Sample. No**	**Total isolate**	**Sample. No**	**Total isolate**
QnrA			**22,31**	**2**
Qnr B	**12,22,31,32,45**	**5**	**3,5,8,9,10,14,15,16,20,22,25,30,31,35**	**14**
Qnr S			**14**	**1**

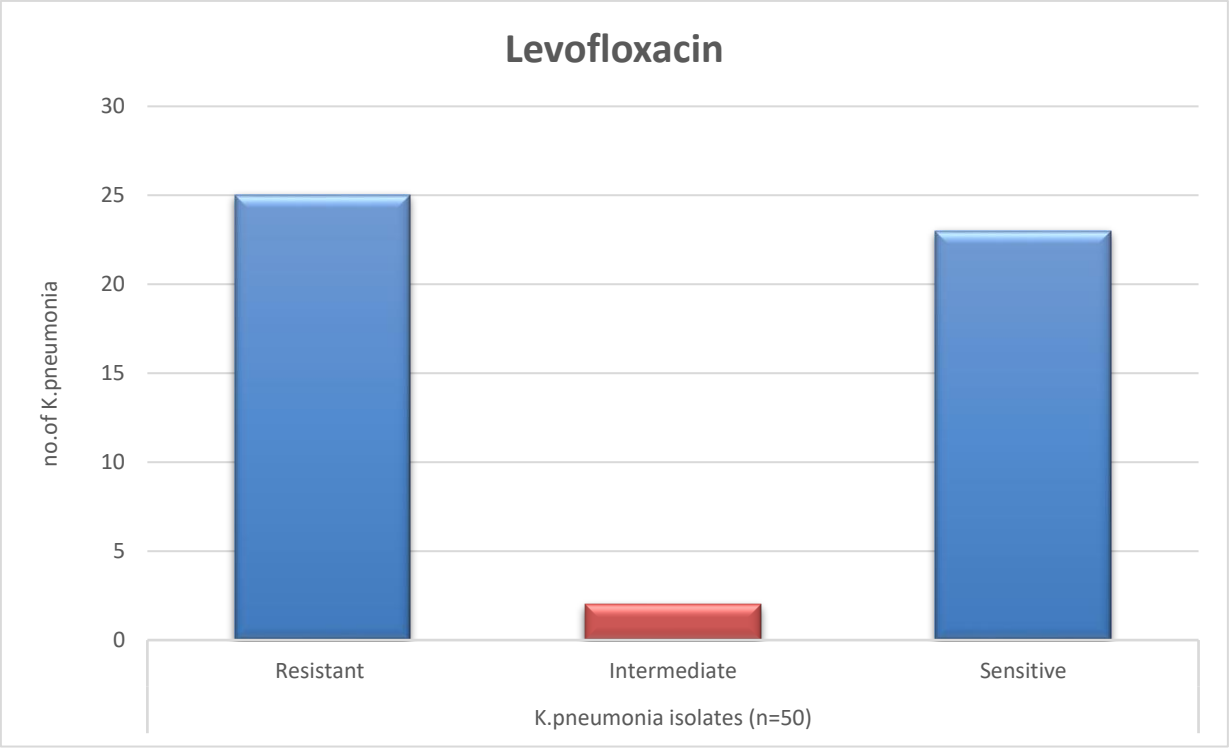

Figure 2: Susceptibility pattern of *Klebsiella pneumonia* towards levofloxacin

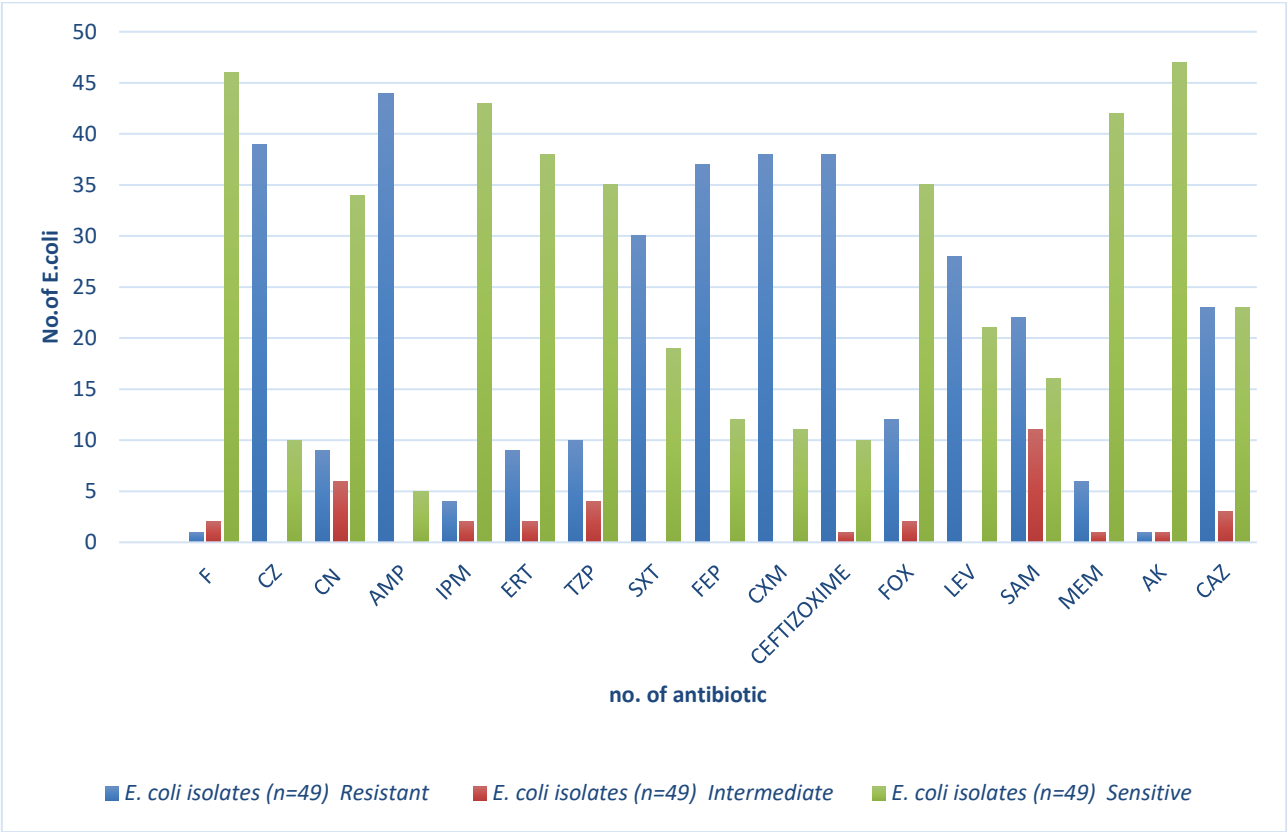

Figure 3: Susceptibility pattern of *Escherichia coli* towards different antimicrobials.

S: sensitive, I: intermediate, R: resistant, AML: Amoxicillin, CAZ: Ceftazidime, CRO: Ceftriaxone, CTX: Cefotaxime, ATM: Aztreonam, FOX: Cefoxitin, CXM: Cefuroxime, CN: Gentamycin, CIPRO: Ciprofloxacin, LEVO: Levofloxacin, NOR: Norfloxacin, OFX: Ofloxacin, C: Chloramphenicol, SXT: Sulphamethoxazole/Trimethoprim, and DO: Doxycycline.

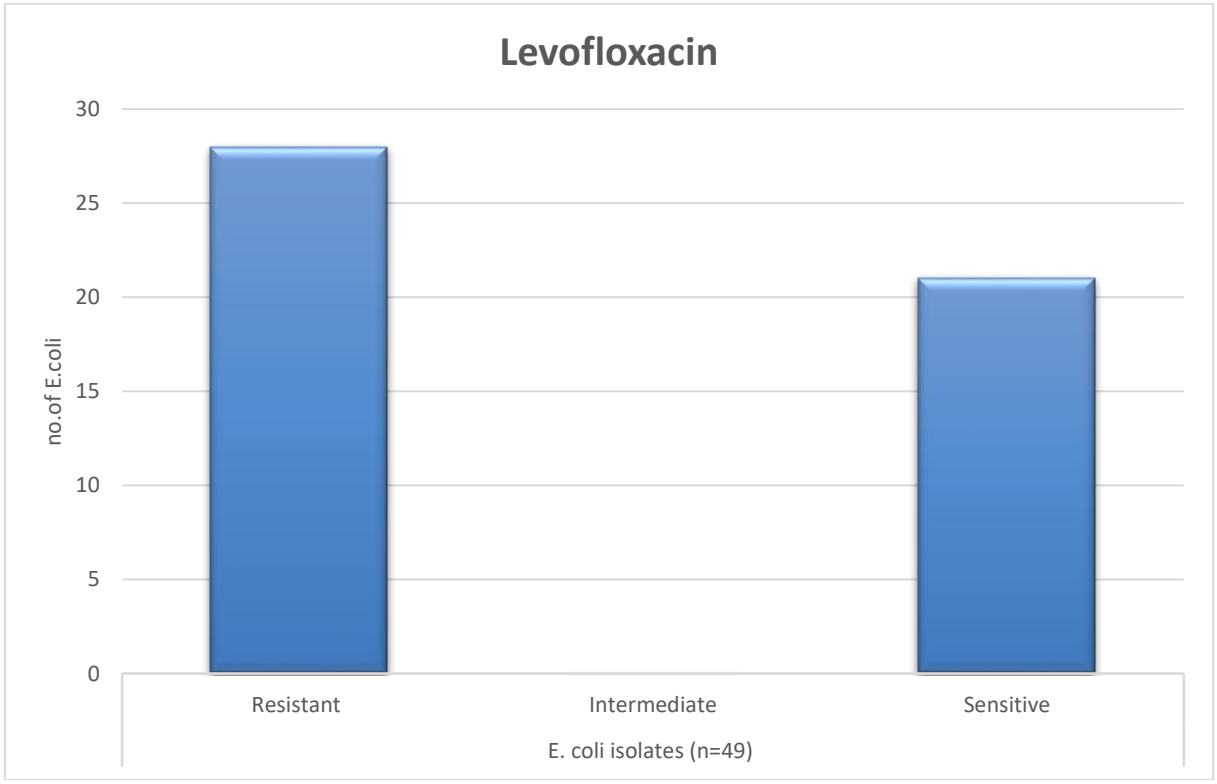

Figure 4: Susceptibility pattern of *E. coli* towards levofloxacin.

Intermediate resistance rates were found with Ampicillin-sulbactam, Cefoxitin and Piperacillin-tazobactam with percentages of 44%, 24% and 20%; respectively. These results agreed with that reported by Du *et al.* [17]. On the other hand, low resistance was reported with Amikacin (2%). This result lower than the study performed by Chen *et al.* [20]. However, resistance rate of *E. coli* isolates against the 3rd generation of cephalosporins (CAZ) with percentage of 48% was lower than results reported by Babypadmini & Appalaraju, they found that tested isolates showed higher resistance to CAZ ranged between 80% -87% [21]. In an investigation performed by Kim *et al.*, the study revealed fluoroquinolone resistance elevated from 42% in 2013 to 48% in 2015, whereas trimethoprim-sulfamethoxazole resistance stayed at 38-39%. [18]. In this study, the data revealed that, ESBL producing isolates are mainly resistant to cefuroxime and ceftizoxime (86% and 80%; respectively) and to other antibiotics such as gentamicin (18%). Our research found that ESBL-producing isolates had a high rate of antibiotic co-resistance, which was consistent with a small difference between nations [22-24]. According to previous study, a case with chronic inflammation of prostate caused by persistent Escherichia coli that produced ESBL and was resistant to antibiotic treatments was quickly healed after receiving treatment with a combination of doxycycline and fosfomycin [25]. According to another investigation, using chloramphenicol and polymyxin B together to treat MDR *K. pneumonia*e improved killing of bacteria and slowed the spread of resistance [26]. M. Al-Kashef *et al.*, found that the observation of ESBL production in 54.6% of isolates supports Egypt's rising ESBL production rate. Selective overuse and other improper uses of these antimicrobial agents are the main causes of the elevated rate of ESBL formation [27]. Plasmid profiling is a useful epidemiologic and typing tool. It correlates with antimicrobial resistance patterns [28] . Plasmid profiling analysis distinguished more strains than antimicrobial. susceptibility patterns [29]. The presence of PMQR genes was in addition to genetic changes encoding gyrA and parC. The drug target was tested in a range of *K. pneumonia*e and *E. coli* FQR isolates. In general, *E. coli* isolates were more resistant to FQs (58%) than *K. pneumonia*e isolates (50%). The most common GyrA mutations were at codons 83 and 87. FQR *E. coli* isolates were found to have mutations. The S83L alteration was found in 98.9% (n = 27) of the FQR isolates, with 11 isolates carrying only S83L and 16 isolates carrying both S83L and D87N substitutions. Several other studies have detected the S83L and D87N alterations in FQR *E. coli* isolates, supporting the concept that these locations are crucial for drug binding and are prone to mutation during resistance emergence [30]. However, more research and confirmatory tests are required to fully understand its relationship to FQ resistance. Furthermore, S80I and E84V alterations in the ParC subunit were found in 55.2% (n = 48) of the FQR *E. coli* isolates.

In a study conducted in Brazil by Minarini *et al.*, 47 *E. coli* samples with mutant gyrA also exhibited a change in parC [31]. Additionally, it was found that the most prevalent ParC mutation in the tested FQR *E. coli* samples was the S80I substitution. The same authors did not find the E84V mutation, and instead found the E84G, E84A, and E84K replacements at this location of Par C [32]. Similarly, many additional investigations have described S80I and E84V amino acid changes in ParC [30]. Also, E84G or E84K changes have also been observed [33]. Our results were consistent with those of other investigations that found distinct mutations, such as S83I and S83Y in parC and D87N in gyrA, in FQR *K. pneumonia*e isolates [33]. S80R, S80I, E84G, and E84K in parC and S83N, S83I, S83Y, D87A, D87G and D87N in gyrA [34]. S83I in gyrA [35], and S83Y, S83I and S80I in gyrA [36]. In the current investigation, PMQR genes were found in 7 (25%) of 28 FQR *E. coli* and 14 (56%) of 25 FQR *K. pneumonia*e isolates. The most prevalent PMQR gene in *E. coli* isolates was qnrB (7/7; 100%). QnrB was found in 4.8% of *E. coli* isolates, according to numerous investigations from various nations [37]. Like other studies [38], In the current investigation, only 25% of the FQR *E. coli* samples had qnrA or qnrB found. The most common PMQR gene in *K. pneumonia*e was qnrB, which was found in 85.7% (12/14) of FQR isolates. Similar findings were additionally reported [38], Two investigations revealed that qnrS was the most prevalent qnr gene in *K. pneumonia*e isolates [39, 40]. In *K. pneumonia*e isolates, PMQR mechanisms, particularly those mediated by the qnr genes, played a more significant role in the establishment of FQ resistance than chromosomal alterations did in *E. coli* isolates where resistance was primarily mediated by mutant gyrA or parC. In fact, 50% of target and 85% PMQR genes, of the FQR *K. pneumonia*e isolates were altered. Despite the possibility that PMQR genes alone do not result in clinical resistance, they can accumulate for resistance. The likelihood that these plasmids may spread to isolates that are FQ-susceptible is the main worry.

5. Conclusions

Enterobacteriaceae that produce fluoroquinolone resistance have spread throughout the world and have become multidrug resistant (MDR), particularly to SXT, aminoglycosides, and fluoroquinolones. The coexistence of ESBLs and PMQR genes is a significant problem because of MDR establishment. These MDR isolate infections are linked to significant public health expenses, therapeutic failures, limitations on the antibacterial medicines that can be utilized, lengthened hospital stays, rising morbidity, and rising death. The most prevalent PMQR is qnrB, and Enterobacteriaceae isolates have a high incidence of PMQR genes. Therefore, for proper empirical medication and infection management, the identification of PMQR determinants among members of the Enterobacteriaceae is crucial.

Conflict of interest

No potential conflict of interest was reported by the authors.

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