ORIGINAL ARTICLE

Prevalence of Quinolone Resistance Genes among Some Clinical Gram-negative Isolates

[1]Nourhan H. El-Subbagh[*], [2]Ramadan Hassan, [2]Abeer M. Abd El-Aziz

[1]Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura; Microbiology & Immunology Department, Faculty of Pharmacy, Horus University, New Damietta, Egypt

[2]Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

Key words:
P. aeruginosa, E. coli, K. pneumoniae, *Plasmid Mediated Quinolone Resistance Genes, Gram Negative Bacteria, Antimicrobial Resistance*

Corresponding Author:
Nourhan H. El-Subbagh
Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura; Microbiology & Immunology Department, Faculty of Pharmacy, Horus University, New Damietta, Egypt
nelsubbagh@horus.edu.eg
dr.light93@hotmail.com
ORCID ID: Nourhan H. El-Subbagh : 0009-0001-1781-8947

ABSTRACT

Background: *Gram-negative bacilli are pivotal in both nosocomial and community-acquired illnesses, making them an essential category of bacteria. Quinolones are the antibiotics of choice for the treatment of both illnesses. Resistance to quinolones has been often documented during the last three decades due to their extensive therapeutic use. The resistance mechanisms were thought to be solely chromosome-encoded, including alterations of molecular targets (DNA gyrase and topoisomerase IV), reduced outer-membrane permeability, and upregulation of naturally existing efflux systems. The establishment of plasmid-mediated quinolone resistance (PMQR) has been documented since 1998.* ***Objective:*** *The aim of this work is to detect antimicrobial susceptibility as well as molecular studies on Gram-Negative clinical isolates.* ***Methodology:*** *A total of 150 clinical isolates were subjected to antimicrobial susceptibility testing using the Kirby Bauer disc diffusion method and the Polymerase chain reaction (PCR) to detect the production of plasmid-mediated quinolone resistance genes (PMQR).* ***Results:*** *Out of the 150 clinical isolates, 102 (68%) strains were resistant to fluoroquinolones; where E. coli-resistant strains were 27 (18%), K. pneumoniae were 30 (20%), and P. aeruginosa were 45 (30%).* ***Conclusion:*** *The high prevalence of PMQR-producing bacteria among various infections underscores the necessity of monitoring them. Additionally, it is imperative to raise the awareness of physicians and healthcare providers in order to mitigate the transmission of these resistant isolates.*

INTRODUCTION

Antimicrobial resistance is a severe worldwide health emergency, since some bacterial strains have developed resistance to almost all antibiotics. In 2017, the World Health Organization (WHO) compiled a list of antibiotic-resistant priority infections. These pathogens were classified into three categories founded upon the urgency for the progression of new antibiotics: critical, high, and medium priority. The overwhelming majority of such organisms are Gram-negative bacteria, known for their high resistance levels and significant impact on global health. Among the WHO's critical priority category are *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*[1].

The opportunistic bacteria *Klebsiella pneumoniae* may cause serious infections in hospital settings, including UTIs, pneumonia, and septicemia[2]. When it comes to nosocomial Gram-negative bacteremia, *Klebsiella pneumoniae* is second only to *Escherichia coli* in terms of frequency[3].

Escherichia coli is often seen in the human gut and can be transmitted to the environment through water or feces, leading to infections inside or outside the body[4,5]. *E. coli* accounts for 50% of hospital-acquired UTIs and 90% of community acquired UTIs[6].

P. aeruginosa is an opportunist bacterium that can cause catastrophic infections in individuals[7]. Its pathophysiology involves host pathway disruption and adhesion. *P. aeruginosa* can create a biofilm to shield itself from the host's immune system and antibiotics[8].

Quinolone and fluoroquinolone compounds are synthetic antibiotics that inhibit type II topoisomerases in a concentration-dependent manner[9]. The most frequent sites of mutation that result in fluoroquinolone resistance are the genetic regions of DNA gyrase (gyrA & gyrB) and topoisomerase IV (parC & parE) that determine quinolone resistance (QRDRs)[10].

Originally known as QnrA1, the quinolone-resistance protein (QNR) confers plasmid-mediated quinolone resistance (PMQR). Seven different Qnr proteins—QnrA, QnrB, QnrC, QnrD, QnrE, QnrVC, and QnrS—have been identified so far, and they all display a wide variety of genetic variations[11]. An AAC (6′)-Ib-cr mutant aminoglycoside-modifying enzyme is involved in the second mechanism of PMQR. An acetyl group may be added to some quinolones by this enzyme, allowing it to alter compounds like ciprofloxacin (CIP) and norfloxacin[12]. The third technique used in PMQR is the

use of active efflux pumps, namely QepA and oqxAB[13]. In contrast, OqxAB (transmissible resistance-nodulation-division) Eflux pump for several drugs) has only just been identified; it decreases vulnerability on nalidixic acid and CIP[14].

This research aimed to determine the incidence of PMQR genes in clinically isolated strains of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. Conducting research on the vulnerability of Gram-negative bacteria to quinolones at university hospitals is essential for providing guidance on treatment procedures and maintaining the safety of patients.

METHODOLOGY

Bacterial isolation and identification:

Patients with infections of the respiratory system, urinary tract, wounds, eyes, and burns were among the 400 clinical specimens first gathered from several institutions affiliated with Mansoura University. All procedures were approved by the Research Ethics Committee of the Faculty of Pharmacy, Mansoura University, Egypt. (codes: 2017-69 and 2023-206). The samples were collected, handled, and cultivated using sterile tubes to prevent contamination during transportation, then isolated and purified on selective and non-selective mediums.

The clinical isolates were determined to be *K. pneumoniae*, *E. coli* and *P. aeruginosa*, using microscopic examination and biochemical tests[15]. In aerobic conditions, the samples were streaked onto nutritional agar (Oxoid, UK) and then place in an incubator set at 37 degrees Celsius for 24 hours. From initial cultivation in Luria-Bertani (LB) medium at 37°C until preservation in 25% (v/v) glycerol at -80◦C for further analysis, all isolates were carefully monitored.

While the colonies that gave mucoid like appearance were expected to be *K. pneumoniae* which for more conformation were sub-cultured on Simmon's citrate agar (Oxoid, UK) that gave blue colonies. In addition to subculturing *E. coli* on MacConkey, which had pink little colonies, Oxoid, UK, provided the eosin methylene blue, which had a distinctive green metallic sheen. The isolates were subsequently subjected to triple sugar iron (TSI) tests and IMViC (indole, methyl red, Voges-Proskauer, and citrate) assays to provide additional confirmation.

Antimicrobial susceptibility testing:

To assess susceptibility to various antibiotics, the Kirby-Bauer disc diffusion technique, as outlined in the Clinical and Laboratory Standards Institute (CLSI) manual, was used[16]. This study utilized antibiotic disks from Bioanalyse®, Turkey, which included the following: Piperacillin (100 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefepime (30 µg), Gentamicin (10µg), Tobramycin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), and Gatifloxacin (5 µg).

The sizes of the inhibition zones were measured and the data were interpreted according to the CLSI breakpoints[17]. When bacteria show resistance in order to get access to agents from three or more categories of antimicrobials, it is considered multidrug resistance (MDR).

Determination of minimum inhibitory concentration of the tested isolates using resazurin-based turbidimetric assay:

Our goal was to find the MICs of ciprofloxacin, levofloxacin, and gatifloxacin against every one of the tested isolates. To do this, we employed the broth microdilution method following the CLSI 2020 standards[16].

After overnight incubation, resazurin was applied to detect color change[18]. By scoring the initial color change from blue to pink, the MIC was calculated.

Molecular detection of plasmid mediated quinolone resistance (PMQR) genes:

The extraction of plasmids was conducted using QIAprep Spin Miniprep Kits (Cat. No. 27104, Qiagen, Inc. USA) from all isolates. The extraction procedure was conducted in accordance with the instructions provided by the manufacturer.

The used primers are listed in Table 1 to conduct PCR tests to determine if any of the isolates of *K. pneumoniae*, *E. coli*, and *P. aeruginosa* contained PMQR genes. The PCR amplification was performed in a 25 µl total volume using the following reaction mixture: 12.5 µl of DreamTaq™ Green PCR master mix [2x] (Thermo scientific, US), 1 µl of plasmid DNA extract, 1 µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), and 9.5 µl of nuclease-free water. An additional negative control did not include a DNA template, was also included. PCR was performed using the Mastercycler EP Gradient S thermocycler (Eppendorf, Mississauga, ON, Canada).

Table 1: Primers sequences used for screening PMQR genes

Primer	Sequence (5′-3′)	Target Gene	Product size (bp)	Reference
QnrA-F	GCCCGCTTCTACAATCAAGT	*QnrA*	347	[19]
QnrA-R	GGCAGCACTATTACTCCCAAG			
QnrB-F	TATGGCTCTGGCACTCGTT	*QnrB*	193	
QnrB-R	GCATCTTTCAGCATCGCAC			
QnrS-F	TCGGCACCACAACTTTTCAC	*QnrS*	255	
QnrS-R	TCACACGCACGGAACTCTAT			
Aac(6′)- Ib-F	CTTGCGATGCTCTATGAGTGG	*aac(6′)-Ib*	480	
Aac(6′)-Ib-R	GAATGCCTGGCGTGTTTGAA			
OqxA-F	CTCTCCTTTCTGCTCGTCGG	*OqxA*	207	[20]
OqxA-R	AATAGGGGCGGTCACTTTGG			
OqxB-F	TAGTGCTGGTGGTGCTGGTA	*OqxB*	512	
OqxB-R	GGGTAGGGAGGTCTTTCTTCG			
QepA-F	TCTACGGGCTCAAGCAGTTG	*QepA*	312	[19]
QepA-R	ACAGCGAACCGATGACGAAG			

F: forward, R: reverse, bp: base pair

Statistical analysis:

Software developed by SPSS and published in Chicago, Illinois, USA, version 25, PASW Statistics for Windows, was used to analyze the data. The Shapiro-Wilk test checked the normality distribution. the qualitative data was presented as percentages and numbers using Monte Carlo and Cochrane tests. For non-normal quantitative data, we determined the median, minimum, and maximum values using the Kruskal-Walli's test and the Mann-Whitney U test. Significance considered at P (≤0.05).

RESULTS

Isolation and identification of clinical isolates and demographic characteristics of patients:

Within this investigation, fifty isolates were determined to be *K. pneumoniae*, 50 as *E. coli* and 50 as *P. aeruginosa* based on microscopic and biochemical identification methods. The prevalence of different Gram-negative bacteria based on clinical sources is shown in (Table 2).

Table 2: Clinical sources of the isolates

Clinical source	No. of specimens (%)	Clinical isolates			Test of significance
		E. coli n=50 (%)	*K. pneumoniae* n=50 (%)	*P. aeruginosa* n=50(%)	
Urine	85(56.7)	26(52.0)	37(74)	22(44.0)	Mc=9.83 P=0.007*
Sputum	32(21.3)	24(48.0)	0	8(16.0)	Mc=35.59 P<0.001*
Burn swab	18(12.0)	0	3(6.0)	15(30.0)	Mc=23.86 P<0.001*
Wound swab	12(8.0)	0	10(20.0)	2(4.0)	Mc=15.22 P=0.004*
Corneal swab	3(2.0)	0	0	3(6.0)	Mc=6.12 P=0.04*

MC: Monte Carlo test, *statistically significant

Antimicrobial susceptibility testing of bacterial strains:

Fig. 1 shows that elevated resistance to CTX (74%), PRL (68%), CRO (66%), and CAZ (66%), were seen in a considerable percentage of *K. pneumoniae* isolates, while the lowest resistance frequency was observed towards AK (28%). *E. coli* isolates exhibited significant resistance to CTX (64%), PRL (60%), CRO (58%), and FEP (52%), while the lowest frequency of resistance was also observed towards AK (24%). *P. aeruginosa* isolates demonstrated a high frequency of resistance to almost all antibiotics, especially CTX (100%), CRO (98%), PRL (96%), and CAZ (94%).

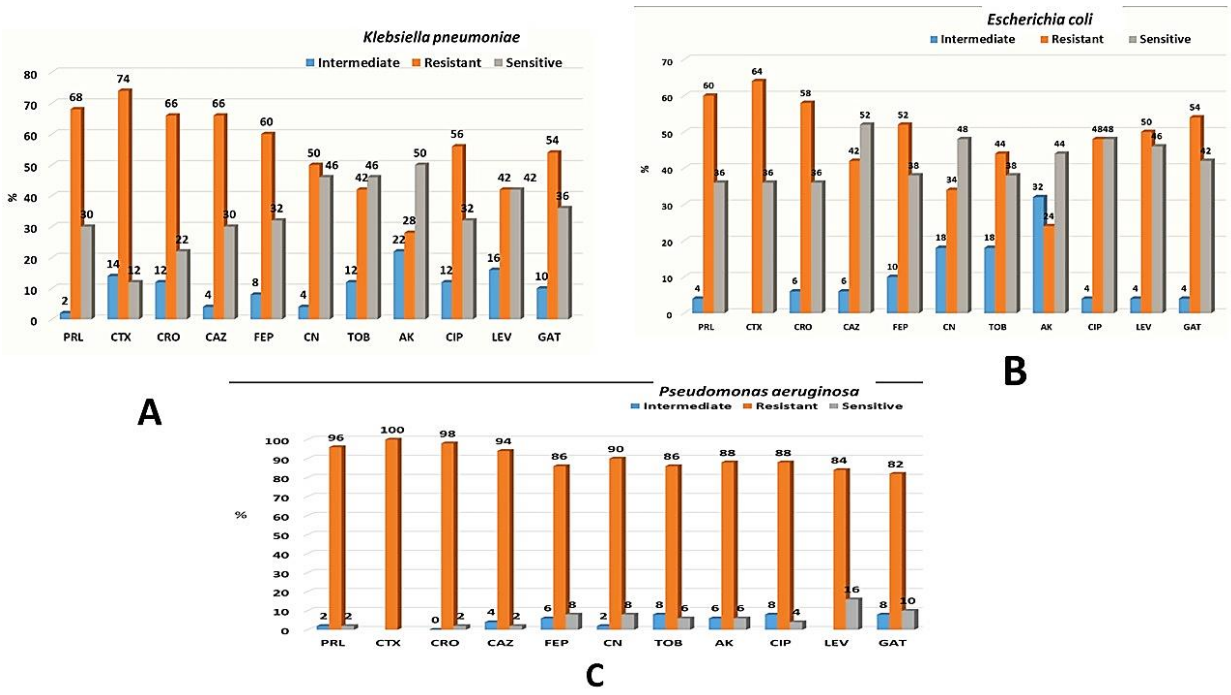

Fig. 1: Susceptibility patterns of A) K. pneumoniae, B) *E. coli* and C) *P. aeruginosa* clinical isolates to different antibiotics. PRL: piperacillin, CTX = cefotaxime, CRO= ceftriaxone, CAZ = ceftazidime, FEP: cefepime, CN: gentamicin, TOB: tobramycin, AK: amikacin, CIP: ciprofloxacin, LEV: levofloxacin, GAT: gatifloxacin.

Susceptibility to ciprofloxacin, levofloxacin, and gatifloxacin by broth microdilution method:

The MIC values for all three fluoroquinolone antibiotics are highest in *P. aeruginosa*. CIP and LEV showed similar antimicrobial activity against *K. pneumoniae* and *E. coli*. GAT may have slightly better efficacy against *E. coli* compared to the other two antibiotics (Figure 2).

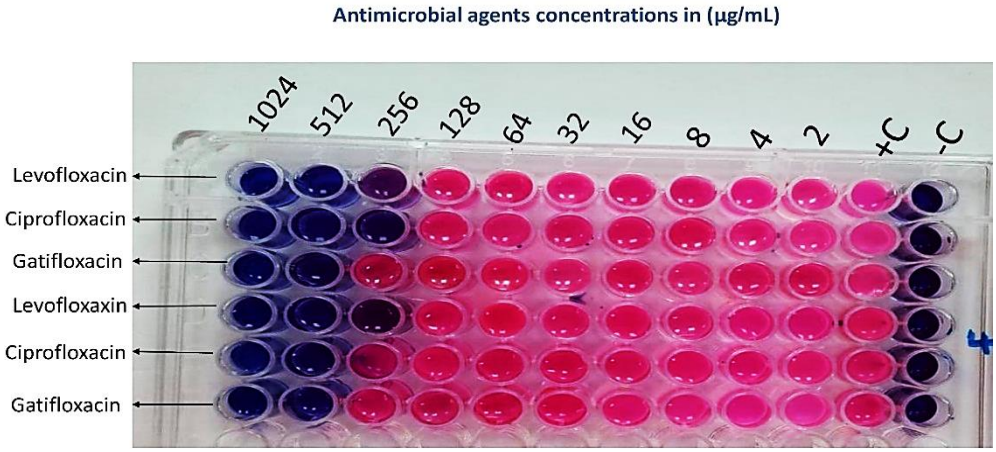

Fig. (2): MIC of levofloxacin, ciprofloxacin and gatifloxacin using resazurin in a microtiter plate for *P. aeruginosa* isolate P21 (the first three rows) and isolate P23 (last three rows along), with positive (+C) and negative (-C) controls.

There are statistically significant variations in the median MIC values for CIP, LEV, and GAT among all three bacterial categories (P<0.001*). The bacteria with the lowest median MIC are *K. pneumoniae*, followed by *P. aeruginosa* and *E. coli* (Table 3).

Table 3: Comparison of MIC between studied groups

	Ciprofloxacin (CIP)	**Levofloxacin (LEV)**	**Gatifloxacin (GAT)**	**Test of significance**
	MIC (µg/ml) Median (min-max)			
K. pneumoniae	32(2-12)	8(2-128)	16(2-256)	KW=51.99 P<0.001*
E. coli	64(2-512)	16(2-256)	16(2-256)	KW=38.71 P<0.001*
P. aeruginosa	256(2-1024)	256(2-512)	384(2-512)	KW=62.98 P<0.001*
	KW=43.98 P=<0.001*	KW=11.48 P=0.003*	KW=23.37 P<0.001*	

KW: Kruskal Wallis test, *statistically significant

The results of antibiotic susceptibility test by the use of disc diffusion and broth dilution method shown in table 4

Table 4: Comparison of disc diffusion method and broth microdilution method between studied groups:

Isolate	**Method**	**Antibiotic**		
K. pneumoniae	Broth dilution	CIP	LEV	GAT
		60%	48%	58%
	Disc diffusion	56%	42%	54%
E. coli	Broth dilution	CIP	LEV	GAT
		50%	46%	48%
	Disc diffusion	48%	50%	54%
P. aeruginosa	Broth dilution	CIP	LEV	GAT
		86%	78%	82%
	Disc diffusion	88%	84%	82%

Molecular detection of quinolone resistance genes using PCR:

The Oxq A gene was the most prevalent gene in *K. pneumoniae*, as evidenced by the PCR amplification of seven quinolone resistance genes. This gene was amplified in 11 isolates (55%). The Qnr S and Qnr A genes were the next most frequent, being amplified in 8 isolates (40%) and 7 isolates (35%) respectively. The Aac(6')-Ib gene was detected in just three isolates (15%), which is the lowest detection rate.

The Qnr S gene was identified in 19 isolates (63.3% of the total) during the PCR investigation, making it the most frequently discovered gene among the *E. coli* strains. Fifteen isolates (50%) tested positive for the QepA gene, while 14 isolates (46.7%) tested positive for the Oxq B gene. Conversely, the Qnr A gene had the lowest detection rate, being present in just 10 isolates (33%).

Among the 50 *P. aeruginosa* isolates, 116 (50%) were positive for the quinolone genes using PCR screening. The molecular test findings revealed that the Oxq A and QepA genes were the most prevalent, accounting for 48% (n = 24) of the samples. This was followed by the Oxq B and Qnr S genes, which were detected in 36% (n = 18) of the samples. The Qnr A gene was present in 32% (n = 16) of the samples.

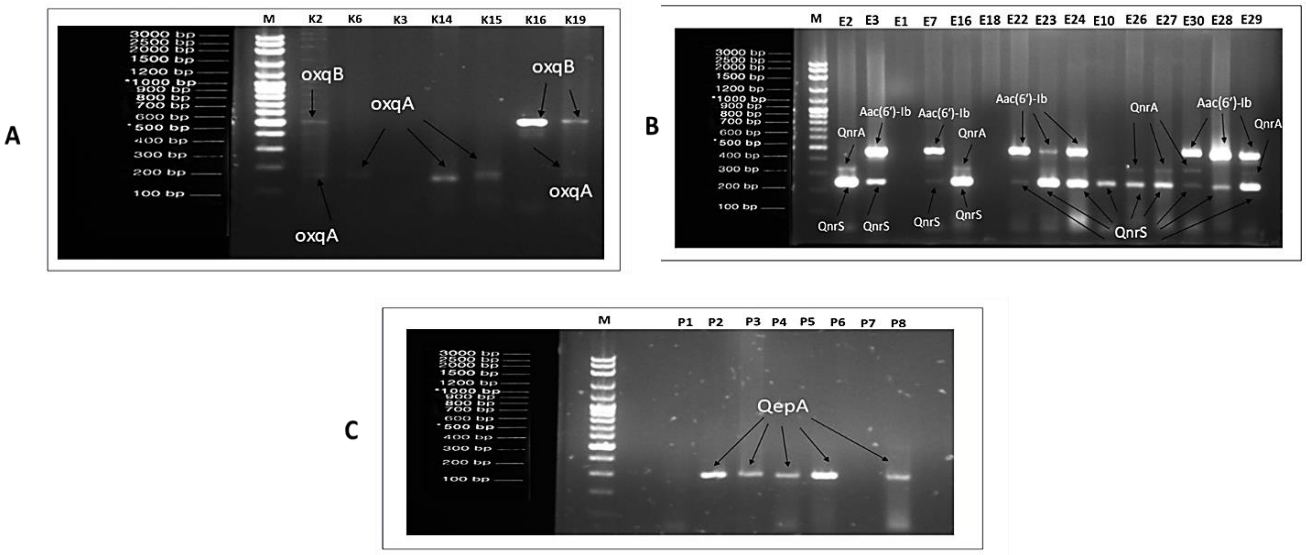

Fig. 3: PCR assay for PMQR genes. Lane M was 3000bp DNA marker. **A.** Agarose gel electrophoresis of *OxqA* (207 bp) and *OxqB* (512 bp) among K. pneumoniae isolates **B.** agarose gel electrophoresis of *QnrA* gene (347 bp), *QnrB* (193 bp), *QnrS* (255 bp), *aac(6`)-Ib-cr* (480 bp) among *E. coli* isolates **C.** agarose gel electrophoresis of *QepA* (312 bp) among *P. aeruginosa* isolates

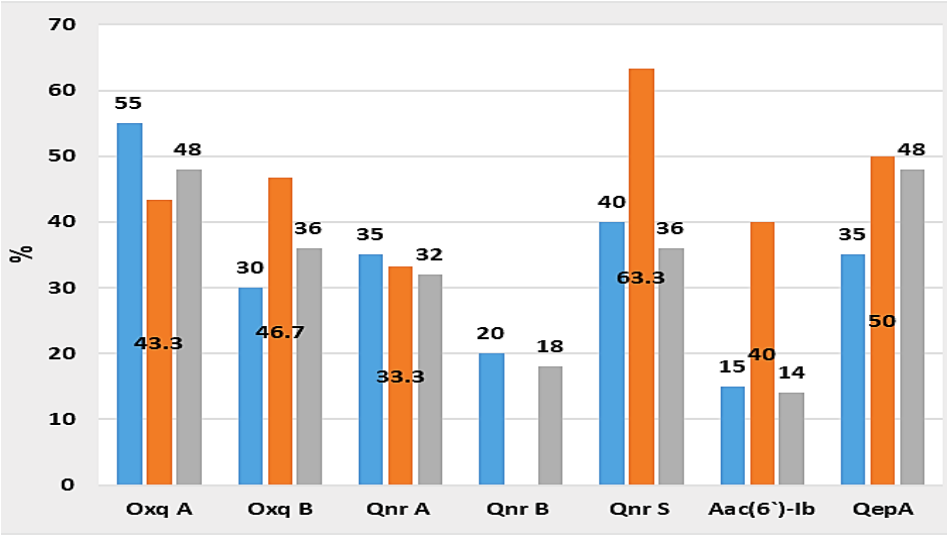

Fig.4: PCR results for harboring of the PMQR genes among the three microorganisms *K. pneumoniae*, *E. coli*, and *P. aeruginosa* respectively, presented by percentage

DISCUSSION

Bacteria are developing resistance to numerous antibiotics, which has resulted in a global health crisis known as antimicrobial resistance. The WHO has identified critical priority pathogens, including *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, as urgent for new antibiotics. These bacteria are known for their high resistance levels and impact on global health. Gram-negative bacteria are common bacterial infections in our society either nosocomial or community acquired[21].

The current investigation produced a total of 150 Gram-negative isolates from a cohort of 400 individuals. The isolation rates for inpatients and outpatients were 78% (117 cases) and 22% (33 cases), respectively. *E. coli* was the most prevalent infection among inpatients, followed by *K. pneumoniae* and finally *P. aeruginosa*. These results were replicated in research had done in Saudi Arabia, which demonstrated that *E. coli* infections were the most prevalent illnesses in the city of Najran[22]. A higher prevalence rate was discovered in another study in Asia-Pacific region[23]. Despite of these similar findings, a study done in Egypt disagrees with them where they reported that *Citrobacter* spp. isolation rate is the highest[24].

The antimicrobial resistance rates of *E. coli* were the highest in our study were found to be against CTX with a percentage of (64%), followed by PRL (60%) then CRO (58%). However, the antimicrobial susceptibility rate against CAZ was the greatest at 52%. While in *K. pneumoniae* rates of resistance were CTX (74%), PRL (68%) and CRO & CAZ come in equality (66%). The highest susceptibility rate was against AK (50%). At last, *P. aeruginosa* has the highest resistance rate against CTX with percentage of (100%), followed by PRL (96%) then CRO (98%). Additionally, it was limited to sensitive to CTX with a rate of (66%). Our results showed that resistance to CTX is common between the 3 microorganisms. This is attributable to the antibiotic policies followed in Egypt which resulted in the dissemination of CTX resistance. These findings were supported in many studies[25].

The substantial resistance levels, especially in *P. aeruginosa*, highlight the challenges presented by antibiotic resistance.

The development of quinolone resistance in Gram-negative bacteria was the primary emphasis of our research. Therefore, for more accuracy we conducted MIC for three different generations of quinolones, one drug of each generation. The drugs were CIP, LEV and GAT.

The resistance rates of the three drugs against the three used microorganisms were much different. The fifty *K. pneumoniae* isolates showed resistance to both CIP and LEV with percentage of (60%) and (48%) and to GAT by (58%). Furthermore, *E. coli* isolates showed resistance to both CIP and LEV by a percentage of (50%) and (46%) respectively while (48%) of the isolates were resistant to GAT. *P. aeruginosa* isolates gave the utmost resistance rates with CIP (86%), LEV (78%) and finally GAT (82%). The results were within the normal range of variation and quite acceptable as in a research done in India to assess colistin susceptibility by using different methods[26].

From the previous results it is obvious that *P. aeruginosa* isolates constitute the major resistance among all isolates but, it remains susceptible to levofloxacin more than the other two quinolones.

The outcomes of a study done in London, UK provide support for the assertion that LEV exhibits around tenfold more bactericidal activity compared to both CIP and ofloxacin, as shown by bactericidal evaluation tests[27]. Another comprehensive American study also reports that CIP remains the most active against *P. aeruginosa*[28].

As per the results obtained from our experiment, we can see that *E. coli* is highly susceptible to levofloxacin (LEV) than any other quinolone for usage in the same experiment. A study conducted in India was to assess the in-vitro activity of GAT against gram-negative bacteria. Additionally, the research revealed that GAT had superior efficacy compared to the other two fluoroquinolones and non-quinolone antibiotics when targeting *Enterobacteriaceae*. But in a research conducted by Sah and Feglo[29], it reported that 29.5% of isolates exhibit resistance to CIP. Among the other quinolones, LEV (24.4%) had the greatest resistance rate (50%).

The efficacy of GAT and CIP against *P. aeruginosa* was determined to be comparable, as our data revealed a minimal difference between the two treatments.

In the current investigation, it was shown that all the Q resistance isolates showed the existence of the plasmid in all *P. aeruginosa* isolates, whereas only 30 out of the *E. coli* isolates and 20 out of the *K. pneumoniae* isolates carried the plasmid. Among the isolates, the QnrS gene stood out as the most prevalent Q resistance gene. Of the *P. aeruginosa, E. coli, and K. pneumoniae* isolates tested positive, it was found in 18(36%), 19(63%), and 8 (40%) cases, respectively.

Our findings were in accordance to the study held in Cairo by Tohamy et al where they also found that the most prominent PMQR gene was QnrS[30]. Another Egyptian study done by Khalifa et al.[31] also supported our findings that QnrS was found in most of the isolates tested for PMQR genes.

However, Kotb et al.[24], mentioned that QnrB was the most common PMQR genes which is different from our findings. Different results could be ascribed to variations in the geographic distribution of Qnr genes, the specific types of clinical isolates, sources of collection, in addition to discrepancies in the methods of detection. OxqA and OxqB genes are two plasmid-mediated genes mostly referred as OxqAB genes which are plasmid-encoded efflux pump genes. Moreover, the existing data about the incidence and epidemiology of OqxAB are significantly restricted compared to the comprehensive information available for other PMQR factors[32].

In our study, about 50% of the plasmid harbored isolates had the gene of oxqA and 39% harbored the gene of oxqBIsolates developing resistance to Q may be related to the efflux pump and its cohabitation partner, OqxAB. The results of the research conducted at the Hamadan hospital in Iran, however, contradict this finding, since they imply that the existence of efflux pump genes is unrelated to the development of Q resistance[20].

CONCLUSION

Patients infected with Gram-negative bacteria at Mansoura hospitals in Egypt are becoming resistant to quinolones, according to the results of the present study.

Coexisting with other genes, including efflux pump genes, gives bacteria resistance to many different types of antimicrobials. Thus, to avoid the vanishing of an important family of antimicrobial medications, such as

quinolones, it is critical to prevent the future emergence of its resistance.

Declaration

Acknowledgements
All appreciation to Mansoura University Hospitals.

Ethics approval and consent to participate
The collection of all specimens followed a procedure that was authorized by the Research Ethics Committee of the Faculty of Pharmacy, Mansoura University, under the ethical code 2023-206.

Disclosure statement
No authors have declared a conflict of interest.

Authors' contributions
The practical work, data analysis, initial paper writing, and final format revisions were all done by N.H. The original draft was corrected by AM. A, who also oversaw the practical work, fixed problems, and improved the data analysis. RH provided feedback on the final article and proposed the study topic.

Availability of data and materials
The paper and additional information files include all the data that were created or analyzed during this investigation are available upon request from the corresponding author.

Funding
No funds.

REFERENCES

1. Breijyeh Z, Jubeh B, Karaman R. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 2020;25(6):1340.
2. Abbas R, Chakkour M, Zein El Dine H, Obaseki EF, et al. General Overview of Klebsiella pneumonia: Epidemiology and the Role of Siderophores in Its Pathogenicity. Biology. 2024;13(2):78.
3. Li D, Huang X, Rao H, Yu H, et al. Klebsiella pneumoniae bacteremia mortality: a systematic review and meta-analysis. Frontiers in Cellular and Infection Microbiology. 2023;13:1157010.
4. Puvača N, de Llanos Frutos R. Antimicrobial Resistance in Escherichia coli Strains Isolated from Humans and Pet Animals. Antibiotics. 2021;10(1):69.
5. Lorenz B, Ali N, Bocklitz T, Rösch P, Popp J. Discrimination between pathogenic and non-pathogenic E. coli strains by means of Raman microspectroscopy. Analytical and Bioanalytical Chemistry. 2020;412:8241-7.
6. Radera S, Srivastava S, Agarwal J. Virulence genotyping and multidrug resistance pattern of Escherichia coli isolated from community-acquired and hospital-acquired urinary tract infections. Cureus. 2022; (9)14.
7. Diggle SP, Whiteley M. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. Microbiology. 2020;166(1):30-3.
8. Tuon FF, Dantas LR, Suss PH, Tasca Ribeiro VS. Pathogenesis of the Pseudomonas aeruginosa biofilm: a review. Pathogens. 2022;11(3):300.
9. Assar S, Nosratabadi R, Khorramdel Azad H, Masoumi J, Mohamadi M, Hassanshahi G. A review of immunomodulatory effects of fluoroquinolones. Immunological Investigations. 2021;50(8):1007-26.
10. Kim JW, Blay V, Mira P, Barlow M, Camps M. Genetic interplay between type II topoisomerase enzymes and chromosomal ccdAB toxin-antitoxin in E. coli. bioRxiv.2021; 2021.09. 24.461737.
11. Ayobola ED ,Oscar WO, Ejovwokoghene EF. Occurrence of plasmid mediated fluoroquinolone resistance genes amongst enteric bacteria isolated from human and animal sources in Delta State, Nigeria. AIMS microbiology. 2021;7(1):75.
12. Bush NG, Diez-Santos I, Abbott LR, Maxwell A. Quinolones: Mechanism, Lethality and Their Contributions to Antibiotic Resistance. Molecules. 2020;25(23):5662.
13. Dehnamaki M, Ghane M, Babaeekhou L. Detection of OqxAB and QepA efflux pumps and their association with antibiotic resistance in Klebsiella pneumoniae isolated from urinary tract infection. International Journal of Infection. 2020;(4):7.
14. Amereh F, Arabestani MR, Shokoohizadeh L. Relationship of OqxAB efflux pump to antibiotic resistance, mainly fluoroquinolones in Klebsiella pneumoniae, isolated from hospitalized patients. Iranian Journal of Basic Medical Sciences. 2023;26(1):93.
15. Ejikeugwu C, Nworie O, Saki M, Al-Dahmoshi HO, et al. Metallo-β-lactamase and AmpC genes in Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa isolates from abattoir and poultry origin in Nigeria. BMC microbiology. 2021;21:1-9.
16. CLSI. Weinstein, M. P. and J. S. Lewis (2020). "The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. Journal of clinical microbiology. 2020;58(3): 10.1128/jcm. 01864-01819.
17. Clinical, Institute LS. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute Wayne, PA; 2020.
18. Garong CJC, Burigsay NPF, Gapultos RS, Pedrosa RMV, Mandawe JLB., & Dayrit GB. Optimization

of resazurin microplate assay (REMA) in evaluating anti-MRSA and anti-MSSA activities. Acta Medica Philippina, (Early Access 2024);58:1-7.

19. Hamed SM, Elkhatib WF ,El-Mahallawy HA, Helmy MM, Ashour MS, Aboshanab KMJSr. Multiple mechanisms contributing to ciprofloxacin resistance among Gram negative bacteria causing infections to cancer patients. Scientific reports. 2018;8(1):12268.
20. Amereh, F., Arabestani, M. R., Hosseini, S. M. & Shokoohizadeh, L. Association of qnr Genes and OqxAB Efflux Pump in Fluoroquinolone-Resistant Klebsiella pneumoniae Strains. International Journal of Microbiology. 2023,(1), 919910.
21. Mir, R. A. & Kudva, I. T. Antibiotic-resistant Shiga toxin-producing Escherichia coli: An overview of prevalence and intervention strategies. Zoonoses and Public Health. 2019, 66, 1-13
22. Masoud, E., Mahdy, M. & Esmat, A. Bacterial prevalence and resistance to antimicrobial agents in southwest, Saudi Arabia. Egyptian Academic Journal of Biological Sciences. 2011, 3, 105-111.
23. Lu P-L, Liu Y-C, Toh H-S, Lee Y-L, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009-2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). International journal of antimicrobial agents. 2012, 40, S37-S43.
24. Kotb DN, Mahdy WK, Mahmoud MS, Khairy RM. Impact of co-existence of PMQR genes and QRDR mutations on fluoroquinolones resistance in Enterobacteriaceae strains isolated from community and hospital acquired UTIs. BMC infectious diseases. 2019;19:1-8.
25. El-Kholy AA, Girgis SA, Shetta MA, Abdel-Hamid DH, Elmanakhly AR. Molecular characterization of multidrug-resistant Gram-negative pathogens in three tertiary hospitals in Cairo, Egypt. European Journal of Clinical Microbiology & Infectious Diseases. 2020, 987-992.
26. Jeram H, Mahajan RK, Chauhan AK. Comparative Assessment of Disk Diffusion, E-test and Broth Dilution Methods for Determining Colistin Susceptibility in Acinetobacter Species. National Journal of Laboratory Medicine. 2021, 10, 10-14.
27. Morissey I, Smith J. Activity of 4-quinolones against Pseudomonas aeruginosa. Arzneimittel-forschung. 1994, 44, 1157-1161.
28. Oliphant, C. M. & Green, G. M. Quinolones: a comprehensive review. American family physician. 2002, 65, 455-465.
29. Sah AK, Feglo PK. Plasmid-mediated quinolone resistance determinants in clinical bacterial pathogens isolated from the Western Region of Ghana: a cross-sectional study. Pan African Medical Journal. 2022;43.
30. Tohamy ST, Aboshanab KM, El-Mahallawy HA, El-Ansary MR, Afifi SS. Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. Infection and drug resistance. 2018; 791-803.
31. Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, et al. High prevalence of antimicrobial resistance in gram-negative bacteria isolated from clinical settings in Egypt: recalling for judicious use of conventional antimicrobials in developing nations. Microbial Drug Resistance. 2019;25(3):371-385.
32. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. Clinical microbiology reviews. 2009;22(4):664-689.