BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

Prevalence of quinolone resistance genes mediated by plasmids in ciprofloxacin-resistant strains Escherichia coli isolates in a sample of .patients with urinary tract infections

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Abstract
Genetic variations in the uniquebacterial genomic region may lead to resistance ofquinolone(bactericidal antibiotic) compounds or via by transmission with plasmids and then possessionof plasmid-mediatedquinolone resistance(PMQR) determinants, involved,*qnrA,qnrB, and.qnrS.*Current study aimed to detect the most frequencyof PMQR genes that may leading to increasing *Escherichia coli (E.coli)* resistanceto ciprofloxacin antibiotic.100 of *E.Coli*isolates were collectedand identified. Molecular detection achieved by 16SrDNA marker. All of *E. coli* isolates were subjected to PCR testing to determine the frequency of the PMQR genes (*qnrA, qnrB, and qnrS*). Concerning antibiotic resistance results revealed that 62% (n=62) isolate were non-susceptible and/or resistance of ciprofloxacin antibiotic. It was found that58% of all *E.Coli* isolates hade one or moreof PMQR genes, Also 41% (n = 41/58) were harbored single form of PMQR genes.Additionally, it was discovered that 29% (n = 12), 32% (n = 13), and 39% (n = 16) of the isolates had the qnrA, qnrB, and qnrS genes respectively..Conclusion; 58% of all isolates had one or more of PMQR genes and the*qnrS* genes were the most recurrence among PMQR genes**.**
Key words: PMQR, *E.coli*, gene,quinolone, resistance.

1. Introduction

One of most quinolone compounds that reported effective systemic activity was ciprofloxacin antibiotic[1,2,3]. Being utilized in clinical settings to treat a variety of urinary tract infections, including those brought on by E. coli[4,5]. E. coli infections of the urinary tract are rather common. Fluoroquinolones were a lot usedof UTIs treatment and improper use of these antibiotics has led to antibiotic resistance. Ciprofloxacin is most commonly fluoroquinolone antibiotic used for the treatment of UTIs[5,6].Previously recorded scientific facts demonstrated that quinolone resistance genes that plasmid-related a large number of them included *qnr* gene alleles have been found carried on plasmid or bacterial chromosome[7,8].
a large number of *qnr* alleles have been found on plasmids or bacterial chromosome. Reported that about one hundred *qnr* genes have been detected and described fromEnterobacteriaceae and classified into five distinct families (*gnrA ,gnrB*, and *gnrS*).The selection of mutant strain and/or gene approved or facilitated by the mechanism of plasmid-mediated quinolone resistance[9,10].

2. Materialand methods

One hundred (100) isolates of Escherichia coli urine were taken from people who had urinary tract infections.Detection of Isolates was achieved through biochemical test , and confirmed VITEK-2 compact system. Molecular detectiondepending on 16SrDNA as a molecular marker. By using the PCR method, the PMQR genes qnrA, qnrB, and qnrS were examined in all E. coli isolates. By using the disk diffusion technique and regular diagnostics, the susceptibility to the antibiotic ciprofloxacin was found. Used concentration of ciprofloxacin antibioticwas (5µl) performed according to Clinical Standards Institute Guideline[11]. PMQR gene detection achieved through DNA isolationof *E.Coli*isolates. Using a kit supplemented by Geneaid (UK), the isolation of bacterial genomic DNA

was carried out. For all PCR isolated DNA was used as templates[12,13]. (Bioneer, Korea) produced the forward and rivers primers[7,8] (Table 1), which were kept at -20°CThe manufacturing company (Bioneer- Korea), claims that PCR was successful in a 25µl total volume. Successful PCR amplifications were confirmed by agarose gel electrophoresis.

Table 1. Show sequences of primers that were used in present study.

Primers	**Sequence of Primers**	**Size product (bp)**	**Ref.**
16SrDNA	F: AGAGTTTGATYMTGGCTCAG R: CTACGGCTACCTTGTTACG	1500	(7)
qnrA	F: ATTTCTCACGCCAGG ATTTG R: GATCGGCAAAGGTTA GGTCA	516 (bp)	(8)
qnrB	F: GATCGTGAAAGCCAG AAAGG R: ACGATGCCTGGTAGTTGTCC-3	469 (bp)	
qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	417 (bp)	

Results

In current study all of 100 isolated clinical samplesare detected as *EschirichiaColi*. After being amplified by a polymerase chain reaction, the E. coli ITS1 regions, which are internally transcribed 16S rDNA spacers, were examined. PCR products (figure 1) were sent to gradient analysis to identify the bacterial isolates.Molecular analysis results revealed that all of hundred isolates of *E. coli*were positive and/or harboredfor 16S rDNA genes.

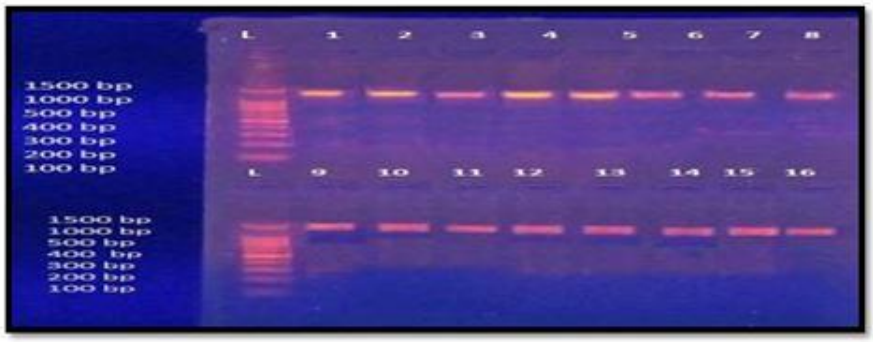

Figure 1: Show PCR product (1500 bp) of *E coli* with 2% agarose. Lane 1-16: PCR products for 16S rDNA genes, Lane L : DNA marker (1500bp).

E.coli Resistance to Ciprofloxacin Antibiotic and its Relation with *qnr* genes:

Current study demonstrated that 58% (n =58) out of 100 isolates harbored one or more of PMQR genes divided to seventeen (17) isolates harbored more than one of PMQR genes and forty one (41) isolates distributed as 29% (n =12), 32% (n =13), and 39% (n =16) were harboring single form of *qnrA, qnrB, and qnrS* respectively (Figure 2,3,4respectively).On the other hand 62% (n =61) out of 100 isolateswere nonsusceptible of ciprofloxacin antibiotic. Allof 58 isolates that had PMQR genes showed ciprofloxacin resistance arranged from high 57% (n=34/58) tolow43% (n= 24/58).There was no significant difference between the intensity of resistance and the type of PMQR(*qnrA, qnrb, qnrS*) genes.

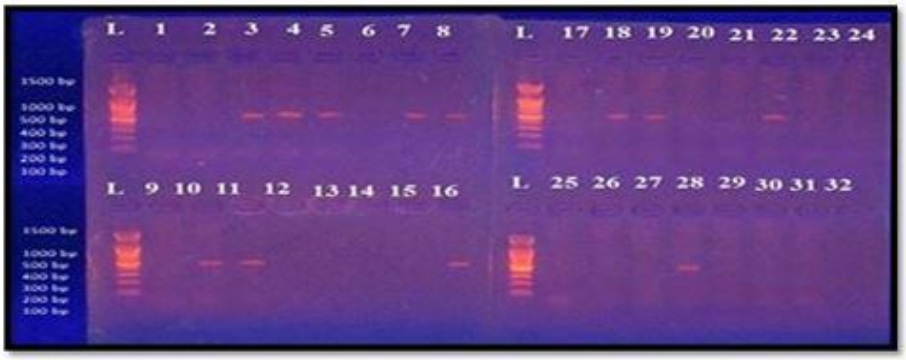

Figure 2. : Show gel electrophoresis of PCR products (1500 bp) L: 1500 bp DNA marker; *qnrA* lane: (3,4,5,7,8,10,11,16,18,19,22,28). PCR size product 516 bp.

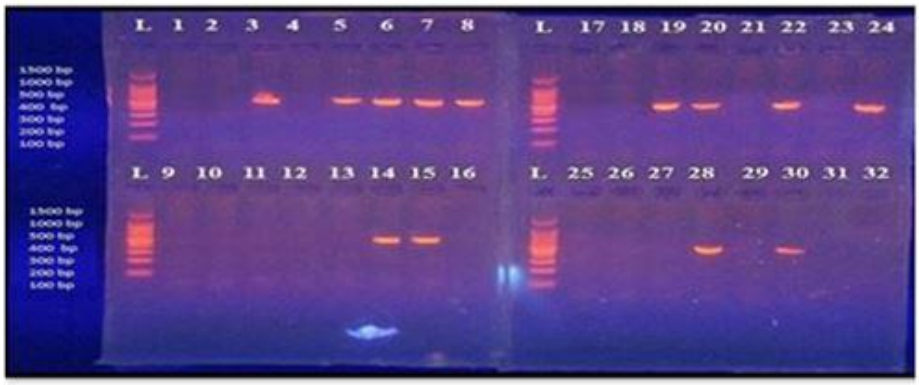

Figure 3: Show gel electrophoresis of PCR products (1500 bp) L: 15000 bp DNA marker; *qnrB* gene lane: (3,5 ,6,7,8,14 ,15,19,20,22,24,28,30). PCR size product was 469 bp.

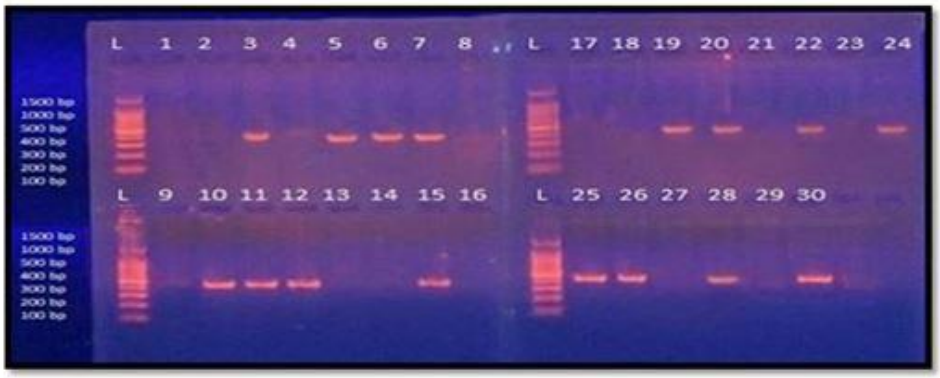

Figure 4. : Show gel electrophoresis of PCR products (1500 bp) L1: 15000 bp DNA marker, *QnrS* gene lane: (3,5,6, 7, 10 ,11, 12, 15,19, 20, 22, 24,25,26,28,30). PCR size product was 417 bp

Discussion

Escherichia coli is the most common cause of urinary tract infections, which are the most common bacterial infection[14]. On the other hand quinolones compounds are most frequently employed antibacterial agents for treating a variety of infections. caused by *Escherichia Coli*. Previous studies reported that plasmid-mediated quinolones resistance is being increasingly especially in Asia[15]. However reports about the prevalence of PMQR genes among Iraqi enterobacterial isolates were limited. So the present study contributed to highlight on the spread of PMQR genes frequencyamong clinical samples of *E.Coli*collected from urine samples of Iraqi urinary tract infection patients..

Present results in current study are consistent with previous studies and do not agree with others. Hog bin kim et al. reported in 2009 that only 10% of E. Coli isolates contained one or more qnr genes[16]. Anotherreports in 2016 among Iranian population showed that *qnrA* and *qnrB* were not found and out of 136 E.Coli isolates just 2.9% were carried *qnrS* gene only[17]. Our findings don't agree with both above studies but it is in line with other study[18]conducted in 2014, which showed that 72.8% (59/81 isolates)of *E.Coli* among Korean population had one or more of PMQR genes.

According to assumptive scientific truths that *qnr* genes encode several proteins that protect DNA gyrase from inhibition by quinolones compounds like ciprofloxacin antibiotics[19,20]and the final result is increasing of bacterial resistance. But the important scientific fact that has been observed through this study and other studies is the significant vary in the prevalence of *qnr* genes from one place to other among worldwide[21,22]. Concerning resistance of ciprofloxacin antibiotic our findings reported that 62% of all *E.Coli*isolates were nonsusceptible of ciprofloxacin antibiotic and these data were in agreement with the results recorded by Naji et al 2017 they have been reported, resistance range arranged from (50 to 70 %) with ciprofloxacin antibiotic[9].The results of the current investigation were also found to be higher than or in conflict with those discovered in Nigeria by Ekwealor et al. in 2016, who reported[10] that the rate of ciprofloxacin resistance was (27%).

Through the current and previous studies, concluded that there is a fact that the *qnr* genes increase the rates of *E. Coli* bacteria resistance, but with great variation from one race to another and from one region to another. Concerning of resistance range in present study, 58% of hundred isolates were *E.coli*it ranged from high to low resistance that correspond with other studies achieved by Hamza et al 2019 and Saenz et al 2015 they have been recorded average resistances of *E.coli* are 36% and 38% respectively of *E.Coli* isolated from urine samples[12,13]. Because that*E.coli* are widelyprevalence in patients who have undergone instrumentation or catheterization of UTIS and resulted in eighty percent (80%) of cases had *Escherichia. coli* among Gram-negative bacteria as a reason of catheter-associated urinary tract infections[23].

The scientific and logical interpretation for such changes in the rates and/or percentage of *E.Coli* resistance to antibiotics and the rates of recurrence of PMQR genes, it is not easy to suggest a single cause or factor, but it may be linked to multiple environmental and bacterial factors that played an important role in creating such variations. On the other hand the time may be one of factors thatcontributed in such variations to occur. Since it was discovered in 1998 that ciprofloxacin antibiotic resistance grew over time (P = 0.001) and the prevalence of PMQR genes has grown, generally increased(P=0.20)[24]. Other suggestion to understanding like such variation also may be because the selectionarea of *E. coli* isolates and the changing in circumstances conditions and may be the diversity of geographical areas. However, other elements such as increased intracellular concentration, overexpression of the efflux pump, and mutations (DNA gyrase enzymes) may also be equally efficient in this regard. Sothe prevalence of PMQR genes and their effecting on bacterial resistance remains great challenge and more studied are needed.

Conclusion

Present study concluded that 58% of *E.Coli* included one or more of PMQE genes and itwas found that *qnrS* gene is the more recurrence among PMQR genes in present study.

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