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Molecular Investigation of Plasmid–Mediated Quinolone Resistant Genes among aminoglycoside-resistant uropathogenic *Escherichia coli* Isolates from Babylon Hospitals, Iraq

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Abstract

Background: Uropathogenic *E coli* is the most frequently causes of UTIs in humans ,accountable 75- 95 % of UTIs. PMQR have been identified in family *Enterobacteriaceae* worldwide. The purpose of this study to examine the dissemination of *aac(6')-Ib-cr* and PMQR genes in isolates of UPEC.

Method : A cross sectional study of mid-stream urine of the participate patients their approval for direction usage their specimen. The susceptibility test by disk diffusion for UPEC were isolated from urine and screening of quinolone resistant by multiplex PCR for *qnrA*, *qnrB* and *qnrS* genes and monoplex PCR for *qunD ,qepA* and *aac(6')-Ib-cr* genes.

Results : A total of 1072 mid-stream urine were collected randomly, overall 132 were identified *E. coli* , among these 60 aminoglycoside resistant UPEC isolates were screened to the five quinolone antibiotics. The resistance proportion to nalidixic acid and ciprofloxacin were 75.0%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7%, 73.3% and 71.7%, respectively. The susceptibility test for antibiotics showed a high incidence of the resistance to the majority of antibiotics class. It was found that 73.3%, 38.3% , and 20.0% of the isolates harbored *aac(6')-Ib-cr, qnrS* and *qnrB* genes ,respectively either alone or in combination, while the *qnrA*. *qunD ,qepA* genes were not detected.

Conclusion : All isolates were identified as multiple antibiotic resistances, only one isolate can be considered as possible PDR and high prevalence of *aac(6')-Ib-cr, qnrS* and *qnrB* genes among isolates**.**

Keywords : *UPEC, aac(6')-Ib-cr qnrB and qnrS , plasmid mediated quinolone PMQR , multidrug resistance, extended drug resistant, pan drug resistance.*

Introduction

UTI is the second most common clinical in primary and secondary care suggestion for experiential antimicrobial treatment[(1)]. *E. coli* can cause both complicated and uncomplicated UTIs[(2)]. fluoroquinolones has represented an alternative therapeutic select for the treatment. But, newly the incidence of fluoroquinolones-resistance between uropathogenic isolates has also been described in different areas[(3)]. Quinolone resistance mechanisms include mutation in target gene , elevated expression of efflux pumps and yielding of modifying enzyme and target protection protein[(4)]. They involve the chromosome encoded and PMQR, three PMQR mechanisms have been identified: target protection by Qnr proteins, drug inactivation by AAC(6)- Ib-cr, and drug efflux by QepA and OqxAB[(5)]. The rapid dissemination of MDR bacteria has become a concern worldwide and complicated the treatment of infections. This phenomenon is a consequence of the ability of bacteria to acquire exogenous genes by mobile elements (e.g. conjugative plasmids, transposons and integrons) [(6)].

Increased use of ciprofloxacin and other fluoroquinolones antibiotics in Iraq may be cause selection of isolates resistant to aminoglycosides by selecting the isolates that possess AAC(6`)-Ib-cr.

The aim of this study to examine the dissemination of *aac(6')-Ib-cr* and PMQR genes in aminoglycosides resistance in UPEC isolates from Babylon hospitals .

Method

Collection of specimens

This cross section study of the participate patients their approval for direction usage their specimen was conducted in in two main hospitals in Babylon hospitals during the period of six months from March to September 2018 in patients clinically suspected to have UTI . The inclusion criteria for the volunteer patients were attended or admitted in the two hospitals .

Isolation and Identification of *E. coli* isolates

Identification of bacterial pathogens was made based on Gram reactions, culture characters, and routine standard biochemical tests. All *E coli* strains were purified on EMB agar plates to confirm that there is no mixed culture. Additionally, *E. coli* isolates were also confirmatory identified by using commercially available API 20 E kit.

Antibiotic Susceptibility Test

Antibiotic susceptibility test was performed by Kirby –Bauer method based to the guideline suggested by the Clinical and Laboratory Standards Institute[7].

Examining for Quinolone Resistance and other Antibiotics

The disk diffusion method was made to identify quinolone resistance in all aminoglycosides resistance UPEC by using Nalidixic acid (30µg),Ciprofloxacin (5µg) Levofloxacin (5µg) Lomefloxacin (10µg), Norfloxacin (10 µg).In addition , all aminoglycosides resistance UPEC were screen for other antibiotics .

Examining for PMQR Genes

Multiplex PCR were used to determine the presence *qnrA*, *qnrB* and *qnrS* genes and monoplex PCR for *qunD* ,*qepA* and *aac(6')-Ib-cr* genes in quinolone resistance UPEC by using T300 (thermo cycle) and Go Taq Green Master Mix (Promega USA). The primer target for each PMQR genes were available elsewhere[8,9,10,11] .

Results

Out of 298 isolates of uropathogen , 132 (44.3%) isolates were recognized as *E. coli* followed by *Klebsiella* spp (22.5%), *Proteus* spp (10.0%), *S. aureus* (9.4%), *S. saprophyticus* (3.4%) , *P.aeruginosa* (3.4%), *S. faecalis* (2.7%), *Enterococcus* spp (1.7%) *Acinetobacter* spp (1.3%) and *Citrobacter* spp (1.3%).

Examining for Quinolone Resistance

Overall, 78.3% (47/60) of the aminoglycoside resistant UPEC isolates were non susceptible to at least one of the five quinolone antibiotics by disk diffusion methods. The resistance proportion to nalidixic acid and ciprofloxacin were 75.0%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7%, 73.3% and 71.7%, respectively.

Co-resistance of *E. coli* to other Antibiotic

Antibiotic susceptibility test of all 60 uropathogenic *E .coli* against 28 antibiotics (belong to twelve classes) displayed high occurrence of resistance to the majority agents of antibiotics. According to the definitions proposed[12], all of the 60 aminoglycoside resistant UPEC isolates (100%) were multiple resistance to antibiotics. Based on their MDR, 49 (81.6%) of the isolates were included, 2 (3.3%), 1 (1.7%), 4 (6.7%), 5 (8.3%), 18 (30%) and 19 (31.6%) were resistant to 3, 5, 6, 7, 8 and 9 antibiotic classes, respectively. While, 10 (16.7%) isolates was considered as XDR organisms , of which 6 (10%) were resistant to 10 antibiotic classes and 4 (6.7 %) were resistant to 11 antibiotic classes. PDR could be detected in only one isolate (1.7%) in this study (Table 1).

Table (1): MDR, XDR and PDR of aminoglycosides resistant UPEC isolate (*n*=60)

Type of resistance	No. of isolates	No. of resistance to antibiotic groups(n=12)
MDR* (n=49, 81.6%)	19 18 5 4 1 2	9 8 7 6 5 3
XDR*(n=10,16.7%)	6 4	10 11
PDR* (n=1, 1.7%)	1	12

*MDR: multidrug resistance; XDR: extended drug resistance; PDR: pan drug resistance

Examining for PMQR Genes

PMQR genes were identified in 78.3% (47/60) of *E. coli*, harbor at least only one PMQR gene, and three types of PMQR (*qnrS*, *qnrB* and *aac(6')-Ib-cr* variant) were recognized alone or in combination (Table 2). The *aac(6')-Ib-cr* variant was present in 73.3% of the isolates, followed by *qnrS* (38.3%) and *qnrB* 20.0% of the isolates. Interestingly, *qnrA*, *qnrD*, and *qepA* were not found in any isolates.

Table (2): Distribution of PMQR genes and their combinations among the UPEC isolate (*n*= 60)

Type of PMQR gene	No. (%) of isolates
aac(6')-Ib -cr	17 (28.3%)
qnrS	2 (3.3%)
qnrB	1 (1.7%)
aac(6')-Ib-cr, qnrS	16 (26.7%)
aac(6')-Ib-cr, qnrB	6 (10%)
aac(6')-Ib-cr, qnrS,qnrB	5 (8.3%)
Total	47(78.3%)

Discussion

Frequency and Resistance of UPEC to Quinolone antibiotic

This study revealed that the UPEC isolates (44.3%) were the major causative agent of significant bacteriuria compared to other uropathogen due to enteric flora , the way of transmission is by fecal contamination and anatomical nearness to the genito–urinary tract in females and the poor hygiene[13].This result is agreement with other authors have the same findings, which *E. coli* was the predominant uropathogen[14,15] .

The resistance rates to nalidixic acid and ciprofloxacin were 75.0%, as well as to lomefloxacin, norfloxacin and levofloxacin were 76.7%, 73.3% and 71.7%, respectively. This may be due to rampant use of quinolones as first line empirical therapy in UTI cases. The data reported in this study is close related with the previous study described that ciprofloxacin resistance rate 79.66% to *E coli* in Saudi Arabia[16]. In the previous report of the ECDC[17] , the presence of UPEC isolates resistant to fluoroquinolones were existent in low numbers in Sweden (7.9%) and Norway (9.0%), but they were dominant in Italy (40.5%) and Slovakia (41.9%) .

Co-resistance of aminoglycosides and quinolones was observed in the UPEC isolates of the present study. The study report in Iraq the identification of a variant of the aminoglycoside modifying enzyme AAC(6`)-Ib, as a common incidence in *Enterobacteriaceae* isolates[18] .

Antibiotic Resistance

All UPEC isolates were identified as multiple antibiotic resistances. This result was considered a high level when compared to other report. Frequency of MDR 81.6%, which was relatively high prevalence when compared to rates reported, in Saudi Arabia it was 67.0%[19] , and in Iran it was 63.0%[20] .The reason of high MDR may be due to the most cases of UTIs are treated empirically in Iraq, where patients frequently cannot afford to consult a clinicians or have a laboratory tests. Therefore, there may be over cases of bacteria that are not responded to treatment.

XDR, 16.7% this finding are alarming since infections with these XDR UPEC isolates leave physicians with only one or two antibiotic treatment options, leading to increased mortality and morbidity[20] . In present study remarked PDR (1.6%) of UPEC. No study investigation PDR-producers among UPEC. However, the prevalence of PDR in *Enterobacteriaceae* is rare in Europe and other countries[21,22]. Although infections by PDR *Enterobacteriaceae*, still rare, but have been associated with a high mortality.

Occurrence of PMQR Genes

PMQR determinants have been identified in family *Enterobacteriaceae* worldwide, with varying frequency rates[23]. 78.3% of the isolates harbored at least one PMQR gene (Table 2). In Saudi Arabia, reported that 26.0% of isolates carried PMQR genes[24]. Previous investigations conducted in Europe described fewer PMQR determinants in ESBL-*E. coli* isolates than found in the current study, 19.0% in Spain, and 10.0% in Sweden and Norway[25,26] .

The AAC(6′)-Ib-cr variant, an enzyme encoded by a plasmid-borne *aac(6')-Ib-cr* gene, that inactivate selected fluoroquinolones (ciprofloxacin and norfloxacin) by acetylation in addition to aminoglycosides[27]. *aac(6')-Ib* PCR positive products were further digested with *Bst*CI to identify *aac(6')-Ib-cr* variants. Present study demonstrated that the most prevalent PMQR gene was *aac(6')-Ib-cr* as 73.3% . This is also identified in other studies (84.0%)[27], (94.0%)[28] of the *aac(6')-Ib* were of the *aac(6')-Ib-cr* variant. Presence of this variant also increased the incidence of selection of chromosomal mutants (quinolone resistance determine region, QRDR) upon exposure to ciprofloxacin[29]. However, Spread of this variant is undesirable because these fluoroquinolones are used as first choice for treatment of UTIs in Iraq.

qnrS was the most prevalent 38.3%, followed by *qnrB* (20.0%). Present findings concurred with a previous European survey that *qnrS* was more frequently detected than other *qnr* genes in clinical *Enterobacteriaceae* isolates[30]. In the USA, among ceftazidime-resistant *Enterobacteriaceae* isolates, 23% were positive for either *qnrA* or *qnrB,* while *qnrS* was absent[31]. In China among ciprofloxacin resistant *E. coli* is 7.5%. *qnrA, qnrB* and *qnrS* were detected in 3.8%, 4.7% and 3.8% of these isolates, respectively[32]. However, variations in distribution of *qnr* genes have been suggested to be attributing to differences in selection criteria or geographic area[33]. None of the *qnrA*, *qnrD* and quinolone specific efflux pumps (*qepA*) were found in present collection.This is not surprising since *qnrD* and *qepA* determinants are rarely found worldwide.

This study was revealed high combination of *aac(6')-Ib -cr* ,*qnrS*, followed by *aac(6')-Ib-cr, qnrB* and *aac(6')-Ib-cr, qnrS*, *qnrB* isolates as shown in Table (2). This could be explained by the coexistence of the *aac(6')-Ib-cr* and *qnr* genes on the same plasmid[34]. The combination is also observed where 35.1% and 1.3% of the *E. coli* isolates carried two and three different PMQR genes, respectively.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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