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Association of Plasmid-Mediated Quinolone Resistance with AmpC- Beta-Lactamase Producing *E. coli* strains from Different Sources

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ABSTRACT

Objectives: this study was devoted to detect the plasmid-mediated quinolone resistance (PMQR) mechanisms (*qnr*, *qepA* and *aac(6')-Ib-cr*) and its association with AmpC- Beta-lactamase production in *E. coli* strains from different sources.

Methods: Twenty-nine *E. coli* isolates from food-producing animals (chicken and sheep) and their by-products, collected from Sharkia province, Egypt were tested for their susceptibilities for different antimicrobial groups. Uniplex PCR was applied using specific primer sets for screening the presence of PMQR and AmpC β –lactamases genes in strains under study.

Results: Out of 29 *E. coli* strains, only 9 isolates were positive for *qnr*A gene (31.03%) and associated with the *amp*C β -lactamase genes (MOX, DHA, ACC, EBC) whereas only one *qnr*B and *qnr*S-like genes (3.45 %) were detected. A *qnr*B gene as well as qnrS was detected in (DHA , EBC) isolate. *qepA* and *aac(6')-Ib-cr* were detected in 41.38% and 3.45% of the *E .coli* isolates, respectively alone or in combination with *qnr* genes. The *amp*C β -lactamase genes were detected in 75.86 % of all strains and in 100% and 66.66 %of the PMQR determinant-positive and -negative strains, respectively, gave amplicons range from 302 bp to 520 bp, that easily distinguished by gel electrophoresis.

Conclusions: A high prevalence of PMQR determinants among AmpC β-lactamase producing *E. coli* isolates from chicken mainly and their by-products was detected in Egypt. Their effect may slightly increase the MIC of quinolone and may related to the development of full resistance to quinolone.

INTRODUCTION

In Egypt, quinolones and β-lactams are among the most commonly used antimicrobials in both human and veterinary clinical medicine. The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain. Infection with antibiotic resistant bacteria negatively impacts on public health, due to an increased incidence of treatment failure and severity of disease *(1)*.

Fluoroquinolone resistance is emerging in Gram-negative pathogens worldwide. Unfortunately, quinolone resistant *E. coli* in animals have increased in numbers after quinolone introduction *(2)*. The traditional understanding that quinolone resistance is acquired only through mutation and transmitted only vertically does not entirely account for the relative ease with which resistance develops in exquisitely susceptible organisms, or for the very strong association between resistance to quinolones and to other agents. The recent discovery of plasmid-mediated horizontally transferable genes encoding quinolone resistance might shed light on these phenomena *(3)*.

However, since the first plasmid-mediated quinolone resistance (PMQR) gene (*qnr*A) was

reported in 1998 for a *Klebsiella pneumoniae* isolate from the United States *(4)*, five different transferable mechanisms of quinolone resistance (TMQRs) have been described. These mechanisms including target protection (*qnr* genes), quinolone modification (*aac(6')-Ib-cr)*, plasmid-encoded efflux systems (*qepA* or OqxAB, amongst others*)*, effect on bacterial growth rates and natural transformation *(5)*.

Qnr proteins belong to the pentapeptide-repeat family that directly protect DNA gyrase and topoisomerase IV from quinolone inhibition *(6)* leading to 8 to 32-fold increase in MICs of quinolones *(7)*. There are at least 6 *qnrA*, 20 *qnrB*, and 3 *qnrS* alleles descriped, with one or more amino acid alterations within each family *(8)*, furthermore, *qnrC* and *qnrD* (one variant for each) were also reported *(9,10);* a database of *qnr* allele designations are maintained at the website http://www.lahey.org/qnrStudies.

Qnr could bind to the gyrase holoenzyme and its respective subunits, GyrA and GyrB, and consequently topoisomerase IV and its subunits, ParC and ParE, such binding does not require the presence of the enzyme–DNA–quinolone complex. It was hypothesized that the formation of Qnr-gyrase / Qnr-topoisomerase IV complex occurs before the formation of the cleavage complex. Furthermore, DNA binding by gyrase / topoisomerase IV decreases when gyrase / topoisomerase IV interacts with Qnr reducing the amount of holoenzyme-DNA targets for quinolone inhibition *(11,12)*.

aac(6')-Ib-cr gene encodes a new variant of common aminoglycoside acetyltransferase. Two single amino acid substitutions, Trp102Arg and Asp179Tyr, in the wild-type allele *aac(6')-Ib* enable the gene product to be capable of N-acetylation of piperazinyl amine of certain fluoroquinolones and thereby reduces their antibacterial activities *(13)*. It was first reported in 2003 and confers 2-4 folds increase in MICs *(14)*.

The QepA determinant is an efflux pump protein putatively belonging to 14-transmembrane-segment major facilitator superfamily of transporters involved in pumping of hydrophilic fluoroquinolones out of bacterial cells. It confers a 32-to 64-fold increase of fluoroquinolone MIC values. The novel *qepA* gene was identified on plasmid pHPA of *Escherichia coli* strain, which was isolated in 2002 from the urine of an inpatient in Japan; this plasmid displayed a multiple-resistance profile for aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams *(15)*.

Today, emerging newer β-lactamase enzymes including extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases are associated with misuse of β-lactam antibiotics resulting in evolution of Beta-lactam resistance in Gram-negative bacteria, especially *Escherichia coli (16)*.

AmpC β-lactamases belong to Ambler class C and, once expressed at high levels, confer resistance to a wide variety of β-lactam antibiotics including penicillins, most of the expanded spectrum cephalosporins, (excluding cefpirome and cefepime) and monobactams *(17)*. Furthermore, AmpC enzymes, with only few exceptions, are not inhibited by the ESBL inhibitor clavulanic acid *(18)* and in a strain with decreased outer membrane permeability, such enzymes can provide resistance to carbapenems *(19,20)*.

Actually, AmpC β-lactamases can be either chromosomal or plasmid mediated. In *E. coli*, the natural chromosomal AmpC is constitutively produced at a very low level because of a transcriptional attenuator coupled with a weak promoter *(21,22)*. However, constitutive overexpression of AmpC can occur due to either the deregulation of the chromosomally encoded ampC gene (derepressed ampC mutants) or by acquisition of a transferable ampC gene, imported from the chromosomal genes, on a plasmid or other transferable elements (plasmid-mediated AmpC) conferring resistance similar to their chromosomal counterparts *(23)*.

Six different groups of plasmid mediated AmpC were identified. These groups include ACC, DHA, CIT and EBC, which originated

from *H. alvei*, *M. morganii*, *C. freundii* and *E. cloacae*, respectively, as well as FOX and MOX (unknown origins) *(23)*. One important difference between *E. coli* and the other members of the family Enterobacteriaceae is that the expression of *ampC* in *E. coli* is not inducible *(24)*.

Association of PMQR determinants with extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases is also noteworthy; as qnr genes were found to be carried on the same plasmid with various extended spectrum or AmpC-type β-lactamase genes *(6)*.

Hence, the objective of this study was to determine the coexistence of PMQR determinants and AmpC beta-lactamases in *E. coli* isolates from livestock animals and their meat products in Egypt to assess their potential role as a reservoir of emerging multidrug resistant bacteria which may subsequently transmit to humans through food chain or human-animal interactions.

MATERIAL AND METHODS

Bacterial strains

E. coli isolates were collected from livestock animal farms (chicken and sheep) and their by-products from different sail shops; each isolate was from a separate animal. Twenty-nine *E. coli* isolates were collected, including 17 from respiratory organs or feacal samples from diseased food- producing animals (13 chickens and 4 sheep, respectively) and 12 isolates from their by-products in Sharkia province, Egypt. The by-product isolates were classified as 8 chicken by-products (4 of each chickens burger and luncheon) and 4 beef by-products (2 burger and one of each sausage and minced meat). The bacterial strains were identified by classical biochemical methods and using rapid API20 E bacterial identification system (Biomerieux, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by Kirby-Bauer standard disk diffusion method onto Mueller-Hinton agar (Difco, USA), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35°C for 16 to 18 h *(25)*. The following antimicrobials were tested: nalidixic acid, ciprofloxacin, norfloxacin, levofloxacin, gatifloxacin, ampicillin, ampicillin-sulbactam, amoxycillin-clavulanic acid, piperacillin, imipenem, cefazolin, cefoxitin, cefuroxime , cefotaxime, ceftriaxone, cefepime, aztreonam, gentamicin, amikacin, erythromycin, tetracycline, chloramphenicol, colistin and sulfamethoxazole-trimethoprim (Oxoid, UK). Strains concomitantly resistant to ≥3 antimicrobial classes were defined as multidrug-resistant (MDR).

Furthermore, the minimum inhibitory concentrations (MICs) of ciprofloxacin (Sigma, USA) were determined by reference broth microdilution method according to ***Clinical and Laboratory Standards Institute (26)*** guidelines using custom-designed 96-well panels (Corning, USA). The interpretive criteria were those published in the relevant CLSI document.

AmpC disc test

The test is based on use of Tris-EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. AmpC disks were prepared in-house by applying 20 µl of a 1:1 mixture of saline and 100X Tris-EDTA (Sigma, USA) to sterile filter paper disks, allowing the disks to dry, and storing them at 2 to 8°C. The surface of a Mueller-Hinton agar plate was inoculated with a lawn of cefoxitin susceptible *E. coli* strain according to the standard disk diffusion method *(26)*. Immediately prior to use, AmpC disks were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disk. A 30µg cefoxitin disk was placed on the inoculated surface of the Mueller-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface. The plate was inverted and incubated

overnight at 35°C in ambient air. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disk with test strain was interpreted as positive for the production of AmpC β-lactamase, while an undistorted zone was considered as negative *(27)*.

Plasmid extraction

Thermo Scientific GeneJET Plasmid Miniprep Kit was used for plasmid DNA extraction from pelleted bacterial cell after harvesting on 1-5 ml LP media. The bacterial pellet was lysed and the plasmid DNA was bound on the silica membrane, which was then washed and eluted by 50 µl of the elution buffer.

Detection of PMQR determinants and AmpC β -lactamase-encoding genes

The isolates were investigated for the presence of *qnrA*, *qnrB*, *qnrS*, *aac(6_)-Ib-cr*, *qepA* and AmpC β-lactamases genes by PCR amplification with the primer sets described in table (1). DNA samples were amplified in a total of 25 µl of the following reaction mixture: 12.5 µl DreamTaq TM Green Master Mix (2X) (Sigma, UK), 1 µl of each primer (10pmole), 2µl template DNA and 8.5 µl water nuclease-free. The amplified PCR product was electrophoresed on a 1.5% agarose gel in Tris-acetate-EDTA buffer. A 100-bp DNA ladder (Invitrogen, Carlsbad, CA) was used as a molecular weight marker.

Table 1. Oligonucleotide primers, amplification cycles and amplicons of PMQR and AmpC β-lactamases in *E. coli* strains

Genes	Primers "5-3"	Amplification cycle			Amplicons (bp)	References
		Denaturation	Annealing	Extension		
qnrA	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	94°C 45 S.	53°C 45 S.	72°C 45 S.	516	*(13)*
qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC				469	
qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC				417	
aac(6')-Ib-cr	F: CCCGCTTTCTCGTAGCA R: TTAGGCATCACTGCGTCTTC	94°C 30 S.	52°C 30 S.	72°C 30 S.	113	*(28)*
qepA	F: CGTGTTGCTGGAGTTCTTC R: CTGCAGGTACTGCGTCATG	94°C 45 S.	50°C 45 S.	72°C 45 S.	403	*(29)*
MOXM (MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11)	F: GCTGCTCAAGGAGCACAGGAT R:CACATTGACATAGGTGTGGTGC	94°C 45 S.	55°C 45 S.	72°C 45 S.	520	*(23)*
CITM (LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1)	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC				462	
DHAM (DHA-1, DHA-2)	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC				405	
ACCM (ACC)	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC				346	
EBCM (MIR-1T ACT-1)	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT				302	
FOXM (FOX-1 to FOX-5b)	F:AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	94°C 30 S.	53°C 30 S.	72°C 45 S.	190	

RESULTS

Antimicrobial susceptibility testing

E. coli strains were tested against 24 antimicrobial agents including in 11 different groups (Table 2). The results showed that 82.76% of the isolates were resistant to nalidixic acid, additionally; high level of resistance was recorded for fluoroquinolones tested as ciprofloxacin (72.4%), norfloxacin and levofloxacin (62%) and gatifloxacin (51.7%). Regarding to beta-lactams, absolute resistance was detected for ampicillin, amoxycillin-clavulanic acid, piperacillin and cefazolin meanwhile, 79.3% of the isolates were resistant to cefuroxime, 72.4% to aztreonam, 69% to cefepime and 65.5% to cefoxitin, cefotaxime, ceftriaxone, and low level of resistance was reported for imipenem (3.4%). Furthermore, all tested *E. coli* strains were considered as multidrug resistant. It is noteworthy that all *E. coli* strains were sensitive to amikacin, which is considered as a drug of choice for treatment (Table 2).

Table 2. Antibiogram for *E. coli* strains using disk diffusion test

AMA / Strain No	NAL	CPFX	NFLX	LVFX	GFLX	AM	AMC	SAM	PRL	IPM	CZ	FOX	CXM	CTX	CRO	FEB	SXT	CN	AK	C	CT	TE	E	ATM
1	R	R	R	R	R	R	R	R	R	S	R	R	R	R	I	S	R	S	S	I	S	R	R	R
2	R	R	R	I	R	R	R	S	R	S	R	R	R	R	S	R	R	S	S	R	S	I	R	R
3	R	S	S	S	S	R	R	I	R	S	R	S	R	R	I	R	R	S	S	S	S	R	R	S
4	R	R	R	R	I	R	R	R	R	S	R	S	R	R	R	I	R	S	S	I	S	R	R	R
5	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	S	S	R	S	R	R	R
6	R	R	R	R	R	R	R	I	R	S	R	R	I	S	I	S	R	R	S	R	S	R	R	S
7	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	S	R	S	S	R	S	R	R	R
8	R	R	R	R	R	R	R	I	R	S	R	S	R	S	I	S	R	S	S	R	S	R	R	R
9	R	R	R	R	R	R	R	R	R	S	R	S	S	S	I	S	R	R	S	R	S	R	R	S
10	R	S	S	S	S	R	R	S	R	S	R	S	S	S	S	S	R	S	S	S	S	R	R	R
11	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	S	I	S	R	R	R
12	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	S	I	S	R	R	S
13	R	S	S	S	S	R	R	R	R	S	R	S	S	S	I	R	R	S	S	S	S	R	R	R
14	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	I	S	R	S	R	R	R	R	R
15	R	R	R	I	R	R	R	R	R	S	R	I	R	R	R	R	I	R	S	R	S	R	R	R
16	R	R	R	R	S	R	R	R	R	S	R	I	R	R	R	S	R	R	S	R	R	R	R	R
17	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	I	S	R	S	R	R	R
18	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R	S	R	S	R	R	R
19	R	R	R	R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	S	R	R	R
20	R	R	I	I	S	R	R	R	R	S	R	R	R	R	R	R	R	S	S	R	R	R	R	R
21	R	R	S	S	S	R	R	R	R	S	R	R	R	R	R	S	R	S	S	R	R	S	R	R
22	S	S	S	S	S	R	R	S	I	S	R	R	R	R	S	R	S	S	S	S	R	S	R	R
23	S	S	S	S	S	R	R	I	I	S	R	S	S	R	S	S	S	S	S	S	S	S	R	S
24	S	S	S	S	S	R	I	S	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	I
25	S	S	S	S	S	R	R	S	I	S	R	R	R	R	S	S	S	S	S	S	S	S	R	S
26	S	S	S	S	S	R	R	R	R	S	R	R	R	S	S	S	R	S	S	S	S	R	R	S
27	R	R	S	S	S	R	R	S	R	S	R	R	R	S	S	S	R	R	S	S	S	R	R	I
28	R	S	S	S	S	R	R	I	S	S	R	R	R	S	S	S	S	S	S	S	S	S	R	S
29	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	S	R	R	S	R	S	R	R	R

AMA: Antimicrobial agent, NAL: nalidixic acid, CPFX: ciprofloxacin, NFLX: norfloxacin, LVFX: levofloxacin, GFLX: gatifloxacin, AM: ampicillin, AMC: amoxycillin-clavulanic acid, SAM: ampicillin-sulbactam, PRL: piperacillin, IPM: imipenem, CZ: cefazolin, FOX: cefoxitin, CXM: cefuroxime, CTX: cefotaxime, CRO: ceftriaxone, FEB: cefepime, SXT: sulfamethoxazole-trimethoprim, CN: gentamicin, AK: amikacin, C: chloramphenicol, CT: colistin TE: tetracycline, E: erythromycin, ATM: aztreonam, R: resistant, S: sensitive, I: intermediate

Incidence of *qepA*, *qnr*, and *aac(6')-Ib-cr* genes

Among the total of 29 *E. coli* strains, PMQR determinants were present in 48.27%, with *qepA*, *qnr*, and *aac(6')-Ib-cr* being detected alone or in combination in 41.38%, 31.03%, and 3.45% of the strains, respectively. The *qnr* genes included 9 *qnrA*, and one of both *qnrB* and *qnrS*. One strain was positive for *aac(6')-Ib-cr* or *qnrB or qnrS* in addition to *qepA*, while seven strains harbored both *qnrA* and *qepA*. *qnrA*, *qnrB*, and *qepA* coexisted in a strain of *E. coli* isolated from respiratory organs of chicken (No.6) as well as *qnrA*, *qnrS*, and *qepA* coexisted in a strain of *E. coli* isolated from chicken burger (No.14). Detailed information on these PMQR determinant-positive isolates is given in Table (3) and Fig (1). Among the 17 isolates from livestock animals, 64.7% strains of chicken origin only carried at least one PMQR determinant; 47.6%, 5.88%, and 52.9% strains were positive for *qnr* genes, *aac(6')-Ib-cr*, and *qepA*, respectively. Sheep feacal strains were not showed any PMQR determinants. Of the 12 isolates from animals by-products, 25% contained one or more PMQR determinants; 25 % strains were positive for *qepA* genes (2 of sheep origin and one of chicken origin), only one of them (chicken origin) was positive for both *qnrA* and *qnrS* (No.14). *qnrB* and *aac(6')-Ib-cr* are not detected.

The incidence of PMQR determinants was significantly higher in livestock animal strains (64.7%) than in by-products strains (25%). The difference contributed to the higher incidence of *qnrA* and *qepA* in animals than in their by-products.

Identification of plasmid-mediated AmpC β-lactamases in *E. coli* strains

AmpC β-lactamases were detected genotypically by PCR in 75.86% of all strains and in 100% and 66.66 % of the PMQR determinant-positive and -negative strains, respectively. Among the *amp*C genes detected in AmpC-positive strains, twenty-one had EBC β-lactamase gene, eighteen had MOX gene, fifteen had DHA gene, nine had ACC gene and three had the CIT β-lactamase gene, showing amplicon sizes of 302 bp, 520 bp, 405 bp, 346 bp and 462 bp respectively (Fig 2), while FOX β-lactamases gene was not detected in any of them . The AmpC β -lactamases present in all 14 strains positive for PMQR determinants are listed in Table (3). PMQR determinants were detected in 63.63 % of 22 isolates positive for AmpC β-lactamases that reflect multidrug resistance within the strains and strong association between *qnr* genes and plasmid carrying *amp* C genes.

The AmpC β -lactamases were detected phenotypically in 8 strains (57.1%) (of 14 ampC-PCR positive strains) only, as a flattening or an indentation the zone of inhibition indicating enzymatic inactivation of cefoxitin as shown in Fig (3).

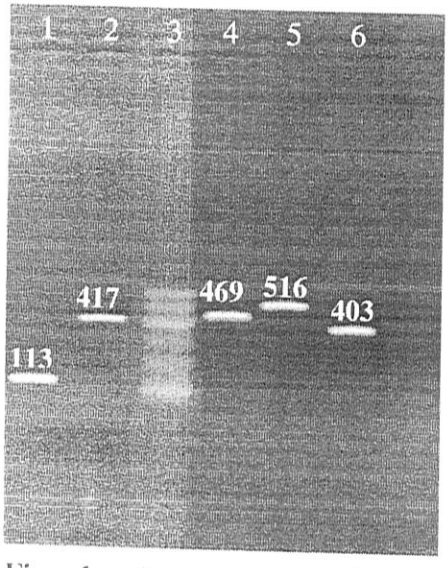

Fig 1. Agarose gel electrophoresis representing amplicons of PMQR determinants in base pairs. Lane 1: *aac(6')-Ib-cr, lane 2: qnrS*, lane *3:* molecular size markers (100 bp), lane 4: *qnrB*, lane 5 *qnrA* and lane 6:

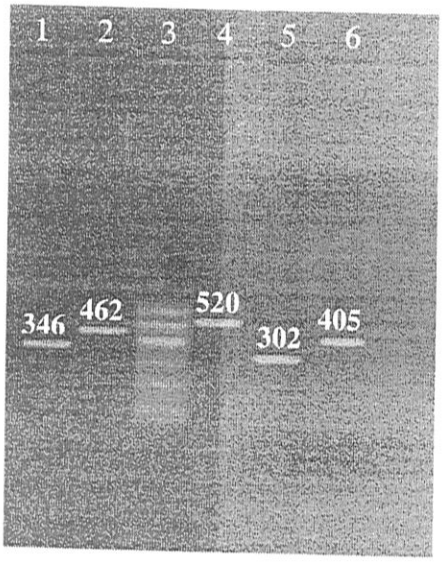

Fig 2. Agarose gel electrophoresis representing amplicons of AmpC β-lactamase genes in base pairs. Lane 1: *ACC*, lane 2: *CIT*, lane 3: molecular size markers (100 bp), lane 4: *MOX*, lane 5 *EBC* and lane 6: *DHA*

Fig 3. AmpC disc test result showing flattening or an indentation the zone of inhibition indicating enzymatic inactivation of cefoxitin.

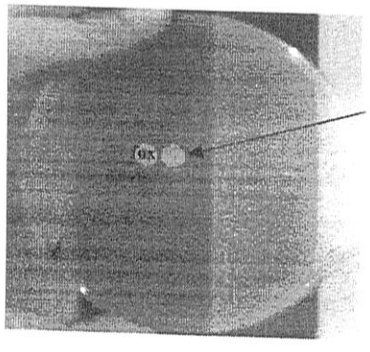

Table 3. Characteristics of PMQR determinants and AmpC β -lactamase of *Ecoli* strains recovered from different sources

Strain No.	Animal source	Origin	CFLX MIC µg/ml	PMQR determinant	AmpC β -lactamase
1	CF	RO	128	*qnrA*, *qepA*	*MOX, DHA, ACC, EBC*
2	CF	RO	256	*qepA*	*MOX, DHA, ACC, EBC*
3	CF	RO	32	ND	*MOX, DHA, ACC, EBC*
4	CF	RO	1024	*qnrA*, *qepA*	*MOX, DHA, ACC, EBC*
5	CF	RO	256	*qnrA*, *qepA*	*MOX, DHA, ACC, EBC*
6	CF	RO	256	*qnrA*, qnrB, *qepA*	DHA, EBC
7	CF	RO	1024	*qnrA*, *qepA*	*MOX, DHA, ACC, EBC*
8	CF	RO	1024	*QepA*, *aac(6')-Ib-cr*	EBC
9	CF	RO	1024	*qepA*	*MOX, DHA, ACC, EBC*
10	CF	RO	1024	*ND*	*MOX, DHA, EBC*
11	CF	RO	1024	*qnrA*	*MOX, DHA, ACC, EBC*
12	CF	RO	1024	*qnrA*	*MOX, DHA, EBC*
13	CF	RO	16	*qnrA*, *qepA*	*MOX, DHA, EBC*
14	C-bp	CB	16	*qnrA*, qnrS, *qepA*	*DHA, EBC*
15	C-bp	CB	1024	*ND*	*DHA, EBC*
16	C-bp	CB	16	*ND*	ND
17	C-bp	CB	8	*ND*	MOX, EBC
18	C-bp	CL	1024	*ND*	MOX, EBC
19	C-bp	CL	32	*ND*	MOX, CIT, EBC
20	C-bp	CL	512	*qepA*	*MOX,CIT, DHA, ACC, EBC*
21	C-bp	CL	1024	*qepA*	MOX, CIT
22	A-bp	BB	1	*ND*	ND
23	A-bp	BB	0.5	*ND*	ND
24	A-bp	BS	1	*ND*	ND
25	A-bp	MM	0.25	*ND*	ND
26	SF	feces	2	*ND*	MOX, EBC
27	SF	feces	1024	*ND*	MOX, EBC
28	SF	feces	1	*ND*	ND
29	SF	feces	32	*ND*	ND

CF: chicken farm, C-bp: chicken by-product, A-bp: animal by products, SF: sheep farms, RO: respiratory organs, CB: chicken burger, CL: chicken luncheon, BB: beef burger, BS: ,beef sausage, MM: minced meat, *ND:*, not detected

DISCUSSION

In the present study, the rates of quinolone resistance in *E. coli* strains were high in Egypt; more than 50% of *E. coli* strains were resistant to quinolones and fluoroquinolones especially in strains producing AmpC beta-lactamase which was in accordance with *(30)*.

Plasmid mediated quinolone resistant determinents were highly prevalent (48.27%) in *E. coli* isolates from different sources in Egypt and in these strains, *qepA* and *qnrA* were more common than other determinants. This percentage was higher than that (34.7%) of enterobacteriaceae isolates (mainly *E. coli* and *K. pneumoniae)* of animals from China, that

included mostly *qepA* and *aac(6')-Ib-cr* genes **(*31*)**. Among 29 *E. coli* strains, *qnr* being detected alone or in combination with *qepA* and *aac(6')-Ib-cr* genes in 9 (31.03 %) isolates and included mainly *qnrA*, and only one of both *qnrB* and *qnrS*. Different result was obtained by *(3)* in United States that, *qnr* genes were significantly more prevalent in *Enterobacter* species (31%) and *K. pneumoniae* (20%) isolates than in *E. coli* isolates (4%) with equivalent frequencies for *qnrA* and *qnrB* while *qnrS* was absent. Also, *(31)* in China recorded that *qnr* genes were prevalent in 7.9% of *E. coli* isolates and were mainly *qnrB* and *qnrS*.

The *qnrS* as well as *aac(6')-Ib-cr* gene were previously found in *E. coli* isolates from China by *(32)* in pig, *(33)* in poultry and swine and *(31)* in poultry and pig.. All of *qnr* positive isolates showed decreased susceptibility to fluoroquinolones, mainly ciprofloxacine (Table 3).

A *qep*A is the most common than other determinants in this study, however the prevalence of *qep*A was low (0.3%) in *E. coli* clinical isolates collected previously from 140 Japanese hospitals in *(34)*

In current report, about 75.86% isolates of *E. coli* carried plasmid-mediated AmpC β-lactamase genes by PCR and 63.63% of them revealed PMQR determinants. This linkage between qnr determinants and AmpC β-lactamases was described in several reports *as ampR* gene which regulates the expression of *ampC* may be present between *qnr* and the 3'CS (*qacE*Δ*1* and *sul1*) or is replaced by *qnr*, which may in turn explain it *(14,35)*

Moreover, a *qnr*A gene was associated with the AmpC (MOX, DHA, ACC, EBC) in nine strains and the data has not been recently reported. Also, a *qnr*B gene was found to be associated with the AmpC β-lactamase (DHA and EBC) (strains No.6). Simillarly, the association between *qnr*B4 variant and plasmid-mediated AmpC DHA-1has been previously detected in *E. coli* and *K.pneumoniae* clinical isolates ***(31,36)***. Additionally, *qnr*S genes in strain No. 14 was associated with the AmpC β -lactamase (DHA and EBC), similar data was recorded previously by *(37,38)* while not recorded by *(31)*.

Eight *E. coli* (57.1%) only out of 14 (ampC -PCR positive) strains were positive for phenotypic confirmation of ampC phenotype that reflect accuracy of PCR as genotypic detection more than phenotypic detection and there is no standard phenotypic method for detection of such enzymes. Similar results was decided by **CLSI.**

Finally, our study showed a high prevalence of PMQR determinants among AmpC-producing *E. coli* isolates that reflect multidrug resistance within the strains and strong association between *qnr* genes and plasmid carrying *amp*C genes. The high prevalence of PMQR determinants and/or AmpC β-lactamases in isolates from livestock animals may related to extensive use of broad spectrum antimicrobial agents resulting in spread and increase detection of these resistance determinents among bacteria of animal origin then of human origin which is important to the public health concern.

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الملخص العربى

ارتباط مقاومة الكينولون عبر البلازميد لعترات الاشريشية القولونية المنتجة للبيتا لاكتاماز المعزولة من مصاد ر مختلفة

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وجهت هذه الدراسه لتحديد طرق واليات مقاومة الكينولون عبر البلازميد (PMQR) مثل (*qnr, qepA* and *aac(6')-Ib-cr*) وارتباطها لعترات الاشريشية القولونيه المنتجة للبيتا لاكتاماز المعزولة من مصاد ر مختلفة. وفد تم جمع ٢٩ معزولة من الاشريشية القولونيه من الحيوانات المنتجة للغذاء (الدجاج والغنم) ومنتجاتهم من محافظة الشرقية بمصروخضعت لاختبار الحساسية لمجموعات مختلفة من المضادات الميكروبية. وتم تطبيق تفاعل انزيم البلمرة المتسلسل الاحادى باستعمال مجموعات بادىء وذلك لفحص وجود جينات المقاومة عبر البلازميد وجينات البيتا لاكتاماز فى هذه العينات محل الدراسة وكانت ٩ عترات من ٢٩ موجبة لجين *qnrA* بنسبة ٣١,٠٣% ومرتبطة بكل من جينات البيتا لاكتاماز الاتيه (MOX, DHA, ACC, EBC) بينما تم تحديد جين واحد لكل من *qnrS* and *qnrB* بنسبة ٣,٤٥%وكانوا مرتبطين بالعترات المحتوية على جينات البيتا لاكتاماز (DHA , EBC) . تم تحديد جينات كل من *qepA* and *aac(6')-Ib-cr* فى عترات الاشريشية القولونية بنسبة٤١,٣٨ % و٣,٤٥% على التوالى متفريدين او مرتبطين بجينات *qnr*. وقد تم تحديد جينات البيتا لاكتاماز فى كل العترات بنسبة ٧٥,٨٦% وفى العترات الموجبة لمقاومة الكينولون عبر البلازميد (PMQR) بنسبة ١٠٠% اما فى العترات السالبة فكانت نسبتها ٦٦,٦٦% على التوالى معطية امبليكونات يمكن فصلها كهربائيا بسهولة ويتراوح حجمها بين 52 bp to 302 bp. والخلاصةانه تم تسجيل نسبة عاليه من مقاومة الكينولون عبر البلازميد لعترات الاشريشية القولونية المنتجة للبيتا لاكتاماز المعزولة من الدواجن ومنتجاتها فى مصر وتأثيرهم ربما يؤدى الى زيادة طفيفة فى التركيز الادنى المثبط للكينولون (MIC of quinolone) وربما يتعلق هذا بتطوير المقاومة كلية للكينولون.