Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (5): 29-35
Published online October 16, 2015 (http://www.cellmolbiol.com)
Received on September 13, 2015, Accepted on September 30, 2015.
doi : 10.14715/cmb/2015.61.5.5

Coexistence of plasmid-mediated quinolone resistance determinants and AmpC-Beta-Lactamases in *Escherichia coli* strains in Egypt

N. K. Abd El-Aziz✉ and A. A. Gharib

Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, Egypt

Corresponding author: N. K. Abd El-Aziz, Mubarak district, Zagazig, Sharkia, Egypt. E-mail: nourhan_vet@yahoo.com or nourhan_vet@zu.edu.eg

Abstract

Three kinds of plasmid-mediated quinolone resistance (PMQR) determinants (*qnr* genes, *qep*A and *aac(6')-Ib-cr*) have been discovered and shown to be widely distributed among clinical isolates. To characterize the prevalence of PMQR determinants among AmpC-producing *E. coli* strains in food-producing animals and animal by-products in Egypt, twenty-nine *E. coli* strains were tested for their susceptibilities to antimicrobials and screened for PMQR determinants and AmpC Beta lactamases using PCR and plasmid profiling. It was found that *qnr* genes being detected alone or in combination with *qepA* or *aac(6')-Ib-cr* genes in 11 (37.9%) strains comprising 9 for *qnrA* and only one for both *qnrB* and *qnrS*. Moreover, *qepA* and *aac(6')-Ib-cr* were detected in 41.38% and 3.45% of *E. coli* strains, respectively. The *amp*C β-lactamase genes were detected in 75.86 % of all strains and in 100% and 53.3% of the PMQR determinant-positive and negative strains, respectively. In several cases, plasmid profiling of *E. coli* strains exhibiting the coexistence of both PMQR determinants and *amp*C genes on a single plasmid as a first report in Egypt that may contribute to rapid spread and increase in bacterial resistance, which is important to public health concern.

Key words: PMQR, AmpC, Beta lactamases, Plasmid profiling.

Introduction

The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant bacteria, which could be transmitted to humans through the food chain. In Egypt, quinolones and β-lactams are among the most commonly used antimicrobials in both human and veterinary clinical medicine (1). Fluoroquinolone resistance is emerging in Gram-negative pathogens worldwide. The traditional understanding that quinolone resistance is acquired only through mutation and transmitted only vertically does not fully account for the relative ease with which resistance develops in exquisitely susceptible organisms, or for the strong association between resistance to quinolones and other antimicrobial agents. Plasmid-mediated horizontally transferable genes encoding quinolone resistance might shed light on these phenomena (2). Five different transferable mechanisms of quinolone resistance (TMQRs) have been described including target protection (*qnr* genes), quinolone modification (*aac(6')-Ib-cr),* plasmid-encoded efflux systems (*qepA* or *Oqx*AB, amongst others*)*, effect on bacterial growth rates and natural transformation (3).

Qnr proteins belong to the pentapeptide-repeat family that directly protect DNA gyrase and topoisomerase IV from quinolone inhibition (2) leading to 8 to 32-fold increase in minimum inhibitory concentrations (MICs) of quinolones (4). There are at least 6 *qnr*A, 20 *qnr*B, and 3 *qnr*S alleles descriped, with one or more amino acid alterations within each family (5), furthermore, *qnrC* and *qnrD* (one variant for each) were also reported (6, 7); a database of *qnr* allele designations are maintained at the website http://www.lahey.org/qnrStudies.

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 (8). It was first reported in 2003 and confers 2-4 folds increase in MICs (9).

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 (10). .

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 resistance in Gram-negative bacteria, especially *E. coli* (11). 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 (12). Furthermore, AmpC enzymes, with only few exceptions, are not inhibited by the ESBL inhibitor clavulanic acid (13) and in a strain with decreased outer membrane permeability, such enzymes can provide resistance to carbapenems (14, 15). 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 (16, 17). However, constitutive over expression of *amp*C can occur due to either the deregulation of the chromosomally encoded *amp*C gene (derepressed *amp*C mutants) or by acquisition of a transferable *amp*C gene, imported from the chromosomal genes, on a plasmid or other transferable

elements (plasmid-mediated AmpC) conferring resistance similar to their chromosomal counterparts (18).

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) (18). 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 (19). Association of PMQR determinants with ESBLs or AmpC beta-lactamases is also noteworthy. Hence, the objective of this study was to determine the coexistence of plasmid mediated quinolone resistance (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.

Materials and methods

Bacterial strains

Twenty-nine consecutive, non-repetitive clinical isolates of *E. coli* recovered from livestock animal farms and animal by-products were investigated. These included 17 isolates from diseased food-producing animals (13 from chickens showing respiratory manifestations and 4 from sheep experiencing diarrhoea) and 12 isolates from animal by-products, which included 8 chicken by-products and 4 beef by-products. Presumptive *E. coli* isolates were confirmed using API-20E identification kits (BioMérieux, Mary l'Etoile, France), serotyped in the Serology Unit, Animal Health Research Institute, Giza, Egypt using commercial antisera (Denka Seiken CO., LTD., Tokyo, Japan) according to the manufacturer's instructions and stored as 20% glycerol stocks at-70°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for *E. coli* strains was performed by Kirby-Bauer standard disk diffusion method as described elsewhere (20). The following antimicrobials were tests: nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), ampicillin (10 µg), ampicillin-sulbactam (20/10 µg), amoxycillin-clavulanic acid (20/10 µg), piperacillin (100 µg), imipenem (10 µg), cefazolin (30 µg), cefoxitin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), gentamicin (10 µg), amikacin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg) and sulfamethoxazole-trimethoprim (23.75/1.25 µg) (Oxoid, Hampshire, England, UK). Strains concomitantly resistant to ≥3 antimicrobial classes were defined as multidrug-resistant (MDR).

Further, the multiple antibiotic resistance (MAR Index) was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested. MAR index higher than 0.2 indicates wide use of this antibiotic in the originating environment of this isolate (21).

Minimum inhibitory concentrations (MICs) of ciprofloxacin (Sigma-Aldrich, St. Louis, USA) were determined by reference broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (22) using custom-designed 96-well panels (Corning, New York, USA). The interpretive criteria were those published in the relevant CLSI document.

Screening for ESBL producers by double disc synergy assay

A Mueller Hinton agar plate was inoculated with standardized inoculum of the test organism to give a semi-confluent growth. Test disks of 3rd generation cephalosporins (ceftazidime, ceftriaxone, cefotaxime) and an aztreonam disk were placed 20 mm apart (center to center) around an augmentin (amoxicillin and clavulanic acid) disk then the plate was incubated at 37°C for 24 hrs. Enhancement of inhibition zone of any one of the test antibiotics towards the augmentin disc was regarded as presumptive ESBL production (23).

AmpC disc test

AmpC disks were prepared by applying 20 µl of a 1:1 mixture of saline and 100X Tris-EDTA (Sigma-Aldrich, St. Louis, 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 (22). 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 (24).

PCR detection of PMQR determinants and AmpC ß-lactamase encoding genes in E. coli strains

Plasmid DNA was extracted using GeneJET Plasmid Miniprep Kit (Thermofisher Scientific, Waltham, Massachusetts, USA) following the manufacturer's recommendations. Oligonucleotide primer sets used for PCR amplification are listed in table 1. PCR was performed with a final volume of 25 µl of the following reaction mixture: 12.5 µl Dream*Taq* TM Green Master Mix (2X) (Sigma, UK), 1 µl of each primer (20 pmole), 2µl template DNA and 8.5 µl water nuclease-free. DNA fragments were analysed by electrophoresis on 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide at 100 V for 30 min on a mini slab horizontal electrophoresis unit (Bio-Rad, California.USA).

Plasmid analysis

Extracted plasmid DNAs were electophoresed using 0.8% agarose gel (Applichem GmbH, Darmstadt, Germany) in 1x TBE buffer at room temperature using gradients of 5V/cm. Gel was stained with 0.1 µg/ml of ethidium bromide for 20 minutes. A geneRuler 1Kb plus DNA ladder (Fermentas, Thermofisher Scientific, Waltham, Massachusetts, USA) was used to determine

Table 1. Primers used for PCR amplification of PMQR determinants and *amp*C genes.

Primer	Target(s)	Oligonucleotide sequences (5′→3′)	Amplicon size (bp)	Anealing temperature (°C)	Reference
QnrA	*qnr*A	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516		
QnrB	*qnr*B	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469	53	(25)
QnrS	*qnr*S	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417		
Aac(6′)-Ib-cr	*aac(6′)-Ib-cr*	CCCGCTTTCTCGTAGCA TTAGGCATCACTGCGTCTTC	113	52	(26)
QepA	*qep*A	CGTGTTGCTGGAGTTCTTC CTGCAGGTACTGCGTCATG	403	50	(27)
MOXM	MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11	GCTGCTCAAGGAGCACAGGAT CACATTGACATAGGTGTGGTGC	520		
CITM	LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	TGGCCAGAACTGACAGGCAAA TTTCTCCTGAACGTGGCTGGC	462		
DHAM	DHA-1, DHA-2	AACTTTCACAGGTGTGCTGGGT CCGTACGCATACTGGCTTTGC	405	55	(18)
ACCM	ACC	AACAGCCTCAGCAGCCGGTTA TTCGCCGCAATCATCCCTAGC	346		
EBCM	MIR-1T ACT-	TCGGTAAAGCCGATGTTGCGG CTTCCACTGCGGCTGCCAGTT	302		
FOXM	FOX-1 to FOX-5b	AACATGGGGTATCAGGGAGATG CAAAGCGCGTAACCGGATTGG	190	53	

the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra, Göttingen, Germany) and the data was analyzed through its computer software (28).

Results

Antimicrobial resistance phenotype

E. coli strains were tested for their susceptibilities to several antimicrobial agents (Table 2). The results showed that 79.3% of tested strains were resistant to nalidixic acid; resistance rates of *E. coli* were recorded for fluoroquinolones as following: ciprofloxacin (65.5%), norfloxacin (58.6%) and levofloxacin (51.7%) and gatifloxacin (48.3%). The highest rates of resistance were observed towards β-lactam antibiotics; ampicillin and cefazolin (100%), amoxycillin-clavulanic acid (96.6%), piperacillin (86.2%), cefuroxime (75.86%), cefotaxime (65.5%), ampicillin-sulbactam (62.1%), cefoxitin (55.2%), ceftriaxone (44.8%) and cefepime (34.5%), while low level of resistance was reported for imipenem (3.45%) and no resistance was recorded for amikacin. It is noteworthy that all *E. coli* strains under study were multidrug resistant, 93% out of them had MAR index

Table 2. Antimicrobial susceptibilities of *E. coli* strains obtained from different sources.

Antimicrobial agent	Source of E. coli strains											
	Chicken organs (13)			chicken products (8)			Sheep diarrhea (4)			Beef products (4)		
	S	I	R	S	I	R	S	I	R	S	I	R
NAL	0	0	13	0	0	8	1	1	2	4	0	0
CPFX	2	1	10	0	1	7	2	0	2	4	0	0
NFLX	3	0	10	1	1	6	3	0	1	4	0	0
LVFX	3	1	9	1	2	5	3	0	1	4	0	0
GFLX	3	1	9	4	0	4	3	0	1	4	0	0
AM	0	0	13	0	0	8	0	0	4	0	0	4
AMC	0	0	13	0	0	8	0	0	4	0	1	3
SAM	2	3	8	0	0	8	0	0	4	3	1	0
PRL	0	0	13	0	0	8	1	0	3	0	3	1
IPM	13	0	0	8	0	0	3	0	1	4	0	0
CZ	0	0	13	0	0	8	0	0	4	0	0	4
FOX	9	0	4	0	2	6	0	1	3	1	0	3
CXM	5	1	7	0	0	8	0	0	4	1	0	3
CTX	7	0	6	0	0	8	3	0	1	0	0	4
CRO	6	3	4	0	0	8	3	0	1	4	0	0
FEB	6	1	6	4	1	3	4	0	0	3	0	1
ATM	4	0	9	0	0	8	2	1	1	2	1	1
SXT	0	0	13	1	1	6	1	0	3	4	0	0
CN	9	0	4	2	1	5	2	0	2	4	0	0
AK	13	0	0	8	0	0	4	0	0	4	0	0
C	3	4	6	0	0	8	3	0	1	4	0	0
TE	0	1	12	2	0	6	1	0	3	4	0	0

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, ATM: aztreonam, SXT: sulfamethoxazole-trimethoprim, CN: gentamicin, AK: amikacin, C: chloramphenicol, TE: tetracycline, S: sensitive, I: intermediate R: resistant.

far greater than 0.2 and are resistant to at least 6 antibiotics (Table 3). Further, MIC of ciprofloxacin using broth microdilution method revealed that amongst 29 examined *E. coli* strains, 19 (65.5%), were found to be highly resistant with MIC values ranged from 32-1024 µg/ml, while 2 strains only exhibited intermediate resistance (Table 3).

Phenotypic characterization of beta lactamases

Of the 29 *E. coli* strains, 8 (27.6%) had a positive screening test for ESBL, while 5 only (17.2%) clearly showed the expression of AmpC enzymes by using AmpC disk test, co-existence of both ESBL and AmpC enzymes in tested strains was not detected.

Detection of PMQR determinants and AmpC β-lactamases

In total, the prevalence of the PMQR determinants among the screened *E. coli* isolates of varying O-serotypes was 48.28% (14/29) with *qnr, qepA,* and *aac(6')-Ib-cr* being detected alone or in combination in 11 (37.9%), 12 (41.38%) and 1 (3.45%) strains, respec-

Table 3. Phenotypic and genotypic characteristics of *E. coli* strains under study.

Strain No.	Serotype	Origin	Resistance pattern	MAR index	CFLX MIC (µg/ml)	PMQR determinants	AmpC beta lactamases	Plasmid size (bp)
1	O115	Chicken organs	NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, ATM, SXT, TE.	0.73	256	*qnrA*, *qepA*	MOX, DHA, ACC, EBC	801, 1232
2	O78		NAL, CPFX, NFLX, GFLX, AM, AMC, PRL, CZ, FOX, CXM, CTX, FEB, ATM, SXT, C.	0.68	128	*qepA*	MOX, DHA, ACC, EBC	16362
3	O27		NAL, CPFX, AM, AMC, PRL, CZ, CXM, CTX, FEB, SXT, TE.	0.5	1	ND	MOX, DHA, ACC, EBC	908
4	O27		NAL, CPFX, NFLX, LVFX, AM, AMC, SAM, PRL, CZ, CXM, CTX, CRO, ATM, SXT, TE.	0.68	1024	*qnrA*, *qepA*	MOX, DHA, ACC, EBC	801, 1232
5	O78		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, FEB, ATM, SXT, C, TE.	0.8	512	*qnrA*, *qepA*	MOX, DHA, ACC, EBC	817, 1258
6	O78		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, PRL, CZ, FOX, SXT, CN, C, TE	0.64	512	*qnrA*, qnrB, *qepA*	DHA, EBC	801
7	O78		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, CRO, ATM, SXT, C, TE.	0.68	1024	*qnrA*, *qepA*	MOX, DHA, ACC, EBC	833, 1258, 3203, 3830, 20735
8	O78		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, PRL, CZ, CXM, ATM, SXT, C, TE	0.64	1024	*qepA*, *aac(6')-Ib-cr*	EBC	817
9	O146		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, SXT, CN, C, TE.	0.64	1024	*qepA*	MOX, DHA, ACC, EBC	801, 1232, 3203, 3942
10	O125		NAL, AM, AMC, PRL, CZ, ATM, SXT, TE	0.36	1	ND	MOX, DHA, EBC	786, 1232
11	Untyped		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, CXM, CTX, CRO, FEB, CN, ATM, SXT, TE.	0.8	1024	*qnrA*	MOX, DHA, ACC, EBC	801, 1215, 3724, 16379
12	Untyped		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, CRO, FEB, SXT, CN, TE.	0.68	1024	*qnrA*	MOX, DHA, EBC	817, 1258, 1978, 5440, 19266
13	O29		NAL, AM, AMC, SAM, PRL, CZ, FEB, ATM, SXT, TE.	0.45	2	*qnrA*, *qepA*	MOX, DHA, EBC	833, 1244
14	O111	chicken products	NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, CN, ATM, C, TE.	0.8	16	*qnrA*, qnrS, *qepA*	DHA, EBC	801
15	O44		NAL, CPFX, NFLX, GFLX, AM, AMC, SAM, PRL, CZ, CXM, CTX, CRO, FEB, CN, ATM, C, TE.	0.77	512	ND	DHA, EBC	793, 16379
16	O44		NAL, CPFX, NFLX, LVFX, AM, AMC, SAM, PRL, CZ, CXM, CTX, CRO, ATM, SXT, CN, C, TE.	0.77	16	ND	ND	ND
17	O44		NAL, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, ATM, SXT, C, TE.	0.77	2	ND	MOX, EBC	817, 1271
18	O125		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, ATM, SXT, CN, C, TE.	0.86	1024	ND	MOX, EBC	817, 1258
19	O78		NAL, CPFX, NFLX, LVFX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, FEB, ATM, SXT, CN, C, TE.	0.86	32	ND	MOX, CIT, EBC	817, 1244, 1978, 3830, 5816, 15003
20	O114		NAL, CPFX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, FEB, ATM, SXT, C.	0.68	32	*qepA*	MOX,CIT, DHA, ACC, EBC	16362
21	O127		NAL, CPFX, AM, AMC, SAM, PRL, CZ, FOX, CXM, CTX, CRO, ATM, SXT, C.	0.64	128	*qepA*	MOX, CIT	825, 1258, 2625, 22205
22	O26	Sheep diarrhea	AM, AMC, SAM, PRL, CZ, FOX, CXM, SXT, TE.	0.4	0.25	ND	MOX, EBC	1232
23	O55		NAL, CPFX, AM, AMC, PRL, CZ, FOX, CXM, SXT, CN, TE.	0.5	1024	ND	MOX, EBC	793, 1244, 2625
24	O125		AM, AMC, CZ, FOX, CXM.	0.23	0.5	ND	ND	786, 1232
25	O146		NAL, CPFX, NFLX, LVFX, GFLX, AM, AMC, SAM, PRL, IPM, CZ, CXM, CTX, CRO, ATM, SXT, CN, C, TE.	0.86	32	ND	ND	ND
26	O26	Beef products	AM, AMC, CZ, FOX, CXM, CTX, FEB, ATM.	0.36	1	ND	ND	ND
27	O128		AM, AMC, CZ, CTX.	0.18	1	ND	ND	ND
28	O124		AM, PRL, CZ, FOX, CXM, CTX.	0.27	0.5	ND	ND	ND
29	O111		AM, AMC, CZ, FOX, CXM, CTX.	0.27	1	ND	ND	900

MAR: multiple antibiotic resistance, MIC: Minimum inhibitory concentration, PMQR: plasmid mediated quinolone resistance, 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, ATM: aztreonam, SXT: sulfamethoxazole-trimethoprim, CN: gentamicin, C: chloramphenicol, TE: tetracycline.

Table 4. Characteristics of positive and negative PMQR *E. coli* strains.

Characteristic	No. of strains with characteristic/total no. of strains with PMQR determinants result (%)		Odds ratio (95% confidence interval)	P value
	Positive (%)	Negative (%)		
-Strain origin:				
Livestock animals	11/14 (78.6)	6/15 (40)	5.50 (1.1-28.4)	0.035
Animal by products[RC]	3/14 (21.4)	9/15 (60)		
-Strain characteristics:				
AmpC producers	14/14 (100)	8/15 (53.3)	25.59 (1.3-506.5)	0.006[F]
Non AmpC producers[RC]	0	7/15 (46.7)		

[F]: Fisher Exact Probability Test was applied as expected cell frequencies are less than 5.
Both probability estimates are non-directional.
[RC]: Reference category.

tively. Among the qnr-positive strains, 9 had *qnrA,* one had *qnrB* and another one harbored *qnr*S. All but one of the PMQR- positive strains (93.3%) were resistant to ciprofloxacin with MICs ranging from 16-1024 µg/ml, the remaining strain was intermediate resistant (MIC 2 µg/ml) according to the CLSI. Among the 17 *E. coli* strains from livestock animals, 11 of chicken origin carried at least one PMQR determinant; 8 (47.6%), 1 (5.88%), and 9 (52.9%) strains were positive for *qnr* genes, *aac(6')-Ib-cr*, and *qepA*, respectively. while sheep feacal strains did not show any of them. Moreover, of the 12 strains obtained from animal by-products, 3 only of chicken origin contained one or more PMQR determinants; all of them harbored *qepA* gene and only one was positive for both *qnrA* and *qnrS* with the absence of *qnrB* and *aac(6')-Ib-cr*. Detailed information on these PMQR determinant-positive strains is given in Tables (3, 4).

Plasmid mediated AmpC β-lactamases were unambiguously detected genotypically in 22 (75.86%) of the 29 tested strains, 14 (63.63%) of them revealed PMQR determinants, of these, 21 (95.45%) contained EBC genes, 18 (81.8%) had MOX genes, 15 (68.2%) harbored DHA genes, 9 (40.9%) had ACC gene and 3 (13.64%) had CIT genes, while FOX genes were not identified in any strain. It was noted that plasmid-mediated AmpC beta-lactamases were detected in 100% (14/14) and 53.3% (8/15) of the PMQR determinant-positive and negative strains, respectively, which reflects the strong association between *qnr* genes and plasmid carrying *amp*C genes *(P*= 0.006 by Fisher Exact Probability Test) which was fully illustrated in Tables (3, 4).

Plasmid profiling

Plasmid profiling of antibiotic resistant *E. coli* strains under study revealed that the strains contained various size plasmids approximately ranging from 786 bp-20735 bp as summarized in Table 3 and Figure 1. Our findings indicated that among the 29 analyzed strains, 8 showed only a single plasmid band, 5 of them exhibiting the coexistence of both PMQR determinants and *amp*C genes on the same plasmid, 9 had double plasmids, 7 harbored 3 plasmids or more while 5 strains don not include any plasmid although they had multidrug resistance. Besides, in several cases, different strains showed very similar migration patterns on agarose gel electrophoresis although they exhibited different antibiotic resistance profiles.

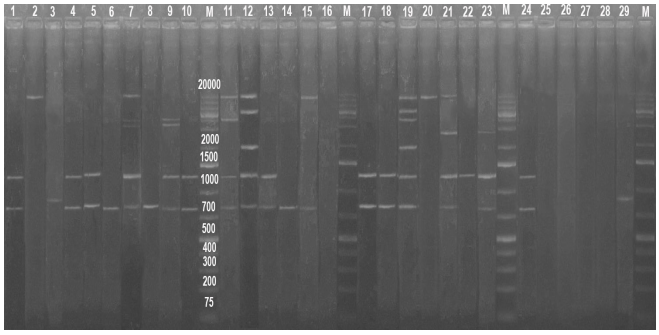

Figure 1. Agarose gel electrophoresis showing plasmid profile of 29 *E. coli* strains. M: Marker (75 to 20000 bp GeneRuler 1 kb Plus DNA Ladder, Fermentas).

Discussion

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 a previous study (29). In current study, PMQR determinants were highly prevalent (48.28%) in *E. coli* strains obtained from different sources in Egypt, among them, *qepA* and *qnrA* were more common than other determinants. This percentage was higher than that recorded in *Enterobacteriaceae* (mainly *E. coli* and *K. pneumoniae)* of animal origin in China (34.7%) which included mostly *qepA* and *aac(6')-Ib-cr* genes (30)**.**

In this study, PMQR determinants were detected in 48.28% of tested *E. coli* strains. A higher percentage was recorded in a recent study in Italy in which PMQR determinants were detected in *E. coli* isolates recovered from retail broiler chicken meat with a percentage of 91% (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 %) strains mainly *qnrA,* and only one of both *qnrB* and *qnrS.* Different result was obtained by another study 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 (25). However, in China, *qnr* genes were prevalent in 7.9% of *E. coli* isolates and were mainly *qnrB* and *qnrS* (30). On the other hand, *aac(6')-Ib-cr* gene was previously found in *E. coli* isolates from China in pig (32), in poultry and swine (33)

and in poultry and pig (30)**.** Further, A *qep*A gene is the most common recorded one 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 2008 (10).

In current report, 22 (75.86%) strains of *E. coli* carried plasmid-mediated *amp*C β- lactamase genes by PCR and 14 (63.63%) of them revealed PMQR determinants among which 5 strains showed only a single plasmid band by plasmid profiling exhibiting the coexistence of both PMQR determinants and *amp*C genes on the same plasmid. 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 (*qacE11* and *sul1*) or is replaced by *qnr,* which may in turn explain it (9, 34)**.**

Moreover, a *qnr*A gene was associated with the *amp*C (MOX, DHA, ACC, and 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) (strain No.6). Similarly, the association between *qnr*B4 variant and plasmid-mediated *amp*C DHA-1has been previously detected in *E. coli* and *K.pneumoniae* clinical isolates (30, 35). Additionally, *qnr*S gene in strain No. 14 was associated with the AmpC β -lactamase (DHA and EBC), similar data was recorded previously (36, 37)**.**

To our knowledge, this is the first report in Egypt that record the presence of PMQR determinants and *amp*C genes on the same plasmid of *E. coli* strains from livestock animals and their by-products. It reflects multidrug resistance within the strains and strong association between PMQR and *amp*C genes thus may collectively transmit to humans so, research is definitely needed to develop novel antibiotics that target or bypass the known resistance mechanisms.

References

1. Walsh, C. and Fanning, S., Antimicrobial resistance in food borne pathogens--a cause for concern? *Curr. Drug Targets* 2008, **9**(9): 808-815. doi: 10.2174/138945008785747761.

2. Robicsek, A., Jacoby, G.A. and Hooper, D.C., The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect. Dis.* 2006a, **6**(10): 629–640. doi: 10.1016/S1473-3099(06)70599-0.

3. Ruiz, J., Pons, M.J. and Gomes, C., Transferable mechanisms of quinolone resistance. *Int. J. Antimicrob. Agents* 2012, **40**(3): 196-203. doi: 10.1016/j.ijantimicag.2012.02.011.

4. Tran, J.H. and Jacoby, G.A., Mechanism of plasmid-mediated quinolone resistance. *Proc. Natl. Acad. Sci. USA* 2002, **99**(8): 5638–5642. doi: 10.1073/pnas.082092899.

5. Jacoby, G., Cattoir, V., Hooper, D., Martínez-Martínez, L., Nordmann, P., Pascual, A., Poirel, L. and Wang, M., *qnr* gene nomenclature. *Antimicrob. Agents Chemother*. 2008*,* **52**(7): 2297-2299. doi: 10.1128/AAC.00147-08.

6. Wang, M., Guo, Q., Xu, X., Wang, X., Ye, X., Wu, S., Hooper, D.C. and Wang, M., New plasmid-mediated quinolone resistance gene, *qnr*C, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob. Agents Chemother.* 2009, **53**(3): 1892-1897. doi: 10.1128/AAC.01400-08.

7. Cavaco, L.M., Hasman, H., Xia, S. and Aarestrup, F.M., *qnr*D, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob. Agents Chemother.* 2009, **53** (2): 603-608. doi: 10.1128/AAC.00997-08.

8. Robicsek, A., Strahilevitz, J., Jacoby, G.A., Macielag, M., Abbanat, D., Park, C.H., Bush, K. and Hooper, D.C., Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat. Med.* 2006b, **12**(1): 83–88. doi: 10.1038/nm1347.

9. Wang, M., Tran, J.H., Jacoby, G.A., Zhang, Y., Wang, F. and Hooper, D.C., Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob. Agents Chemother*. 2003, **47**(7): 2242-2248. doi: 10.1128/AAC.47.7.2242-2248.2003

10. Yamane, K., Wachino, J., Suzuki, S. and Arakawa, Y., Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrob. Agents Chemother*. 2008, **52**(4): 1564-1566. doi: 10.1128/AAC.01137-07.

11. Jacoby, G.A., AmpC beta-lactamases. *Clin. Microbiol. Rev.* 2009, **22**(1): 161-182. doi: 10.1128/CMR.00036-08.

12. Mammeri, H., Nazic, H., Naas, T., Poirel, L., Léotard, S. and Nordmann, P., AmpC β- lactamase in an *Escherichia coli* clinical isolate confers resistance to Expanded-Spectrum cephalosporins. *Antimicrob. Agents Chemother.* 2004, **48**(10): 4050–4053. doi: 10.1128/AAC.48.10.4050-4053.2004.

13. Philippon, A., Arlet, G. and Jacoby, G.A., Plasmid-determined AmpC-type β- lactamases. *Antimicrob. Agents Chemother.* 2002, **46**(1): 1–11. doi: 10.1128/AAC.46.1.1-11.2002.

14. Poirel, L., Héritier, C., Spicq, C. and Nordmann, P., In vivo acquisition of high-level resistance to Imipenem in *Escherichia coli*. *J. Clin. Microbiol.* 2004, **42**(8): 3831–3833. doi: 10.1128/JCM.42.8.3831-3833.2004.

15. Mammeri, H., Nordmann, P., Berkani, A. and Eb, F., Contribution of extended-spectrum AmpC (ESAC) beta-lactamases to carbapenem resistance in *Escherichia coli*. *FEMS Microbiol. Lett.* 2008, **282**(2): 238–240. doi: 10.1111/j.1574-6968.2008.01126.x.

16. Mulvey, M.R., Bryce, E., Boyd, D.A., Ofner-Agostini, M., Land A.M., Simor, A.E. and Paton, S., Molecular characterization of cefoxitin-resistant *Escherichia coli* from Canadian hospitals. *Antimicrob. Agents Chemother.* 2005, **49**(1): 358–365. doi: 10.1128/AAC.49.1.358-365.2005.

17. Tracz, D.M., Boyd, D.A., Hizon, R., Bryce, E., McGeer, A., Ofner-Agostini, M., Simor, A.E., Paton, S. and Mulvey, M.R., *amp*C gene expression in promoter mutants of cefoxitin-resistant *Escherichia coli* clinical isolates*. FEMS Microbiol. Lett.* 2007, **270**(2): 265–271. doi: 10.1111/j.1574-6968.2007.00672.x.

18. Pérez-Pérez, F.J. and Hanson, N.D., Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* 2002, **40**(6): 2153–2162. doi: 10.1128/JCM.40.6.2153-2162.2002

19. Honoré, N., Nicolas, M.H. and Cole, S.T., Inducible cephalosporinase production in clinical isolates of *Enterobacter cloacae* is controlled by a regulatory gene that has been deleted from *Escherichia coli*. *EMBO J.* 1986, **5**(13): 3709–3714.

20. Ortez, H.J., Test methods: disk diffusion testing, In, Coyle B. M. (ed.): Manual of Antimicrobial Susceptibility Testing, American Society for Microbiology, 2005, 39-52 p.

21. Krumperman, P.H., Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Envir. Microbiol.* 1983, **46** (1): 165-170.

22. Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, CLSI document M100-S21, Wayne, Pennsylvania, USA, 2011, **30**(15):42-46.

23. Jarlier, V., Nicolas, M.H., Fournier, G. and Philippon, A., Extended spectrum β-lactamases conferring transferable resistance to

newer β- lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.* 1988, **10**(4): 867–878. doi: 10.1093/clinids/10.4.867.

24. Black, J.A., Moland, E.S. and Thomson, K.S., AmpC Disk Test for Detection of Plasmid-Mediated AmpC β-Lactamases in *Enterobacteriaceae* Lacking Chromosomal AmpC β –Lactamases. *J. Clin. Microbiol.* 2005, **43**(7): 3110–3113. doi: 10.1128/JCM.43.7.3110-3113.2005.

25. Robicsek, A., Strahilevitz, J., Sahm, D.F., Jacoby, G.A. and Hooper, D. C., *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. *Antimicrob. Agents Chemother.* 2006c, **50**(8): 2872–2874. doi: 10.1128/AAC.01647-05.

26. Lunn, A.D., Fàbrega, A., Sanchez-Céspedes, J. and Vila, J., Prevalence of mechanisms decreasing quinolone-susceptibility among Salmonella spp. clinical isolates. *Int. Microbiol.* 2010, **13**(1): 15-20. doi: 10.2436/20.1501.01.107.

27. Cattoir, V., Poirel, L. and Nordmann, P., Plasmid-mediated quinolone resistance pump *Qep*A2 in an *Escherichia coli* isolate from France. Antimicrob. Agents Chemother. 2008, **52**(10): 3801-3804. doi: 10.1128/AAC.00638-08.

28. Gomes, A.K., Akhter, H., Mahmud, B., Khan, S.I. and Begum, A., Prevalence and characterization of multi-drug resistant *Escherichia Coli* from urine samples. *Dhaka Univ. J. Biol. Sci.* 2011**, 20**(1): 23–30. doi: 10.3329/dujbs.v20i1.8834.

29. Han, C., Yang, Y., Wang, M., Wang, A., Lu, Q., Xu, X., Wang, C., Liu, L., Deng, Q. and Shen, X., The prevalence of plasmid-mediated quinolone resistance determinants among clinical isolates of ESBL or AmpC-producing *Escherichia coli* from Chinese pediatric Patients. *Microbiol. Immunol.* 2010, **54(3)**: 123-128. doi: 10.1111/j.1348-0421.2010.00200.x.

30. Ma, J., Zeng, Z., Chen, Z., Xu, X., Wang, X., Deng, Y., Lü, D., Huang, L., Zhang, Y., Liu, J. and Wang, M., High Prevalence of Plasmid-Mediated Quinolone Resistance Determinants *qnr*, *aac(6`)-Ib-cr*, and *qepA* among Ceftiofur-Resistant *Enterobacteriaceae* Isolates from Companion and Food-Producing Animals. *Antimicrob. Agents Chemother.* 2009, **53**(2): 519-524. doi: 10.1128/AAC.00886-08.

31. Ghodousi, A., Bonura, C., Di Noto, A.M. and Mammina, C., Extended-Spectrum ß-Lactamase, AmpC-Producing, and Fluoroquinolone-Resistant *Escherichia coli* in Retail Broiler Chicken Meat, Italy. *Foodborne Pathog. Dis.* 2015, **12**(7): 619-625. doi: 10.1089/fpd.2015.1936.

32. Liu, J.H., Deng, Y.T., Zeng, Z.L., Gao, J.H., Chen, L., Arakawa, Y. and Chen, Z.L., Coprevalence of plasmid-mediated quinolone resistance determinants *Qep*A, *Qnr*, and *AAC(6`)-Ib-cr* among 16S rRNA methylase RmtB producing *Escherichia coli* isolates from pigs. *Antimicrob. Agents Chemother.* 2008, **52**(8): 2992–2993. doi: 10.1128/AAC.01686-07.

33. Yue, L., Jiang, H.X., Liao, X.P., Liu, J.H., Li, S.J., Chen, X.Y., Chen, C.X., Lu,¨D.H. and Liu, Y.H., Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine clinical isolates of *Escherichia coli*. *Vet. Microbiol.* 2008, **132**(3-4): 414–420. doi: 10.1016/j.vetmic.2008.05.009.

34. Mammeri, H., Van De Loo, M., Poirel, L., Martinez-Martinez, L. and Nordmann, P., Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob. Agents Chemother*. 2005, **49**(1): 71-76. doi: 10.1128/AAC.49.1.71-76.2005.

35. Wu, J.J., Ko, W.C., Wu, H. M. and Yan, J.J., Prevalence of *Qnr* determinants among bloodstream isolates of *Escherichia coli* and *Klebsiella pneumoniae* in a Taiwanese hospital 1999–2005. *J. Antimicrob. Chemother*. 2008, **61**(6): 1234-1239. doi: 10.1093/jac/dkn111.

36. Park, C.H., Robicsek, A., Jacoby, G.A., Sahm, D. and Hooper, D.C., Prevalence in the United States of *aac(6_)-Ib-cr* encoding a ciprofloxacin modifying enzyme. *Antimicrob. Agents Chemother.* 2006, **50**(11): 3953-3955. doi: 10.1128/AAC.00915-06.

37. Strahilevitz, J., Jacoby, G.A., Hooper, D.C. and Robicsek, A., Plasmid-Mediated Quinolone Resistance: a Multifaceted Threat. *Clin. Microbiol. Rev.* 2009, **22**(4): 664-689. doi: 10.1128/CMR.00016-09.