JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian *Escherichia coli* isolates from Algeria

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Abstract

Introduction: The emergence and spread of quinolone-resistant *Escherichia coli* in poultry products puts consumers at risk of exposure to the strains of *E. coli* that resist antibiotic treatment. The objective of this study was to define the prevalence and virulence potential of poultry-associated nalidixic acid (NAL)-resistant *E. coli* in the Annaba city, Algeria.

Methodology: In total, 33 samples of retail chicken meat were purchased from various butcher shops and examined for bacterial contamination with NAL-resistant *E. coli*. These isolates were subjected to antimicrobial susceptibility testing and were also investigated for the presence of plasmid-mediated quinolone resistance (PMQR) genes and virulence genes using conventional polymerase chain reaction (PCR) and DNA sequencing. Phylogenetic grouping of the NAL-resistant *E. coli* isolates was determined by the conventional multiplex PCR method.

Results: Twenty-nine (87.8%) products yielded NAL-resistant *E. coli*. Antibiograms revealed that 96.55% of NAL-resistant *E. coli* isolates were multidrug resistant (MDR). Resistance was most frequently observed against sulfamethoxazole-trimethoprim (96.6%), tetracycline (96.6%), ciprofloxacin (72%), and amoxicillin (65.5%). Group A was the most prevalent phylogenetic group, followed by groups D, B1, and B2. The PMQR determinants were detected in three isolates with *qnrB72* and *qnrS1* type identified. Four (13.8%) isolates carried one of the *Shiga* toxin *E. coli*-associated genes *stx1*, *stx2*, and *ehxA* alleles.

Conclusions: The high prevalence of NAL-resistant *E. coli* isolated from retail chicken meat with detection of MDR *E. coli* harboring *Shiga* toxin genes in this study gives a warning signal for possible occurrence of foodborne infections with failure in antibiotic treatment.

Key words: retail chicken meat; NAL-resistant *E. coli*; phylogenetic group; virulence; PMQR; Algeria.

J Infect Dev Ctries 2017; 11(2):143-151. doi:10.3855/jidc.8643

(Received 29 April 2016 – Accepted 27 September 2016)

Introduction

Escherichia coli is one of the most frequently encountered bacterial species of animal and human commensal intestinal flora. It can cause healthcare- and community-acquired infections, systemic infections [1-4], and can lead to serious complications and death [5], based upon its virulence gene content and antibacterial drug resistance. Due to its ubiquity, *E. coli* has become one of the bacterial species that are commonly resistant to antibiotics and can transmit antibiotic-resistance genes from other Enterobacteriaceae species in the environment [6-8].

In many countries, *E. coli* is used as a sentinel for monitoring antimicrobial drug resistance and as an indicator of selective pressure imposed by the antibiotics used in the production of food animals and treatment of humans. Quinolones have been used on farms to treat and prevent diseases in animals [8,9]. They are also used extensively in sub-therapeutic doses to promote growth and increase body weight by improving feed utilization. Quinolones will select for quinolone- and fluoroquinolone-resistant bacteria, which in turn will spread by direct contact or through food, water, and animal waste application to farm fields [7,10]. Quinolone-resistant *E. coli* are now relatively common and often exhibit multidrug resistance [11,12].

In many countries, the rate of quinolone-resistant *E. coli* increased in human infections and among bacteria isolated from food animals such as cattle, pigs, and poultry, and particularly in retail chicken meat [12-15].

In developing countries, such as Algeria, there are very little data about antimicrobial resistance of *E. coli* isolated from the production and environment of food animals. Messai *et al.* [16] showed that the frequency of resistance to quinolone and fluoroquinolone in *E. coli* isolated from broiler chickens in east Algeria was 96.7% and 72.2%, respectively.

The chromosomal mutations in genes encoding DNA gyrase and topoisomerase IV are the main mechanisms of quinolone resistance [6], followed by upregulation of efflux pumps and/or decreased expression of outer membrane porins [6]. Plasmid-mediated quinolone resistance (PMQR) determinants have added a new dimension to quinolone resistance. These transferable determinants confer a low level of quinolone resistance on their own, and they facilitate the acquisition of high-level resistance among initially susceptible strains. PMQR includes Qnr proteins (QnrA, QnrB, QnrS, QnrC, and QnrD), the aminoglycoside acetyltransferase variant, *aac(6')-Ib-cr*, and the fluoroquinolone-specific efflux pump protein, QepA [6].

The aim of the present study was to describe the prevalence of quinolone-resistant *E. coli* isolated from raw retail chicken meat on sale in Annaba, Algeria, and to determine the phylogenetic background, antimicrobial resistance profile, virulence factors, and the PMQR determinants in these isolates.

Methodology

Study area

This study was conducted in Annaba city. Annaba city is located in the northeast corner of Algeria, near the Seybouse River at latitude 36°54′N and longitude 7°46′E. Its altitude is 3 meters above sea level. The minimum and maximum temperatures of the area are -4°C and 47.5°C, respectively. The area receives a bimodal rainfall pattern with an annual precipitation rate of 675.5 millimeters. The total human population of the town is estimated to be 257,359.

Study design

A cross-sectional study was conducted from February to June 2012 to estimate the prevalence of nalidixic acid (NAL)-resistant *E. coli* isolates and their virulence and susceptibility to other antimicrobial agents. The retail chicken meat used in this study was collected in almost all butcher shops operating in Annaba city during the study period.

Sample collection and transportation

In this study, 33 fresh raw chicken meat samples were collected randomly from different parts of the chicken carcass, following most purchasers' preference to keep uniformity. Before collecting chicken meat samples, the external surfaces were disinfected with 70% alcohol to minimize surface contamination. Using sterile scissors and tissue forceps, pieces of the meat were collected separately in sterile bags. The samples were then transported to the microbiology laboratory of the University of Badji Mokhtar in a cold box. Retail meat was kept intact until the boxes were aseptically opened in the laboratory at the start of examination.

Sample processing and isolation of E. coli

E. coli from whole meats were isolated as previously described [17]. Briefly, 25 g portions (breast and thigh muscles) of each sample were taken aseptically by scalpel excision, placed into a separate stomacher bag with 225 mL of sterile peptone water (Difco, Detroit, USA), and homogenized at 230 rpm for 2 minutes. From the homogenate, 100 µL of aliquots was plated onto MacConkey agar (Difco, Detroit, USA) and incubated for 24 hours at 44°C. Three to five lactose-fermenting colonies from separate regions of multiple plates were subcultured onto eosin-methylene blue agar (Difco, Detroit, USA). Only one *E. coli* isolate was selected from each food sample. Colonies that showed a dark blue color with a characteristic metallic sheen were selected from each of the agar plates and identified as *E. coli* by API 20E commercial strips (bioMérieux, Paris, France).

Isolation of NAL-resistant E. coli and antimicrobial susceptibility test

All *E. coli* isolates were screened for NAL resistance by using the disk diffusion method following guidelines established by the French Society for Microbiology (http://www.sfm-microbiologie.org/).

The NAL-resistant *E. coli* strains were tested for their susceptibility to other antibiotics (Bio-Rad, Marne-la-Coquette, France): amoxicillin-clavulanic acid (20/10 µg), imipenem (10 µg), amoxicillin (25 µg), cefoxitin (30 µg), cefepime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), fosfomycin (200 µg), ciprofloxacin (5 µg), tetracycline (30 µg), and sulfamethoxazole-trimethoprim (23.75/1.25 µg).

The minimum inhibitory concentrations (MICs) of nalidixic acid and ciprofloxacin were determined for

PMQR, and virulence gene-positive strains were determined using the Etest.

Phenotypic detection of ESBL production

Extended-spectrum beta-lactamase (ESBL) production was screened by double disk with a synergy test and agar diffusion test between a central amoxicillin/clavulanic acid disk (20/10 µg) and a third-generation cephalosporin (cefotaxime 30µg and ceftazidime 30 µg) (Bio-Rad, Marne-la-Coquette, France), placed at a distance of 30 mm (center to center) as described previously [10].

The standard strains *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 (Manassas, USA) were used as negative and positive controls of ESBL production, respectively.

Preparation of DNA template for PCR

DNA templates for polymerase chain reaction (PCR) process were generated by suspending five colonies of an overnight culture of *E. coli* isolates growing on Luria-Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500 µL of DNase- and RNase-free water (Invitrogen, Paisley, UK). The suspension was boiled at 100°C for 10 minutes in a thermal block (Bioblock Scientific, Illkirch, France), then centrifuged at 19,000g for 5 minutes. An aliquot of 1 µL of the supernatant was used as DNA template for PCR.

Phylogenetic genotyping

Phylogenetic grouping of the *E. coli* isolates was determined by a PCR-based method developed by Clermont *et al.* [18], which uses a combination of three DNA markers (*chuA, yjaA*, and an anonymous DNA fragment, TspE4.C2). Strains were assigned to phylogenetic groups on the basis of presence or absence of the three DNA fragments: *chuA*-, TspE4.C2-, group A; *chuA*-, *yjaA*-, Tspe4.C2+, group B1; *chuA*+, *yjaA*+, group B2; and *chuA*+, *yjaA*-, group D (Figure 1).

To increase strain discrimination, subgroups or phylotypes were determined as follows: group A subgroup A_0, *chuA*-, *yjaA*-, Tspe4.C2-; group A subgroup A_1, *chuA*-, *yjaA*+, Tspe4.C2-; group B2 subgroup $B2_2$, *chuA*+, *yjaA*+, Tspe4.C2-; group B2 subgroup $B2_3$, *chuA*+, *yjaA*+, Tspe4.C2+; group D subgroup D_1,*chuA*+, *yjaA*-, Tspe4.C2-, and group D subgroup D_2,*chuA*+, *yjaA*-, Tspe4.C2+ [19].

Detection of PMQR genes

All ESBL-producing strains were screened by multiplex PCR for *qepA* and *qnr* genes (*qnrA, qnrB, qnrD, qnrC,* and *qnrS*) [20]. PCR amplification of *aac(6')-Ib-cr,* a fluoroquinolone-modifying aminoglycoside acetyltransferase, was performed using primers that amplify all *aac(6')-Ib* variants. Isolates positive for *aac(6')-Ib* were sequenced to identify *aac(6')-Ib-cr* as described by Barguigua *et al.* [20].

Detection of virulence genes

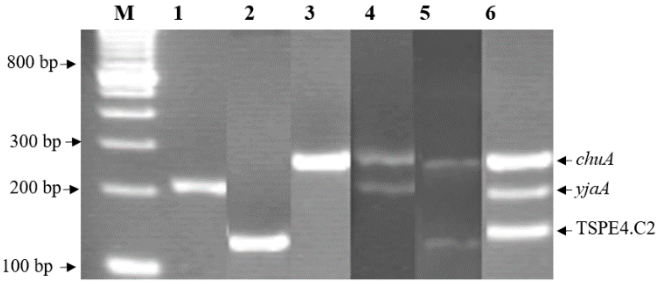
All NAL-resistant *E. coli* isolates were screened for the presence of *Shiga* toxin *E. coli* (STEC)-associated genes *stx1*, *stx2*, *ehxA*, and *eae* by PCR as described by Paton *et al*. [21], with some modifications. In brief, the multiplex PCR mixture of 25 µL contained 1X PCR buffer, 1.5 mM of $MgCl_2$, each primer within the 4 primer sets at a concentration of 0.4 µM, 200 µM each of dNTPs, 1U of *Taq* DNA polymerase, and 2 µL of template DNA. The PCR reaction was performed in a thermal cycler (Thermo Electron, Dreieich, Germany) using specific primers and the following standard cycling procedure: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 60°C for 1 minute and extension at 72°C for 1 minute, and a final extension at 72°C for 7 minutes.

All primers used in this work are shown in Table 1.

Sequencing of resistance genes

All amplified products obtained were sequenced to validate their identities. Both strands of the purified amplicons were sequenced with a Genetic Analyzer 3130x1 sequencer (Applied Biosystems, Foster City, USA), with the same primers used for PCR amplification (Table 1). The nucleotide and deduced protein sequences were analyzed with software available online at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Figure 1. The PCR profiles specific for *E. coli* phylogenetic groups. Each combination of *chuA* and *yjaA* gene and DNA fragment TSPE4.C2 amplification allowed phylogenetic group determination of a strain.



Lane 1: group A; lane 2: group B1; lanes 3 and 4: group D; lanes 5 and 6: group B2; lane M: molecular weight marker.

Results

Isolation of NAL-resistant E. coli

Of the 33 chicken samples cultured, all yielded *E. coli* on non-selective media. NAL-resistant *E. coli* was recovered from 29 (87.87%) of the 33 *E. coli*-positive samples.

Antimicrobial susceptibility patterns of NAL-resistant E. coli isolates

Of the total 29 NAL-resistant *E. coli* isolates subjected for antimicrobial susceptibility test, 28 (96.6%), 28 (96.6%), 20 (65.5%), 18 (72%), 7 (6.9%), 3 (10.3%), and 1 (3.4%) exhibited resistance to sulfamethoxazole-trimethoprim, tetracycline, amoxicillin, ciprofloxacin, amoxicillin/clavulanic acid, cefoxitin, and tobramycin, respectively (Table 2). One isolate was resistant to ceftazidime and none were ESBL producers.

The worrying aspect of the current study is that 28 (96.55%) of the isolates were multidrug resistant, of which 19 (65.51%) were resistant to four antibiotic families (Table 3). Interestingly, all the isolates were susceptible to amikacin, gentamicin, aztreonam, cefotaxime, imipenem, and fosfomycin (Table 2).

PMQR genes

Three (10.34%) NAL-resistant *E. coli* isolates (E27, E29, and E40 isolate) were positive for *qnr* genes; *qnrB*- and *qnrS*-type alleles were detected in two and one *E. coli* isolates, respectively. These were found to be *qnrB72* and *qnrS1* alleles by sequencing of PCR products (Table 4). No NAL-resistant *E. coli* isolates were positive for the *aac(6')-Ib* gene.

Table 1. Summary of primer sets used for the amplification of the plasmid-mediated quinolone resistance genes, virulence genes, and phylogenetic grouping.

Gene or location	Primers	Oligonucleotide sequence (5'–3')	References
qnrA	qnrA(+)	TTCTCACGCCAGGATTTGAG	[2]
	qnrA(-)	TGCCAGGCACAGATCTTGAC	
qnrB	qnrB(+)	TGGCGAAAAAATT(GA)ACAGAA	[2]
	qnrB(-)	GAGCAACGA(TC)GCCTGGTAG	
qnrS	qnrS(+)	GACGTGCTAACTTGCGTGAT	[2]
	qnrS(-)	AACACCTCGACTTAAGTCTGA	
aac(6')-Ib	aac(6')-Ib(+)	ATGACTGAGCATGACCTTG	[2]
	aac(6')-Ib(-)	AACCATGTACACGGCTGG	
qepA	qepA(+)	TGGTCACGCCATGGACCTCA	[2]
	qepA(-)	TGAATTCGGACACCGTCTCCG	
qnrC	qnrC(+)	GGGTTGTACATTTATTGAATC	[37]
	qnrC(-)	TCCACTTTACGAGGTTCT	
qnrD	qnrD(+)	CGAGATCAATTTACGGGGAATA	[37]
	qnrD(-)	AACAAGCTGAAGCGCCTG	
stx1	stx1 F	ATAAATCGCCATTCGTTGACTAC	[20]
	stx1 R	AGAACGCCCACTGAGATCATC	
stx2	stx2 F	TTAACCACACCCCACCGGGCAGT	[20]
	stx2 R	GGATATTCTCCCCACTCTGACACC	
eaeA	SK1	CCCGAATTCGGCACAAGCATAAGC	[20]
	SK2	CCCGGATCCGTCTCGCCAGTATTCG	
ehxA	hylA F	GCATCATCA AGCGTACGTTCC	[20]
	hylA R	AATGAGCCAAGCTGGTTAAGCT	
chuA	ChuA1	GACGAACCAACGGTCAGGAT	[17]
	ChuA2	TGCCGCCAGTACCAAAGACA	
yjaA	YjaA1	TGAAGTGTCAGGAGACGCTG	[17]
	YjaA2	ATGGAGAATGCGTTCCTCAAC	
TspE4.C2	TspE4.C2.1	GAGTAATGTCGGGGCATTCA	[17]
	TspE4.C2.2	CGCGCCAACAAAGTATTACG	

Table 2. Antimicrobial susceptibility patterns of *E. coli* isolates.

Antimicrobials	*E. coli* isolates (n = 29)		
	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
CIP	15 (51.7)	3 (10.3)	14 (48.3)
TET	28 (96.6)	0	1 (3.4)
AN	0	0	29 (100)
TM	1 (3.4)	0	28 (96.6)
GM	0	0	29 (100)
AMC	2 (6.9)	5 (17.2)	22 (75.9)
AMX	19 (65.5)	1 (3.4)	9 (31)
ATM	0	0	29 (100)
FEP	0	0	29 (100)
CTX	0	0	29 (100)
CAZ	0	1 (3.4)	28 (96.6)
FOX	3 (10.3)	0	26 (89.7)
IMP	0	0	29 (100)
FOS	0	0	29 (100)
SXT	28 (96.6)	0	1 (3.4)

AMC: amoxicillin-clavulanic acid; IMP: imipenem; AMX: amoxicillin; FOX: cefoxitin; FEP: cefepime; CAZ: ceftazidime; CTX: cefotaxime; AZT: aztreonam; GM: gentamicin; AN: amikacin; TM: tobramycin; CIP: ciprofloxacin; FOS: fosfomycin; TET: tetracycline; SXT: sulfamethoxazole-trimethoprim.

Table 3. Drug-resistance profiles NAL-resistant *E. coli* isolates.

Resistance profile	Isolates with resistance profile n (%)	Resistance category
NAL, SXT, TE	1 (3.4)	Multidrug resistant
NAL, CIP, TE	1 (3.4)	Drug resistant
NAL, SXT, TE	2 (6.9)	Multidrug resistant
NAL, SXT, CIP, AMX	1 (3.4)	Multidrug resistant
NAL, SXT, TE, FOS	1 (3.4)	Multidrug resistant
NAL, SXT, CIP, TE	3 (10.3)	Multidrug resistant
NAL, SXT, TE, AMX	6 (20.6)	Multidrug resistant
NAL, SXT, CIP, TE, AMX	1 (3.4)	Multidrug resistant
NAL, SXT, CIP, TE, AMX	6 (20.6)	Multidrug resistant
NAL, SXT, TE, AMC, AMX, FOX	3 (10.3)	Multidrug resistant
NAL, SXT, CIP, TE, AMC, AMX	2 (6.9)	Multidrug resistant
NAL, SXT, CIP, TE, TM, AMC, AMX	1 (3.4)	Multidrug resistant
NAL, SXT, CIP, TE, AMC, CAZ, AMX	1 (3.4)	Multidrug resistant

NAL: nalidixic acid; AMC: amoxicillin-clavulanic acid; AMX: amoxicillin; FOX: cefoxitin; CAZ: ceftazidime; GM: gentamicin; CIP: ciprofloxacin; TET: tetracycline; SXT: sulfamethoxazole-trimethoprim.

Table 4. Plasmid-mediated quinolone resistance and *Shiga* toxin *E. coli*-associated virulence genes detected in NAL-resistant *E. coli* isolated from raw chicken meat in Algeria.

Code	PMQR	Virulence genes	Phylogenetic		MIC (µg/mL)		Resistance profile
			Group	Subgroup	NAL	CIP	
27	-	*stx1*	A	A_0	> 256	> 32	NAL, SXT, CIP, TE, AMX
28	-	-	A	A_1	> 256	0.25	NAL, SXT, TE, AMX
29	*qnrB72*	*stx2*	A	A_0	> 256	0.25	NAL, SXT, TE, AMC, AMX, FOX
30	-	*stx1*	A	A_1	> 256	8	NAL, SXT, CIP, TE, AMX
31	*qnrS1*	-	D	D_2	> 256	6	NAL, SXT,CIP, TE, AMX
40	*qnrB72*	-	A	A_0	> 256	0.38	NAL, SXT ,TE, AMC, AMX , FOX
52	-	*ehxA*	D	D_2	> 256	6	NAL, SXT, CIP, TE

MIC: minimum inhibitory concentration; NAL: nalidixic acid; AMC: amoxicillin-clavulanic acid; AMX: amoxicillin; FOX: cefoxitin; CAZ: ceftazidime; GM: gentamicin; CIP: ciprofloxacin; TET: tetracycline; SXT: sulfamethoxazole-trimethoprim.

The three PMQR-producing isolates presented a high level of resistance to nalidixic acid (> 256 µg/mL). The E27 *qnrS1*-positive isolate present a high level of resistance to ciprofloxacin (6 µg/mL), whereas the *qnrB72*-positive isolates (E29 and E40) presented low-level resistance to ciprofloxacin, with MIC values equal to 0.25 µg/mL and 0.38 µg/mL, respectively (Table 4).

Phylogenetic genotyping of NAL-resistant E. coli isolates

According to the classification method by Clermont *et al*. [17], later modified by Escobar-Páramo *et al*. by addition of subgroups [22], eight (27%) isolates belonged to phylogenetic group A_0, eight (27%) to group A_1, four (14%) to group B_1, four (14%) to group D_2, three (10%) to group D1, and two (7%) to group $B2_2$.

Virulence genes

Altogether, four (13.8%) isolates retrieved in this study carried one of the STEC-associated *stx* and *ehxA* alleles (Table 4). Two isolates from the phylogenetic subgroup A_0 were positive for STEC virulence gene *stx1*and *stx2*; one isolate from the phylogenetic subgroup A_1 carried the *stx1* allele, and a further one isolate from the phylogenetic subgroup D_2 was positive for *ehxA* alleles.

Discussion

Quinolone resistance is currently a worldwide problem in human and veterinary medicine, both in developed and developing countries [6]. Several foods and environmental sources harbor bacteria that are resistant to quinolone or fluoroquinolone antibiotics used in human or veterinary medicine and in the production of food animals [6,7,9,23,24].

E. coli of poultry origin are potentially hazardous to humans from the perspective of antimicrobial resistance [4,25]. As described in many developing countries, the contamination of retail meat by *E. coli* could be related to the food-handling practices of workers and hygienic conditions of butcher shops' premises and utensils [26]. Unfortunately, these factors were not studied in this work. To limit these contaminations, the World Health Organization and Food and Agriculture Organization of the United Nations recommend that surfaces of floors and walls should be made of impervious materials; floors should be constructed to allow adequate drainage and cleaning; walls should have a smooth surface up to a height appropriate to the operation; and that ceilings and overhead fixtures should be constructed and finished to minimize the build-up of dirt and condensation and the shedding of particles [26].

The aim of this study was to examine samples of raw chicken meat as potential reservoirs of virulence and quinolone-resistant *E. coli*; 87.87% of the *E. coli* isolates were resistant to nalidixic acid (quinolone). This could reflect the over-use of fluoroquinolones for the prevention and/or treatment of infections, or the use of less active quinolones and fluoroquinolones [23,27,28]. The use of short-term treatment with fluoroquinolones could also have been a contributory factor in the selection of mutant isolates [27,28]. To our knowledge, no studies of quinolone resistance of commensal *E. coli* spread in food products intended for humans have been performed by other investigators in Algeria. The high recovery rate of quinolone-resistant *E. coli* from raw chicken meat samples in Algeria was troubling, but not surprising with the insufficiency in the application of legislation relating to antibiotic use and given the routine application of quinolones and fluoroquinolones at sub-therapeutic doses for prophylactic and therapeutic purposes by farmers without prescription and for treatment by veterinary prescription in the absence of documented laboratory findings. An Algerian study showed strong contamination (85.51%) of the poultry meat samples analyzed by antibiotic residues and confirmed the misuses and non-compliance of the withdrawal period between the administration of antibiotics in chickens and their slaughter [24].

In the present study, among 29 NAL-resistant *E. coli* isolates, 3 (10.34%) chicken isolates were found *qnrB* and *qnrS* positive by PCR. It is already known that *qnr* genes increase resistance to fluoroquinolones and nalidixic acid [29]. This result and the high rate of PMQR-negative but nalidixic acid- and ciprofloxacin-resistant *E. coli* strains lead us to consider other known antimicrobial resistance mechanisms, such as chromosomal mutations in genes encoding DNA gyrase and topoisomerase IV [6]. These mutations were not investigated in the current study, but several reports have identified these mutational hotspots in sequences corresponding to amino acid positions Ser83 and/or Asp87 of the *gyrA*, while substitutions at positions Ser80 and Glu84 are frequently associated mutations in the *parC* [30,31] gene.

Previous studies from several countries exhibited the prevalence of *qnr* genes in veterinary clinical *E. coli* isolates, which varies in different regions of the world, and revealed that *qnr* genes in conjugative plasmids confer resistance to quinolones [12,13,15,32,33]. In Algeria, there is no data about PMQR in NAL-resistant

E. coli strains isolated from animals, with the exception of one study in humans, which reported a prevalence of 10% [3]. The prevalence of PMQR genes reported in this study (10.34%) was higher than that reported in the United States (0.7%) [15], the Czech Republic (4%) [11], Turkey (5.3%) [32], and Portugal (5.5%) [34], but lower than that reported in Tunisia (81.8%) [13] and Italy (90%) [12].

The *qnrB1* and *qnrS1* types were the most frequent enzymes detected in NAL-resistant *E. coli* isolated from raw chicken meat [12,13,15,32]. The *qnrB1*, *qnrB4*, and *qnrS1* enzymes have been the three *qnr* types identified in Algerian hospitals [3,35]; in this study, we described the first report of *qnrB72* in NAL-resistant *E. coli* isolated from raw chicken meat worldwide.

The results demonstrated frequent resistance to antimicrobial agents of different classes, particularly aminopenicillins, tetracycline, and cotrimoxazole. The status of the antimicrobial susceptibility of *E. coli* isolated from poultry origin obtained by other authors depended on the country [15,36,37]. For instance, in Iran among NAL-resistant *E. coli* isolates, resistance was observed to sulfamethoxazole (70.7%), ampicillin (66.8%), and ciprofloxacin (41.9%), and fewer isolates were resistant to gentamicin (7.2%) and tobramycin (6.1%) [36]. In the United States [15] among NAL-resistant avian *E. coli* isolates, the most frequent resistance was recorded to sulfamethoxazole (93.1%), tetracycline (86.2%), gentamicin (62%), and ampicillin (55.1%) (the same antimicrobial agents recorded in our study, but with more resistant isolates).

In the present study, resistance to other antibiotics, including cefoxitin and ceftazidime, was also found. Possible sources could be the contamination of meat during manipulation after the slaughtering process and/or the different antimicrobials used as therapy in live birds [12,23,25].

E. coli antimicrobial drug resistance is strongly related to phylogenetic grouping [38]. It has been observed that extraintestinal *E. coli* isolates typically belong to phylogenetic groups B2 and D, whereas commensal isolates belong to group A [18,22]. The majority of our isolates belonged to the reportedly less virulent group A, followed by groups D, B1, and B2. This pattern is comparable with those noted in some previous studies on *E. coli* isolated from raw chicken meat [38,39]. In other studies, the resistant *E. coli* involved in raw chicken meat showed a phylogenetic shift from group B1 to group D [12].

In the present study, 13.8% of the *E. coli* isolates were screened for STEC-associated virulence genes. It is well documented that STEC strains vary in their capacity to cause serious diseases in humans or animals, and this is associated with the type or amount of *stx* produced [1]. Strains that are positive for only the *stx2* allele are reported to be potentially more virulent and are more frequently associated with hemolytic uremic syndrome than those harboring only *stx1* or both alleles [1,40]. One isolate from our collection was found to carry the plasmid-encoded enterohemolysin gene *ehxA*. *ehxA* is an important STEC-associated virulence gene, which has the highest incidence of hemolytic uremic syndrome worldwide [1]. Our results confirmed the presence of virulence genes, including *stx* and *ehxA* in multidrug-resistant (MDR) *E. coli* isolated from Algerian chicken meat. Therefore, the consumption of raw or undercooked chicken meat can cause diseases such as hemolytic uremic syndrome and hemorrhagic colitis in humans.

Conclusions

The present report demonstrates a high prevalence of NAL-resistant *E. coli* in raw chicken meat. These isolates are commonly resistant to different classes of antibiotics, including those that are critically important for humans. These results highlight the importance of consumer awareness of safe handling and cooking of chicken meat. The *Shiga* toxin-producing *E. coli*-associated virulence genes *stx* and *ehxA* were detected in MDR *E. coli* isolates (13.8%). The routine screening of antimicrobial resistance among foodborne pathogenic and commensal organisms from such commodities is of great importance to help identify and manage the emerging resistance problem in the food supply. This study is the first report of the *qnrB72* gene in these strains in Algeria and in medical veterinary practice. The integration of virulence potential, antimicrobial susceptibility, and genetic analysis provides costly information on acquisition and spread of virulence and resistance genes in foodborne bacteria.

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Conflict of interests: No conflict of interests is declared.