Virulence gene profiles, antimicrobial resistance and phylogenetic groups of fecal Escherichia coli *strains isolated from broiler chickens in Algeria*

Chahinez Messaili, Yamina Messai and Rabah Bakour*

Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, PB 32 El-Alia, Bab Ezzouar, 16111 Algiers, Algeria

*Corresponding author at: Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, PB 32 El-Alia, Bab Ezzouar, 16111 Algiers, Algeria. Tel.: +213 21 24 79 13, fax: +213 21 24 72 17, e-mail: bakourrabah@gmail.com, rbakour@yahoo.fr.

Veterinaria Italiana 2019, **55** (1), 35-46. doi: 10.12834/Vetlt.799.3865.2
Accepted: 07.07.2016 | Available on line: 31.03.2019

Keywords
Avian fecal *E. coli*, Broiler chickens, Phylogenetic groups, Virulence, Antimicrobial resistance.

Summary
The objective of the study was to determine the virulence and antimicrobial resistance traits of 100 fecal *E. coli* strains isolated from clinically healthy chickens in Algeria. Most of isolates belonged to phylogroups A (45%) and B1 (37%) and showed a great diversity in DNA profiles. The genes *fimH, tsh, entB, iutA, irp2, fyuA, iroN, sitA, etsA, etsB, eitA, iss, traT, ompT, hlyF, vat, ibeA, cvaA, cvaB5', cvaB3', cvaC, cma* and *cbi* were detected. Combinations of virulence genes defined 67 virulence profiles. High resistance rates (62-97%) were noted for amoxicillin, amoxicillin-clavulanic acid, cefazolin, fluoroquinolones, tetracycline, trimethoprim, sulfonamides and sulfamethoxazole/ trimethoprim, and 93% of strains were multidrug-resistant. Combinations of resistance phenotypes defined 59 resistance patterns. The genes bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1}$, *tetA, tetB, qnrB, qnrS1, sul1, sul2, sul3, dfrA1, dfrA7, dfrA12* and *dfrA14* were identified and class 1 integrons were detected in 49% of isolates. A rate of 37% of strains was resistant to mercury, with the presence of *merA* gene. The study reports the presence in the avian strains isolated from fecal swabs of virulence genes of plasmid origin characteristic of ExPEC strains associated with high resistance to first-line antibiotics and class 1 integrons, this augurs a risk for human and animal health.

Geni di virulenza, resistenza antimicrobica e correlazione filogenetica di ceppi di E. coli *isolati da allevamenti di* broiler *in Algeria*

Parole chiave
E. coli fecale aviare, Gruppi filogenetici, Polli, Resistenza antimicrobica, Virulenza.

Riassunto
L'obiettivo dello studio è stato quello di determinare i caratteri di virulenza e di resistenza antimicrobica di 100 ceppi di *E. coli* fecali isolati da polli clinicamente sani in Algeria. La maggior parte degli isolati apparteneva ai filogruppi A (45%) e B1 (37%) e mostrava una grande diversità nei profili genetici. Sono stati rilevati i geni *fimH, tsh, entB, iutA, irp2, fyuA, iroN, sitA, etsA, etsB, eitA, iss, traT, ompT, hlyF, vat, ibeA, cvaA, cvaB5', cvaB3', cvaC, cma* e *cbi*. Le combinazioni tra i geni di virulenza hanno permesso di definire 67 profili di virulenza. Sono stati rilevati alti tassi di resistenza (62-97%) per amoxicillina, amoxicillina-acido clavulanico, cefazolina, fluorochinoloni, tetraciclina, trimetoprim, sulfonamidi e sulfametossazolo/ trimetoprim e il 93% dei ceppi presentavano resistenza multipla. Sono stati identificati i geni bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1}$, *tetA, tetB, qnrB, qnrS1, sul1, sul2, sul3, dfrA1, dfrA7, dfrA12* e *dfrA14*; gli integroni di classe 1 sono stati rilevati nel 49% degli isolati. Una percentuale del 37% dei ceppi era resistente al mercurio, con la presenza del gene merA. Lo studio riporta la presenza, nei ceppi aviari isolati da tamponi fecali, di geni di virulenza di origine plasmidica caratteristici dei ceppi ExPEC associata ad un'elevata resistenza agli antibiotici di prima linea e di integroni di classe 1: un rischio per la salute umana e animale.

Introduction

Most *Escherichia coli* are commensal bacteria present in the gut of humans and warm-blooded animals. However, some strains can harbor virulence genes and might be associated with various intestinal (InPEC strains) or extraintestinal (ExPEC strains) infections in humans and animals (Bélanger *et al.* 2011). Different combinations of virulence factors define pathotypes, which are specific of a type of infection. The ExPEC group includes UPEC (uropathogenic), NMEC (newborn meningitic), SePEC (septicaemia associated) and APEC (avian pathogenic) strains (Bélanger *et al.* 2011). Eight phylogenetic groups are now recognized in *E. coli*: seven (A, B1, B2, C, D, E, F) belong to *E. coli sensu stricto*, whereas the eighth is the *Escherichia* cryptic clade I (Clermont *et al.* 2013). A and B1 groups generally include commensal strains and certain intestinal pathogens while B2 and to a lesser extent D groups are characteristic of extraintestinal pathogens (Ewers *et al.* 2007, Mellata 2013). Avian colibacillosis caused by APEC strains is one of the major causes of economic losses in the poultry industry in Algeria and throughout the world; it manifests by various injuries as airsacculitis, peritonitis, polyserositis and sepsis. APEC strains are characterized by the expression of various virulence factors including adhesins, toxins, iron uptake systems and serum resistance (Johnson *et al.* 2008, Bélanger *et al.* 2011, Mellata 2013). There are genotypic similarities between APEC and human ExPEC, mainly UPEC and NMEC, suggesting zoonotic potential among APEC strains (Bélanger *et al.* 2011, Mellata 2013). In addition to the virulence factors, antibiotic resistance of bacteria determines their impact in infectious diseases. The increase of antibiotic resistance has worldwide reached alarming proportions; it is inherent in large part to the widespread use of antibiotics in intensive livestock, especially poultry (Mellata 2013). Healthy poultry is considered the main reservoir of virulence genes and antibiotic resistance (Rodriguez-Sieck *et al.* 2005, Ewers *et al.* 2009). Pathogenic strains result generally from commensals by the acquisition of infectious capacity through horizontal transfer of virulence genes (Johnson and Nolan 2009, Mellata *et al.* 2010). In addition to inter-animal transfer, virulent and/or resistant bacteria and genes can even reach humans via contaminated environment and food chain (Graham *et al.* 2008, Vincent *et al.* 2010). In order to better understand and monitor the emergence of pathogens and antibiotic-resistant strains from healthy animal reservoir, it is necessary to know the presence and prevalence of virulence factors and antibiotic resistance. These investigations are very important in Algeria, because of the lack of data on this issue in this country, especially since veterinary practices, farming conditions and environment have a considerable impact on the evolution of intestinal flora in terms of virulence and antimicrobial resistance. In this context, the objective of this study was to assess the prevalence of virulence factors and antimicrobial resistance and to determine the phylogenetic groups in fecal *E. coli* strains isolated from clinically healthy broiler chickens.

Materials and methods

Bacterial strains

One hundred non-repetitive avian fecal *E. coli* strains (one isolate, one chicken) were recovered from intestines of 45-47 day old clinically healthy chickens using sterile cotton swabs. Chickens were from five poultry farms in the center of Algeria: Rouiba, Shaoula, Lakhdaria, Tizi-Ouzou, Bejaia. Strains were identified using API 20E identification system (bioMérieux, France) and by PCR detection of *iudA* gene (beta-glucuronidase) (Clermont *et al.* 2013).

Phylogenetic grouping and genotyping of isolates

Phylogenetic groups of strains were determined as previously described (Clermont *et al.* 2013). Molecular typing of strains was performed by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) using primer ERIC2 (Messai *et al.* 2008). ERIC profiles are compared visually and those dissimilar by one band or more were considered as different.

PCR detection of virulence-associated gene

The DNA template for PCR was extracted using the boiling method (Feria *et al.* 2002). All isolates were screened for the presence of 31 virulence associated genes (VAGs) by multiplex and simplex PCR (Table I). *E. coli* EC7372, ECOR66 and CFT073 were used as control strains.

Biofilm formation assay

Biofilm formation (BF) ability was assessed as previously described (Naves *et al.* 2008). Briefly, a 100 fold dilution in LB medium of an overnight bacterial culture was distributed in 96-well polystyrene microplate. After incubation at 37 °C for 24 h, the optical density (OD) of bacterial growth was measured at 630 nm. Plate was then emptied, washed with sterile distilled water, air dried for 20 min and stained with 130 µl of 1% crystal violet for 5 min. Dye was discarded, and plate was washed five times to remove excess dye and air dried for 1 hour. The biofilm-bound dye was eluted with 130 µl of 95% ethanol and the absorbance was measured at 570

nm. The Specific Biofilm Formation index (SBF) was determined as follow: SBF = T - C/G. T is OD_{570} value of wells containing strains to be tested; C is OD_{570} of control wells containing bacteria-free medium and G is OD_{630} of bacterial growth. Strains were classified as weak biofilm producer (SBF ≤ 1) or strong biofilm producer (SBF > 1).

Antimicrobial susceptibility testing

Antibiotic susceptibility was tested by the disk diffusion method according to guidelines of antibiogram committee of the French Society for Microbiology (CA-SFM 2013) (www.sfm-microbiologie.org). The following disks of antibiotics (Bio-Rad, Marnes la Coquette, France) were used (µg/ disk): amoxicillin (AMX) (25 µg), amoxicillin/clavulanic acid (AMC) (20 µg/10 µg), cefazolin (CZ) (30 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg), nalidixic acid (NA) (30 µg), ciprofloxacin (CIP) (5 µg), pefloxacin (PEF) (5 µg), ofloxacin (OFX) (5 µg), tetracycline (TE) (30 µg), kanamycin (K) (30 µg), netilmicin (NET) (30 µg), gentamicin (GM) (15 µg), sulfonamides (SSS) (200 µg), trimethoprim (TMP) (5 µg), sulfamethoxazole/trimethoprim (SXT) (1.25 µg/23.75 µg) and colistin (CS) (50 µg). *E. coli* ATCC 25922 was used as a control strain.

Multiple antibiotic resistance index (MAR) was used to check the antibiotic resistance. MAR is calculated as a ratio a/b, 'a' is the number of antibiotics to which the isolate is resistant and 'b' is the total number of antibiotics to which it is exposed.

Table I. *All primer sequences used in PCR for detecting* E. coli *virulence associate genes. — cont'd*

Gene	Primer sequence (5'-3')	Annealing temp. (C°)	Expected size (bp)	Reference
		Adhesion		
papA	atggcagtggtgtcttttggtg cgtcccaccatacgtgctcttc	63°	720	Johnson and Stell 2000
fimH	tgcagaacggataagccgtgg gcagtcacctgccctccggta	63°	508	Johnson and Stell 2000
afa/draBC	ggcagagggccggcaacaggc cccgtaacgcgccagcatctc	63°	559	Johnson and Stell 2000
bmaE	atggcgctaacttgccatgctg agggggacatatagccccttc	63°	507	Johnson and Stell 2000
sfa/focDE	ctccggagaactgggtgcatcttac cggaggagtaattacaaacctggca	63°	410	Johnson and Stell 2000
tsh	gggaaatgacctgaatgctgg ccgctcatcagtcagtaccac	60°	420	Johnson *et al.* 2006a
		Protectins		
kpsMTII	gcgcatttgctgatactgttg catccagacgataagcatgagca	63°	272	Johnson and Stell 2000
kpsMTIII	tcctcttgctactattccccct aggcgtatccatccctcctaac	63°	392	Johnson and Stell 2000
		Serum resistance		
iss	cagcaacccgaaccacttgatg agcattgccagagcggcagaa	63°	323	Johnson *et al.* 2008
traT	ggtgtggtgcgatgagcacag cacggttcagccatccctgag	63°	290	Johnson *et al.* 2008
		Iron-Related		
entB	atttcctcaacttctggggc agcatcggtggcggtggtca	57°	371	El Fertas-Aissani *et al.* 2013
iroN	aagtcaaagcaggggttgcccg gacgccgacattaagacgcag	63°	667	Johnson *et al.* 2006a
fyuA	gcgacgggaagcgatgattta taaatgccaggtcaggtcact	56°	547	El Fertas-Aissani *et al.* 2013
irp2	aaggattcgctgttaccggac tcgtcgggcagcgtttcttct	57°	287	El Fertas-Aissani *et al.* 2013
iutA	ggctggacatcatgggaactgg cgtcgggaacgggtagaatcg	63°	300	Johnson and Stell 2000
sitA	agggggcacaactgattctcg taccgggccgttttctgtgc	59°	608	Johnson *et al.* 2006a
eitA	acgccgggttaatagttgggagatag atcgatagcgtcagcccggaagttag	60°	450	Johnson *et al.* 2006a
etsA	caactgggcgggaacgaaatcagga tcagttccgcgctggcaacaacctac	60°	284	Johnson *et al.* 2006a
etsB	cagcagcgcttcggacaaaatctcct ttccccaccactctccgttctcaaac	60°	380	Johnson *et al.* 2006a

continued

Table I. Table I. *All primer sequences used in PCR for detecting* E. coli *virulence associate genes. — cont'd*

Gene	Primer sequence (5'-3')	Annealing temp. (C°)	Expected size (bp)	Reference
		Toxins		
vat	aacggttggtggcaacaatcc agccctgtagaatggcgagta	58°	420	Restieri *et al.* 2007
hlyA	aacaaggataagcactgttctggct accatataagcggtcattcccgtca	63°	1177	Johnson and Stell 2000
hlyF	ggccacagtcgtttagggtgcttacc ggcggtttaggcattccgatactcag	63°	450	Johnson *et al.* 2008
cnf1	aagatggagtttcctatgcaggag cattcagagtcctgccctcattatt	63°	498	Johnson and Stell 2000
		Colicins		
cvaA	atccgggcgttgtctgacgggaaagttg accagggaacagaggcacccggcgtatt	63°	319	Johnson *et al.* 2006a
cvaB5'	tggccacccgggctctttcactggagtt atgcgggtctgcagggtttccgactgga	63°	550	Johnson *et al.* 2006a
cvaB3'	ggcccgtgccgcctcctatttta tcccgcaccggaagcaccagttat	63°	247	Johnson *et al.* 2006a
cvaC	cacacacaaacgggagctgtt cttcccgcagcatagttccat	63°	679	Johnson and Stell 2000
cbi	acaagacagcaccagttatgggtatt gttgttggttttgttggcgtagttat	63°	430	Johnson *et al.* 2006b
cma	cagcgccattaccccataaatagtga ggttcgttcgccggtgtaagcgttag	63°	498	Johnson *et al.* 2006b
		Miscellaneous		
ompT	tcatcccggaagcctccctcactactat tagcgtttgctgcactggcttctgatac	63°	496	Johnson *et al.* 2008
ibeA	aggcaggtgtgcgccgcgtac tggtgctccggcaaaccatgc	63°	170	Johnson and Stell 2000

The screening of isolates for ESBL production was performed by the Double-Disc Synergy Test (DDST) (Messai *et al.* 2008).

Heavy metal susceptibility was assessed by the agar-dilution method on Mueller Hinton medium, the following heavy metal concentrations were tested: $HgCl_2$: 2.7, 13.57, 27.15 and 54.3 µg/ml; $CuCl_2$ and $ZnCl_2$: 100, 200, 400, 800, 1600 and 3,200 µg/ml; $Pb(NO3)_2$: 400, 800, 1,600, 2,400 and 3,200 µg/ml; $Cd(NO_3)_2$. $4H_2O$: 7, 12.5, 25, 50, 100, 200, 400 and 800 µg/ml. MIC values indicative of metal resistance were: 200 µg/ml for Cd^{2+}, 1,600 µg/ml for Zn^{2+}, 3,200 µg/ml for Cu^{2+} and Pb^{2+} (Calormiris *et al.* 1984) and 27.15 µg/ml for Hg^{2+} (Edlund *et al.* 1996).

Detection of antibiotic resistance genes and integrons

Simplex and multiplex PCR were used for the detection of following resistance genes as previously described: β-lactamases bla_{TEM}, bla_{SHV} and bla_{CTX-M} (Messai *et al.* 2008); plasmid mediated quinolone resistance (PMQR) genes *qnrA, qnrB, qnrS*, and *qepA* (Figueira *et al.* 2011); aminoglycoside-modifying enzyme *aac(6')-Ib* (Figueira *et al.* 2011); tetracycline efflux pumps *tetA* and *tetB* (Guardabassi *et al.* 2000); dihydropteroate synthases *sul1*, *sul2* and *sul3* (Frank *et al.* 2007, Messai *et al.* 2008); dihydofolate reductase *dfrA1, dfrA5, dfrA7, dfrA8* and *dfrA12* (Šeputienė *et al.* 2010); mercuric reductase *merA* (Bass *et al.* 1999).

Class 1 integrons were searched by multiplex PCR targeting *int1, sul1* and *qacEΔ1* genes (Messai *et al.* 2008).

PCR products of positive reactions for *qnrB, qnrS,* bla_{CTX-M}, *dfrA1, dfrA5, dfrA7* and *dfrA12* were sequenced and analyzed with the BLAST and FASTA programs of the National Center of Biotechnology Information (www.ncbi.nlm.nhi.gov).

Conjugation assay and plasmid analysis

Conjugation assay was performed for the cefotaxime resistant strain using sodium azide resistant *E. coli* BM21 as recipient. Exponential culture of donor isolate (1 volume) and recipient (2 volumes) were inoculated as a spot on Brain Heart Infusion Agar (BHIA). After overnight incubation at 37 °C, transconjugants were selected on Mueller Hinton agar supplemented with cefotaxime (4 µg/ml) and sodium azide (300 µg/ml). Plasmid DNA was extracted by alcalin lysis method (Kado and Liu 1981) and analyzed by electrophoresis on 0.7% (wt/vol) agarose gels at 5 volts/cm. Plasmid size was estimated by using reference plasmids, pRK2013 (48 kb) and pIP173 (126.8 kb).

Plasmid incompatibility group was determined by a PCR-based replicon typing method (Carattoli *et al.* 2005).

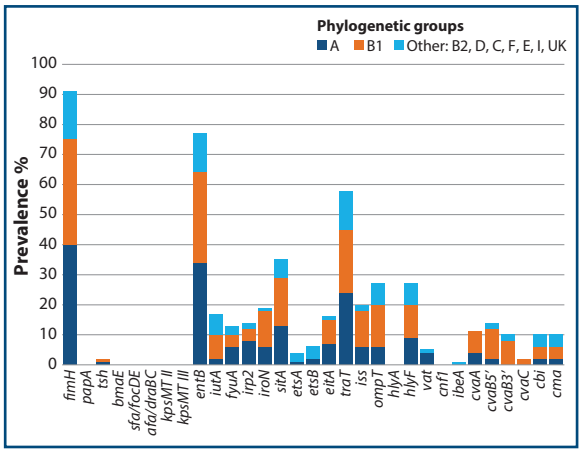

Figure 1. *Prevalence of virulence-associated genes and their distribution according to phylogenetic groups.*

Statistical analysis

For comparison of rates, Fisher's exact test was used, $p < 0.05$ was considered significant.

Results

Phylogenetic analysis allowed to assign isolates to phylogroups A (45%), B1 (37%), B2 (3%), C (1%), D (3%), E (4%), F (4%), I (1%) and unknown (2%). Molecular typing of strains by ERIC-PCR showed 80 different genetic profiles. The phenotypic and genotypic screening for 31 virulence-associated genes (Figure 1) showed the presence of *fimH* gene in almost all strains (91%), while the other adhesin genes (p*apA, bmaE, sfa/focDE, afa/drABC*) were absent. The temperature-sensitive hemagglutinin gene *tsh* was detected in 2% of strains. Genes of iron acquisition and transport, *entB, iutA, irp2, fyuA, iroN, sitA, etsA, etsB* and *eitA* were found in 77%, 17%, 14%, 13%, 19%, 35%, 4%, 6% and 16% of strains, respectively. Serum resistance-associated genes, *iss* and *traT*, were present at rates of 20% and 58%, respectively. Capsular genes *kpsMTII* and *kpsMTIII* were not found. A rate of 27% of strains harbored *ompT* and avian hemolysin *hlyF* genes. The *vat* gene was present in 5% of strains and, hemolysin A and cytotoxic necrotizing factor 1 genes, *hlyA* and *cnf1*, were absent. The invasion gene *ibeA* was detected in a single strain (1 %). The colicin V, B and M operon genes, *cvaA, cvaB5', cvaB3', cvaC, cma* and *cbi*, were present in 12%, 14%, 10%, 2%, 10% and 10% of strains, respectively, revealing a total of 24% of strains harboring a presumptive plasmid ColV and/or ColBM (ColV: 14%, ColBM: 6%, ColV+ColBM: 4%). All strains had a biofilm formation (BF) ability, of which 20% were strong biofilm producers. The combinations of the different virulence factors allowed to distinguish 67 virulence profiles including 0 to 16 virulence genes, and based on the gene combination "*iutA, hlyF, iss, iroN, ompT*", 5% of strains could be assigned to APEC pathotype (Johnson *et al.* 2008) (Table II). The Fisher's exact test showed significant association between *colV* operon and phylogenetic group B1, with a rate of 29.7% in B1 strains versus 11.1% in the non-B1 strains ($P = 0.021$).

Antibiotic susceptibility testing showed high level of resistance to amoxicillin (97%), amoxicillin-clavulanic acid (72%), cefazolin (73%), nalidixic acid (97%), ofloxacin (78%), ciprofloxacin (62%), pefloxacin (68%), tetracycline (90%), trimethoprim (75%), sulfonamides (75%) and sulfamethoxazole/ trimethoprim (69%). Resistance rates of 53%, 6% and 5% were observed

Table II. *Virulence and antimicrobial resistance patterns of some representative* E. coli *strains. — cont'd*

Phylo-group	DNA profile	Strain	Virulence gene profile	Antimicrobial resistance pattern	Resistance and integron genes
A	E40	S67	*fimH entB traT hlyF colV*	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SXT	bla_{TEM} *tetA*
	E73	S111	*fimH entB traT eit vat BF*	AMX AMC CZ NA CIP PEF TE K SSS TMP SXT	bla_{TEM} *tetA sul2 sul3*
	E38	S65	*fimH entB yers hlyF colV*	AMX NA CIP PEF OFX TE K SSS	*tetA tetB sul2*
	E53	S84	*fimH entB sitA iss ompT hlyF*	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SSS TMP SXT	bla_{TEM} bla_{SHV}
	E80	S118	*fimH iroN sitA iss ompT hlyF*	AMX AMC CZ NA CIP PEFOFX	bla_{TEM}
	E22	S37	*fimH entB yers sitA traT ompT hlyF*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT Hg	*tetA sul1 sul2 dfrA12 qacEΔ1 merA*
	E39	S66	*fimH entB iroN sitA iss hlyF colBM*	AMX CZ NA CIP PEF	bla_{TEM}
	E8	S14	*fimH entB iutA sitA ets traT iss colBM colV*	AMX AMC CZ NA CIP PEF OFX TE K SSS Hg	bla_{TEM} bla_{SHV} *tetB sul2 int1*
	E78	S116	*fimH entB iroN sitA eit traT iss ompT hlyF*	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SXT	bla_{TEM} bla_{SHV} *tetA sul2 qacEΔ1 int1*
	E45	S72	*fimH tsh entB iroN iutA sitA ets eit traT iss ompT hlyF colV*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} bla_{SHV} *tetB sul1 sul2 dfrA7 int1 qacEΔ1*

continued

Table II. *Virulence and antimicrobial resistance patterns of some representative* E. coli *strains. — cont'd*

Phylo-group	DNA profile	Strain	Virulence gene profile	Antimicrobial resistance pattern	Resistance and integron genes
B1	E47	S75	*fimH entB iutA sitA traT*	AMX AMC NA CIP PEF OFX TE K SSS	bla_{TEM} *tetB sul2*
	E8	S12	*fimH entB iroN ompT hlyF* BF	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} *tetA dfrA5 int1*
	E29	S53	*fimH sitA traT iss ompT colV*	AMX AMC NA CIP PEF OFX TE K SSS TMP SXT Hg	bla_{TEM} *tetA sul2 dfrA5 merA int1*
	E37	S63	*fimH entB iroN sitA traT iss ompT*	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SXT Hg	bla_{TEM} bla_{SHV} *tetA sul2 dfrA1 dfrA5 merA int1*
	E69	S103	*fimH entB iroN sitA iss ompT hlyF*	AMX CZ NA CIP PEF OFX TE	bla_{TEM} bla_{SHV} *tetA*
	E59	S93	*fimH entB sitA eit iss ompT hlyF colV*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} *tetA sul2 dfrA5 merA int1*
	E31	S55	*fimH entB iroN sitA traT ompT colBM colV*	AMX AMC NA TE K	bla_{TEM} bla_{SHV} *tetA*
	E64	S98	*fimH entB iroN sitA traT iss ompT hlyF colV*	AMX AMC CZ NA CIP PEF TE SSS TMP SXT Hg	bla_{TEM} bla_{SHV} *tetA sul2 dfrA5 merA int1*
	E52	S83	*fimH iroN iutA sitA eit iss ompT hlyF colV*	AMX AMC CZ NA OFX TE K	bla_{TEM} *tetA dfrA5 int1*
	E63	S97	*fimH entB iutA sitA traT iss ompT hlyF colV*	AMX AMC CZ NA CIP PEF TE K SSS TMP SXT	bla_{TEM} bla_{SHV} *tetA tetB sul2 dfrA7 int1*
	E19	S31	*fimH entB iroN sitA traT iss ompT hlyF colBM*	AMX AMC NA CIP PEF OFX TE K SSS TMP SXT Hg	*tetA sul1 dfrA12 qacEΔ1*
	E50	S81	*fimH entB iroN iutA sitA traT ompT hlyF colV*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} bla_{SHV} *tetA sul2 dfrA5 merA int1*
	E16	S27	*fimH tsh entB iroN iutA sitA traT iss colBM colV*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} *tetB sul1 sul2 sul3 dfrA7 qacEΔ1*
	E51	S82	*fimH iroN iutA sitA eitA traT iss ompT hlyF colV*	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SXT	bla_{TEM} bla_{SHV} *tetA sul2 dfrA5 int1*
	E72	S110	*fimH entB iroN fyuA irp2 iutA sitA eit traT iss ompT hlyF colV* BF	AMX AMC CZ NA CIP PEF OFX TE SSS TMP SXT Hg	*tetB sul2 dfrA5 merA int1*
D	E12	S19	*fimH IutA sitA ets ompT hlyF colBM*	AMX NA CIP PEF TE SSS TMP SXT	bla_{SHV} *tetA sul2*
B2	E33	S58	*fimH entB*	AMX AMC OFX	*qnrB*
	E20	S35	*fimH entB traT*	AMX AMC CZ NA CIP PEF OFX TE SSS	bla_{TEM} *tetA*
	E37	S64	*fimH entB ompT*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{SHV} *tetA sul3 dfrA5 int1*
F	E54	S86	*iutA ibeA sitA colV* BF	AMX AMC CZ NA CIP PEF OFX TE K NET SSS TMP SXT	bla_{TEM} *tetB sul1 sul2 dfrA7 qacEΔ int1*
	E61	S95	*fimH entB iutA sitA ets traT ompT hlyF* BF	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} *tetA sul2 dfrA5 int1*
	E60	S94	*fimH entB iutA sitA ets eit traT ompT hlyF colV*	AMX CZ NA CIP PEF OFX TE K SSS TMP SXT	bla_{TEM} *tetA sul2 dfrA5 int1*
	E30	S54	*fimH entB iutA fyuA sitA ets traT ompT hlyF vat colBM*	AMX AMC CZ NA CIP PEF OFX TE K SSS TMP SXT Hg	bla_{TEM} bla_{SHV} *tetA sul2 dfrA5 merA int1*
E	E9	S15	*fimH iroN IutA sitA traT iss ompT hlyF colBM colV*	AMX AMC CZ CTX NA CIP PEF TE K SSS TMP SXT Hg	bla_{TEM} bla_{SHV} $bla_{CTX-M-1}$ *tetA sul1 sul2 sul3 dfrA12 qacEΔ1 merA*
C	E5	S6	*fimH entB fyuA, irp2 iutA traT iss ompT hlyF colBM*	AMX AMC NA OFX TE Hg	bla_{SHV} *tetA*

for kanamycin, netilmicin and gentamicin. One strain (1%) was resistant to cefotaxime and positive for DDST. All strains were susceptible to ceftazidime, amikacin and colistin (Figure 2). Molecular identification by PCR and sequencing of resistance genes (Figure 3) revealed the presence of broad-spectrum beta-lactamase (BSBL) genes bla_{TEM}, bla_{SHV} and extended-beta-lactamase (ESBL) $bla_{CTX-M-1}$ allele in 70%, 50% and 1% of isolates, respectively. ESBL allele $bla_{CTX-M-1}$ and, amoxicilline, cefazolin and cefotaxime resistance phenotypes of the cefotaxime-resistant strain were transferable by conjugation in association with an *IncI1* plasmid of about 118 kb. Tetracycline resistance genes *tetA* and *tetB* were detected in 74% and 12% of the strains. Plasmid mediated quinolone resistance determinants *qnrB* and *qnrS1* were present in 12% and 1% of strains. Sulfonamide resistance genes *sul1*, *sul2* and *sul3* were identified in 14%, 53% and 10% of strains and trimethoprim resistance genes *dfrA1*, *dfrA7*, *dfrA12* and *dfrA14* were present in 5%, 7%, 13% and 43% of strains, respectively. The combinations of resistance phenotypes allowed distinguish 59 antibiotic resistance patterns

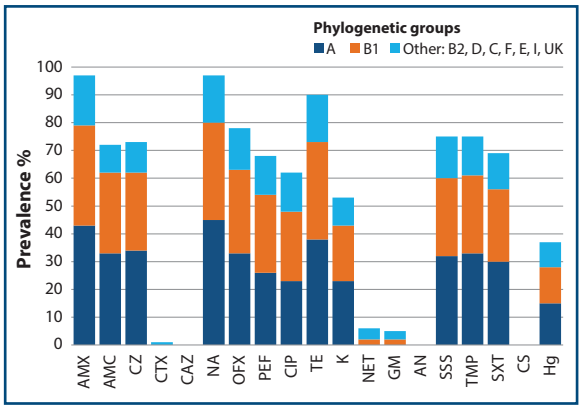

Figure 2. *Prevalence of* E. coli *antimicrobial resistance phenotypes and their distribution according to phylogenetic groups.*

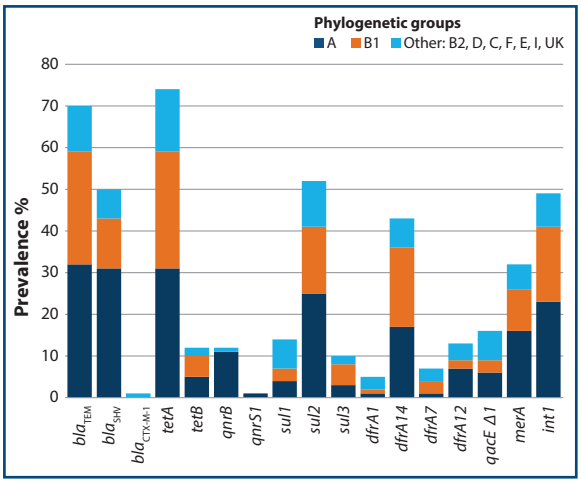

Figure 3. *Prevalence of* E. coli *antimicrobial resistance genes and their distribution according to phylogenetic groups.*

(resistance to 1-7 antibiotic families); 93% of strains had a multidrug-resistance (MDR) phenotype, they resisted to at least three antibiotic classes, and 95% had a MAR index from 0.27 to 0.72. No statistically significant association was found between antibiotic resistance and phylogenetic groups. Class 1 integrons were detected in 49% of strains, fourty five (91.8%) of them lacked the 3'-conserved sequence (3'-CS) that contains *qacEΔ1* and *sul1* and one lacked only *sul1*. In these cases, sulfonamides resistance was conferred by *sul2* and/or *sul3*. Furthermore, 10% of strains contain *qacEΔ1* gene in absence of integrons.

Agar dilution MICs of Cd^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+} were, respectively, from 25 µg/ml to 50 µg/ml, 100 µg/ml to 400 µg/ml, 800 µg/ml to 1,600 µg/ml, 3,200 µg/ml and 2.7 µg/ml to > 54.3 µg/ml. A total of 37 strains were resistant to mercury, of which 26 (70.2%) harbored *merA* gene, and 69.2% of *merA* were associated with class 1 integrons. All strains were susceptible to the other heavy metals tested.

There were significant associations between antimicrobial resistance and virulence factors.

Table III. *Association between* E. coli *virulence genes and antimicrobial susceptibility phenotypes (P < 0.05).*

Virulence gene (%)	% of virulence gene among resistance strains - % of virulence gene among susceptible strains *P* Antimicrobials (% resistance , % susceptibility)										
	AMC (72, 28)	**CZ (73, 27)**	**CIP (62, 38)**	**PEF (68, 32)**	**OFX (78, 22)**	**TE (90, 10)**	**K (53, 47)**	**SSS (75, 25)**	**TMP (75, 25)**	**SXT (69, 31)**	**Hg (37, 63)**
iroN (19)	22.22-10.71	20.53-14.81	**25.8-7.89 0.035***	18.60-9.37	19.23-18.18	18.89-20	18.87-19.15	18.67-20	18.67-20	20.29-16.13	18.91-19.05
fyuA (13)	12.5-14.28	**8.21-25.92 0.039****	11.29-15.79	10.29-18.75	**8.97-27.27 0.034****	14.44-0	9.43-17.02	**8-28 0.016****	10.66-20	7.25-25.80	**24.32-6.35 0.014***
irp2 (14)	11.11-21.43	**13.51-33.33 0.001****	9.68-21.05	10.29-21.05	10.26-27.27	15.55-0	9.43-19.15	**8-32 0.005****	13.33-16	7.24-29.03	**27.03-6.35 0.006***
iutA (17)	20.83-7.14	19.18-11.11	**24.19-5.26 0.014***	22.06-6.25	17.95-13.64	18.89-0	24.53-8.51	20-8	17.33-16	18.84-12.90	13.51-19.05
sitA (35)	40.28-21.43	36.99-29.63	**48.39-13.15 0.0004***	**44.11-15.62 0.006***	37.18-27.27	36.67-20	43.39-25.53	40-20	37.33-28	40.57-22.58	35.13-34.92
traT (58)	58.33-57.14	56.16-62.96	58.06-57.89	52.94-68.75	53.85-72.73	**63.33-10 0.001***	**67.92-46.81 0.043***	62.66-44	64-40	62.32-48.39	67.57-52.38
iss (20)	23.61-10.71	21.91-14.81	**29.03-5.26 0.004***	**26.47-6.25 0.017***	20.51-18.18	20-20	18.87-21.28	20-20	18.67-24	20.29-19.35	21.62-19.05
ompT (27)	31.94-14.28	28.77-22.22	**38.70-7.89 0.0009***	**35.29-9.37 0.007***	26.92-27.27	28.89-10	32.07-21.27	**29.33-20 0.001***	**29.33-20 0.001***	**31.88-16.13 0.001***	27.03-26.98
hlyF (27)	27.78-25	28.77-22.22	**37.09-10.53 0.004***	**33.82-12.5 0.030***	24.36-36.36	28.89-10	26.41-27.66	20.67-28	26.67-28	27.54-25.81	24.32-28.57
cvaA (12)	15.28-3.57	15.07-3.70	**17.74-2.63 0.027***	16.18-3.12	12.82-9.09	13.33-0	16.98-6.38	14.67-4	12-12	13.04-9.68	10.81-12.69
cvaB5'(14)	**19.44-0 0.004***	16.44-7.40	19.35-5.26	17.65-6.25	14.10-13.63	15.55-0	**22.64-4.25 0.009***	16-8	14.67-12	15.94-9.68	13.52-14.28

Positive* and negative** significant association between virulence gene and antimicrobial resistance ($P < 0.05$).

Resistance to ciprofloxacin was distinguished by its association to 7 virulence genes (*iutA, iroN, sitA, iss, ompT, hlyF, cvaA*) (Table III).

Discussion

Most of our isolates (82%) belonged to A and B1 phylogenetic groups in accordance with many previous studies (Johnson *et al.* 2008, Bonnet *et al.* 2009, Hiki *et al.* 2014). The clonal relationship investigated by ERIC-PCR showed a large genetic diversity among strains. Almost all of our isolates (99%) possessed at least one of the examined virulence genes. Type 1 fimbriae are ubiquitous among *E. coli* strains including APEC, they are involved in the initiation of the colonization of respiratory tract epithelium (Wooley *et al.* 1998). According to our result (2%), *tsh* was reported at low frequency (10%-11.2%) in avian fecal *E. coli*, while it was described at higher rates (49.7%-97.7%) in APEC strains; it would have a role in the colonization of tracheal mucosa and in the development of lesions in the air sacs, it was proposed as a marker of APEC (Dozois *et al.* 2000, Dozois *et al.* 2003, Amabile de Campos *et al.* 2005, Bonnet *et al.* 2009). Seven iron-related genes were detected; enterobactin was reported among pathogens and commensals; however, it was found in our study at a rate higher (77%) than in commensals (3%-13.2%) and close to those in APEC (40%-75%) (Amabile de Campos *et al.* 2008, Bonnet *et al.* 2009). The transformation of enterobactin to salmochelin (C-glucosylation) mediated by *iroBCDEN* gene cluster can prevent siderocalin binding (Dozois *et al.* 2003, Garénaux *et al.* 2013). Gene *iroN* representative of this gene cluster was detected at rate (19%) close to that reported in fecal strains by Johnson and colleagues (21%) (Johnson *et al.* 2008), but lower than in study of Bonnet and colleagues (62.4%) (Bonnet *et al.* 2009). Aerobactin and Yersiniabactin were reported at high frequency in pathogenic strains (Amabile de Campos *et al.* 2008, Bonnet *et al.* 2009), their significant role in APEC virulence was demonstrated (Dozois *et al.* 2003, Tuntufye *et al.* 2012). Yersiniabactin allowed evasion of siderocalin and prevents reactive oxygen species production by innate immune cells. The rate of aerobactin was close to those of fecal *E. coli* in certain studies (12.2%-15%) (Amabile de Campos *et al.* 2008, Bonnet *et al.* 2009, Kemmett *et al.* 2013) but lower than in others (25.9%, 35.5%) (Rodriguez-Sieck *et al.* 2005, Johnson *et al.* 2008). The prevalence of yersiniabactin (14%) is consistent with that reported in fecal *E. coli* by Amabile de Campos and colleagues (13%) (Amabile de Campos *et al.* 2008) and Kemmett and colleagues (11%) (Kemmett *et al.* 2013), whereas it was lower than in studies of Rodriguez-Sieck and colleagues (30.1%) (Rodriguez-Sieck *et al.* 2005), Johnson and colleagues (30%) (Johnson *et al.* 2008) and Bonnet and colleagues (32%) (Bonnet *et al.* 2009). The gene *sitA* belongs to *sitABCD* operon encoding an iron and manganese ABC transport system, whose role in virulence and resistance to oxidative stress of APEC was demonstrated (Sabri *et al.* 2008). The gene *sitA* was mostly associated with pathogenic strains than fecal ones; however, its rate in our strains (35%) was higher than those reported in fecal strains (27%, 19%) (Amabile de Campos *et al.* 2008, Kemmett *et al.* 2013). Genes *eitA* and *etsA/etsB* are located in the *eitABCD* and *etsABC* operons encoding putative iron ABC transporters identified in high pathogenic APEC and induced *in vivo* during infection in chickens (Johnson *et al.* 2008, Tuntufye *et al.* 2012); their prevalence (16%, 4%, 6%) was relatively lower than that reported by Johnson and colleagues in commensal strains (43%, 43%, 44%) (Johnson *et al.* 2008). Serum resistance is one of the pathogenicity mechanisms of APEC strains, there is a correlation between serum resistance and the ability of bacteria to persist in body fluids and internal organs (Mellata *et al.* 2003). Genes *iss* and *traT* involved at least in part in serum resistance, were detected at rates below (20%, 58%) those reported in fecal *E. coli* by Bonnet and colleagues (*traT*, 86.3%) (Bonnet *et al.* 2009) and Johnson and colleagues (*iss*, 60%) (Johnson *et al.* 2008), consistent with results of Hiki and colleagues (*iss*, 20.5%) (Hiki *et al.* 2014) and higher than in Kemmett and colleagues (*iss*, 10%) (Kemmett *et al.* 2013). The avian hemolysin gene *hlyF*, epidemiologically associated to the most virulent APEC, was found at prevalence (27%) close to that of Hiki and colleagues (28.2%) (Hiki *et al.* 2014). The serine protease gene *ompT*, involved in providing defense against cationic antimicrobial peptides secreted by the epithelial cells and macrophages, was present at rate (27%) close to result of Hiki and colleagues (29.5%) (Hiki *et al.* 2014), but under those recorded by Rodriguez-Sieck and colleagues (45.2%) (Rodriguez-Sieck *et al.* 2005), Johnson and colleagues (42%, 47%) (Johnson *et al.* 2008) and Bonnet and colleagues (46.7%) (Bonnet *et al.* 2009). The invasion-related gene *ibeA* was present in our strains at low rate (1%) compared to that previously reported in fecal strains (7.7%, 16%) (Rodriguez-Sieck *et al.* 2005, Kemmett *et al.* 2013). The prevalence of *vat* gene (5%) matches that reported by Kemmett and colleagues (6%) (Kemmett *et al.* 2013) in fecal strains. Biofilm is a form of bacterial resistance to antimicrobials, opsonization and phagocytosis. Rate of strong biofilm producers among our strains was lower (20%) than the 30% reported by Skyberg and colleagues (Skyberg *et al.* 2007).

Most of the detected genes (*iutA, fyuA, irp2, iroN, fimH, cvaC, traT, iss, sitA, ompT, hlyF, cvaA, etsA, etsB, eitA, tsh*) have been described in pathogenicity islands associated with virulence plasmids in APEC, of which ColV and ColBM plasmids as pAPEC-O1,

pAPEC-O2-ColV and pAPEC-O1-ColBM (Johnson and Nolan 2009, Mellata *et al.* 2010). The simultaneous presence of several of these plasmid-borne virulence-associated genes and, operon ColV (*cvaA, cvaB, cvaC*) and/or colB/M (*cbi, cma*) genes in 24% of our strains augurs that the latter harbored ColV and/or ColBM plasmids. These virulence plasmids have an important role in pathogenicity, evolution from commensal to pathogenic state and zoonotic risk (Johnson and Nolan 2009, Mellata *et al.* 2010). In addition to plasmid genes, some of our isolates possessed certain chromosomal genes (*fyuA, vat, ibeA*), which characterize APEC strains (Johnson *et al.* 2008).

The phylogroup B2 strains were characterized by the presence of little virulence genes, mainly chromosomal and ubiquitous (*entB, fimH*), compared to other groups and the majority of strains carrying presumptive ColV plasmids belonged to phylogroup B1 (61.1%), this finding is not in accordance with previous reported data (Johnson *et al.* 2008). No statistical association with phylogenetic groups was noted for the remaining virulence factors.

The combination of virulence factors showed a diversity in virulence profiles (n = 67). Five percent of our isolates possessed the gene combination "*iutA, hlyF, iss, iroN, ompT*" defined as the most significantly genes associated with highly pathogenic APEC strains (Johnson *et al.* 2008), and 12% harbored at least 4 genes of this combination. These findings were in agreement with results from Hiki and colleagues (Hiki *et al.* 2014).

The prevalence of virulence factors found among our strains differ for some of them from those reported in other countries, environmental conditions (feed, production systems, veterinary practices) can modulate the distribution of virulence determinants (Amabile de Campos *et al.* 2008, Bonnet *et al.* 2009, Kemmet *et al.* 2013)

High resistance rates were observed for amoxicillin, amoxicillin-clavulanic acid, cefazolin, fluoroquinolones, tetracycline, trimethoprim, sulfonamides and sulfamethoxazole/ trimethoprim. These results reflect the general trend worldwide both for fecal and APEC strains; however, resistance rates vary by country. In comparison, our rates are significantly higher than those recorded in the USA (Johnson *et al.* 2012), Europe (de Jong *et al.* 2012), Canada (Bonnet *et al.* 2009), Japan (Hiki *et al.* 2014), and lower than those from Egypt (Mohamed *et al.* 2014), China (Wang *et al.* 2013). The range of antibiotics used in Algeria for prophylaxis, therapy and growth promotion covers various families; this can directly affect antimicrobial resistance of endogenous bacteria. Furthermore, the environment can also be a source of resistant organisms and resistance genes for animals (Bélanger *et al.* 2011). Links between antimicrobial resistance and ExPEC strains in animal food products, specifically chicken meat, and human infections were observed (Vincent *et al.* 2010).

BSBL genes bla_{TEM} and bla_{SHV} were detected in a large number of strains, as already reported (Gyles 2008, Bonnet *et al.* 2009, Wang *et al.* 2013), they would be the cause of resistance to amoxicillin, amoxicillin-clavulanic acid and cefazolin in our strains. Conversely, ESBL $bla_{CTX\text{-}M\text{-}1}$, located in transferable IncI1plasmid, was found in the single cefotaxime-resistant strain (1%). The prevalence of broad cephalosporin resistance and ESBLs varies by country, our results were in accordance with those of studies from Europe and China (de Jong *et al.* 2012, Wang *et al.* 2013). CTX-M-1 ESBL and IncI1 plasmids are among the most widespread, particularly in animal strains including poultry (Caratolli 2009, Johnson and Nolan 2009); however, it should be noted that CTX-M-1 type is reported for the first time in Algeria in this study. Plasmid mediated quinolone resistance genes *qnrB* (12%) and *qnrS1* (1%) were detected, their presence among avian *E. coli* strains was reported with varying frequency by country. Their transfer from animal to human was reported, they contribute to the emergence of highly quinolone-resistant bacteria mainly due to mutations in the DNA gyrase and topoisomerase IV genes (Szmolka and Nagy 2013). A rate of 92% of tetracycline-resistant strains had *tetA* and/or *tetB*, they are the most frequently involved among avian strains with a predominance of *tetA* (Guerra *et al.* 2003, Bonnet *et al.* 2009, Johnson *et al.* 2012). The three dihydropteroate synthase genes *sul1, sul2* and *sul3* were detected in 72.3% of sulfonamides-resistant strains; they play an important role in sulfonamide resistance and are significantly related to integrons and transposons. Consistent with our result, *sul2* gene was the most widely distributed in avian *E. coli* (Guerra *et al.* 2003, Wang *et al.* 2013). Genes *dfrA1, dfrA7, dfrA12* and *dfrA14* were detected alone or in combination in 77.3% of trimethoprim-resistant strains; *dfrA1, dfrA12, dfrA14* and *dfrA17* were the most commonly identified, inside integrons (Guerra *et al.* 2003, Machado *et al.* 2008). Integrons are important contributors in the emergence and dissemination of antimicrobial resistance, half of our strains carried class 1 integrons, the most frequently detected in avian *E. coli*. Our prevalence is equal to that reported in Greece (49.6%) (Vasilakopoulou *et al.* 2009) and higher than those recorded in Portugal (22.5%) (Machado *et al.* 2008) and Germany (36%) (Guerra *et al.* 2003). The majority of detected integrons lacked *sul1* and *qacEΔ1*, this truncated structure was already described, it generally contains *sul3* at the 3'-end and is linked to IS26, which probably is the cause of the 3'-CS deletion (Dawes *et al.* 2010, Sáenz

et al. 2010). The presence of the gene *qacEΔ1* in the absence of integrons would probably be the result of a selection pressure by quaternary ammonium compounds which are, among disinfectants, the most used in the poultry industry. The mercury resistance (37%) can result from an anthropogenic selection pressure or co-selection by antibiotics. The detection of *merA* gene associated in the majority of cases to class 1 integrons is indicative of the presence of the transposon Tn21 which carries mercury resistance operon (*mer*) and an integron (In2). This transposon allows co-selection by antibiotics and mercury. This finding is in agreement with that already reported among avian strains (Bass *et al.* 1999, Johnson *et al.* 2012).

Many associations between antimicrobial resistance and virulence factors were noted, the most remarkable was resistance to ciprofloxacin that was statistically associated with seven virulence genes. The combination of ExPEC virulence factors and antibiotic resistance was reported (Pitout 2012); however, in contrast to our results for ciprofloxacin, previous studies demonstrated that ciprofloxacin resistance was associated with fewer virulence genes in comparison to ciprofloxacin-susceptible strains (Graziani *et al.* 2009). The molecular mechanisms underlying association between resistance and virulence remains to understand.

This study, the first in Algeria devoted to virulence and antimicrobial resistance of fecal strains from healthy broiler chickens, reported the presence of ExPEC virulence genes typically found in pathogenicity islands located on plasmids, particularly ColV and ColBM plasmids. High prevalence of MDR phenotype was observed, with resistance to first line antibiotics including amoxicillin-clavulanate, fluoroquinolones and trimethoprim-sulfamethoxazole, as well as various plasmidic resistance genes and class1 integrons. Intensive chicken farming in the current conditions in Algeria really constitutes a source of virulence and antimicrobial resistance genes that may spread and exacerbate virulence and resistance of animal and human pathogenic strains. This situation should incite to take measures at the level of farming conditions and veterinary practices.

References

Amabile de Campos T., Stehling E.G., Ferreira A., Castro A.F.P., Brocchi M. & Silveira W.D. 2005. Adhesion properties, fimbrial expression and PCR detection of adhesin-related genes of avian *Escherichia coli* strains. *Vet Microbiol*, **106**, 275-285.

Amabile de Campos T., Lago J.C., Nakazato G., Stehling E.G., Brocchi M., Castro A.F.P. & Silveira W.D. 2008. Occurrence of virulence-related sequences and phylogenetic analysis of commensal and pathogenic avian *Escherichia coli* strains (APEC). *Pesq Vet Bras*, **28**, 533-540.

Bass L., Liebert C.A., Lee M.D., Summers A.O., White D.G., Thayer S.G. & Maurer J.J. 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrob. Agents Chemother*, **43**, 2925-2929.

Bélanger L., Garénaux A., Harel J., Boulianne M., Nadeau E. & Dozois C.M. 2011. *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. *FEMS Immunol Med Microbiol*, **62**, 1-10.

Bonnet C., Diarrassouba F., Brousseau R., Masson L., Topp E. & Diarra M.S. 2009. Pathotype and antibiotic resistance gene distributions of *Escherichia coli* isolates from broiler chickens raised on antimicrobial-supplemented diets. *Appl Environ Microbiol*, **75**, 6955-6962.

Calormiris J., Armstrong J.L. & Seidler R.J. 1984. Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Appl Environ Microbiol*, **47**, 1238-1242.

Carattoli A., Bertini A., Villa L., Falbo V., Hopkins K.L. & Threlfall E.J. 2005. Identification of plasmids by PCR-based replicon typing. J *Microbiol Methods*, **63**, 219-228.

Carattoli A. 2009. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother*, **53**, 2227-2238.

Clermont O., Christenson J.K., Denamur E. & Gordon D.M. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep*, **5**, 58-65.

Dawes F.E., Kuzevski A., Bettelheim K.A., Hornitzky M.A., Djordjevic S.P. & Walker M.J. 2010. Distribution of class 1 integrons with IS26-mediated deletions in their 3'-conserved segments in *Escherichia coli* of human and animal origin. *PLoS ONE*, **5**, e12754. doi:10.1371/journal.pone.0012754.

de Jong A., Thomas V., Simjee S., Godinho K., Schiessl B., Klein U., Butty P., Vallé M., Marion H. & Shryock T.R. 2012. Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother*, **67**, 638-665.

Dozois C.M., Dho-Moulin M., Breé A., Fairbrother J.M., Desautels C. & Curtiss III R. 2000. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the Tsh genetic region. *Infect Immun*, **68**, 4145-4154.

Dozois C.M., Daigle F. & Curtiss III R. 2003. Identification of pathogen specific and conserved genes expressed in vivo by an avian pathogenic *Escherichia coli* strain. *Proc Natl Acad Sci USA*, **100**, 247-252.

Edlund C., Björkman L., Ekstrand J., Sandborgh-Englund G. & Nord C.E. 1996. Resistance of the normal human microflora to mercury and antimicrobials after exposure to mercury from dental amalgam fillings. *Clin Infect Dis*, **22**, 944-950.

El Fertas-Aissani R., Messai Y., Alouache S. & Bakour R. 2013. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. *Pathol Biol*, **61**, 209-216.

Ewers C., Li G., Wilking H., Kiessling S., Alt K., Antáo E.M., Laturnus C., Diehl I., Glodde S., Homeier T., Böhnke U., Steinrück H., Philipp H.C. & Wieler L.H. 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they?. *Int J Med Microbiol*, **297**, 163-176.

Ewers C., Antao E.M., Diehl I., Philipp H.C. & Wieler L.H. 2009. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. *App Environ Microbiol*, **75**, 84-192.

Feria C., Ferreira E., Correia J.D., Gonçalves J. & Caniça M. 2002. Patterns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *J Antimicrob Chemother*, **49**, 77-85.

Figueira V., Vaz-Moreira I., Silva M. & Manaia C.M. 2011. Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res*, **45**, 5599-5611.

Frank T., Gautier V., Talarmin A., Bercion R. & Arlet G. 2007. Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in *Enterobacteriaceae*, Central African Republic (CAR). *J Antimicrob Chemother*, **59**, 742-745.

Garénaux A., Houle S., Folch B., Dallaire G., Truesdell M., Lépine F., Doucet N. & Dozois C.M. 2013. Avian lipocalin expression in chickens following *Escherichia coli* infection and inhibition of avian pathogenic *Escherichia coli* growth by Ex-FABP. *Vet Immunol Immunopathol*, **152**, 156-167.

Graham J.P., Leibler J.H., Price L.B., Otte J.M., Pfeiffer D.U., Tiensin T. & Silbergeld E.K. 2008. The animal-human interface and infectious disease in industrial food animal production: rethinking biosecurity and biocontainment. *Public Health Rep*, **123**, 282-299.

Graziani C., Luzzi I., Corro M., Tomei F., Parisi G., Giufre M., Morabito S., Caprioli A. & Cerquetti M. 2009. Phylogenetic background and virulence genotype of ciprofloxacin-susceptible and ciprofloxacin-resistant *Escherichia coli* strains of human and avian origin. *J Infect Dis*, **199**, 1209-1217.

Guardabassi L., Dijkshoorn L., Collard J.M., Olsen J.E. & Dalsgaard A. 2000. Distribution and *in-vitro* transfer of tetracycline resistance determinants in clinical and aquatic *Acinetobacter* strains. *J Med Microbiol*, **49**, 929-936.

Guerra B., Junker E., Schroeter A., Malorny B., Lehmann S. & Helmuth R. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. *J Antimicrob Chemother*, **52**, 489-492.

Gyles C.L. 2008. Antimicrobial resistance in selected bacteria from poultry. *Anim Health Res Rev*, **9**, 149-158.

Hiki M., Usui M., Akiyama T., Kawanishi M., Tsuyuki M., Imamura S., Sekiguchi H., Kojima A. & Asai T. 2014. Phylogenetic grouping, epidemiological typing, analysis of virulence genes, and antimicrobial susceptibility of *Escherichia coli* isolated from healthy broilers in Japan. *Ir Vet J*, **67**, 14.

Kado C.I. & Liu S.T. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol*, **145**, 1365-1373.

Johnson T.J., Siek K.E., Johnson S.J. & Nolan L.K. 2006a. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. *J Bacteriol*, **188**, 745-758.

Johnson T.J., Johnson S.J. & Nolan L.K. 2006b. Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids. *J Bacteriol*, **188**, 5975-5983.

Johnson J.R. & Stell A.L. 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis*, **181**, 261-272.

Johnson T.J., Wannemuehler Y., Doetkott C., Johnson S.J., Rosenberger S.C. & Nolan L.K. 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. *J Clin Microbiol*, **46**, 3987-3996.

Johnson T.J. & Nolan L.K. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. *Microbiol Mol Biol Rev*, **73**, 750-750.

Johnson T.J., Logue C.M., Johnson J.R., Kuskowski M.A., Sherwood J.S., Barnes H.J., DebRoy C., Wannemuehler Y.M., Obata-Yasuoka M., Spanjaard L. & Nolan L.K. 2012. Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal *Escherichia coli* from humans and poultry. *Foodborne Pathog Dis*, **9**, 37-46.

Kemmett K., Humphrey T., Rushton S., Close A., Wigley P., Nicola J. & Williams N.J. 2013. A longitudinal study simultaneously exploring the carriage of APEC virulence associated genes and the molecular epidemiology of faecal and systemic *E. coli* in commercial broiler chickens. *PLoS ONE*, **8**, e67749. doi: 10. 1371/journal. pone.0067749.

Machado E., Coque T.M., Canton R., Sousa J.C. & Peixe L. 2008. Antibiotic resistance integrons and extended spectrum beta-lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal. *J Antimicrob Chemother*, **62**, 296-302.

Mellata M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog Dis*, **10**, 916-932.

Mellata M., Ameiss K., Mo H. & Curtiss III R. 2010. Characterization of the contribution to virulence of three large plasmids of avian pathogenic *Escherichia coli* chi7122 (O78:K80:H9). *Infect Immun*, **78**, 1528-1541.

Mellata M., Dho-Moulin M., Dozois C.M., Curtiss III R., Brown P.K., Arné P., Breé A., Desautels C. & Fairbrother J.M. 2003. Role of virulence factor in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. *Infect Immun*, **71**, 536-540.

Messai Y., Iabadene H., Benhassine T., Alouache S., Tazir M., Gautier V., Arlet G. & Bakour R. 2008. Prevalence and characterisation of extended-spectrum beta-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). *Pathol Biol*, **56**, 319-325.

Mohamed M.A., Shehata M.A. & Rafeek E. 2014. Virulence genes content and antimicrobial resistance in *Escherichia coli* from broiler chickens. *Vet Med Int*, 1-6. Article ID 195189, http://dx.doi.org/10.1155/2014/195189.

Naves P., del Prado G., Huelves L., Gracia M., Ruiz V., Blanco J., Dahbi G., Blanco M., Ponte M.D.C. & Soriano F. 2008. Correlation between virulence factors and *in vitro* biofilm formation by *Escherichia coli* strains. *Microb Pathog*, **45**, 86-91.

Pitout J.D.D. 2012. Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. *Front Microbiol*, **3**, 9. doi: 10.3389/fmicb.2012.00009.

Restieri C., Garriss G., Locas M.C. & Dozois C.M. 2007. Autotansporter-encoding sequences are phylogenetically distributes among *Escherichia coli* clinical isolates and reference strains. *Appl Environ Microbiol*, **73**, 1553-1562.

Rodriguez-Siek K.E., Giddings C.W., Doetkott C., Johnson T.J. & Nolan L.K. 2005. Characterizing the APEC pathotype. *Vet Res*, **36**, 241-256.

Sabri M., Caza M., Proulx J., Lymberopoulos M.H., Brée A., Moulin Schouleur M., Curtiss III R. & Dozois C.M. 2008. Contribution of the SitABCD, MntH, and FeoB metal transporters to the virulence of avian pathogenic *Escherichia coli* O78 strain chi7122. *Infect Immun*, **76**, 601-611.

Sáenz Y., Vinué L., Ruiz E., Somalo S., Martínez S., Rojo-Bezares B., Zarazaga M. & Torres C. 2010. Class 1 integrons lacking qacEDelta1 and sul1 genes in *Escherichia coli* isolates of food, animal and human origins. *Vet Microbiol*, **144**, 493-497.

Šeputienė V., Povilonis J., Ruzauskas M., Pavilonis A. & Suziedeliene E. 2010. Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. *J Med Microbiol*, **59**, 315-322.

Skyberg J.A., Siek K.E., Doetkott C. & Nolan L.K. 2007. Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny. *J Appl Microbiol*, **102**, 548-554.

Szmolka A. & Nagy B. 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Front Microbiol*, **4**, 258. doi: 10.3389/fmicb.2013.00258.

Tuntufye H.N., Lebeer S., Gwakisa P.S. & Goddeerisa B.M. 2012. Identification of avian pathogenic *Escherichia coli* genes that are induced *in vivo* during infection in chickens. *Appl Environ Microbiol*, **78**, 3343-3351.

Vasilakopoulou A., Psichogiou M., Tzouvelekis L., Tassios P.T., Kosmidis C., Petrikkos G., Roma E.S., Charvalos E., Passiotou M., Avlami A. & Daikos G.L. 2009. Prevalence and characterization of class 1 integrons in *Escherichia coli* of poultry and human origin. *Foodborne Pathog Dis*, **6**, 1211-1218.

Vincent C., Boerlin P., Daignault D., Dozois C.M., Dutil L., Galanakis C., Reid-Smith R.J., Tellier P.P., Tellis P.A., Ziebell K. & Manges A.R. 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg Infect Dis*, **16**, 88-95.

Wang C.G., Lv J.C. & Zhang T. 2013. Detection of resistance phenotype and genotype of avian *Escherichia coli* in Hebei Province. *Poult Sci*, **92**, 2326-2332.

Wooley R.E., Gibbs P.S., Brown T.P., Glisson J.R., Steffens W.L. & Maurer J.J. 1998. Colonization of the chicken trachea by an avirulent avian *Escherichia coli* transformed with plasmid pHK11. *Avian Dis*, **42**, 194-198.