Austral J Vet Sci **54, 115-125 (2022)**

ORIGINAL ARTICLE

Virulence and antimicrobial resistance of *Escherichia coli* isolated from chicken meat, beef, and raw milk

Djamel Boudjerda[a,b], Mesbah Lahouel[a]*

ABSTRACT. Two hundred and thirty-five strains of *E. coli* were recovered from beef, chicken meat, and raw milk sold in butcher shops and markets in the town of Jijel, East Algeria. A PCR investigation revealed the predominance of bacterial strains with extraintestinal pathogenic *E. coli* (ExPEC) genes (19.91%). Enteroaggregative (EAEC) and enteroinvasive (EIEC) bacteria were also detected. Strains from phylogenetic groups A and B1 were the most common; they accounted for 62.35% and 21.17% in chicken meat, 67.53% and 16.88% in raw milk and 64.38% and 25.97% in beef, respectively, followed by the strains from phylogenetic groups B2 and D. Moreover, *E. coli* from phylogenetic group D was more abundant in chicken than in beef or raw milk samples ($P<0.05$). Antibiogram results revealed the presence of three major antibiotic-susceptibility groups and more than a hundred antibiotypes. Between 50% and 70% of strains were resistant to antibiotics of the first group (ampicillin, amoxicillin, trimethoprim, sulfonamide and tetracycline), 20% to 50% to antibiotics of the second group (amoxicillin plus clavulanic acid, kanamycin, streptomycin, ciprofloxacin, flumequine and neomycin) and less than 20% to those of the third group (cephalothin, gentamicin and colistin). Additionally, the resistance to flumequine, trimethoprim, sulfamethoxazole-trimethoprim and tetracycline was more frequent in chicken meat strains than in those from beef and milk. It is important to point out that the strains carrying more than two virulence factors belonged to the B2 or D phylogenetic groups and had weaker resistance to antibiotics. The strains from the A and B1 groups had fewer virulence factors and showed high resistance to antibiotics.

Keywords: Chicken meat, beef, raw milk, *Escherichia coli*, virulence factor, phylogeny, antibiotic resistance.

INTRODUCTION

Foodborne diseases and microbial food safety are becoming global public health concerns. Most foodborne diseases are generally caused by the consumption of contaminated beverages or food products like raw milk, beef and chicken meat. A variety of pathogens are involved in this type of infections, such as pathogenic *E. coli* strains of zoonotic origin (Rivera-Betancourt *et al.*, 2004). Furthermore, pathogenic *E. coli* strains were detected in beef-processing plants as reported in several studies (Johnson *et al.*, 2005; Holko *et al.*, 2006). *E. coli* are natural inhabitants of the digestive tract of humans and animals. However, some strains can be pathogenic for humans and animals (Kaper *et al.*, 2004; Holko *et al.*, 2006). Pathogenic *E. coli* can be categorised as intestinal pathogenic *E. coli* or extraintestinal pathogenic *E. coli* (ExPEC) (Russo & Johnson, 2000). Among the intestinal pathogenic *E. coli*, enterohemorrhagic *E. coli* (EHEC) are responsible for severe clinical symptoms, such as haemorrhagic colitis and the potential lethal haemolytic uremic syndrome (Karmali *et al.*, 2010). EHEC strains are zoonotic pathogens because domestic ruminants, mainly cattle, sheep, and goats have been considered as major natural reservoirs for EHEC (Ferens & Hovde, 2011). Pathogenic *E. coli* strains with common genetic characteristics have been found in humans and animals (Clermont *et al.*, 2011). These pathogenic strains have been divided into numerous categories or pathotypes on the basis of their distinct virulence properties and the clinical symptoms of the hosts. The intestinal strains include enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), and enterohaemorrhagic *E. coli* (EHEC). Extraintestinal infections (sepsis, urinary tract infections and neonatal meningitis strains) are caused by ExPEC (extraintestinal pathogenic *E. coli*) (Rodriguez-Siek *et al.*, 2005). Generally, virulence genes are used as targets to determine the pathogenic potential of any given *E. coli* isolate (Holko *et al.*, 2006; Cheng *et al.*, 2020; Kim *et al.,* 2022). Moreover, the virulence factors and virulence genes are similar in strains of the same pathotype. It has been reported that human and animal pathogenic *E. coli* strains can also be assigned to one of the main phylogenetic groups, A, B1, B2 and D (Clermont *et al.*, 2000) and share common genetic backgrounds (Clermont *et al.*, 2011). Whether animals are a source for human pathogenic *E. coli* or not is still a matter of debate. Nevertheless, *E. coli* strains with virulence genes have been detected in food products of avian and cattle origin.

Antimicrobial resistance (AMR) is recognised as a global problem in human and veterinary medicine. The indiscriminate use of antimicrobials in both medicines, as well as their use as growth-promoting factors in husbandry, has caused an increase in antibiotic residues in the environment and they have also been found in food products and pathogenic samples of human and animal origin. The accumulation of these residues promotes selective pressure, enhancing the selection of resistant

Received: 15.02.2022.

Accepted: 08.06.2022.

[a]Laboratory of Molecular Toxicology, University of Jijel, Jijel, Algeria.

[b]Toulouse Inserm Umr1043, Inra Usc1360, Université de Toulouse, INP, ENVT, France.

*Corresponding author: M. Lahouel; BP 98 Ouled Aissa Jijel, 18000 Jijel, Algeria; lahouelmesbah@yahoo.fr

bacteria, since several genes coding for antibiotic resistance are located on mobile genetic elements (Cheng *et al.*, 2020). Therefore, animal food of animal origin can constitute an ideal environment for the emergence of new pathogenic and resistant bacterial strains by the acquisition of different virulence and resistance determinants (Kim *et al.*, 2022). The analysis of bacteria present in dairy food products can thus provide information on the bacterial gene reservoir that may be useful for predicting risk for human populations. Finally, to estimate the extent of the antimicrobial resistance (AMR) problem and follow its evolution, surveillance programs have been established in many countries worldwide.

The main objectives of the present study were (i) to make a collection of *E. coli* strains isolated from three types of food products (chopped chicken, beef and raw milk) sold in butcher and traditional milk shops in Jijel (Eastern Algeria), (ii) to screen virulence genes and determine the phylogenetic group for each strain, (iii) to evaluate the resistance of the isolated strains to commonly used antimicrobial agents in human and veterinary medicine in Algeria.

MATERIAL AND METHODS

SAMPLE COLLECTION

Three hundred samples of chicken meat, beef and raw milk were collected randomly (at least one per month) from 10 butcher shops localised in the wilaya of Jijel, a region located in eastern Algeria. The samples consisted of 1 kg of bovine meat, 1 liter of milk and a whole broiler. All the samples were purchased from butcher and milk sales outlets located in the Jijel region. All animals came from jijlians herds and the sampling period was from September 2017 to June 2019. Collected samples were sent to the laboratory in sterile bags on ice and were processed on the same day at the Microbiological Laboratory of the Microbiology and Biochemistry Department (Mohamed Seddik Benyahia University, Jijel, Algeria).

ISOLATION AND PRIMARY IDENTIFICATION

E. coli were isolated from the samples as previously described by Zhao *et al.* (2001). Briefly, 25g portions of chicken meat or beef and 25ml samples of raw milk were aseptically taken from collected samples. After homogenisation in 225 ml of sterile buffered Peptone Water (Institut Pasteur, Algiers, Algeria (IPA)), 1 ml of the suspension of each sample was plated onto approximately 15 ml of Violet Red Bile Glucose Agar (VRBG, IPA) and incubated at 37 °C for 24 hours. Primary identification procedures involved subculturing the presumptive *E. coli* colonies in Brilliant Green Bile Broth (BGBB, IPA) containing an inverted Durham tube and in Tryptone Water without indole (IPA). The samples were incubated at 37 °C for 24 h. After the incubation, the produced gas, visible as a bubble in the inverted Durham tube, indicated a positive result. Kovacs (IPA) reagent (0.2-0.3 ml) was added to Tryptone Water to detect indole production; the appearance of a red colour on the upper layer indicated a positive reaction. The presumptive *E. coli* was recovered and confirmed by Gram staining and biochemical tests by using API 20E test (BioMérieux, Marcy l'Etoile, France) (Badri *et al.*, 2009). It has been shown that an arbitrarily selected *E. coli* colony has an 86% probability of representing the quantitatively predominant clone in the sample (Lidin-Janson *et al.*, 1978). Two hundred thirty-five *E. coli* strains (85 from chicken meat, 73 from beef, and 77 from raw milk) were collected, identified and grown separately with agitation at 37 °C in Luria Bertoni broth (LB). They were then stored at -80 °C in LB broth containing 30% glycerol in 96-well microtiter plates for further analysis.

SCREENING FOR POTENTIALLY PATHOGENIC *E. coli*

A total of 235 *E. coli* strains was screened for the presence of three virulence genes associated with EHEC (*eae, stx1 and stx2*), ten genes associated with ExPEC (*f17A, cnf, papEF*, *afa/draBC*, *fyua*, *clbN*, *hlyf*, *kpsMT(K1)*, *hlyA*, and *sfa/focDE*), one with EIEC (*ipah*) and three virulence genes associated with EAEC (*AAprobe, aap* and *aggr*). DNA was extracted from overnight cultures using NaOH and subjected to multiplex and uniplex PCR (Diallo *et al.*, 2013).

A triplex PCR was performed to detect *eae, stx*$_1$ and *stx*$_2$ genes as described previously (Paton & Paton 1998). EHEC O157:H7 RIMD 050992 (Sakaï) (Hayashi *et al.*, 2001) was used as a positive control. For the ExPEC, triplex PCRs were performed to detect *sfa/focDE* (Le Bouguenec *et al.*, 1992), *kpsMT* K1 (Johnson & Stell, 2000), and *hlyA* (Johnson & Stell, 2000) and *papEF* (Yamamoto *et al.*, 1995) *afa/draBC*, and *fyua* (Johnson & Stell, 2000). A duplex PCR was conducted to detect *clbN* (Johnson & Stell, 2000) and *hlyF* (Moulin-Schouleur *et al.*, 2007). A uniplex PCR was performed for the *f17A* gene (Bertin *et al.*, 1996) and another to detect the *cnf* gene (Yamamoto *et al.,* 1995). For EAEC, a triplex PCR was used to identify *AAprobe*, *aap*, and *aggr,* as described by Cerna *et al.* (2003). Finally, for EIEC, a uniplex PCR was performed to detect *ipah* (Aranda *et al.*, 2007). The following control strains were used: J96 for *sfa/focDE*, *hlyA*, *papEF*, and *fuyA* (Johnson *et al.*, 1997), SP15 for *kpsMT K1*, and *hlyF* (Johnson *et al.*, 2000), A30 for *afa/draBC* (Johnson and Stell 2000), IHE3034 for *clbN* (Korhonen *et al.*, 1985), 25KH9 for *f17A* (Girardeau *et al.*, 1988), S5 for *cnf* (Smith, 1974), O42 for *AAprobe*, *aap*, and *aggr* and EDL1284 for *ipah* (Aranda *et al.*, 2007) (table 1).

PHYLOGENETIC GROUP CLASSIFICATION

Phylogenetic grouping of *E. coli* strains was conducted using multiplex PCR with the *chuA* and *yjaA* genes and

Table 1. PCR conditions and the different multiplex and uniplex carried out for the detection of virulence genes of isolated *E. coli* strains.

	GENES CIBLES	SEQUENCES (3'-5')		Strains (+)	Target (bp)	Denaturation	Hybridation	Elongation	cycles	And	REFERENCES
ExPEC	*f17A*	P1(322) P2(323)	*GCAGAAAATTCAATTTATCCTTGG* *CTGATAAGCGATGGTGTAATTAAC*	*25KH9*	537	95/2 then 95/45	56/45	72/1,5	35	72/10	Bertin *et al.*, 1996
	cnf	cnfxbiss308 cnfxbisas309	*CAA TGG CAA CAA AAA TAC CTT* *GAA CGA CGT TCT TCA TAA GTA TC*	*28C*	1147	95/2 then 95/45	56/45	72/1,5	35	72/10	Yamamoto *et al.*, 1995
	papEF	pap3 pap4	*GCA ACA GCA ACG CTG GTT GCA TCA T* *AGA GAG AGC CAC TCT TAT ACG GAC A*	*J96*	336	94/4 then 94/1	63/0,5	72/2	30	72/7	Yamamoto *et al.*, 1995
	afa/draBC	Afa f Afa r	*GGC AGA GGG CCG GCA ACA GGC* *CCC GTA ACG CGC CAG CAT CTC*	*A30*	559						J.R. Johnson., 2000
	fyuA	fyua/f fyua/r	*TGA TTA ACC CCG CGA CGG GAA* *CGC AGT AGG CAC GAT GTT GTA*	*J96*	880						J.R. Johnson., 2000
	clbN	69	*GTT TTG CTC GCC AGA TAG TCA TTC* *CAG TTC GGG TAT GTG TGG AAG G*	*IHE3034*	733	95/2 then 95/45	56/45	72/1,5	35	72/10	J.R. Johnson., 2008
	hlyF	70	*TCG TTT AGG GTG CTT ACC TTC AAC* *TTT GGC GGT TTA GGC ATT CC*	*SP15*	444						Moulin-Schouler, 2007
	kpsMT (K1)	k1.f kapsII r	*TAG CAA ACG TTC TAT TGG TGC* *CAT CCA GAC GAT AAG CAT GAG CA*	*SP15*	153	94/4 then 94/1	63/0,5	72/2	30	72/7	J.R. Johnson., 2000
	hlyA	hly1 hly2	*AAC AAG GAT AAG CAC TGT TCT GGC T* *ACC ATA TAA GCG GTC ATT CCC GTC A*	*J96*	1177						J.R. Johnson., 2000
	sfa/focDE	sfaD(595) sfaE(596)	*CTC CGG AGA ACT GGG TGC ATC TTA C* *CGG AGG AGT AAT TAC AAA CCT GGC A*	*J96*	410						Yamamoto *et al.*, 1995
EHEC	*eae*	eaeB52 eaeB53	*GAC CCG GCA CAA GCA TAA GC* *CCA CCT GCA GCA ACA AGA GG*	Sakai O157H7	384 Kb	Need special program (In every cycle conditions must be changed automatically)					Paton & Paton (1998)
	stx1	slt-I B54 slt-I B55	*ATA AAT CGC CAT TCG TTG ACT AC* *AGA ACG CCC ACT GAG ATC ATC*		180 Kb						
	stx2	slt-II B56 slt-II B57	*GGC ACT GTC TGA AAC TGC TCC* *TGC CCA GTT ATC TGA CAT TCT G*		255 Kb						
EAEC	*AAprobe*	*EAEC1* *EAEC2*	*CTG GCG AAA GAC TGT ATC AT* *CAA TGT ATA GAA ATC CGC TGT T*	*O42*	629	94/4 then 94/1	63/0,5	72/2	30	72/7	Cerna *et al.*, 2003
	aap	*Aap1* *Aap2*	*CTT GGG TAT CAG CCT GAA TG* *AAC CCA TTC GGT TAG AGC AC*		310						
	aggR	*AggR1* *AggR2*	*CTA ATT GTA CAA TCG ATG TA* *AGA GTC CAT CTC TTT GAT AAG*		457						
EIEC	*ipah*	ipaH1 ipaH2	*GTT CCT TGA CCG CCT TTC CGA TAG CGT C* *GCC GGT CAG CCA CCC TCT GAG AGT AC*	*EIEC 85b*	600	94/4 then 94/1	63/0,5	72/2	30	72/7	Cerna *et al.*, 2003

the DNA fragment *TSPE4.C2*, according to the method described by Clermont *et al.* (2000). Representative *E. coli* reference collection strains were used as template control.

PCR was performed in 0.5 ml Eppendorf tubes on a Techne Progene (UK) thermal cycler with a reaction of 50 µl volume. The DNA template (5 µl containing100-200 pg of DNA) was added to 45 µl reaction mixture containing 0.1 mM each dATP, dCTP, dGTP, and dTTP (Invitrogen); 5 µl buffer solution 10X (Biolabs, New England); PCR primers (Tbl1), 1.5 U of Taq DNA polymerase (Biolabs, New England) were added to the reaction. The amplification products were analysed by gel electrophoresis on a 2.0% agarose gel (ROTI1 Garose, Roth GmbH, Germany), stained with ethidium bromide, and photographed at UV exposure. PCR Conditions, primers and programs were summarised in table 1.

ANTIBIOTIC SUSCEPTIBILITY TESTING

All *E. coli* strains were subjected to antimicrobial susceptibility testing. The tests were performed using the disk diffusion method according to the CLSI standards (CLSI, 2009) on Mueller-Hinton agar (Bio-Rad Laboratories). *E. coli* ATCC 25922 was used as the control strain. The 16 antibiotic disks (Bio-Rad Laboratories) used in this study were Ampicillin (Amp) 10 µg, (Amoxicillin + alvulanic acid (Amx+AC) 20+10 µg), Amoxicillin (Amx) 25 µg, Cephalothin (CF) 30 µg, Gentamicin (Gn) 10 µg, Kanamycin (K) 30 µg, Streptomycin (S) 10 µg, Colistin (CT) 50µg, Neomycin (N) 30 µg, Ciprofloxacin (Cip) 5 µg, Flumequin (UB30) 30 µg, Trimethoprim (TMP) 5 µg, Sulfamethoxazole-Trimethoprim (Sxt) 23.75 µg + 1.25 µg, Sulphonamid (SSS) 300 µg, Tetracycline (Tet) 75 µg, Chloramphenicol (C) 30 µg. Susceptibility breakpoints for all the antimicrobials followed the recommendations of CLSI (CLSI, 2009). Strains were classified as multiresistant when they exhibited resistance to three or more classes of antimicrobial agents (Schwarz *et al.*, 2010). It should be noted that identification tests, PCR tests and antibiotic sensitivity tests were carried out at Inserm Umr1043, Inra Usc1360, University of Toulouse, INP, ENVT, France.

STATISTICAL ANALYSIS

The different phylogenetic groups and antimicrobial resistance data were compared using the chi-square test (R. software version 2.14.1 (2011-12-22) for Windows) and ANOVA (Statistica software 8.1) test was used to compare de prevalence of antibiorestance versus prevalence of virulence factors. A P value of < 0.05 was considered statistically significant.

RESULTS

E. coli STRAINS COLLECTION

Three hundred samples were purchased and analysed (100 samples of each food product) and two hundred thirty-five (235) *E. coli* strains were collected (85 strains from chicken meat, 73 from beef, and 77 from raw milk).

CHARACTERISATION OF *E. coli* STRAINS FROM FOOD PRODUCTS

Prevalence of pathogenic E. coli. All the two hundred thirty-five recovered strains were submitted to PCR to detect 17 virulence genes. Ninety-seven potentially virulent strains were identified (41.27% of investigated strains). The comparisons between the samples of different origins showed that 47 (55.25%) of chicken meat samples, 25 (34.24%) of beef samples and 29 (37.66%) of milk samples contained the bacteria with virulence genes.

The prevalence of virulence genes ranged from 0% for *eae*, *stx1*, *sfa/focDE*, and *kpsMT* (*K1*) to 19.91% (46 strains) for *hlyf*. Globally, the adhesin-coding genes, *papEF* 5 (2.16%), *afa/draBC* 5 (2.16%), and *f17* 3 (1.20%) were the most prevalent, followed by *ipaH* (two strains) and *aggr* (two strains). The *fyuA* gene, found in 20 strains (8.65%), was more common than *clbN* (seven strains, 3.03%) and *cnf* (three strains, 1.2%), *hlyA* (one strain), and stx2 (one strain) (table 2). The *hlyf* and *fyua* genes were detected more frequently in the strains isolated from chicken meat than beef and raw milk samples ($P<0.05$). The *eae, stx1, sfa/focDE, kpsMT (K1), and afa/draBC* were not amplified in any isolated strains. Potentially virulent *E. coli* strains tended to show few antibiotic-resistance patterns. However, our study clearly showed that chicken meat, beef, and raw milk act as reservoirs for *E. coli* strains carrying virulence factors associated with resistance to many antibiotics. The distribution of the various targeted sequences revealed 30 virulence gene patterns (labelled with EC followed by arabic number, from one (EC1) to thirty (EC30). Our results reveal that certain strains belonging to phylogenetic groups A, B1, B2, or D and carrying at least 1 virulence gene are at the same time resistant to at least 5 antibiotics, such is the case respectively of phylogenetic group A with 31 strains distributed over 3 patterns (EC1, EC2, EC5), phylogenetic group B1 with 11 strains distributed over 3 patterns (EC1, EC13, EC28), phylogenetic group D with 5 strains distributed over 4 patterns (EC11, EC12, EC13, EC17) and finally the B2 phylogenetic group with 11 strains spread over 3 patterns (EC15, EC19, EC21).

Phylogenetic group. It was shown that the phylogenetic group A was the most common; 67.53% (52/77) in raw milk, 64.38% (47/73) in beef and 62.35% (53/85) in chicken meat, followed by group B1 in beef (25.97%, 20/73), in chicken meat (21.17%, 18/85) and in raw milk (16.88%,

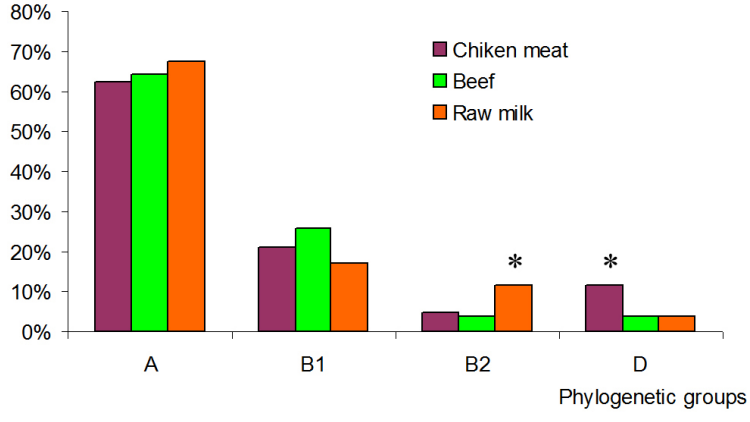
Table 2. Prevalence of virulence factors among 235 *E. coli* strains isolated from chicken meat, beef, and raw milk samples.

		Chicken meat n = 85	Beef n = 73	Raw milk n = 77	Total no. of positive strains n = 235	Stat.
EHEC	*stx1*	–	–	–	–	ns
	stx2	–	1 (1.36%)	–	1 (0.4%)	
	eae	–	–	–	–	
ExPEC	*f17*	–	1 (1.36%)	1 (1.29%)	2 (0.8%)	ns
	cnf	–	2 (2%)	1 (1.29%)	3 (1.2%)	
	hlyF	24(28.23%)	9 (12.32%)	13 (16.88%)	46 (19.91%)	
	papEF	4 (4.7%)	1 (1.36%)	–	5 (2.12%)	
	afa/draBC	3 (3.52%)	2 (2%)	–	5 (2.12%)	
	sfa/focDE	–	–	–	–	
	hlyA	1 (1.17%)	–	2 (2.59%)	3 (1.2%)	
	kpsMT (K1)	–	–	–	–	
	fyua	10 (11.76%)	5 (6.84%)	5 (6.49%)	20 (8.51%)	
	clbN	2 (2.35%)	3 (4.10%)	4 (5.19%)	7 (2.97%)	
EIEC	*ipah*	–	–	1 (1,29%)	1 (0,4%)	ns
EAEC	*aggr*	1 (1.17%)	1 (1.36%)	–	2 (0.8%)	ns
	aap	1 (1.17%)	–	1 (1.29%)	2 (0.8%)	

-: Absent= (0%), ns: No Significant (P>0.05).

13/77). The phylogenetic group B2 was significantly more frequent in raw milk 11.68% (9/77) than chicken meat 4.7% (4/85) and beef 3.98% (3/73) samples (P<0.05). The phylogenetic group D was more common in chicken meat (11.76%, 10/85) than in beef (3.89%, 3/73) and in raw milk (3.89%, 3/77) (P<0.05) (figure 1). Moreover, the comparison of the percentages of strains carrying Vfs and belonging to phylogenetic group B2 (68.75%) showed that they are higher than those recorded in strains belonging to phylogenetic groups D (37.5%) A (30.26%) and B1 (27.45) respectively (table 3).

Antibacterial susceptibility. Figure 2 shows the results of the susceptibility of the isolated 235 *E. coli* strains to 16 antibiotics. The highest prevalence of resistance was recorded for tetracycline with 64.25% (151/235 strains), followed by amoxicillin with 54.04% (127/235 strains), ampicillin with 53.61% (126/235 strains), sulfonamide with 43.82% (103/235 strains), trimethoprim with 37.87% (89/235 strains), trimethoprim-sulfamethoxazole with 37.44% (88/235 strains), streptomycin with 31.06% (73/235 strains) oxacillin plus clavulanic acid with 19.14% (45/235 strains), ciprofloxacin with 17.87% (42/235 strains),



*= Significant difference (P<0.05)
- (Chicken meat, Beef), (Chicken meat, Raw milk) Significant difference (P<0.05)
- (Raw milk, Beef), (Raw milk, Chicken meat) Significant difference (P<0.05)

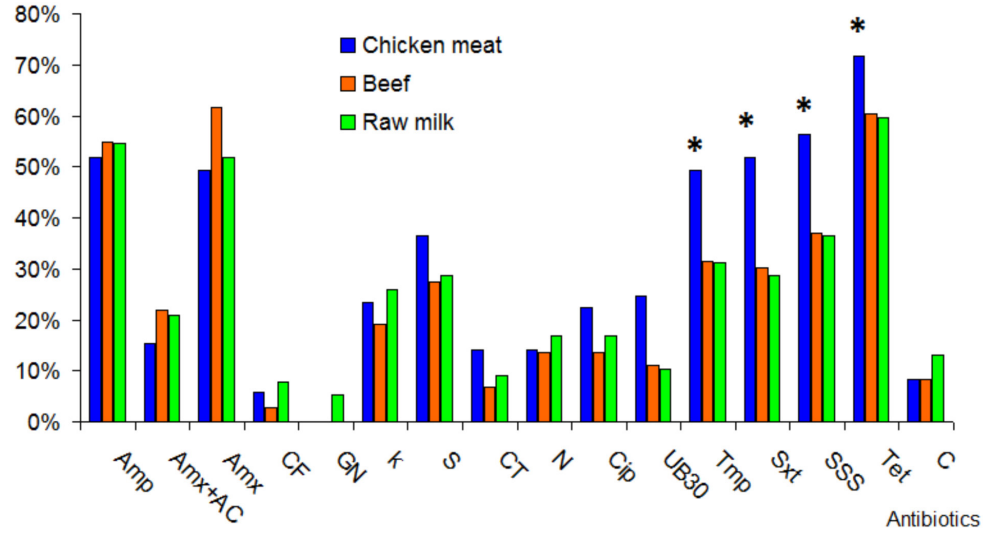
Figure 1. Phylogenetic group classification of *E. coli* strains isolated from chicken meat (n = 85), beef (n = 73), and raw milk (n = 77).

Table 3. Virulence, phylogeny, and antibiotic-resistance patterns identified among 235 *E. coli* strains isolated from chicken meat, beef, and raw milk samples.

	Patterns	Virulence gene carriage	Phylogeny group				ATB
			A n=(152)	B1 n=(51)	B2 n=(16)	D n=(16)	
Chicken meat	*EC1*	*hlyf*	14 (9%)	5 (9.8%)		3 (18.75%)	Amp., Amx., Amx+AC., GN., K., N., Cip., UB30., SSS., Tmp., Sxt Tet., C.
	EC2	*ipah*	1 (0.6%)				Amp,. Amx., K., C., Sxt., Tmp., Cip., UB30., SSS., Tet
	EC3	*hlyA*	1 (0.6%)				–
	EC4	*fyua*	3 (1.9%)				S., Tet., SSS.
	EC5	*papEF*	1 (0.6%)				Amp. k., S., Tet., SSS.
	EC6	*afa/draBC*	3 (1.9%)				
	EC7	*aggr*	1 (0.6%)				–
	EC8	*hlyF-fyuA*	2 (1.3%)				Amp., Amx., K., N., S., Cip., Sxt., SSS., Tet.
	EC9	*clbN- fyuA*			2 (1.25%)		S., Tet., SSS.
	EC10	*papEF- fyua*	2 (1.3%)				Amx+AC., Amx.
	EC11	*hlyf- aap-*				1 (6.25%)	S, Tmp, Sxt, UB30, SSS
	EC12	*papEF- fyua*				1 (6.25%)	Amp., Amx+AC., C., Tet., Tmp., Sxt., Cip., SSS., Amx.
Beef	*EC13*	*hlyF*	4(2.6%)	2 (3.9%)		1 (6.25%)	Amp., Amx., S., Tet., Tmp., SXT., SSS.
	EC14	*afa/draBC*	2 (1.3%)				Amx+AC., Aux., S., Tet., Tmp., SXT., SSS.
	EC15	*f17*			1 (6.25%)		Amp., Amx., Amx+AC., SSS., Tmp., Sxt., Tet., Aux.
	EC16	*aggr*	1 (0.6%)				–
	EC17	*fyua*				2	Amp., Amx., Tet., S., SSS., Tmp., Sxt.
	EC18	*Stx2*	1 (0.6%)				Amp., Amx., K., N., S., Cip., Sxt., SSS., Tet.
	EC19	*clbN- Cnf- fyua*			1 (6.25%)		Amp., K., N., S., Tet., C., SSS., Amx.
	EC20	*clbN- papEF - fyua*			1 (6.25%)		Tet.
	EC21	*clbN- cnf- fyua- ipah*			1 (6.25%)		Amp, K, N, S, Tet, SSS, Amx
Raw milk	*EC22*	*hlyA*	2 (1.3%)				–
	EC23	*f17*			1 (6.25%)		Amp., Amx., Amx+AC., SSS., Tmp., Sxt., Tet., Aux.
	EC24	*hlyF*	7	5 (9.8%)		1 (6.25%)	Amp., Amx., Amx+AC., GN., N., Cip., UB30., SSS., Tmp., Sxt., Tet., C.
	EC25	*fyua*	1 (0.6%)	1 (1.9%)			Amx+AC, Amx. Tet
	EC26	*clbN- hlyF*			1 (6.25%)		–
	EC27	*clbN- fyuA*			1 (6.25%)		–
	EC28	*aap*		1 (1.9%)			Amp., Amx+AC., K., N., S., Tet., C., Tmp,. CT., SSS., Amx.
	EC29	*clbN- cnf- fyua-*			1 (6.25%)		–
	EC30	*clbN- f17- fyua-*			1 (6.25%)		S., SSS.
Tot			46 (30.26%)	14 (27.45%)	11 (68.75%)	6 (37.5%)	

-: Sensitive to all tested antibiotics.

flumequine with 15.74% (37/235 strains), neomycin with 14.89 (35/235 strains) and colistin with 10.21% (24/235 strains). Resistance to cephalothin, trimethoprim, trimethoprim-sulfamethoxazole and sulfonamide was significantly higher for strains from chicken meat than beef and raw milk samples (P<0.05) (figure 2). In addition, 62.12% (146/235) of strains were resistant to at least one antibiotic. The prevalence of multiresistant strains ranged from 1 isolate resistant to 13 antibiotics (representing 0.42%) to 23 strains resistant to 3 antibiotics (9.78%). It can also be noted that thirteen antibiotypes were observed (table 4). All antibiotypes were present in chicken meat samples. Eighteen strains (7.65%) were resistant only to tetracycline. Nine strains (3.82%) were resistant to three antibiotics (tetracycline, ampicillin and amoxicillin) and four to 7 antibiotics (1.87%). Moreover, some strains showed simultaneous resistance to fluoroquinolone, gentamycin, streptomycin, and chloramphenicol.



(Amp: Ampicillin), (Amx+AC: Amoxicillin plus Clavulanic acid), (Amx: Amoxicillin), (CF: Cephalothin), (Gn: Gentamicin), (K: Kanamycin), (S: Streptomycin), (CT: Colistin), (N: Neomycin), (Cip: Ciprofloxacin) , (UB30: flumequine), (TMP: Trimethoprim), (Sxt: Sulfamethoxazole-Trimethoprim), (SSS: Sulfonamide), (Tet: Tetracycline), (C: Chloramphenicol).
* = (Chicken meat, beef) (P<0.05)
- (Chicken meat, raw milk) Significant difference (P<0.05)

Figure 2. The antibiotic susceptibility of the 235 *E. coli* strains isolated from chicken meat, beef, and raw milk.

Table 4. The most frequent antibiotic resistance patterns in the 235 *E. coli* strains isolated from food products (chicken meat, beef, and raw milk)

Resistance patterns	Chicken meat n = 85	Beef n = 73	Raw milk n = 77	Total n = 235
	b(%)[a]	b(%)[a]	b(%)[a]	b(%)[a]
Tet.	7(8.23)	6(8.21)	5(6;49)	18(7.76)
Amp., Amx+AC.	1(1.17)	4(5.47)	2(2.59)	7(2.97)
Tet., Amp., Amx.	1(1.17)	6(8.21)	2(2.59)	9(3.82)
Tet., Tmp., SXT., SSS.	2(2.35)	–	2(2.59)	4(1.70)
Amp., Amx., Tmp., Sxt., SSS.	3(3.52)	–	–	3(1.27)
Am., Amx+AC., SSS., Tm., Sxt., Tet..	2(2.35)	–	–	2(0.85)
Amp., Amx., Tet., S., SSS., Tmp., Sxt.	1(1.17)	2(2.73)	1(1.29)	4(1.70)
Amp., Amx., Cip., UB30., SSS., Sxt., K., Tet.	1(1.17)			1(0.42)
Amp., Tmp., Sxt., Tet., SSS., Amx., UB30., Cip., C.	1(1.17)	1(1.36)	1(1.29)	3(1.29)
Amp,. Amx., K., C., Sxt., Tmp., Cip., UB30., SSS., Tet.	1(1.17)		1(1.29)	2(0.85)
Amp., Amx., Amx+AC., Cip., Tmp., Sxt., SSS., K., S., T., C.	2(2.35)	–	–	2(0.85)
Amp., Amx., K., N., S., Cip., UB30., Tmp., Sxt., SSS., Tet., C.	1(1.17)	–	–	1(0.42)
Amp., Amx., Amx+AC., GN., K., N., Cip., UB30., SSS., Tmp., Sxt., Tet., C.	1(1.17)	–	–	1(0.42)

a: Number of the most frequent antibiotic-resistance patterns.
b: N° antibiotic-resistance pattern.

DISCUSSION

The consumption of animal products such as meat and milk is the origin of many health problems, as revealed by several disease outbreaks and investigations of food products sold in different countries. Therefore, determining the microbial content of raw meat and milk products is an important concern for the meat and milk industries. Although many researchers have attempted to identify the foodborne pathogens in meat and raw milk, relatively few reports are available on the prevalence of commensal *E. coli* as a faecal contamination indicator (Schlundt, 2002; Aslam *et al.*, 2003).

The main objective of the present study was to demonstrate that commensal *E. coli* might be a source of contamination of food products of animal origin. To our knowledge, this study is the first to report an in-depth analysis of *E. coli* strains isolated from chicken meat, beef, and raw milk sold in Jijel's area, Eastern Algeria. We performed systematic phylogenetic grouping, screening of virulence genes and the evaluation of resistance to different antimicrobial agents. Two hundred and thirty-five *E. coli* strains isolated from these food products were classified into four phylogenetic groups (A, B1, B2, and D) using the triplex PCR method of Clermont *et al.* (2000). Most *E. coli* strains are belonged to the A and B1 phylogenetic groups. Other authors have reported similar results (Unno *et al.*, 2009; Lagerstrom & Hadly, 2021; Lozica *et al.*, 2021). However, in contrast to the results obtained by Soufi *et al.* (2009), we found in the present study that the number of strains classified in the phylogenetic group D was higher in chicken meat than in beef and raw milk. The large number of A and B1 strains recorded in the present study could be explained by the fact that the samples were obtained from healthy animals, and they were probably of faecal origin. These groups are generally associated with commensal strains, whereas in most cases, enteropathogenic strains are assigned to group D and uropathogenic strains are assigned to group B2 (Lozica *et al.*, 2021). We should mention here that enterohemorrhagic *E. coli* O157H7 belongs to the phylogenic group A.

The PCR screening showed that the ExPEC bacteria of group B2 (in all sample types) account for 5.53% of the total. The ExPEC have been associated with both human and animal diseases (Manges & Johnson, 2012). It has been reported that foods of animal origin, such as the samples analysed here, are important vehicles for these bacteria and could be implicated in infections of humans and dairy animals (Joanne *et al.*, 2011; Amer *et al.*, 2020). It is important to note that the prevalence of ExPEC recorded in this study was much higher than that recorded in Spain by Quinto and Cepeda (1997) (0.4%) and in Ontario (Canada) by Steele *et al.* (1997) (0.87%). However, our results were slightly lower than that reported by Johnson *et al.* (2005) (46%) in poultry samples from 10 retail markets in the Minneapolis-Saint Paul area (USA) between 2001 and 2003. Moreover, in our study, the prevalence of *hlyF* in chicken meat was higher than in beef and raw milk as reported by James *et al.* (2009).

Here, only one isolate of *E. coli* from beef encoded Shiga-toxin (*stx2*) gene. This result is in agreement with the report of Abdullah *et al.* (2010). It should also be noted that Garcia-Aljaro *et al.* (2009) reported that Stx2 which is of phage origin can be the cause of carriage and dissemination of antibiotic resistance genes such as chloramphenicol, tetracycline, sulfamethoxazole, streptomycin, trimethoprim, and trimethoprim-sulfamethoxazole.

Usually, the *E. coli* strains belonging to B2 and D phylogroups carry more virulence-associated genes and have lower antimicrobial resistance rates than the so-called nonpathogenic commensal strains (phylogenetic group A and B1) (Cocchi *et al.*, 2007; Cortes *et al.*, 2010). The present study found the virulence-associated genes in the strains belonging to phylogenetic groups B1, B2, D and one strain in the A group. Most of our strains carrying two or more virulence-associated genes belonged to group D or B2, as previously observed by Cortes *et al.* (2010). Moreover, it should be noted that our results reveal the presence of strains carrying gene virulence can also be multiresistant and belonging to the phylogroups recognised as regrouping the most pathogenic strains like B1, B2 and D. Such is the case of the patterns cited in the results section (table 3). This observation should alert us to the probable carrying of antibiotic resistance genes which may be associated with virulence genes.

Antibiotic resistance is also a global problem that encourages researchers to survey the evolution of antibiotic resistance of different microbial pathogens. It is important to preserve the efficacy of the antibiotics used in human and veterinary medicine and prevent the dissemination of antimicrobial resistance genes in the environment. The appearance of resistance profiles is usually caused by the extensive use of antibiotics, leading to the spread of resistance genes among the bacteria (Smith *et al.*, 2007; Gyles, 2008). The association between the use of antibiotics in animal feeds and the emergence of resistant bacteria has been known for a long time (Levy *et al.*, 1976; Aarestrup *et al.*, 1998; Lathers, 2001; Barbieri *et al.*, 2017; Messaili *et al.*, 2019). Therefore, examining resistant clones in foods of animal origin is important to determine the impact of on-farm use of antimicrobials and the possible food-borne transmission of the resistant clones to humans and is an idea that should be considered in our future work (Lerminiaux *et al.*, 2019; Thomrongsuwannakij *et al.*, 2022).

Our results reveal the presence, in food products, of strains carrying virulence genes and resistant to the various antibiotics prescribed in human and animal medicine and even some of them are prohibited from use in Algeria either due to their toxicity or are reserved for the treatment of certain serious bacterial infections such as cholera disease, e.g. Chloramphenicol. The obtained results agree with previously published studies on chicken meat (Amara

et al., 1995; Hammoudi & Aggad, 2008; Aggad *et al.*, 2010; Messaili *et al.*, 2019) and beef samples (Schwaiger *et al.*, 2012).

Our data also revealed the presence of three major antibiotic-resistance groups. In the first group, between 50% and 70% of strains were resistant to Amp, Amx, Tmp, SSS and Tet. In the second one, 20% to 50% of resistant strains were observed (Amx+AC, K, S, Cip, UB30, and N) and the third group included less than 20% of resistant strains (CF, GN, and CT). Furthermore, antibiotic resistance to UB30, Tmp, Sxt and Tet was higher in chicken-derived strains than that observed in beef and milk strains. Similar results have been observed in Saudi Arabia as reported by Abdullah et al (2010). We should also note the appearance of multiresistance as most strains (63.7%) were resistant to at least 2 antibiotics.

In Algeria, numerous antibiotics are often administrated concomitantly for prophylaxis or to fight infections. The high incidence of antibiotic resistance and multiresistance of *E. coli* is possibly caused by the extensive and indiscriminate use of antibiotics and the likely dissemination of resistance genes in the poultry industry and bovine herds in Algeria. Such practices, especially without prior antibiotic sensitivity testing of bacterial strains, may lead to the development of a pool of antibiotic-resistant genes and the selection of increasing numbers of resistant *E. coli* gene clones as reported by Chique *et al.* (2019). Antibiotic resistance is frequently encoded by conjugative plasmids or transposons; thus, *E. coli* of avian origin could be a source of antibiotic resistance in other bacterial species, including human and other animal pathogens (Miles *et al.*, 2006). An increase in the pool of antibiotic-resistant bacteria could heavily impair the treatment of human and animal diseases.

Finally, in Algeria, beef, chicken meat, and raw milk represent the major sources of animal proteins for a large part of the population. The poor hygiene associated with the food processing plants and the sale of the products in butcher shops and other inadequately controlled outlets carries a high risk of microbial contamination. In addition, gene exchange may also contribute to the rising prevalence of pathogens in foods as reported by previous studies (Cohen & Karib, 2006; Garcia-Aljaro *et al.*, 2009; Murase *et al.*, 2016; Kim *et al.*, 2022). Pathogenic bacteria such as *E. coli* may be transferred to raw milk, which should be considered as a potential vehicle for transmitting bacterial pathogens (Mohamed-Zeinhom & Gihan, 2014). Since many people still drink uncooked milk, especially in rural areas, some effort should be put into informing the population about the health risks associated with the consumption of raw unpasteurized milk or poultry consumption (Manges *et al.*, 2007; Johar *et al.*, 2021).

We can conclude that the phylogenetic groups A and B1 were the most prevalent among the 235 *E. coli* strains isolated from three different food products (beef, chicken meat and raw milk), followed by groups B2 and D. The screening of the virulence genes for the isolated and identified strains allowed us to reveal the presence of pathogenic strains belonging to the different groups of pathovars. A high occurrence of the ExpEC was recorded with the predominance of the *Hlyf* gene, followed by the EPEC with the presence of EIECs and EAEC and a single strain affiliated with the EHEC pathovars. Antibiotic sensitivity tests revealed the presence of polyresistant strains to antibiotics in the different types of analysed food product samples. Also, our results show that several strains carrying virulence genes are resistant to at least two types of antibiotics and even to chloramphenicol, which is normally prohibited for use in veterinary medicine. Finally, the obtained results should be taken into account in future studies, focusing on the role of meat products and raw milk bacteria as vectors for the transmission of virulence genes and genes that are responsible for antibiotic resistance.

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