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ANTIBIORESISTANCE, PHYLOGENY AND VIRULENCE MARKERS OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM CHICKEN MEAT COMMERCIALISED IN JIJEL AREA (EAST ALGERIA)

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Abstract

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This study was conducted to investigate the prevalence, phylogeny, virulence markers and antibioresistance of commensal *Escherichia coli*. In order to get this objective Eighty-five (85) *Escherichia coli* isolates were obtained from 100 chicken meat samples commercialised in butcheries localised in Jijel area (East Algeria). Our results showed that strains belonged to phylogenetic groups A and B1 were the most common 67.5% and 16.9% respectively followed by B2 4.7% and D 11.76% groups. Antibiogramme results revealed three great antibiotic groups and more than one hundred antibiotype, the first include antibiotics which have between 50% to 70% of resistant strains (Ampicillin, Amoxicillin, Trimethoprim, Sulphonamid, Tetracycline), the second one, have 20% to 50% of resistant strains (Amoxicillin plus Clavulanic acid, Kanamycin, Streptomycin, Ciprofloxacin, Flumequin, Neomycin) and the third group include less than 20% of resistant strains (Cephalothin, Gentamicin, Colistin). Furthermore, antibioresistance to Flumequin, Trimethoprim, Sulfamethoxazole-Trimethoprim, and Tetracycline are more frequent in analysed chicken meat samples. PCR investigation shown the predominance of strains with Extraintestinal pathogenic *E. coli* virulence factors related genes (2.4%) and the presence of strains with EAEC and EIEC genes. We noted also that strains carrying more than two virulence factors are belonged to B2 or D phylogenetic groups and present less resistance to antibiotics whereas strains belonged to group A or B1 present less virulence factors and high rate of antibioresistance.

Key words: chicken meat, *Escherichia coli,* virulence factors, antibioresistance, phylogeny

Introduction

Microbial food safety and food-borne infections are an important public health concern in many countries worldwide. Some food-borne diseases are resulting from consumption of animals contaminated food products like milk and meat. Several pathogens involved in this type of infections such as pathogenic *E. coli* strains have a zoonotic origin (Rivera-Betancourt et al., 2004; James et al., 2007). Moreover many studies reported that, pathogenic *E. coli* strains were detected in beef-processing plants. These contaminations are related to the deficiencies in hygiene and meat handling practices in slaughters and in butcheries (Johnson et al., 2005; Holko et al., 2006).

E. coli is a normal commensal inhabitant of the intestinal tract of humans and animals (Holko et al., 2006). Strains of this species can be assigned to one of the main phylogenetic groups A, B1, B2 and D (Clermont et al., 2000). Moreover, pathogenic strains of *E. coli* can harbour common genetic character in human and animals (Clermont et al., 2011).

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Pathogenic *E. coli* strains were divided into numerous categories or pathotypes on the basis of their distinct virulence properties and the clinical symptoms of the host. The intestinal *E. coli* strains include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (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-Sieket al., 2005).

Generally; virulence factors and virulence genes are similar in strains of the same pathotype. Moreover, the virulence genes are used as targets for the determination of the pathogenic potential of any given *E. coli* isolate (Aslam et al., 2003; Holko et al., 2006). It has been reported that domestic animals can be asymptomatic vectors of virulent strains (Chapmann et al., 1993), where, ruminants are frequently colonised by EHEC whereas other animals like avian populations carry especially ExPEC strains, the reason(s) for this is still unknown (Cornick et al., 2002).

In Algeria, the use of antibiotics is not controlled and sometimes they may be commercialised without the veterinary surgeon notice. This situation promotes the appearance of the multiresistant strains. Several studies reported that the antibiotic selection pressure is responsible for high proportion of resistant bacteria (Smith et al., 2007).

In Algeria, chicken meat represents the major source of animal proteins for a large part of population and are the most consumed products. Furthermore, epidemiological data about characterisation of *E. coli* strains in food products of animal origin are not available in Algeria and this appears to be the first study that investigates at the same time virulence, phylogeny and antibioresitance of *E. coli* isolates from chicken meat.

In order to obtain data to help for bacterial risk assessment for consumers, our objectives were (i) to make a collection of *E. coli* strains isolated from chicken chopped which is commercialised in butcher and traditional shops in Jijel (city in the East of Algeria), (ii) to make a screening of virulence genes and to determine phylogenic group of each strain, (iii) to evaluate the resistance of these *E. coli* strains to different antimicrobial agents used in human and in veterinary medicine in Algeria.

Materials and Methods

Samples collection

Respectively, 100 samples of chicken meat, were randomly taken (Two per month) from 10 retails markets localised in the town of Jijel. All samples were sent to the laboratory in sterile bags on ice and processed the same day at Microbiological Laboratory of the Microbiology and Biochemistry Department (University Mohamed Sedik BEN YAHIA, Jijel, Algeria).

Culture and isolation

E. coli were isolated from samples as previously described (Zhao et al., 2001). Briefly, 25g portions of chicken meat were taken aseptically. After homogenization in 225 mL of sterile buffered peptone water (Institut Pasteur, Algeria (IPA)), for each sample 1 ml of the suspension was plated onto approximately 15 ml of Violet Red Bile Agar (VRBG; IPA) and incubated at 37°C for 24 hours.

Primary identification procedures involved subculturing of the presumptive *E. coli* colonies into brilliant green bile broth (IPA) containing an inverted Durham tube, as well as into tryptone water without indol (IPA). These were incubated at 37°C for 24 h. After incubation, gas production in the form of a bubble in the inverted Durham tube showed a positive result. Kovacs (IPA) reagent (0.2–0.3 ml) was added to tryptone water to detect indol production; a positive reaction is seen by a red surface layer. One Presumptive *E. coli* is recovered and confirmed by Gram coloration and biochemical tests. Previous data show 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). Identified strains were stored at –80°C (30% Glycerol). In order to process to molecular and antibioresistance studies at the National Veterinary School of Toulouse – UMR 1043, a total of 85 *E. coli* isolates were cultured on Muller Hinton agar medium (IPA) and stored at ambient temperature during the travel.

Screening for potentially pathogenic E. coli

The collection of 85 isolated was screened for the presence of 3 virulence genes associated EHEC with (*eae, stx1 and stx2*), and 10 virulence genes associated ExPEC with (*f17A, cnf, papEF, afa/draBC, fyua, clbN, hlyf, kpsMT(K1), hlyA and sfa/focDE*), one virulence gene associated EIEC with (*ipah*) and 3 virulence genes associated EAEC with (*AAprobe, aap and aggr*). DNA was extracted from overnight cultures using NaOH and was subjected to multiplex and uniplex PCR sets. A triplex PCR was performed to detect *eae,* stx_1 and stx_2 genes as already described by Paton and Paton, 1998. EHEC O157:H7 RIMD 050992 (Sakaï) (Hayashi et al., 2001) was used as a positive control. Concerning the ExPEC, one triplex PCR was performed to detect *sfa/focDE* (Le Bouguenec et al., 1992)*, kpsMT* K1 (Johnson and Stell, 2000) and *hlyA* (Johnson and Stell, 2000), one triplex PCR to detect *papEF* (Yamamoto et al., 1995) *afa*/*draBC* and *fyua*

(Johnson and Stell, 2000), one duplex PCR to detect *clbN* (Johnson and Stell, 2000) and *hlyF* (Moulin-Schouleur et al., 2007), one uniplex PCR to detect *f17A* gene (Bertin et al., 1996) and one to detect *cnf* gene (Yamamoto et al., 1995). For EAEC, a triplex PCR was performed to detect *AAprobe, aap and aggr* as describe by Cerna *et al.*(Cerna et al., 2003) and finally, for EIEC, one 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., 2002), 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 et al., 1974), O42 for *AAprobe, aap and aggr* and EDL1284 for *ipah* (Aranda et al., 2007).

Phylogenetic group classification

All *E. coli* isolates were screened for phylogenetic groups by using multiplex PCR with the *chuA* and *yjaA* genes and the DNA fragment *TSPE4.C2* according the method described by Clermont et al. (Clermont et al., 2000). Representative *E. coli* Reference Collection strains were used as template control.

Antibiotic sensitivity tests

85 *E. coli* strains were subjected to antimicrobial susceptibility testing. Antimicrobial susceptibility tests were performed by using a disk diffusion method according to the CLSI standards (CLSI, 2009a) 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, Amoxicillin plus Clavulanic acid, Amoxicillin, Cephalothin, Gentamicin, Kanamycin, Streptomycin, Colistin, Neomycin, Ciprofloxacin, Flumequin, Trimethoprim, Sulfamethoxazole-Trimethoprim, Sulphonamid, Tetracycline, Chloramphenicol susceptibility breakpoints for all antimicrobials were those recommended by CLSI (CLSI, 2008, 2009b). Isolates were classified as multiresistant when they exhibited resistance to three or more classes of antimicrobial agents (Schwarz et al., 2010).

Results

E.coli strains collection

The prevalence of *E. coli* in all Chicken meat samples was 85% (85/100). Eighty five *E. coli* strains isolated were submitted to PCR for the detection of 17 virulence genes. Thirty seven strains (43.52%) (37/85) were identified as potentially pathogenic because of the identification of one or more virulence genes.

PCR screening of phylogenetic groups

Phylogenetic group A *E. coli* strains were the most common in chicken meat 53 (62.35%), followed by group B1 strains 18 (21.17%), group D 10(11.76) strains and group B2 4 (4.7%) strains (Figure 1).

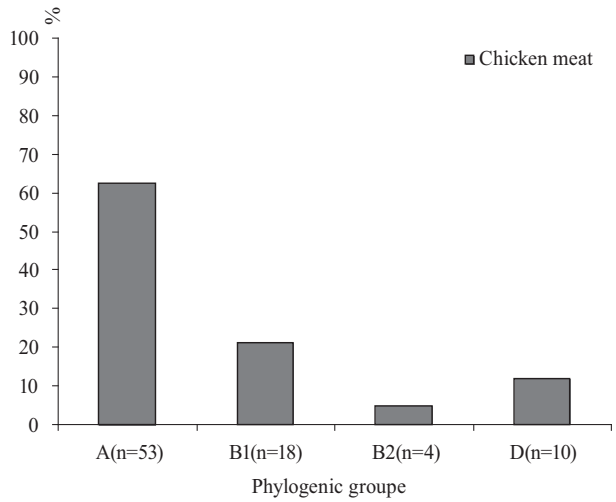

Fig. 1. Phylogenetic groups repartition of *E.coli* strains isolated from chicken meat

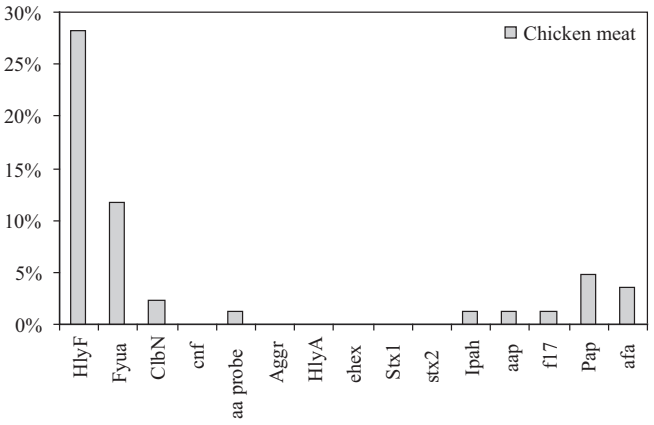

Fig. 2. Distribution of genes encoding virulence factors in *E. coli* strains isolated from chicken meat
(Tet : Tetracycline); (Amp: Ampicillin); (Sxt : Sulfamethoxazole-Trimethoprim); (SSS : Sulphonamid); (Amx : Amoxicillin); (TMP : Trimethoprim); (S : Streptomycin); (UB30 : Flumequin); (K : Kanamycin); (Cip : Ciprofloxacin); (Amx+AC : Amoxicillin plus Clavulanic acid); (CT : Colistin); (N : Neomycin); (C : Chloramphenicol); (CF : Cephalothin); (Gn: Gentamicin).

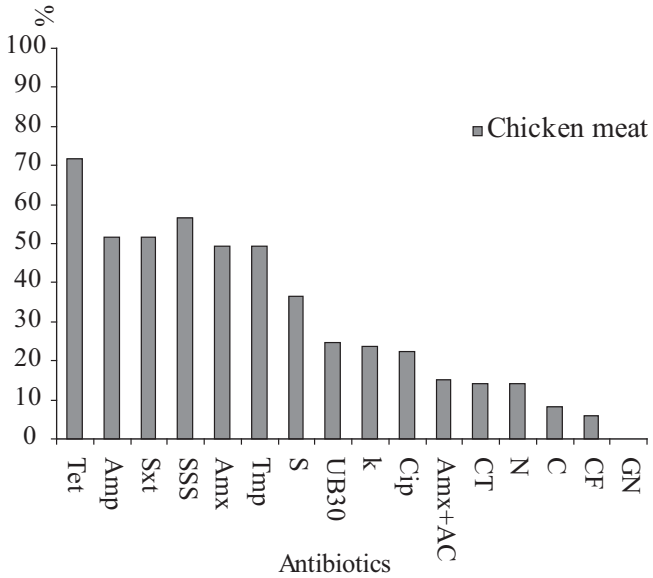

Fig. 3. Results of antibiotic sensitivity tests of *E. coli* strains isolated from chicken meat

Table 1
Virulence patterns and associated phylogenetic group among *E. coli* strains isolated from chicken meat

Virulence pattern	Genes Association	Number of *E. coli* strains	Phylogenetic Group
EC1	*Pap, fyua*	1	A
EC2	*hlyF, fyua*	3	B2
EC3	*hlyF, aap, Aaprobe*	1	B1
EC4	*hlyF, Ipah*	1	D
EC5	*ClbN, fyua*	2	B2
EC6	*Pap, afa, fyua*	2	D

Virulence genes screening by conventional PCR

The prevalence of virulence genes ranged from 0% for *stx1,stx2, eae, aggr, cnf, , sfa/focDE, kpsMT (K1*), *and afa/draBC*, to 28.23% (24/85) strains for hlyf. The adhesine coding genes, pap (4.76%) (4/85), *afa* (3.52%) (3/85) were the most prevalently detected, followed by *f17* and *ipaH,* (1.17%) (1/85) strain. The *fyuA* gene, encoding the yersiniabactin receptor 11.76% (10/85) strains was more prevalent than clbN 2.35% (2/85) strains (Figure 3). Moreover the studied strains exhibited six (6) different virulence genes patterns (Table 1).

Antibiotic sensitivity tests

The susceptibility of the 85 isolated *E. coli* strains to 16 antibiotics is shown in Figure 3.

The highest prevalence of resistance was detected for Tetracycline with 96.41% (82/85), followed by Amoxicillin and Sulphonamide 51.76% (44/85), Ampicillin 50.58% (43/85), Sulfamethoxazole-trimethoprim 48.23% (41/85), Trimethoprim 44.70% (38/85), Streptomycin 34.11% (29/85), Kanamycin and Flumequin 25.88% (22/85), Ciprofloxacin 18.82% (16/85), Amoxicillin+Calvulanic acid and Neomycin 15.29% (13/85), Colistin 11.76% (10/85), Chloramphenicol 10.58% (9/85), Cephalotin 3.52% (3/85) (Figure 2). All strains isolated form chicken meat samples were susceptible to gentamicin. In addition, 71.86% (61/85) of strains were resistant to at least one antibiotic. The prevalence of multiresistant isolates ranged from one (1/85) isolate which was resistant to 13 antibiotics (represent about 1.17%) to (20/85) isolates which were resistant to 2 antibiotics (represent about 23.52%). However, results showed that strains belonged to phylogenetic group A present a higher prevalence of multiresistance patterns (41/85 isolates) than B1 (16.47%) (14/85), B2 (3.52%) (3/85) and D (5.88%) (5/85).

A total of 13 major antibiotypes present in chicken meat samples could be distinguished (Table 2). A total of (8.23%) (7/85) strains were resistant only for tetracycline, (4.7%) (4/85) strains were resistant for the 3 antibiotics: Tetracycline, Trimethoprim and Sulfamethoxazole-Trimethoprim followed by those resistant to 5 antibiotics (3.52%) (3/85). Some strains exhibited resistance at the same time for Fluoroquinolone, Gentamycin, Streptomycin ant Chloramphenicol.

Discussion

Many health problems are related to the consumption of animal products like meat and milk, as supported by several investigations and outbreaks carried out on food products commercialised in markets in different countries. Moreover, determination of microbial content of raw meat is an important concern for the meat industries. Although, many researchers have attempted to identify the occurrence of foodborne pathogens in meat, whereas, relatively few reports are available on the prevalence of commensal *E. coli* as a faecal contamination indicator as compared with *Campylobacter*, *Salmonella* and pathogenic *E. coli* like STEC (Aslam et al., 2003).

In the present study, our objective is to demonstrate that commensal *E. coli* may be a source of contamination of animal food products. Furthermore, this study appears as the first report that compare the systematically phylogenetic grouping, screening of virulence genes and evaluation of the resistance to different antimicrobial agents of *E. coli* strains isolated from chicken meat, commercialised in Algeria. 85 *E. coli* strains isolated from chicken meat commercialised in the urban areas of Jijel (East of Algeria), were classified into

Table 2
Most frequent antibiotic resistance patterns in Escherichia coli strains isolated from chicken meat (n=85)

Resistance patterns	Number of isolates	Percentage of isolates
Tet	7	8.23
Amp-Amx+AC	1	1.17
Tet-Tmp-STX.	4	4.70
Tet-Tmp-SXT-SSS	2	2.35
Amp-Amx-Tmp-Sxt-SSS	3	3.52
Am-Amx+A-SSS-Tm-Sxt-Tet	2	2.35
Amp-Amx-Tet-S-SSS-Tmp-Sxt.	1	1.17
Amp-Amx-Cip-UB30-SSS-Sxt-K-Tet	1	1.17
Amp-Tmp-Sxt-Tet-SSS-Amx-UB30-Cip-C	1	0.43
Amp-Amx-K-C-Sxt-Tmp-Cip-UB30-SSS-Tet	1	1.17
Amp-Amx-Amx+AC-Cip-Tmp-Sxt-SSS-K-S-T-C	2	2.35
Amp-Amx-K-N-S-Cip-UB30-Tmp-Sxt-SSS-Tet-C.	1	1.17
Amp-Amx-Amx+AC-GN-K-N-Cip-UB30-SSS-Tmp-Sxt-Tet-C	1	1.17

(Tet : Tetracycline); (Amp: Ampicillin); (Sxt : Sulfamethoxazole-Trimethoprim); (SSS : Sulphonamid); (Amx : Amoxicillin); (TMP : Trimethoprim); (S : Streptomycin); (UB30 : Flumequin); (K : Kanamycin); (Cip : Ciprofloxacin); (Amx+AC : Amoxicillin plus Clavulanic acid); (CT : Colistin); (N : Neomycin); (C : Chloramphenicol); (CF : Cephalothin); (Gn: Gentamicin)

four phylogenetic groups (A, B1, B2 and D) using the triplex PCR method of Clermont *et al.,* 2000. The most number of *E coli* isolates are belonged to the phylogenetic groups A and B1, followed by B2 and D phylogenetic groups, respectively. Similar results have been reported by (Unno et al., 2009). In contrast to the results obtained by Soufi et al. (2009), the number of strains belonged to phylogenitic group D in this study was higher. The higher proportion of A and B1 isolates in the present study could be explained by the fact that they were from healthy animals, and they were probably of faecal origin. These groups are generally associated with commensal isolates, whereas in most cases, enteropathogenic isolates are assigned to group D and uropathogenic isolates are assigned to group B2 (Johnson et al., 2005). We should mention here that enterohemorrhagic *E. coli* O157H7 is belonged to the phylogenic group A.

It was reported that exPEC have been associated with human and animal diseases (Manges, A., and Johnson, J.R., 2012) and foods of chicken meat origin are the important vehicles for exPEC and could be implicated in extra-intestinal infections of humans and dairy animals (Joanne et al., 2011). The prevalence of exPEC showed in our study was much higher than that registered in Spain (0.4%) by Quinto and Cepeda (1997) and in Ontario (Canada) (0.87%) by Steele et al. (1997). Meanwhile, our result was slightly lower than those reported by Johnson et al. (2005) (46%, in poultry) in 10 retail markets in the Minneapolis–St.Paul area (USA) during 2001–2003.

Regarding to phylogenetic groups, it have been previously reported that *E. coli* isolates belonging to B2 and D phylogroups usually carry more virulence-associated genes and present less antimicrobial resistance rates than the so-called non-pathogenic commensal strains (phylogenetic group A and B1) (Boyd and Hartl, 1998; Cocchi et al., 2007 and Cortes et al., 2010). In the present study, virulence-associated genes was found in the *E. coli* isolates that belonged to phylogenetic groups B2 and D and only one strain belonged to A phylogenetic group and tow strains to B1. Most of our isolates carrying tow or more virulence associated genes belonged to group D or B2 as previously observed by Cortes et al. (2010).

Throughout the world, microbiologists are encouraged to survey the evolution of antibiotic resistances of different microbial pathogens, to preserve the efficacy of the used antibiotic molecules in human and veterinary medicine and the dissemination of antimicrobial resistance genes in the environment. The appearance of resistance profiles are usually determined by the extensive use of antibiotics and the spreading of resistance genes in the environment (Gyles, 2008; Smith et al., 2007). The relationship between the use of antimicrobial agents in animal foods and the emergence of resistant bacteria has long been reported (Levy et al., 1976, Aarestrup, 1998; Lathers, 2001). Therefore, carriage of resistant clones by foods of animal origin is of considerable importance in determining the impact of on-farm antimicrobial use and the possible foodborne transmission of these resistant clones to humans. In Algeria, the use of antibiotics is not so much controlled, and only prescription of chloramphenicol is prohibited. Our results are in accordance with previous published studies carried out on chicken

pathological samples (Aggad et al., 2010; Hammoudi and Aggad, 2008; Amara et al., 1995) and imported bovine frozen meat (Barka and Kihal, 2010). Particularly, our results revealed the presence of three major antibiotic groups, the first one include antibiotics which have between 50% to 70% of resistant strains (Amp, Amx, Tmp, SSS, Tet), the second one, have 20% to 50% of resistant strains (Amx+AC, K, S, Cip, UB30, N) and the third group include less than 20% of resistant strains (CF, GN, CT). Moreover, Antibioresistance to UB30, Tmp, Sxt, and Tet are highest in chicken meat. Similar results were reported in Saudi Arabia by Abdullah et al. (2010). We should note that multiresistance appeared as a serious problem as the majority of strains (63.7%) was resistant to at least 2 antibiotics. Indeed, numerous antibiotics are administrated often concomitantly for prophylaxis or in infections. This indicates that the abusive and indiscriminate use of antibiotics is probably at the origin of the high incidence of antibioresistances and multiresistances of *E. coli* and the dissemination of resistance genes in poultry industry herds in Algeria. Such practices, especially without prior antibiotic sensitivity testing of bacterial isolates, may lead to the development of a pool of antibiotic-resistant genes and to the selection of increasing numbers of resistant *E. coli* clone genes. Antibiotic resistances are frequently encoded by conjugative plasmids or transposons, thus *E. coli* of avian origin could act as a possible source for the transfer of antibiotic resistance to other bacterial species including human and other animal pathogens (Miles et al., 2006). Otherwise, an increase in the reservoir of antibiotic resistant bacteria could heavily impair the treatment of human and animal diseases.

The results reported in this present study are a survey of the incidence of potentially polyresistant and virulent *E. coli* strains in food products of animal origin in Jijel, Algeria. Conventional microbiological diagnostics in food control includes only determination of *E. coli* numbers per gram without any other characterization of isolated strains. According to the obtained results, the incidence of potentially virulent strains of *E. coli* was important and the diagnostic based only on the determination of the number is probably not sufficient to make a decision on the quality and the safety of the product; consequently the authorities should take the necessary measures to protect consumer health by obligating the food control laboratory to use advanced and adequate methods to investigate microbial pathogens.

Comparing with other countries, Algerian experience in the novel culinary traditions is not newer, but there is an obvious tendency to adopt international standards for processed foods. Such tendency combined with poor hygienic practices may contribute to increase the prevalence of pathogens in foods, thereby increasing the risk of food borne disease for consumers (Cohen and Karib, 2006). Moreover, in the light of recent epidemiological findings, it was found that urinary tract infections in humans may be associated with poultry consumption (Manges et al., 2007).

Conclusion

Our data show that phylogenetic groups A and B1 were the most prevalent among the 85 *E. coli* isolates, followed, respectively by those of group B2 and D in chicken meat. A higher prevalence of exPEC was shown in our study. Moreover, our results revealed a high prevalence of *hlyF* and a high level of *E. coli* antibioresistance was recorded in strains isolated from chicken meat samples. These findings support the need for more rigorous surveillance and improved farming practices that can reduce the carriage of antibiotic resistance and virulence genes. Further studies are required to demonstrate the role of meat products bacteria as vectors in transmitting drug resistance. Attention needs to be paid to personnel hygiene in processing and handling of raw chicken food products and to these likely sources of resistant bacteria in Algeria.

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