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ORIGINAL ARTICLE

Clonal relationship between human and avian ciprofloxacin-resistant *Escherichia coli* isolates in North-Eastern Algeria

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Abstract The objectives of this study were to determine rates, patterns, and mechanisms of antibiotic resistance, and to assess connections between chicken commensal, human commensal, and pathogenic ciprofloxacin-resistant *Escherichia coli* isolates. All *E. coli* isolates collected from chickens, their farmers, and patients in the Constantine region (North-east Algeria) were analyzed for *bla* and plasmid-mediated quinolone resistance (PMQR) gene contents, phylogroups, Rep-PCR profiles, and multilocus sequence types. A high prevalence of resistance to fluoroquinolones (51.4 % to ciprofloxacin) was recorded in avian isolates. Of these, 22.2 % carried the *aac(6')-Ib-cr* gene, whereas lower resistance levels to these antibiotics were recorded in chicken farmers' isolates. None of the commensal isolates harbored the *qnr, qepA*, or *oqxAB* genes. One human pathogenic isolate was ertapenem-resistant and harbored the bla_{OXA-48} gene, 84 showed an extended-spectrum β-lactamase phenotype, with $bla_{CTX-M-15}$ gene prevalent in 87.2 % of them. Seventy isolates were resistant to fluoroquinolones, with *aac(6')-Ib-cr* present in 72.8 %, *qnrB* in 5.7 %, and *qnrS* in 10 %. Three Rep-PCR profiles were common to chicken commensal and human pathogenic isolates (phylogroups D and B1; ST21, ST48, and ST471 respectively); one was found in both chicken and chicken-farmer commensal strains (D; ST108), while another profile was identified in a chicken-farmer commensal strain and a human pathogenic one (B1; ST19). These findings suggest clonal and epidemiologic links between chicken and human ciprofloxacin-resistant *E. coli* isolates and the important role that poultry may play in the epidemiology of human *E. coli* infections in the Constantine region.

Introduction

Escherichia coli are commensal bacteria found in the gastrointestinal tract of humans and many animal species. However, some strains are pathogenic and frequently encountered in clinical practice. In poultry production, colibacillosis (caused by avian pathogenic *E. coli*: APEC) continues to be a devastating disease all over the world.

Antimicrobial therapy is an important tool to reduce both the incidence of this pathology and its economic impact [1]. Nevertheless, used therapeutically or given sub-therapeutically as feed additives, antibiotics seem to have a profound impact on the development of antibiotic resistance in commensal bacteria, which are potential genetic partners in the transfer of resistance genes to pathogenic ones [2, 3]. Over many years, the multiplication of bacterial resistance to antibiotics in animals has become a major concern in both animal and public health, as resistant bacteria can pass through the food chain to people [4]. In several surveys, the use of antibiotics in animal production has been held responsible for the

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increase of rebellious human infections due to resistant bacteria. The association between deficiency in new marketed antibiotics and accelerated evolution of bacterial resistance in both animals and humans represents a real "emergency", and is more considered as an ecosystem problem, especially for commensal microfloras [5].

Many studies have demonstrated that some APEC strains could belong to the same clones as human extraintestinal pathogenic ones (ExPEC), and may act as potential pathogens for humans as they often possess similar virulence genes [6–9]. However, few studies have been investigating the relationship between avian non-clinical bacteria and human pathogenic ones.

The present study was designed to assess in detail rates, patterns, and mechanisms of antibiotic resistance in chicken commensal, human commensal, and pathogenic *E. coli* strains in the Constantine region (North-East of Algeria), and to evaluate the connection between them.

Material and methods

Chicken and chicken farmers' commensal isolates

Fourteen independent farms keeping chicken flocks (A to N, aged 35 days or more) from various regions of Constantine were randomly enrolled from March 2011 through December 2012. On each farm, a mixture of 20 fresh poultry droppings was collected as well as a stool sample from a consenting chicken farmer. At the laboratory, samples were diluted and cultured on MacConkey agar (BioMerieux, France). Suspected colonies were identified using the API 20E system (BioMérieux) and from each sample five *E. coli* colonies were arbitrarily selected for antibiotic susceptibility testing.

The following information was also recorded: flock size, age, health status and antibiotics used for birds, other animals kept at the farm, recent hospital stay and antibiotics taken by poultry farmers, their family members, or their companion animals during the last 3 months.

Human pathogenic isolates

We selected 94 (consecutive non-repetitive) human pathogenic *E. coli* isolates obtained from various clinical samples and collected from different wards of the Military Hospital of Constantine from March 2011 to December 2012. Epidemiological and clinical data were collected for each patient.

Antimicrobial susceptibility testing and ESBL detection

All isolates were subjected to susceptibility testing against 31 selected antibiotics important in clinical practice by the disc

diffusion method. Results were interpreted according to the SFM/EUCAST recommendations [http://www.sfm-microbiologie.org]. ESBL-production was screened by the double-disk synergy test and fluoroquinolones-resistant isolates were selected for further molecular analyses at the INSERM U1047 (France).

Characterization of β-lactamase-encoding and PMQR genes

Plasmid or chromosomal DNA was extracted from the isolates using the EZ1 DNA Tissue kit on the BioRobot EZ1 extraction platform (QIAGEN, France). Genes encoding for the most clinically prevalent carbapenemases (bla_{KPC}, $bla_{OXA-48-like}$, bla_{VIM}, bla_{IMP}, and bla_{NDM}) and ESBLs (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) were detected by PCR and confirmed by sequencing the PCR products [10–12]. Similarly, plasmid-mediated quinolone resistance (PMQR) genes ($qnrA$, $qnrB$, $qnrS$, $qepA$, $aac(6')$-Ib-cr, and $oqxAB$) were screened and confirmed (by PCR and sequencing) in all the quinolone-resistant isolates [13–16].

Phylotyping and O25b subgrouping

Phylogenetic groups of the *E. coli* isolates and the O25b subgroup of the ESBL-producers were determined by PCR as previously described [17, 18].

Clonality analysis

The genetic relationship of all the human and avian ciprofloxacin-resistant isolates was studied using Rep-PCR (DiversiLab system, BioMérieux). Isolates with identical patterns were considered indistinguishable if their similarity percentage was ≥95 %.

MLST

Multilocus sequence typing (MLST) was carried out by PCR amplification and sequencing of eight housekeeping genes: *dinB, icdA, pabB, polB, putP, trpA, trpB*, and *uidA*. Allelic profile and sequence type determinations were assigned according to the Institut Pasteur's MLST web site scheme (www.pasteur.fr/mlst).

Statistical analysis

Comparisons of antimicrobial resistance rates distribution and the resistance genes characteristics of the different *E. coli* isolated from poultry (P), their farmers (F) and clinical samples (C) were performed by calculating Fisher's exact test (at 95 % CI and $p < 0.05$ %) using SAS/ETS® version 9.1 (SAS Institute Inc., Cary, NC, USA, 2004).

Results

Drug resistance profiles and phylogenetic grouping in chicken commensal *E. coli*

The resistance phenotypes confirmed that among the 70 avian commensal *E. coli*, 44.3 % (belonging to all flocks except B and F) were resistant to three or more antimicrobial classes.

Resistance levels to nalidixic acid (88.6 %), ofloxacin (80 %) and ciprofloxacin (51.4 %) were very frequent, and all the strains were susceptible to 3rd generation cephalosporins and many other antibiotics (Table 1).

Among the ciprofloxacin-resistant isolates ($n=36$) none harbored *qnr, qepA*, or *oqxAB* genes, but 22.2 % of them carried the *aac(6')-Ib-cr* gene. These isolates were distributed among the four main phylogenetic groups: B1 (41.7 %), D (30.6 %), A (25 %), and B2 (2.7 %) (Table 2). They were detected from all the poultry farms except the F one, and 59.6 % of them belonged to flocks where quinolones had not been used.

Drug resistance profiles and phylogenetic grouping in chicken farmers commensal *E. coli*

These isolates showed lower resistance levels to nalidixic acid (15.7 %), ofloxacin (11.4 %), and ciprofloxacin (5.7 %), and all were susceptible to 3rd generation cephalosporins and other drugs (Table 1). None of the chicken farmers was treated with antibiotics, and 64.3 % of them had pets.

Half of the ciprofloxacin-resistant isolates were collected from farmers who did not use these drugs for their poultry. These isolates harboured no PMQR genes. They belonged to phylogroups A (25 %), B1 (25 %), and D (50 %) (Table 2), and were isolated in poultry farms B, D, F, and N.

Drug resistance profiles and phylogenetic grouping in human pathogenic *E. coli*

Among the 94 clinical isolates, one exhibited resistance to ertapenem (1.1 %), 84 (89.4 %) showed an ESBL phenotype, and 70 (74.5 %) were insensitive to ciprofloxacin.

Resistance to antibiotics was significantly more prevalent in clinical isolates than in farmers or poultry isolates (22/31 antibiotics tested), in particular to ciprofloxacin ($p<0.001$) (Table 1).

The ertapenem-resistant strain harboured the bla_{OXA-48} gene in association with bla_{TEM-1} and *aac(6')-Ib-cr*. Among the 84 ESBL-producers, 82 harboured the $bla_{CTX-M-15}$ gene and the two remaining strains carried bla_{TEM-24} and bla_{TEM-3}. These ESBLs were significantly associated with strains isolated from patients ($p<0.001$). Co-resistance to ciprofloxacin was shown by 78.1 % of the $bla_{CTX-M-15}+$ isolates (Table 2).

The *aac(6')-Ib-cr* gene was identified in 51 ciprofloxacin-resistant strains (72.9 %), *qnrS* in seven (10 %) and *qnrB* in five (7.1 %). The presence of the *aac(6')-Ib-cr* gene was significantly associated with clinical strains as compared to poultry or farmer ones ($p<0.001$). The *qnr*+strains also harboured *aac(6')-Ib-cr* gene in six of the *qnrS*+ and two of the *qnrB*+ strains. Ciprofloxacin-resistant isolates were scattered among the four major phylogenetic groups: D (40 %), A (24.3 %), B1 (18.6 %), and B2 (17.1 %) (Table 2).

Clonality analysis and MLST of ciprofloxacin-resistant *E. coli* strains

The obtained dendrogram showed that the 110 fluoroquinolones-resistant isolates were grouped in 38 Rep-PCR patterns (data not shown). Only seven profiles included five or more isolates: patterns XXIII ($n=14$; phylogroup D; ST3), XXXIII ($n=13$; B2; ST43), VI ($n=10$, B1; ST471), XXXVI ($n=10$; D; ST477), XIII ($n=8$; A; ST2), XVI ($n=5$; A; ST2), and XIX ($n=5$; A; ST347). The residual patterns contained two to four isolates (ten Rep-PCR patterns) or single isolates (21 Rep-PCR patterns). Cluster of the sixth profile was very diverse, intermixing isolates of different origins (hospital and several farms: A, C, D, and J), and all the clonally related isolates (determined by Rep-PCR) were of the same sequence-type defined by MLST. Three clonal groups (VI, X, and XXXI) connected chicken commensal isolates to human pathogenic ones. They belonged to phylotypes B1 and D and ST21, ST48, and ST471 (Fig. 1). One pattern (XXX) was common to chicken and chicken-farmer commensal strains (D; ST108), and another (I) was identified in a chicken-farmer commensal strain and a human pathogenic one (B1; ST19) (Fig. 1). The clustered isolates exhibited slight variations independently of their origin, year of isolation, resistance pattern, or sample nature. This suggests a link between the poultry *E. coli* isolates and the humans' ones.

None of the 12 human B2 O25b-ST131 strains clustered with any avian strain, suggesting the absence of any chicken reservoir for this worldwide clone. The same observation could be made for the $bla_{OXA-48}+$ strain that belonged to ST5.

Discussion

The findings of the present study provide evidence of a genetic homogeneity and a clonal link between *E. coli* from poultry, farmers, and clinical cases in the Constantine region, suggesting that avian commensal, human commensal, and pathogenic strains are from the same origin. However, the exact routes of transmission remain uncertain.

Chicken-to-human transmission of bacteria during farming has been largely demonstrated. Johnson et al. [19, 20] showed that ciprofloxacin-resistant *E. coli* strains from human blood

Table 1 Resistance profiles of *E. coli* isolated from poultry (P), their farmers (F) and patients (C)

Antibiotics	Origin of isolates			P		
	Poultry (n=70) n (%)	Farmers (n=70) n (%)	Clinical samples (n=94) n (%)	P vs F	P vs C	F vs C
Amoxicillin	55 (78.5)	10 (14.3)	93 (98.9)	<0.001	<0.001	<0.001
Ticarcillin	55 (78.5)	10 (14.3)	93 (98.9)	<0.001	<0.001	<0.001
Piperacillin	3 (4.3)	0 (0)	92 (97.8)	NS	<0.001	<0.001
Amoxicillin/clavulanic acid	0 (0)	0 (0)	76 (80.8)	NS	<0.001	<0.001
Ticarcillin/clavulanic acid	2 (2.9)	1 (1.4)	83 (88.3)	NS	<0.001	<0.001
Piperacillin/tazobactam	0 (0)	1 (1.4)	8 (8.5)	NS	NS	NS
Cefalotin	0 (0)	0 (0)	88 (93.6)	NS	<0.001	<0.001
Cefamandole	3 (4.3)	0 (0)	90 (95.7)	NS	<0.001	<0.001
Ceftazidime	0 (0)	0 (0)	78 (82.9)	NS	<0.001	<0.001
Cefotaxime	0 (0)	0 (0)	87 (92.5)	NS	<0.001	<0.001
Cefepime	0 (0)	0 (0)	76 (80.8)	NS	<0.001	<0.001
Cefoxitin	0 (0)	0 (0)	17 (18.1)	NS	<0.001	<0.001
Cefixime	0 (0)	0 (0)	82 (87.2)	NS	<0.001	<0.001
Aztreonam	0 (0)	0 (0)	84 (89.3)	NS	<0.001	<0.001
Mecillinam	16 (22.8)	3 (4.3)	68 (72.3)	0.002	<0.001	<0.001
Moxalactam	0 (0)	0 (0)	0 (0)	NS	NS	NS
Ertapenem	0 (0)	0 (0)	1 (1.1)	NS	NS	NS
Imipenem	0 (0)	0 (0)	0 (0)	NS	NS	NS
Meropenem	0 (0)	0 (0)	0 (0)	NS	NS	NS
Amikacin	0 (0)	0 (0)	4 (4.2)	NS	NS	NS
Netilmicin	0 (0)	0 (0)	29 (30.8)	NS	<0.001	<0.001
Tobramycin	0 (0)	0 (0)	62 (65.9)	NS	<0.001	<0.001
Gentamicin	0 (0)	0 (0)	54 (57.4)	NS	<0.001	<0.001
Nalidixic acid	62 (88.7)	11 (15.7)	75 (79.7)	<0.001	NS	<0.001
Ofloxacin	56 (80)	8 (11.4)	74 (78.7)	<0.001	NS	<0.001
Ciprofloxacin	36 (51.4)	4 (5.7)	70 (74.4)	<0.001	0.003	<0.001
Minocycline	8 (11.4)	0 (0)	32 (34.1)	<0.001	0.001	<0.001
Cotrimoxazole	58 (82.8)	9 (12.9)	80 (85.1)	<0.001	NS	<0.001
Nitrofurantoin	2 (2.9)	2 (2.9)	2 (2.1)	NS	NS	NS
Fosfomycin	0 (0)	0 (0)	0 (0)	NS	NS	NS
Colistin	0 (0)	0 (0)	0 (0)	NS	NS	NS

and fecal samples were almost identical to resistant isolates from geographically linked chickens, whereas drug-susceptible human *E. coli* strains were genetically distinct from avian ones, suggesting a possible dissemination via the poultry. *E. coli* strains could also be transmitted from poultry to humans through meat consumption and environmental contamination with manure or faeces of wild or domestic birds [21, 22]. Another route of antimicrobial-resistant *E. coli* entry into the community is through occupational exposure to *E. coli* from live-animal contact in the broiler chicken industry [23]. This had been confirmed by our findings, as we observed clonality between chicken and chicken farmers' strains, and between the latter and human pathogenic ones.

The sporadic nature and the appearance of new pathogenic *E. coli* strains by virtue of novel STs suggests that these strains may be commensal that have acquired the antibiotic resistance encoding plasmids (especially ESBL) [24]. This is in agreement with our findings, as we observed clonality between a $bla_{CTX-M-15}+$ ExPEC strain and one chicken-farmer commensal strain and another $bla_{CTX-M-15}+$ ExPEC strain with two chicken commensal ones (Fig. 1). Acquisition of resistance genes may also account for the differences in drug resistance between our clonal avian–human strains and clonal commensal–pathogenic strains, as the drug selection pressure in clinical environments is the major cause of the accumulation of resistance determinants against antibiotics. According to

Table 2 Resistance gene characteristics of ciprofloxacin-resistant *E. coli* strains from poultry, their farmers, and patients

|	Origin of isolates			P		
	Poultry (P) (n=36)	Farmers (F) (n=4)	Clinical samples (C) (n=70)	P vs F	P vs C	F vs C
	n (%)	n (%)	n (%)			
ESBL genes						
$bla_{CTX-M-15}$	0 (0)	0 (0)	65 (92.8)	NS	<0.001	<0.001
bla_{TEM-24}	0 (0)	0 (0)	0 (0)	NS	NS	NS
bla_{TEM-3}	0 (0)	0 (0)	0 (0)	NS	NS	NS
Carbapenemase genes						
bla_{OXA-48}	0 (0)	0 (0)	1 (1.4)	NS	NS	NS
PMQR genes						
qnrA	0 (0)	0 (0)	0 (0)	NS	NS	NS
qnrB	0 (0)	0 (0)	5 (7.1)	NS	NS	NS
qnrS	0 (0)	0 (0)	7 (10)	NS	NS	NS
qepA	0 (0)	0 (0)	0 (0)	NS	NS	NS
oqxAB	0 (0)	0 (0)	0 (0)	NS	NS	NS
aac(6')-Ib-cr	8 (22.2)	0 (0)	51 (72.8)	NS	<0.001	<0.001
Phylogenetic group						
A	9 (25)	1 (25)	17 (24.3)	NS	NS	NS
B1	15 (41.6)	1 (25)	13 (18.5)	NS	NS	NS
B2	1 (2.7)	0 (0)	12 (17.1)	NS	NS	NS
D	11 (15.7)	2 (50)	28 (40)	NS	NS	NS
O25b-ST131 clone	0 (0)	0 (0)	12 (17.1)	NS	0.007	0.007
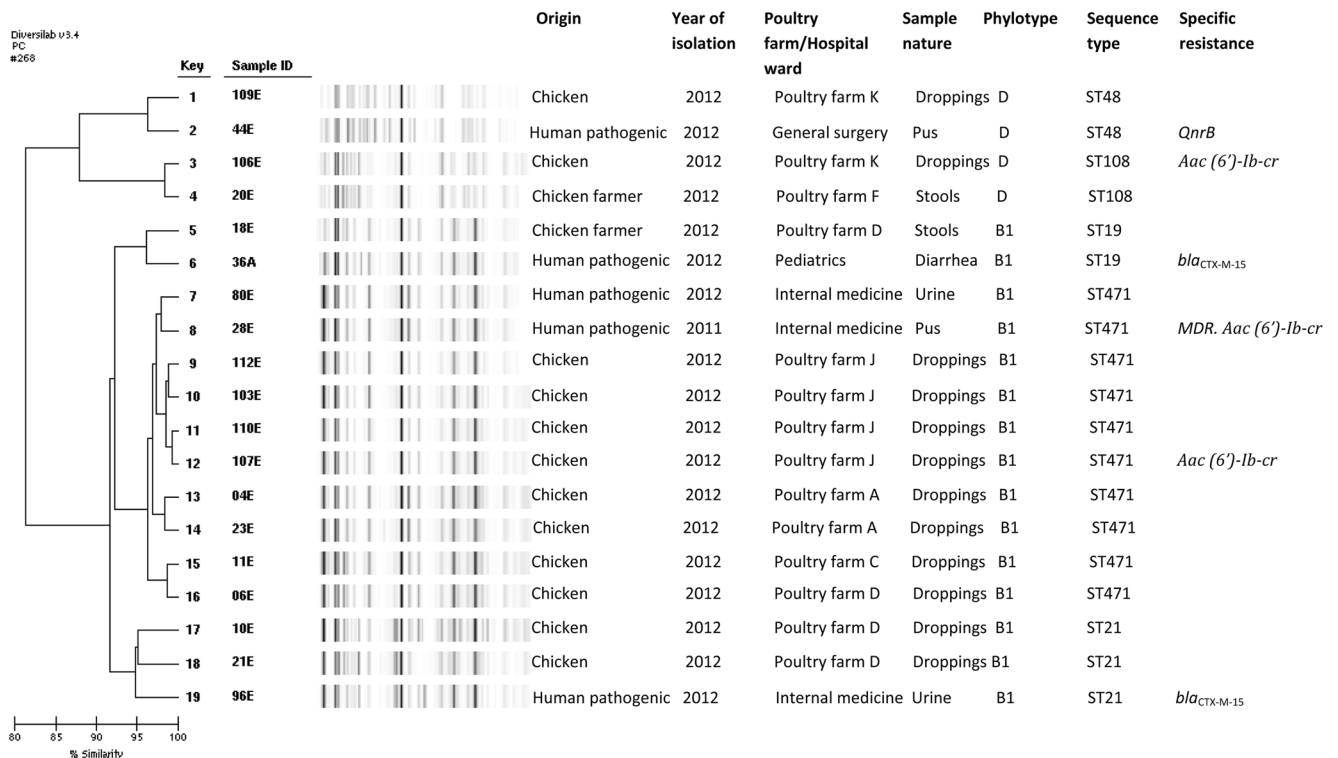
Fig. 1 DiversiLab dendrogram representing the clonal strains with their corresponding characteristics

Kolar et al. [25], β-lactamases of the $bla_{CTX-M-9}$ group are the most frequently found in poultry. bla_{OXA-48} had been reported in *K. pneumoniae* and *E. cloacae* from companion animals (especially dogs, cats, and horses) but not birds [26].

Ciccozzi et al. [27] suggested that the potential transmission of multidrug-resistant *E. coli* clones from poultry to humans seems to involve phylogroups A and B1 rather than B2 and D. Phylogroups A and B1 are known to be broad host-spectrum and could be found in all vertebrate hosts, whereas B2 and D are narrow host-spectrum and largely restricted to endothermic vertebrates, with B2 isolates as the most host-adapted [28]. In the adaptation process, mutation is the main driving evolutionary course in the *E. coli* group A, whereas recombination is more prevalent in B1, B2, and D [29]. Phylogroups A, B1, and D are more likely to have derived from the ancestral *E. coli* lineages [30, 31], and the minor genetic differences observed between strains from the different source groups could be related to their further evolutionary changes associated with host specificity [32]. This is another explanation of our results, as we found clonal human and avian strains belonging mainly to phylogroups B1 and D.

Interestingly, we did not find any correlation between the most important worldwide drug-resistant and virulent *E. coli* O25:H4-B2-ST131 clone (mainly responsible for urinary tract infections) and any avian strains. This is an important finding, since the ST131 clone has been previously isolated from clinical cases and the environment in Algeria [33, 34]. In contrast, a 90.9 % similarity was found between avian and human O25b:H4-ST131 *ibeA-bla*$_{CTX-M-9}$+ strains in Spain [35]. According to Giufrè et al. [36] and Cortès et al. [37], ST131 clone is strongly predominant in human ciprofloxacin-resistant *E. coli* strains, but less in avian ones in which it has not necessary to produce an ESBL, and the acquisition of these enzyme-encoding plasmids was probably achieved in a later stage of its evolution [35, 38, 39]. The fact that a big number of our ExPEC isolates belong to clusters exclusive to humans suggests that most of human pathogenic strains may not be derived from extra-human (avian) ones. However, the clonality among several avian and human isolates supports the idea of their evolution from a recent common ancestor.

Conforming to Lee et al. [40], most fluoroquinolone-resistant *E. coli* in poultry are commensals that harbour mutations in *gyr*A or less frequently *par*C. These chromosomally encoded genes were not investigated in our study, and instead we focused on PMQRs because of plasmids' extreme flexibility in the acquisition and transfer of resistance. The same ciprofloxacin-resistant strains common in both chickens and humans could also be acquired from the environment or from companion animals (dogs and cats) [26, 41].

Conclusion

This study highlights a genetic proximity among avian commensal, human commensal, and pathogenic *E. coli* strains, suggesting that poultry may play an important role in the epidemiology of human *E. coli* infections in Constantine region. These findings may help us to understand the acquisition and the evolution of antimicrobial resistance, and may therefore help us to block its transmission pathways. Efforts are required (i) to understand the ecology of bacterial communities present in animals and their environments, and (ii) to elucidate the transmission dynamics to humans.

Compliance with ethical standards

Conflict of interest The authors report no conflicting interests.

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