BMC Veterinary Research

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# Epidemiological and molecular characteristics of extraintestinal pathogenic escherichia coli isolated from diseased cattle and sheep in Xinjiang, China from 2015 to 2019

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## Abstract

*Escherichia coli* has become a common causative agent of infections in animals, inflicting serious economic losses on livestock production and posing a threat to public health. *Escherichia coli* infection is common and tends to be complex in Xinjiang, a major region of cattle and sheep breeding in China. This study aims to explore the current status and molecular characteristics of *Escherichia coli* infection in cattle and sheep in Xinjiang, as part of the disease prevention and control strategy. Herein we isolated Extraintestinal pathogenic *Escherichia coli* (ExPEC) from the liver, spleen, lung, heart, and lymph nodes of infected cattle and sheep (Xinjiang, China), and phylogenetic grouping, serotyping, and multilocus sequence typing were performed to determine epidemic and molecular characteristics. We also assessed their biofilm formation ability. A total of 132 strains of ExPEC were identified from diseased cattle and sheep, belonging to 7 phylogenetic groups. A and B1 are advantageous groups. Further, 22 serogroups were found, with O101 (26/132), O154 (14/132), and O65 (8/132) being the predominant ones. Among the seven sequence types identified by multilocus sequence typing, ST10 was the most common, followed by ST23 and ST457. Of 132, 105 (79.5%) strains were able to form biofilms: 15 strains (11.4%) were strong, 28 (21.2%) were medium, and 62 (47%) were weak biofilm producers. These findings will contribute to a better understanding of the molecular epidemiology of ExPEC in Xinjiang, China, and can be applied to the development, prevention, and disease control of future diagnostic tools and vaccine.

**Keywords** ExPEC, Phylogenetic grouping, Serotype, Multilocus sequence typing, Biofilm

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## Introduction

*Escherichia coli* (*E. coli*), a Gram-negative intestinal bacterium, is a common cause of infections in humans and animals and is known to cause global foodborne outbreaks [1–3]. Due to the presence of many virulence factors, *E. coli* can cause not only intestinal infections in humans, such as diarrhea, but also extraintestinal organ infections, such as encephalitis, urinary tract infections, and sepsis [4]. In animals, it is the causative agent of encephalitis, liver pericarditis, balloon inflammation, and pneumonia [5].

*E. coli* infection in domestic animals can cause substantial economic losses; in addition, infected animals can serve as a reservoir of drug-resistant *E. coli* strains, posing a threat to public health [6, 7]. *E. coli* from different sources carry different types of factor genes, and *E. coli* strains can be classified into seven groups according to the different combination of genes: A, B1, B2, C, D, E, and F [8, 9]. Clermont et al.. found through studying the evolutionary background of pathogenic *E. coli* from animal and human sources that the types and quantities (≥2) carrying virulence genes *pap* (P pili), *sfa*/*foc* (S/F1C pili), *afa*/*dra* (Dr binding adhesin), *iutA* (ferritin receptor), and *kpsMT II* (type II capsule polysaccharide) can be preliminarily identified as Extraintestinal pathogenic *E. coli* (ExPEC) [9]. Multilocus sequence typing (MLST) combines high-throughput sequencing and bioinformatics, and this approach uses genes that are conserved across strains or species, but may show regions of nucleotide variability. MLST has been applied to study variation and evolution of bacterial populations, and also for monitoring epidemics and classifying microorganisms. *E. coli* ST131 is an extended spectrum β-lactamase (ESBL) producer and most commonly associated with epidemics [10–12]. Soon after its isolation from a clinical patient, it was found that *E. coli* ST131 can rapidly infect various animals, including poultry, cattle, pigs, and wild and companion animals [13, 14]. O antigen is the polysaccharide part of the lipopolysaccharide in the outer membrane of Gram-negative bacteria and plays a key role in pathogenicity. To date, approximately 196 *E. coli* O antigens have been recognized through traditional serogrouping [15–17]. Biofilm (BF) is another important element that is directly related to an increase in bacterial pathogenicity and drug resistance. BFs can protect bacterial cells from environmental stresses and adverse conditions, such as ultraviolet radiation, osmotic pressure, and antibiotics [18, 19]. The metabolic activity of bacteria in BFs considerably varies depends on their localization within the BF and is influenced by their access to nutrients; accordingly, bacteria deepest in the BF can remain dormant for a long period of time, eventually gaining tolerance to a wide range of antimicrobials [20]. Thus, BF formation can lead to infection aggravation in the clinical environment [21] and foodborne disease outbreaks in the food industry [22].

In this study, we investigated the prevalence of extraintestinal organs E. coli isolates from cattle and sheep isolated from 7 different cities in Xinjiang China from 2015 to 2019 and determined the phylogenetic grouping, serogrouping, MLST and BF formation ability of these isolates. but provides effective suggestions for the direction of vaccine prevention and control, as well as the development of more effective diagnostic reagents, to prevent and control E. coli infections in livestock. This will help reduce economic losses and protect public health safety.

## Materials and methods

### *E. Coli* isolation and identification

The strains in this article were isolated, identified, and stored for a long time in our laboratory at an early stage. This article does not conduct direct experimental research on animals. Through biochemical identification and sequencing data analysis, a total of 132 strains of *E. coli* were identified, of which 116 *E. coli* strains were isolated and identified in an early study [23], so overall, presumptive 132 strains from cattle and sheep were subjected to further investigations The specific source of the sample is shown in Table 1.

The samples were inoculated into a nutrient broth and cultured at 37 °C and 180 rpm for 6–8 h. Subsequently, the cultures were inoculated onto eosin-methylene blue agar, MacConkey agar, and blood agar plates and cultured at 37 °C for 10–15 h. In addition, were purified and sequenced using 16 S rRNA (Table 2), verifying the possibility of certain bacterial species, and were further characterized using VITEK 2 Compact GP ID Card (bioMérieux, Marcy l'Etoile, France). Previous studies

**Table 1** Source of isolate

| Area | Strains | Times | Strains | Organ tissue | Strains | Species | Strains |
|---|---|---|---|---|---|---|---|
| Shihezi | 51 | 2015 | 17 | Liver | 31 | Sheep | 28 |
| Tacheng | 8 | 2016 | 20 | Spleen | 26 | Cattle | 104 |
| Altay | 7 | 2017 | 48 | Lung | 35 | | |
| Yili | 25 | 2018 | 19 | Heart | 13 | | |
| Urumqi | 18 | 2019 | 28 | Brain | 7 | | |
| Shawan | 15 | | | Lymph nodes | 20 | | |
| Tumshuk | 8 | | | | | | |

**Table 2** Primer sequences for quadruplex phylogrouping of *E. Coli*

| Genes | Primer name | Primer sequence (5′→3′) | Size (bp) | Reference |
|---|---|---|---|---|
| *16Sr RNA* | *16Sr RNA F* | AGAGTTTGATCMTGGCTCAG | 1500 | |
| | *16Sr RNA R* | TACGGYTACCTTGTTACGACTT | | |
| *arpA* | *arpAF* | AACGCTATTCGCCAGCTTGC | 400 | [4] |
| *(Quadruplex)* | *arpA R* | TCTCCCCATACCGTACGCTA | | |
| *chuA* | chuA F | GACGAACCAACGGTCAGGAT | 288 | |
| (Quadruplex) | chuAR | TGCCGCCAGTACCAAAGACA | | |
| *yjaA* | yjaA F | TGAAGTGTCAGGAGACGCTG | 211 | |
| (Quadruplex) | yjaA R | ATGGAGAATGCGTTCCTCAAC | | |
| *TspE4.C2* | TspE4.C2F | GAGTAATGTCGGGGCATTCA | 152 | |
| (Quadruplex) | TspE4.C2 R | GCGCCAACAAAGTATTACG | | |
| Group*CtrpA* | GroupCtrpA F | AGTTTTATGCCCAGTGCGAG | 219 | |
| | GroupCtrpAR | TCTGCGCCGGTCACGCCC | | |
| Group*EarpA* | GroupEarpA F | GATTCCATCTTGTCAAAATATGCC | 301 | |
| | GroupEarpA R | GAAAAGAAAAAGAATTCCCAAGAG | | |
| Internalcontrol*trpA* | Internal control F | CGGCGATAAAGACATCTTCAC | 489 | |
| | Internal control R | GCAACGCGGCCTGGCGGAAG | | |

detected six virulence genes and found that the carrier rates of *papC* and *iutA* were high at 57.58% and 92.42%, respectively, while the carrier rates of *kpsMII* and *afa* were 3.79% and 9.85%, respectively. Through the detection of special genes, these were determined to be ExPEC [23].

### Phylogenetic grouping

Based on the method reported by Clermont et al. [8, 9], the phylogenetic groups A, B1, B2, C, D, E, and F were determined using PCR depending on the combination of genes carried by various *E. coli* isolates (Table 2).

### O-antigen serogrouping

The O-antigen of *E. coli* isolates was determined by performing slide agglutination assay according to manufacturer instructions (Tianjin Biochip Technology Co., Ltd., Tianjin, China).

### MLST

Thirty-eight representative *E. coli* isolates were chosen for MLST. The following housekeeping gene fragments were assessed: *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *mdh* (malate dehydrogenase), *purA* (adenylate succinate dehydrogenase), *icd* (isocitrate/isopropylmalate dehydrogenase), and *recA* (ATP/GTP-binding motif). Each unique sequence determined an allele, and a unique combination of alleles was used to constitute an allele map or ST. We then compared ST data to those of other *E. coli* strains in the EcMLST database [23]. Detailed protocols for MLST, including PCR conditions, primers, and allele type and ST assignment methods, are available from the MLST database on the Warwick University Network (http://mlst.warwick.ac.uk/mlst).

### Biofilm formation ability assessment

BF formation was measured on the polystyrene surface of 96-well microplates. Isolates were cultured at a dilution of 1:100 in fresh media, and 200 µL bacterial culture was then added to each well (8 wells/strain), followed by incubation at 37℃ for 36 h. Subsequently, the culture media was discarded, and the microplate was washed three times with 200 µL PBS. After staining with 200 µL of 1% crystal violet for 20 min, the unbound dye was washed four times with PBS. Finally, the BF was dissolved in 95% ethanol, and BF formation abilities of isolates were quantified by measuring absorbance at $OD_{570}$. The amount of BF formation was calculated as follows: average OD + 3 × SD (ODc) (blank readings or negative control). All isolates were accordingly classified into four categories [24]: no (OD ≤ ODc), weak (ODc < OD ≤ 2 × ODc), medium (2 × ODc < OD ≤ 4 × ODc), and strong (4 × ODc < OD) BF producers.

## Results

### Phylogenetic grouping

In previous studies, they have been identified as ExPEC [23], According to the classification scheme proposed by Clermont et al. [8, 9]. , 132 ExPEC strains were divided into seven phylogenetic groups: 54 belonged to group A (40.9%), 48 to B1 (36.4%), 2 to B2 (1.5%), 8 to C (6.1%), 9 to D (6.8%), 3 to E (2.3%), and 8 to F (6.1%) (Fig. 1).

### Serogroup

Among the 132 ExPEC strains, 95 (71.97%) were identified as O serogroup; the remaining strains could not be defined by the O antigen (21 strains) or were rough strains (16 strains). These typeable strains belonged to 22 different serogroups, with the most common ones being O101 (26 strains), O154 (14 strains), and O65 (8

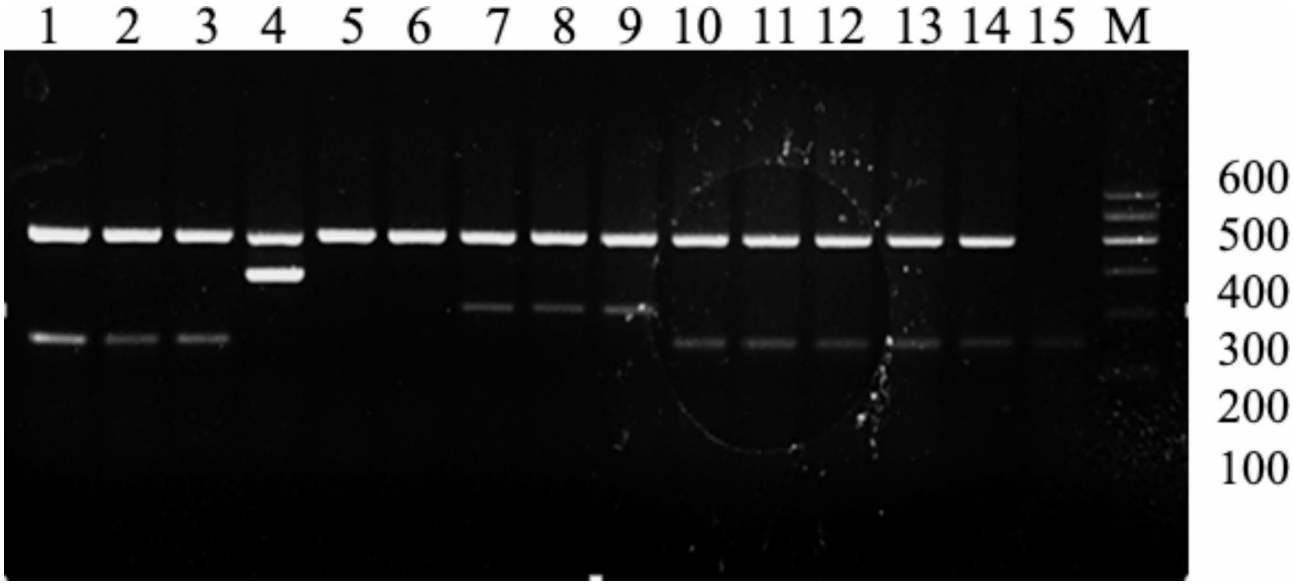


**Fig. 1** Clustering results of some *E. coli* isolates by quadruple PCR system. M: DNA Marker DL600; Lane 1, 2, 3, 10, 11, 12, 13, 14: group B1(+--+); Lane 4: group D or E (++--), screen using E-specific primers, if E + then E, else D; Lane 5, 6: group A (+---) ; Lane 7, 8, 9; group A or C (+-+-), screen using C-specific primers, if C + then C, else A; Lane 15: (---+) Unknown, perform MLST

strains). A few strains carried more than one O antigen: EC XJ-32 carried O1 and O154, EC XJ-48 carried O138 and O147, EC XJ-85 carried O5 and O65, EC XJ-106 carried O4 and O11, and EC XJ-109 carried O4, O15, and O138 (Table 3).

### MLST

MLST led to the identification of 16 sequence types (STs), including seven clonal complexes and nine single nucleotide sequences. Among them, ST10 was the most common, accounting for 18.4% (7/38) isolates, followed by ST23 at 13.2% (5/38) and ST457 at 10.5% (4/38) isolates (Table 3) (Fig. 2).

### Biofilm formation ability

After 24-h incubation at 37℃, 105 (79.5%) strains showed the ability to produce BFs, of which 15 (11.4%) were strong (OD > 4ODc), 28 (21.2%) were medium (2ODc > OD < 4ODc), and 62 (47%) were weak (ODc > OD < 2ODc) BF producers; further, two strains produced thicker BFs and a complete network structure at 6 h, which was maintained for 72 h. The BF-forming rate of cattle strains was 80.8% and that of sheep strains was 75%; no significant differences were observed in BF formation abilities of strains isolated from cattle and sheep, indicating that this ability is not closely related to the source of strains.

### Association between phylogenetic groups and biofilm formation

Research has shown that *E. coli* strains belonging to phylogenetic groups B2 and D are more likely to form BF than strains A, B1, C, E, and F [23]. In this study, the BF formation rate of *E. coli* strains B2 and D was 100%, and most of them were medium or strong BF producing bacteria, with rates of 100% and 88.9%, respectively, indicating a correlation between phylogenetic group B2 and biofilm production ability. The isolated bacteria belonging to groups A, B1, C, E, and F were 18.5%, 47.9%, 37.5%, 33.3%, and 0%, respectively. Overall, the OD values of BF produced by isolates belonging to groups B2 and D were significantly higher than those of isolates belonging to other phylogenetic groups (Fig. 3). Comparison of the STs results of the biofilm positive strains yielded no defined correlations between serovar and STs. There also didn't exist a correlation among BF formation and serovar.

## Discussion

Over the years, various molecular typing methods for *E. coli* have enhance contributed to enhancing our understanding of the molecular epidemiology of *E. coli* infections. In contrast to other regions in China, the livestock industry in Xinjiang is primarily dominated by cattle and sheep. In recent years, the incidence of *E. coli* infections in cattle and sheep has been increasing in Xinjiang [25–27]. During 2015–2019, we isolated and identified 132 ExPEC strains (104 from cattle and 28 from sheep) from the liver, spleen, lung, heart, and lymph nodes of animals with diarrhea, fever, depression, limb weakness, neurological symptoms, septicemia, and respiratory tract infection.

In this study, the majority of the 132 ExPEC strains isolated from diseased cattle and sheep in Xinjiang also

**Table 3** Molecular typing results

| Strain | Phylogenetic classification | | | | Species | Tissue | |
|---|---|---|---|---|---|---|---|
| | Phylogenetic group | ST | ST Cplx | Serotype | | | Year of isolation |
| EC XJ-3 | C | 5706 | - | O20 | Cattle | liver | 2017 |
| EC XJ-4 | C | 5706 | - | O20 | Cattle | lung | 2017 |
| EC XJ-6 | B1 | 156 | 156 | O20 | Cattle | Lymph nodes | 2016 |
| EC XJ-15 | B1 | 156 | 156 | O30 | Cattle | Spleen | 2017 |
| EC XJ-18 | A | 10 | 10 | O101 | Cattle | lung | 2017 |
| EC XJ-20 | A | 10 | 10 | O101 | Cattle | liver | 2017 |
| EC XJ-23 | F | 648 | 648 | O22 | Cattle | Lymph nodes | 2015 |
| EC XJ-24 | F | 457 | - | O11 | Cattle | liver | 2015 |
| EC XJ-28 | A | 457 | - | - | Cattle | liver | 2017 |
| EC XJ-29 | F | 457 | - | O11 | Cattle | Lymph nodes | 2017 |
| EC XJ-32 | B1 | 162 | 469 | O1/154 | Cattle | Lymph nodes | 2017 |
| EC XJ-39 | A | 10 | 10 | O101 | Cattle | Lymph nodes | 2017 |
| EC XJ-40 | A | 10 | 10 | O101 | Cattle | Lymph nodes | 2017 |
| EC XJ-43 | A | 10 | 10 | O15 | Cattle | Lymph nodes | 2019 |
| EC XJ-50 | D | 69 | 69 | self-solidifying | Sheep | lung | 2017 |
| EC XJ-51 | A | 162 | 469 | O5 | Sheep | lung | 2018 |
| EC XJ-55 | A | 10 | 10 | O107 | Sheep | lung | 2018 |
| EC XJ-57 | B1 | 3721 | - | O107 | Sheep | lung | 2016 |
| EC XJ-60 | B1 | 3721 | - | O78 | Sheep | lung | 2016 |
| EC XJ-62 | B1 | 156 | 156 | O13 | Cattle | lung | 2016 |
| EC XJ-63 | B1 | 58 | 155 | O65 | Cattle | spleen | 2017 |
| EC XJ-68 | C | 90 | 23 | O15 | Cattle | Lymph nodes | 2017 |
| EC XJ-69 | B1 | 1196 | - | O91 | Cattle | spleen | 2015 |
| EC XJ-70 | B1 | 448 | 448 | O15 | Sheep | spleen | 2015 |
| EC XJ-71 | B1 | 224 | - | O4 | Cattle | lung | 2018 |
| EC XJ-72 | B1 | 1148 | - | O30 | Cattle | Lymph nodes | 2018 |
| EC XJ-73 | D | 69 | 69 | O15 | Cattle | liver | 2017 |
| EC XJ-74 | A | 10 | 10 | O101 | Cattle | lung | 2017 |
| EC XJ-75 | F | 457 | - | O11 | Cattle | Lymph nodes | 2017 |
| EC XJ-81 | E | - | | O154 | Cattle | heart | 2017 |
| EC XJ-82 | D | 69 | 69 | - | Cattle | spleen | 2017 |
| EC XJ-83 | E | - | | O154 | Cattle | liver | 2017 |
| EC XJ-88 | C | 23 | 23 | O154 | Cattle | Joint effusion | 2017 |
| EC XJ-89 | C | 23 | 23 | O154 | Cattle | spleen | 2019 |
| EC XJ-90 | C | 23 | 23 | O154 | Cattle | Lymph nodes | 2019 |
| EC XJ-91 | C | 23 | 23 | O154 | Cattle | liver | 2019 |
| EC XJ-92 | C | 23 | 23 | O154 | Cattle | lung | 2019 |
| EC XJ-94 | F | 457 | - | O154 | Cattle | lung | 2019 |

ST, sequence type

ST Cplx, ST complex, based on the identity in at least six of the seven gene loci analyzed, between strains of at least three different STs

self-solidifying, Rough type, where the O antigen polysaccharide chain on the surface of the bacterial body is missing, and self coagulation can occur in physiological saline

- represents nontypeable strains

belonged to groups A and B1, and the minority belonged to groups B2, C, D, E, and F. It appears that the distribution of strains belonging to different phylogenetic groups varies depending on animals, regions, or even organs of the same animal. *E. coli* can be either symbiotic or pathogenic, and the phylogenetic positions of these bacteria are determined by different gene cluster combinations that are critical for understanding the pathogenesis of *E. coli* and host–*E. coli* interactions [28]. Research on *E. coli* genomes has indicated that phylogenetic groupings of strains may be related to isolation sources [9]. Previous studies have shown that pathogenic *E. coli* from cattle and non-cattle mainly belong to groups A and B1 [28].

Despite the rapid development of molecular typing methods, O-antigen-based serogrouping remains the standard method to detect pathogenic *E. coli* in food or environmental samples [29]. For the ExPEC strains in this study, of them, 95 strains (71.97%) were identified

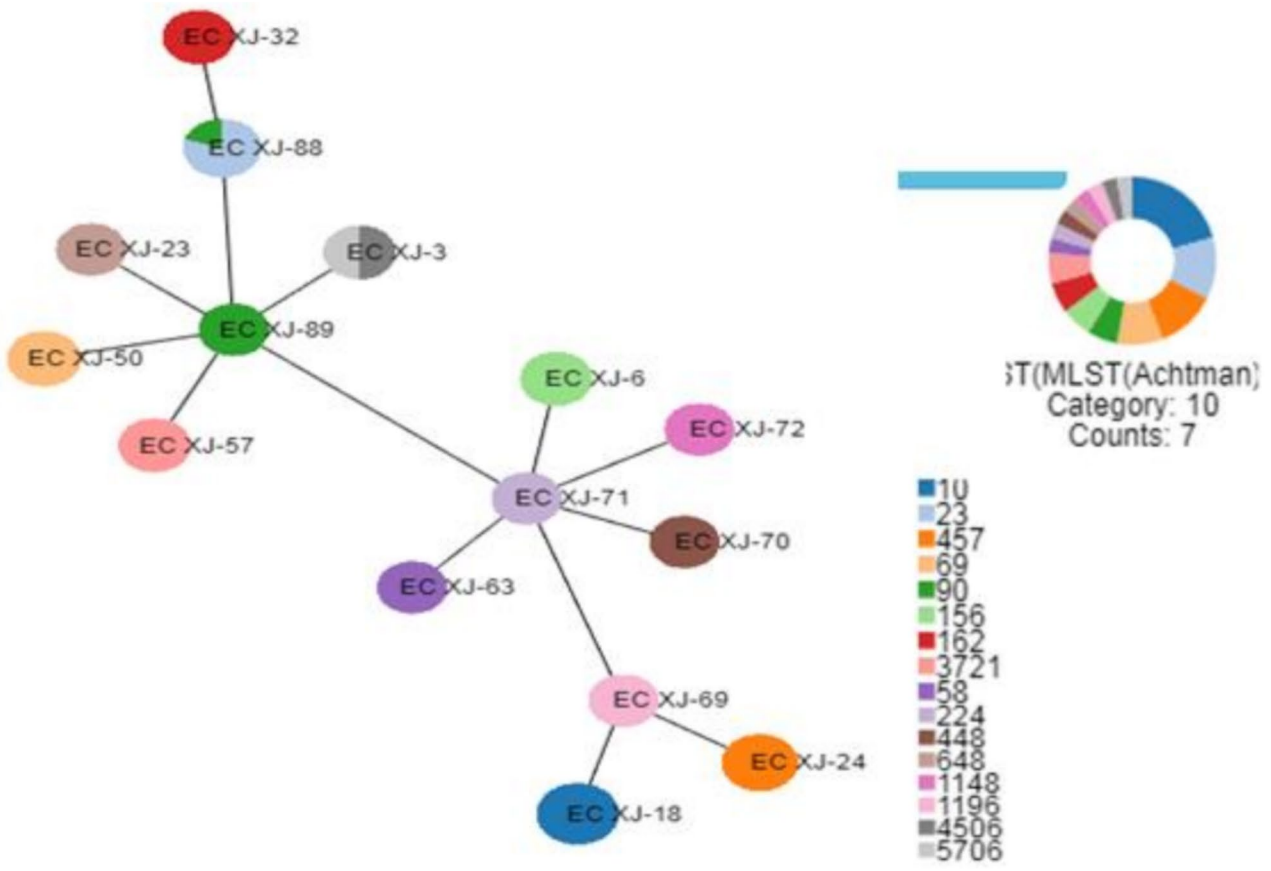


**Fig. 2** Minimum MLST spanning tree of 38 *E. coli* strains. The tree was calculated and generated using the goeBURST full MLST algorithm in Phyloviz 2.0. Node size represents the number of isolates with a specific MLST profile. Numbers in the node indicate strain name. Node colors represent different STs. MLST, multilocus sequence typing; ST, sequence type

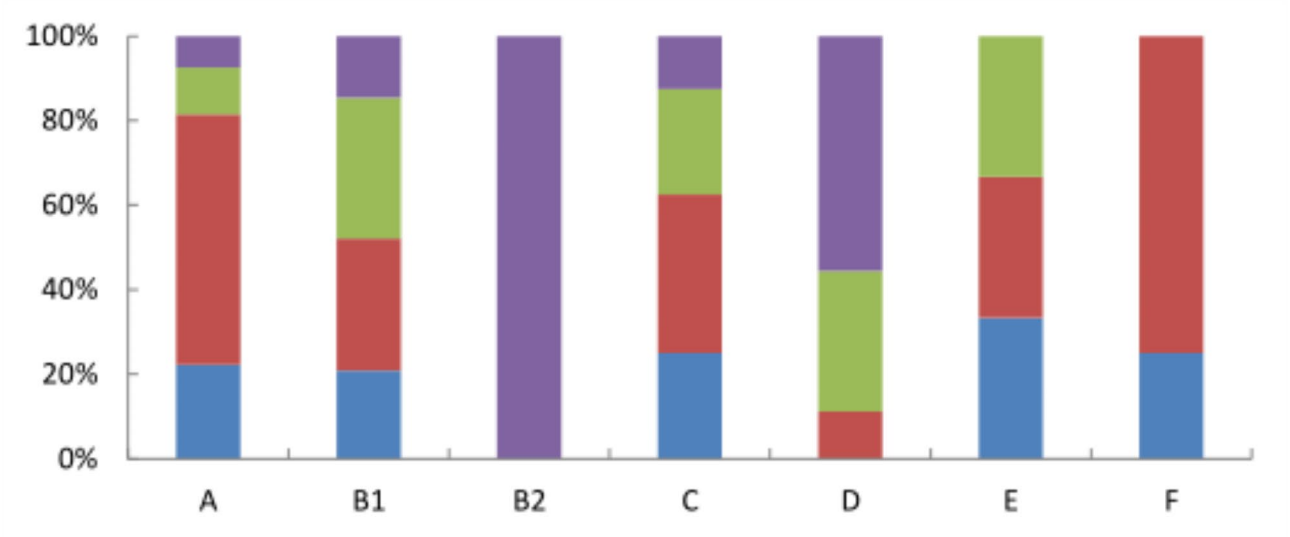


**Fig. 3** Classification of biofilm formation rate according to the revised phylogenetic typing scheme of Clermont et al. Biofilm formation ability of non- (blue), weak (red), medium (green), and strong (purple) biofilm producers

as O serogroups, while others could not be defined by the O antigen (21 strains) or were rough strains (16 strains). O101 was the most prevalent, accounting for 19.7% (26/132) of the 95 *E. coli* strains, followed by O154 (10.6%, 14/132). Serotype O was widely distributed in these isolates. O101 has been previously found in bovine pathogenic *E. coli* strains in France, China, Iran, and Japan, and it was considered to be the most common serogroup of pili F5 [30–33]. In this study, O101 had the highest frequency of occurrence, which is consistent with previous research. According to previous studies, the reason of *E. coli* based on O antigen mixed serotype is amino acid mutations, which cause changes in the characteristics of transmembrane proteins, eventually altering the O-antiglycosyl chain [34]. Some strains have been reported to show mixed serotypes, but there were only a few such strains among the 132 *E. coli* isolates in this study.

Herein we found that 18.4% (7/38) isolates belonged to ST10, followed by 13.2% (5/38) to ST23 and 10.5% (4/38) to ST457. ST10 Cplx is commonly found in the intestinal samples of animals or humans. *E. coli* strains belonging to ST10 have been found to carry a relatively higher number of antibiotic resistance genes than other STs. Further, ST10 is reportedly an extraintestinal pathogenic *E. coli* ST, being responsible for various foodborne infections [35]. Shepard et al. [36] found that the majority of porcine enterotoxigenic *E. coli* isolates belonged ST10, ST23, and ST169 multilocus sequencing types. The ST10 clone belongs to the group with worldwide spread. According to Fuga et al. [37] —which studied *E. coli* isolated from humans, animals, food, and the environment—this lineage has been circulating in Brazil since 1989, predominantly in environmental isolates. Here, ST10-Cplx and ST23-Cplx are evidently associated with few drug resistance-related elements, such as AmpC type β-lactamases and other extended-spectrum β-lactamases. A-ST10-O101 and C-ST23-O154 were dominant in this study. As evident from Table 1, there was a one-to-one correspondence and clear correlation between the A and ST10 phylogenetic groups; in addition, there was a similar relationship between the C and ST23 phylogenetic groups. However, no such relationship was found between a group and serotype. Serotypes have obvious regional advantages, indicating that phylogroups were related to MLST, but there was no special relationship with serotypes.

We herein found that 79.5% *E. coli* isolates showed the ability to produce BFs, and strong BF producers mainly belonged to the A, B1, and D groups; the percentage of strong BF producers belonging to the D group was higher than that of those belonging to the A and B1 groups. *E. coli* D-ST69 (also known as group A or CGA clone) is found in different hosts; it often causes urinary tract infections and shows antibiotic resistance [38]. The B2 group is considered as the sister group of the F group [39]. The BF formation rate was significantly different between the B2 and F groups. *E. coli* strains belonging to the B2 group showed stronger BF formation ability. Further, the source of the strain had no significant impact on BF formation ability in vitro. *E. coli* is a highly adaptive microorganism, and its ability to form BFs under certain conditions is critical to its virulence and pathogenicity [40, 41]. We found that the 132 *E. coli* strains showed different BF formation abilities. Besides, BF formation ability seems to be related to the phylogenetic group; for example, the highly pathogenic *E. coli* strains belonging to the B2 and D group were found to have a strong ability to form BFs.

The distributions of clonal complexes were similar to other extraintestinal or commensal *E. coli* from humans and other animals, suggesting a zoonotic potential. Combining previous research findings [23], the diverse and various combinations of virulence genes implied that the infections were caused by different mechanisms and infection control will be challenging. Moreover, this study has some limitations, as the sampling area is not wide enough and the sample size is limited, making it difficult to see the association and correlation between molecular subtypes. Further in-depth research is needed.

## Conclusion

This study reports the carrier status of suspected ExPEC from cattle and sheep in some areas of Xinjiang. The molecular typing of *E. coli* has greatly expanded our understanding of its geographical distribution and phylogenetic relationships. The A-ST10-O101 and C-ST23-O154 sublines have become important groups of *E. coli* isolated from cattle and sheep in Xinjiang, China. In conclusion, this study has provided information on the diseased cattle and sheep structure of ExPEC lineages needed to track pandemic lineages and guide infection disease control practices in line. And will help investigate and design control strategies for *E. coli* disease originating from cattle and sheep.

### Abbreviations

| | |
|---|---|
| *E. coli* | *Escherichia coli* |
| ExPEC | Extraintestinal pathogenic *Escherichia coli* |
| MLST | Multilocus sequence typing |
| BF | Biofilms |
| ST | Sequence type |

### Author contributions

Conceptualization: Gu XX; Data curation: Wu Q; Formal analysis: Zhou X, Huang X, Wu TZ; Funding acquisition: Zhou X, Huang X, Zhong FG; Methodology: Gu XX; Project administration: Zhou X, Huang X, Zhong FG; Software: Chai YJ, Han ML, Zhang XX; Supervision: Zhou X, Huang X; Validation: Wu Q, Chai YJ; Visualization: Gu XX, Zhou X; Writing - original draft: Gu XX, Zhou X; Writing - review & editing: Gu XX, Zhou X, Huang X.

**Funding**
This work was funded by The National Key Laboratory of Provincial and Ministerial co-construction for Sheep Genetic Improvement and Healthy Special program for scientific and technological key project of corps: Xinjiang Production and Construction Corps key areas of scientific and technological research plan (2019AB029, 2021AB012), Special program for excellent young and middle-aged personnel training and guidance plan (SKLSGIHP2016A02; SKLSGIHP2017A03), Regional Innovation Guide Plan of Xinjiang production and Construction Corps (2017BA038, 2018BB036), and the Major Science and Technology Project of Xinjiang production and Construction Corps (2017AA006-04).

**Data availability**
Data is provided within the manuscript or supplementary information files.

## Declarations

**Ethics approval and consent to participate**
All animal experiments were performed according to the Chinese Regulations of Laboratory Animals - The Guidelines for the Care of Laboratory Animals (Ministry of Science and Technology of People's Republic of China) and Laboratory Animal Requirements of Environment and Housing Facilities (GB 14925 – 2010, National Laboratory Animal Standardization Technical Committee). The animal experiments were reviewed and approved by the ethics committee of the Hospital (approval number: A2019-149-01).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 20 October 2024 / Accepted: 16 January 2025
Published online: 30 January 2025

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