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A virulent escherichia coli O121-B2-ST131 strain causes hemorrhagic pneumonia in mink: evidence from pathogenicity and animal challenge experiments

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Abstract

In recent years, rapid fatal hemorrhagic pneumonia (HP) has been increasingly reported in mink. In several studies, the virulence factors of strains isolated from diseased tissues have been identified as extraintestinal pathogenic *Escherichia coli* (ExPEC). The molecular characteristics of the strains were also analyzed, but whether ExPEC is the etiological agent of HP has not been confirmed in an animal challenge model. In this study, we characterized the antibiotic resistance, virulence characteristics, and pathogenicity of a bacterial strain isolated from a typical case of mink HP, and designated it L1. Our study revealed that isolate L1 has high levels of antibiotic resistance, to multiple antibiotics, including ampicillin, tylosin, kanamycin, and so on. Numerous virulence genes were detected in isolate L1, including those encoding adhesins (*focG, afa/draB, mat, crl*), invasins (*ibeA, einv*), and toxin (*cnf1*). ExPEC isolate L1 belongs to the O121 serogroup and was classified in the B2 phylogroup and sequence type 131 (ST131). Animal experiments showed that L1 is highly pathogenic to mice, and induced fatal HP in mink. A mouse model of isolate L1 infection showed lethargy, depression, and then death. The sick minks showed similar clinical signs and died soon after nasal bleeding and hematemesis, with a large amount of congestion and consolidation in the lungs. Using animal challenge experiments based on Koch's postulates, we demonstrate for the first time that ExPEC is a causative agent of rapid fatal HP in minks. Our research provides important insights into the identification and control of rapid fatal HP in minks and effective antibiotic treatments for infected animals.

Keywords *Escherichia coli*, Extraintestinal pathogenic *E. coli*, Hemorrhagic pneumonia, Mink diseases, Phylogenetic analysis, Virulence factors

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Introduction

Minks are widely farmed in Europe, North America, and China for fur. This fur industry is worth more than US$11 billion, with potentially large domestic and overseas markets. However, the mink is highly susceptible to infection by various pathogens and to environmental elements [1]. This is especially true during intensive farming, which involves a high-density animal environment, facilitating the rapid spread of pathogens with pandemic potential. To date, Aleutian disease virus, influenza A viruses, SARS-CoV-2, mink orthoreoviruses, COVID-19, canine distemper virus, *Minacovirus*, and *Pseudomonas aeruginosa* have been shown to cause respiratory diseases in mink [1–6], resulting in the death of the mink and huge economic losses.

Hemorrhagic pneumonia (HP) is a respiratory disease first detected in mink in 1983 in China, which has become a major cause of mink deaths between September and early December [7]. Without drug control, the fatality rate attributable to HP can reach 100%. Pathogens commonly associated with mink HP are *P. aeruginosa* and *Klebsiella pneumoniae*, which cause death and massive economic losses [8, 9]. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is present in air, water, soil, and animals, including companion animals [10–12]. In recent years, ExPEC has caused highly infectious respiratory system diseases. For instance, common strains of porcine *E. coli* can cause swine respiratory diseases [13] and *Escherichia fergusonii* cause pneumonia in beef cattle [14]. ExPEC also causes fatal pneumonia in dogs [15] and induces acute necrotizing pneumonia in cats [16, 17]. However, little information is available about ExPEC in minks, in which it causes HP.

For the past few years, there have been outbreaks of mink HP in the northern region of China, when ExPEC was isolated from the mink and the molecular and phenotypic characteristics of some strains were described [18, 19]. However, until now, no animal experiments have confirmed that ExPEC is the etiological agent of this disease. According to Koch's postulates, the condition required for identification as a pathogenic organism is that when an isolated disease agent is returned to the same animal, the same clinical symptoms are observed. Therefore, animal challenge experiments are required to confirm whether ExPEC causes HP in mink.

The prevalence of HP in mink in northern China has been monitored by our laboratory since 2016. More than 10 typical cases were detected in 2016, with which ExPEC was always associated, whereas no *P. aeruginosa* was isolated from the HP-affected minks during the period of continuous monitoring. To identify ExPEC as the etiological agent of HP in mink, a representative strain from one of the infected animals was selected randomly for the experiment due to the same source with typical clinical symptoms. In this report, we investigated the disease characteristics, serogroup, multilocus sequence type (MLST), antimicrobial susceptibility, genotypic features, and virulence factor profile of the isolate. Most importantly, an animal challenge experiment was performed, which replicated the classic symptoms of HP in mink. Therefore, in this study, pathogenicity tests and the fulfilment of Koch's postulates confirmed that ExPEC is a cause of mink HP.

Materials and methods

Sample collection

Since 2016, HP in minks has been observed increasingly in northern China, highlighting its potential impact on mink farming. With ongoing surveillance, multiple acute deaths caused by HP were identified across different mink farms in the region. A major outbreak occurred in 2016 at a mink farm in Harbin, Heilongjiang Province, upon which approximately 8,000 minks were housed. During this outbreak, we isolated bacteria from 12 deceased minks that showed typical clinical signs of HP, such as nasal bleeding and hematemesis. Similar symptoms and lesions were observed in multiple animals across the farm. No *P. aeruginosa* was detected in more than 10 typically HP-affected minks, and only ExPEC was detected. Therefore, this farm was chosen as a typical subject of study. The onset of the disease was fast and death was acute. The average course of the disease was 1–3 days. In some cases, sudden death occurred without any symptoms. According to local veterinary clinicians, about 60% of the minks displayed clinical signs. More than 300 minks died within the first 3 days. Stress, poisoning, and feed were eliminated as possible causes of death. With the farmer's consent, local veterinary clinicians sampled the mink carcasses, including the brains, hearts, livers, spleens, lungs, kidneys, intestines, and pleural fluid, after death and immediately submitted them for analysis at our laboratory. Lung samples of the dead minks were used for bacterial isolation.

Bacterial isolation and analysis

Samples of the brains, hearts, livers, spleens, lungs, kidneys, intestines, and pleural fluid of the carcasses were examined for common bacterial and viral pathogens, and lung samples were forwarded for histopathological characterization. The sera of the sick minks were examined for canine distemper virus, canine adenovirus (CAV-1 and CAV-2), canine distemper virus (CDV), and canine parvovirus (CPV) with a colloidal gold immunochromatography strip (Huayi Biotechnology Co., Ltd, Changchun, China). Several kinds of samples from each mink were homogenized for the detection of

mink enteritis virus (MEV), Aleutian mink disease virus (AMDV), CAV, and pseudorabies virus with PCR. The samples were tested for *Mycoplasma* with the TaKaRa PCR Mycoplasma Detection Set (TaKaRa Biotechnology Co., Ltd, Dalian, China), according to the manufacturer's instructions.

To isolate the bacteria, the lung tissues were pierced with inoculation loops, which were then streaked onto Luria Bertani (LB) broth and incubated at 37 °C for 24 h. A few representative colonies were picked and streaked onto eosin–methylene blue (EMB) agar medium (Aoboxing Biotechnology Co., Ltd, Beijing, China), incubated aerobically or anaerobically at 37 °C for 48 h, and then examined for unambiguous species verification. Gram staining, a conventional method for bacterial classification, was performed and the isolate examined with the triple sugar iron agar, sucrose, lactose, glucose, maltose, mannitol, citrate, the Voges–Proskauer, methyl red, indole, and hydrogen sulfide tests to identify the bacterium.

DNA extraction and PCR

Bacterial DNA was extracted with the TIAamp Bacteria DNA Kit (Tiangen, Beijing, China), according to the manufacturer's instructions. To distinguish typical and atypical ExPEC, we initially detected *kpsMII*, *papA/papC*, *sfa/focD*, *iutA*, and *afa/draB*, and the isolate was identified with multiplex PCR [20]. The *chuA*, *yjaA* genes and a DNA fragment (TspE4.C2) of the strain were then analyzed to determine its phylogroup [21]. PCR assays were performed to detect the genes that define the serogroup, phylogroup, MLST (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) [22] and presence of genes encoding different virulence factors. Conventional serogrouping was performed based on the following 15 serogroups: O1, O2, O6, O8, O9, O26, O45, O78, O103, O111, O113, O121, O128, O145, and O157 [23–27]. The virulence genes investigated included those encoding adhesins (*focG, papC, mat, crl, fimH, eae, f5, f41*), invasins (*ibeA, einv),* toxins (*hlyA, hlyD, sat, cdtB, sta, stb, LT, stx1, stx2, cnf1, cnf2, eagg, st1*), iron acquisition proteins (*fyuA, ireA, irp2*), capsular antigen (*iss*), and a pathogenicity island marker (*malx*). All the PCR primers used for bacterial DNA amplification described in this study are listed in Supplementary Table 1 and were synthesized by Comate Bioscience Co., Ltd (Jilin, China) [28–36]. PrimeSTAR GXL DNA Polymerase, dNTP mixture, and PrimeSTAR GXL buffer for PCR were all supplied by TaKaRa Biotechnology Co., Ltd. 16S rDNA and the ST131-specific gene (region 19) were amplified with PCR [37, 38], and produced fragments of 1,590 bp and 580 bp, respectively. The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and then cloned into the pMD-18 T vector (TaKaRa) for sequencing (Sangon, Shanghai, China). The sequences were compared with known sequences in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analyses of the bacterial 16S rDNA and ST131-specific gene (region 19) were performed to identify the bacterial species and determine whether the isolate belonged to the ST131 lineage. To investigate the potential origin and genetic relatedness of the isolate, ST131 reference sequences from human and environmental (water) sources were retrieved based on the BLAST results of homology and were included on the phylogenetic tree. The neighbor-joining method was used to construct phylogenetic trees with the MEGA 7 software.

Antimicrobial susceptibility and biofilm-forming ability

Following the Clinical and Laboratory Standards Institute (CLSI) guidelines outlined in the 2024 edition of Performance Standards for Antimicrobial Susceptibility Testing (CLSI Supplement M100), the isolate was tested for susceptibility to 21 antimicrobial agents using the Kirby–Bauer disk diffusion method. A bacterial suspension was prepared from a fresh culture grown overnight on Mueller–Hinton agar at 37 °C. The optical density at a wavelength of 600 nm (OD_{600}) was adjusted to 0.5 in LB broth prior to inoculation. The adjusted suspension was then evenly spread on Mueller–Hinton agar plates using sterile cotton swabs.

Antimicrobial susceptibility testing was conducted with commercially prepared antibiotic discs (Bio-kont, Wenzhou, China), according to the manufacturer's instructions. Escherichia coli ATCC 25922 was used as a quality control strain. The antimicrobial agents tested were: ampicillin, cefalexin, ceftriaxone, imipenem, tylosin, gentamicin, kanamycin, streptomycin, ciprofloxacin, tetracycline, polymyxin, trimethoprim, amoxicillin, ofloxacin, chloramphenicol, penicillin, nitrofurantoin, clindamycin, fosfomycin, cefmondoxil, and azithromycin. After incubation at 37 °C for 18–24 h, the diameters of the inhibition zones around each disc were measured in millimeters. The results were interpreted as susceptible (S), intermediate (I), or resistant (R) based on the CLSI 2024 criteria.

Biofilm production was evaluated with the method described by Peeters et al., and the ability to form biofilms was scored according to the criteria described earlier [39]. All tests were repeated independently three times and the average result taken.

Experimental challenges

According to Koch's postulates, when a cultured microorganism is introduced into a healthy organism susceptible

to the disease under investigation and the typical clinical symptoms are observed, the microorganism is considered pathogenic. Therefore, animal challenge experiments were required to further confirm whether ExPEC causes HP in mink.

Animal experiments involving mice and mink were performed to verify the pathogenicity of the isolate. In total, 30 6-week-old female BALB/c mice (purchased from Changsheng Biotech, Liaoning, China) were divided equally into six groups. Each mouse was then inoculated with an intraperitoneal (i.p.) injection of the bacterial isolate at concentrations of 10^9, 10^8, 10^7, 10^6, or 10^5 colony-forming units (CFU) in 0.2 mL of sterile PBS. The negative control group was inoculated with phosphate-buffered saline (PBS). The mice were monitored three times daily, and their health status recorded. Similarly, six healthy, 6-month-old mink were purchased from a local farm. Three of the six minks (purchased from Jilin Zhongte Agricultural Technology Co., Ltd) were infected with the bacterial isolate (1×10^9 CFU) by intramuscular (i.m.) injection into the back leg muscles, and the remaining three were injected with sterile PBS as the controls. The clinical signs of the animals were recorded and necropsies were performed within 1 h of the animal's death. At the end of the experiment, the remaining mice and minks were euthanized with pentobarbital sodium (100–150 mg/kg), in accordance with the approval of the Animal Ethics Committee.

Samples were collected from the lungs, liver, spleens kidneys, and lymph nodes of each mink for etiological examination. These tissue samples were cultured on 5% blood agar plates (Huankai Microbial Technology Co., Ltd, Guangdong, China) to isolate and identify bacterial pathogens, using the procedures used for the clinical samples. Gross pathological lesions were recorded. For histopathological analysis, lung tissues were fixed in 4% neutral-buffered formalin, embedded in paraffin, and sectioned to 4–5 μm thickness. Gross pathological lesions were recorded [38]. The lung sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Nikon, Japan) to evaluate microscopic tissue changes.

Results

Clinical signs and pathological lesions

The onset of the disease was rapid, with more than 300 minks dying within 3 days of onset. The minks that died suddenly were asymptomatic. The clinical signs observed in the infected minks included depression, anorexia (loss of appetite), severe respiratory distress, and wheezing. These minks died after nasal bleeding and hematemesis were observed (Fig. 1A). On postmortem examination, the minks displayed large amounts of congestion and consolidation in the lungs, with large amounts of red fluid flowing from the sections (Fig. 1B), brain congestion, and enlarged spleens. Approximately 40–50 mL of red serosanguinous fluid was present in the chest (Fig. 1C). The isolate was identified as *E. coli* with the Biolog Microbial ID System (Biolog Inc., Hayward, CA, USA). To test for viral agents, we screened for AMDV, PRV, CDV, CAV-2, and MEV with PCR or a colloidal gold immunochromatography strip, and for *Mycoplasma* (Fig. 1D). None of these pathogens were detected in the sample.

In the samples from the minks, abundant and purely facultatively anaerobic bacterial growth was observed in the brains, lungs, and pleural fluid. No other significant bacterial growth was detected. The isolated bacterium showed the typical colony morphology of *E. coli* on EMB plates, with a metallic sheen with a dark center (Fig. 2A). A microscopic examination of L1 showed it to be a Gram-negative bacillus (Fig. 2B). The tube inoculated with L1 displayed glucose fermentation, evident as gas production and a color change to yellow on both the slant and butt, indicating acid production (Fig. 2C). The isolate tested positive for lactose, glucose, maltose, mannitol, methyl red, and indole (Fig. 2D). It tested negative for sucrose, citrate utilization, and hydrogen sulfide (H_2S) production, and on the Voges–Proskauer (VP) test. This biochemical profile identified the isolate as *E. coli.*

Serogroup and virulence gene analyses

After nutrition, environmental factors, stress, poisoning, and other causes were excluded as the etiological agent of HP, *E. coli* was determined to be the causative agent of fatal HP in the minks based on bacterial isolation and identification. Because L1 simultaneously contained genes *kpsMTII*, *focG*, and *afa*, it was identified as ExPEC (Fig. 3A), according to the description of Johnson et al. [20], based on the presence of two or more of five virulence markers (*kpsMII*, *papA/papC*, *Sfa/focD*, *iutA*, *afa/drab*). L1 carries the *chuA* and *yjaA* genes, so it belongs to phylogenetic group B2 (Fig. 3B). PCR with 16S-rDNA-specific primers produced the expected target band (Fig. 3C). An MLST analysis not only provides other information, but also phylogenetic relationships. We compared our sequence results with the MLST database (http://pubmlst.org/) and combined them with the results of PCR (Fig. 3D), the sequence type of L1 was ST131. The ST131 lineage has been reported to be associated with infections in birds [40, 41], dogs [42], humans [43], and one reported case in mink [19]. However, the strain identified in that mink was genetically distinct from the L1 isolate recovered in this study [19]. The isolate L1 was identified as serotype O121 (Fig. 3E) and classified within phylogenetic group B2.

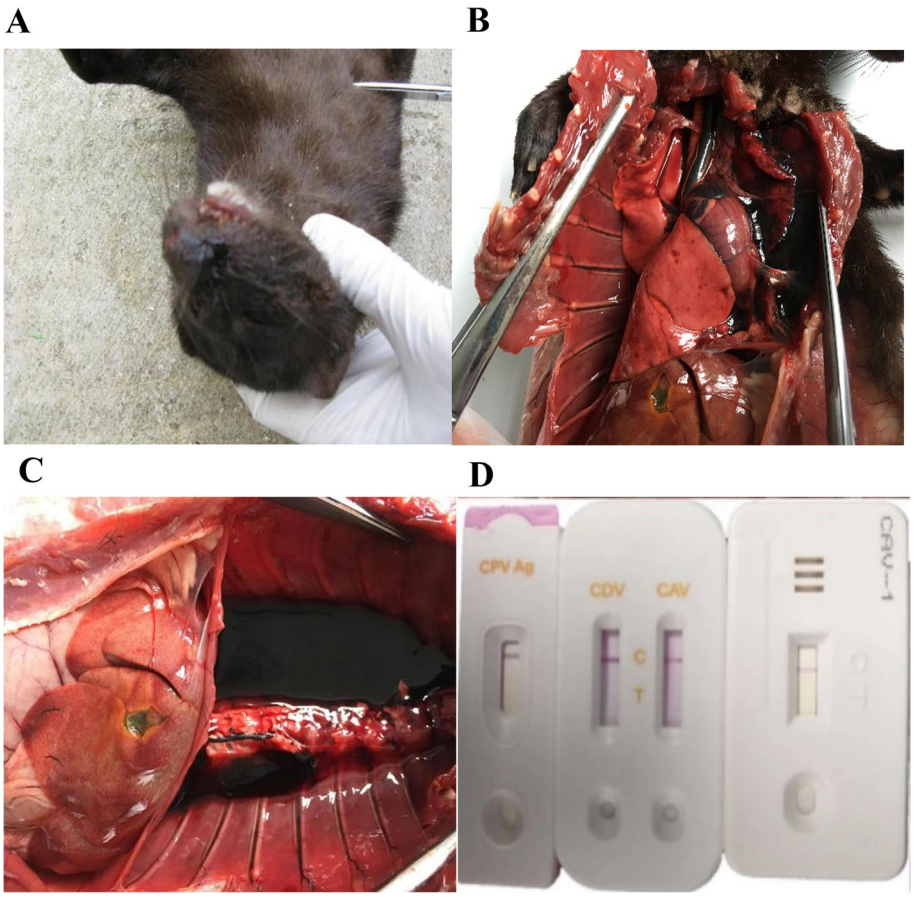

Fig. 1 Clinical signs in mink with ExPEC infection in Harbin, northeastern China. **A** Infected mink showed bleeding from mouth and nose. **B** Necropsy of mink that died from infection. On postmortem examination, gross lesions were mainly concentrated in the lungs. **C** Approximately 40–50 mL of red serosanguinous fluid was present in the chest. **D** Canine distemper virus (CDV), canine adenovirus 1 (CAV-1 and CAV-2) and canine parvovirus (CPV) were screened with a colloidal gold immunochromatography strip (Genbody Inc.), but none of the tested agents was detected in any sample

Isolate L1 contains a wide range of virulence-associated genes, including those encoding adhesins (f*ocG, afa/draB, mat, crl*), invasins (*ibeA, einv*), a toxin (*cnf1*), iron acquisition systems (*fyuA*, *ireA*, *irp2*), capsular antigen protection protein (*kpsMII*), and a pathogenicity island marker (*malX*) (Fig. 3F).

Phylogenetic analysis

The 16S rRNA gene (GenBank PP190255) and ST131-specific gene (region 19) (GenBank PP236910) sequences of L1 were determined and compared against the NCBI database with BLAST. The highest sequence similarity value for 16S rDNA (> 99%,) and the highest ST131 sequence similarity value (> 98%) was to *E. coli* isolated from human. The sequences were used to construct a phylogenetic tree with the neighbor-joining method in the MEGA 7 software (Fig. 4). On the phylogenetic tree, bacterial isolate L1 showed 99.87% sequence identity in 16S rDNA and 100% in ST131 with the corresponding sequences in human ExPEC strains.

Antimicrobial susceptibility and biofilm-forming ability

Antibiotic susceptibility testing showed that L1 is resistant to several antimicrobials: ampicillin, tylosin, kanamycin, streptomycin, ciprofloxacin, trimethoprim, amoxicillin, penicillin, and clindamycin. It is susceptible to ceftriaxone, imipenem, polymyxin, ofloxacin, chloramphenicol, nitrofurantoin, fosfomycin, cefmondoxil, and azithromycin. It showed intermediate sensitivity to the other antimicrobial drugs tested (Table 1). The biofilm-forming ability of L1 is moderate. Numerous bacterial species have been reported to form biofilms, and it seems that bacterial biofilm formation enhances bacterial survival in various environments.

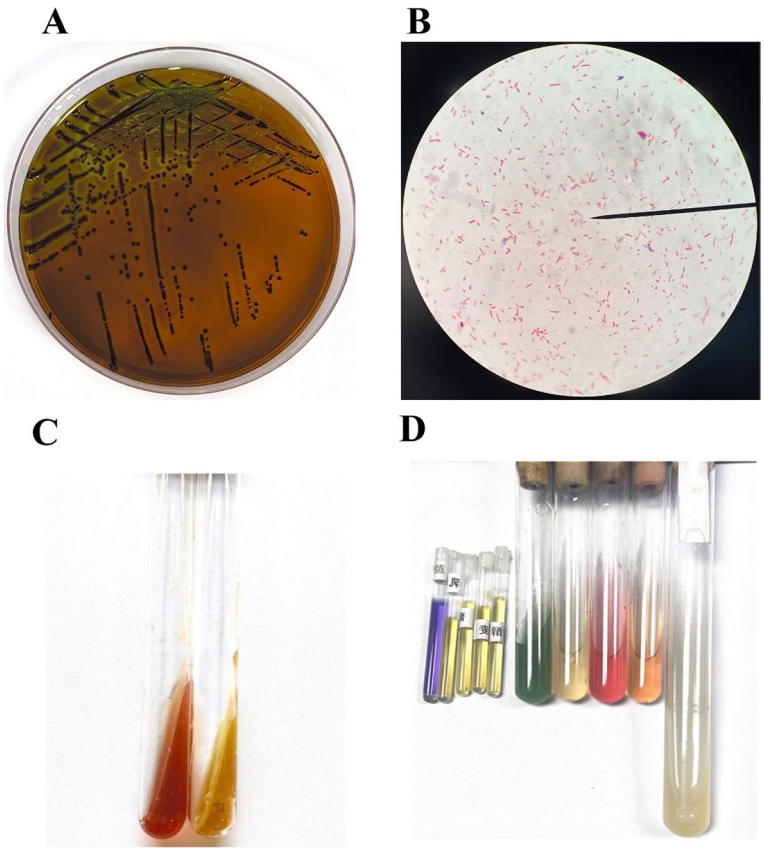

Fig. 2 Colony isolation and identification. **A** Colonies with a metallic green sheen and dark centers on EMB agar were initially judged to be *E. coli*. **B** Gram-negative rod-shaped bacteria. **C** Uninoculated triple sugar iron agar (left) showed no change. Tube inoculated with L1 (right) showed glucose fermentation (gas produced) and a color change to yellow on a triple sugar iron agar medium slant (acid produced). **D** L1 was positive for lactose, glucose, maltose, mannitol, methyl red, and indole, and negative for sucrose, citrate, Voges–Proskauer, and hydrogen sulfide

Pathogenicity analysis

To assess whether the pathogenicity of L1 in mink extends to other animals, 6-week-old female BALB/c mice were infected with the same strain. Samples were collected from the dead mice. In the mouse model of infection, a similar disease was observed in all L1-inoculated groups. The mice first showed lethargy, depression, anorexia, and huddling together at 6–18 h post inoculation. The mice then began to die from severe infection, with mortality rates of 100%, 100%, 80%, and 60% in the four groups inoculated with 10^9, 10^8, 10^7, or 10^6 CFU, as shown in Fig. 5. No more deaths occurred in the other groups (10^5, 10^6, and 10^7 CFU) after 54 h and no clinical symptoms were observed in the control group throughout the entire experimental period. The pathology observed in the experimentally infected mice included liver bleeding, lung edema, subcutaneous jelly-like exudates, and subcutaneous bleeding. The infections observed in mink were consistent with our clinical observations of the minks. The most frequent symptoms in the mink were breathing difficulties, lethargy, weakness, anorexia, and edema. The most significant findings were typical widespread pulmonary consolidation and congestion in the dorsal regions of the lungs in the dead mink. Microscopic lesions included capillary congestion in the alveolar wall, interstitial pneumonia with hyperemia, and serous exudation in the lungs, with a more diffuse distribution than the control tissue. Serious hemorrhage and interstitial broadening were observed and the predominant type of inflammation involved a mixture of macrophages and neutrophils.

The gross and histopathological lesions observed in the experimental animals were consistent with those seen in clinical samples (Fig. 6A, B). Histopathological examination revealed diffuse interstitial pneumonia accompanied by tissue necrosis and inflammatory cell infiltration (Fig. 6C–F). The gross and microscopic pulmonary lesions observed in both minks and mice closely resembled those reported in previous studies of HP caused by *E. coli* [9] The bacterium was reisolated from the lungs of the experimentally infected minks, mirroring the findings from clinical cases. Based on its

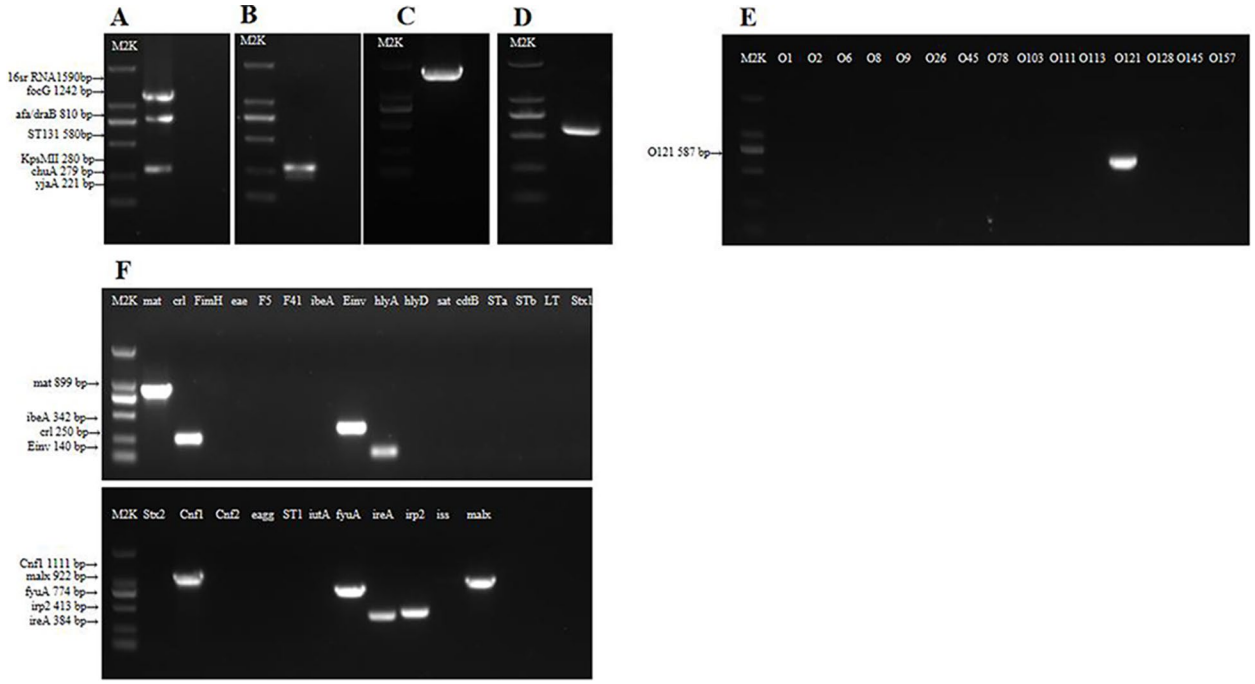

Fig. 3 Amplification products of PCR assays. **A** Multiplex PCR profiles specific for ExPEC and the phylogroup of L1 (**B**). **C** Result of L1 16S rDNA amplification. Agarose gel electrophoresis of the PCR products of L1 confirm ST131 (**D**), serogroup (**E**), and virulence genes (**F**). M2K: DL2000 molecular standard marker with the TaKaRa

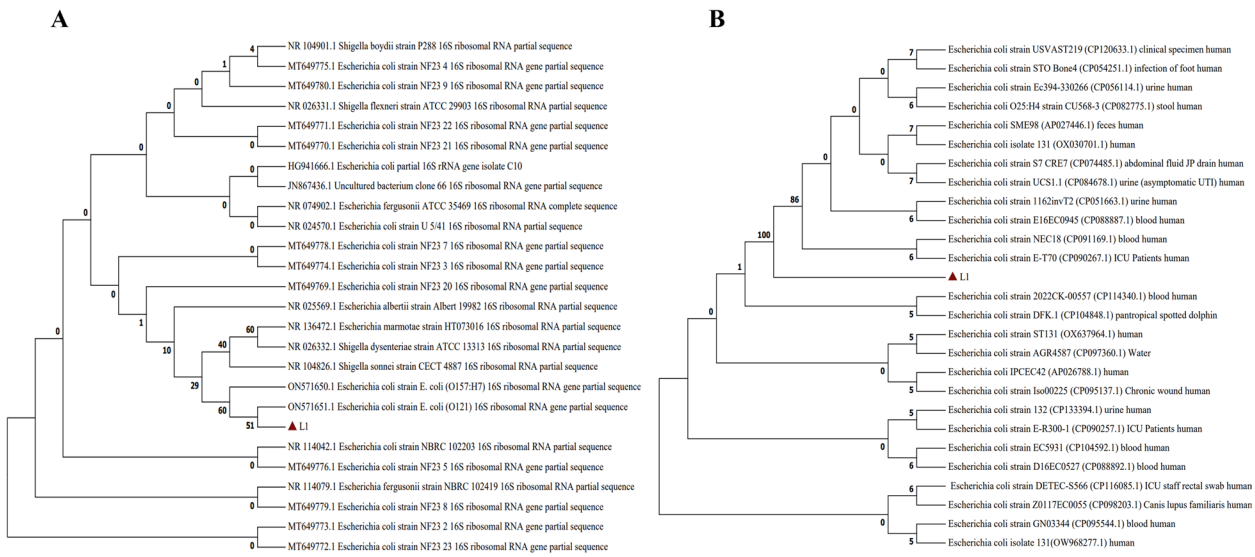

Fig. 4 Results of gene homology comparison. **A** 16S rRNA gene sequence dendrogram showing the position of isolate L1. **B** ST131 gene sequence dendrogram showing the source of isolate L1. Bootstrap values (1,000 replicates) are given at each node

morphological characteristics, tissue distribution, and biochemical profile (data not shown), the isolate was preliminarily identified as the causative agent of HP in mink. Furthermore, ExPEC isolate L1 demonstrated

Table 1 Results of antibiotics susceptibility testing

Antibiotic	Concentration	Diameter (mm)	Result	Antibiotic	Concentration	Diameter(mm)	Result
Ampicillin	10 μg	0	R	Trimethoprim	5 μg	10	R
Cefalexin	30 μg	17	I	Amoxicillin	10 μg	0	R
Ceftriaxone	30 μg	22	S	Ofloxacin	5 μg	24	S
Imipenem	10 μg	22	S	Chloramphenicol	30 μg	22	S
Tylosin	30 μg	0	R	Penicillin	10 μg	0	R
Gentamicin	10 μg	13	I	Nitrofurantoin	300 μg	19	S
Kanamycin	30 μg	12	R	Clindamycin	2 μg	0	R
Streptomycin	12 μg	0	R	Fosfomycin	200 μg	20	S
Ciprofloxacin	5 μg	13	R	Cefmondoxil	30 μg	20	S
Tetracycline	30 μg	17	I	Azithromycin	15 μg	24	S
Polymyxin	300 μg	32	S				

R Resistant, *S* susceptible, *I* Intermediate

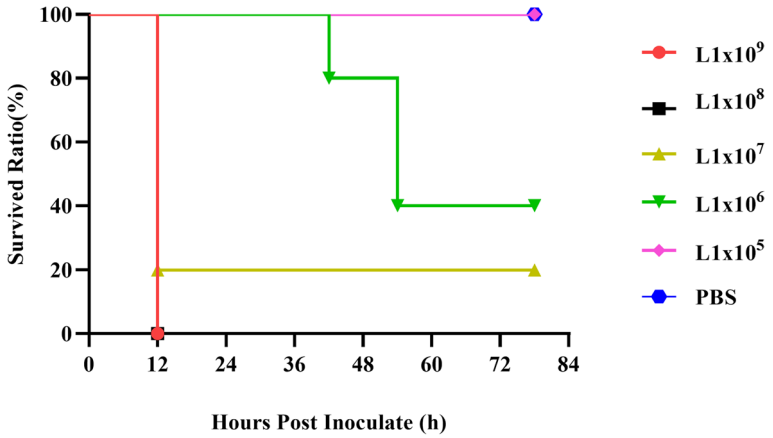

Fig. 5 Percentage survival in BALB/C mice after infection with L1. Thirty 6-week-old female BALB/C mice were assigned to six groups with five mice each. Some mice were injected with L1 and others with PBS (as controls), as described in the Materials and Methods. All the mice in the control group survived, but the infected mice died with severe disease, with mortality rates of 100%, 100%, 80%, and 60% in the four different groups inoculated with $> 1 \times 10^6$ CFU

high pathogenicity in both minks and mice under identical experimental conditions.

Discussion

Escherichia coli is a common bacterium that colonizes the gastrointestinal tracts of humans and many animal species [44], receiving nutrients from the host while providing the host with nutrients and other benefits [45]. Extraintestinal pathogenic *E. coli* (ExPEC), a group of *E. coli* strains, causes most extraintestinal *E. coli* infections. ExPEC includes opportunistic pathogens of great importance to public health, causing diseases characterized by high morbidity and high mortality, and the loss of several billion dollars annually through diverse clinical syndromes [46]. The virulence traits of ExPEC strains allow them to invade, colonize, and induce diseases in parts of the body beyond the gastrointestinal tract, thus increasing their resistance to antimicrobial drugs and the pathogenic potential of extraintestinal diseases. A previous study showed that ExPEC can cause both human illnesses and extraintestinal infections in domestic animals and pets. That study revealed that the ExPEC present in humans and other animals shared virulence factors, suggesting that ExPEC is zoonotic [47]. ExPEC strains cause many human diseases when they leave the original site of colonization and enter sterile extraintestinal sites at many anatomic locations. These include a variety of urinary-tract infections (UTIs), newborn meningitis, abdominal sepsis, meningitis, septicemia, and circulatory system infections [48–50].

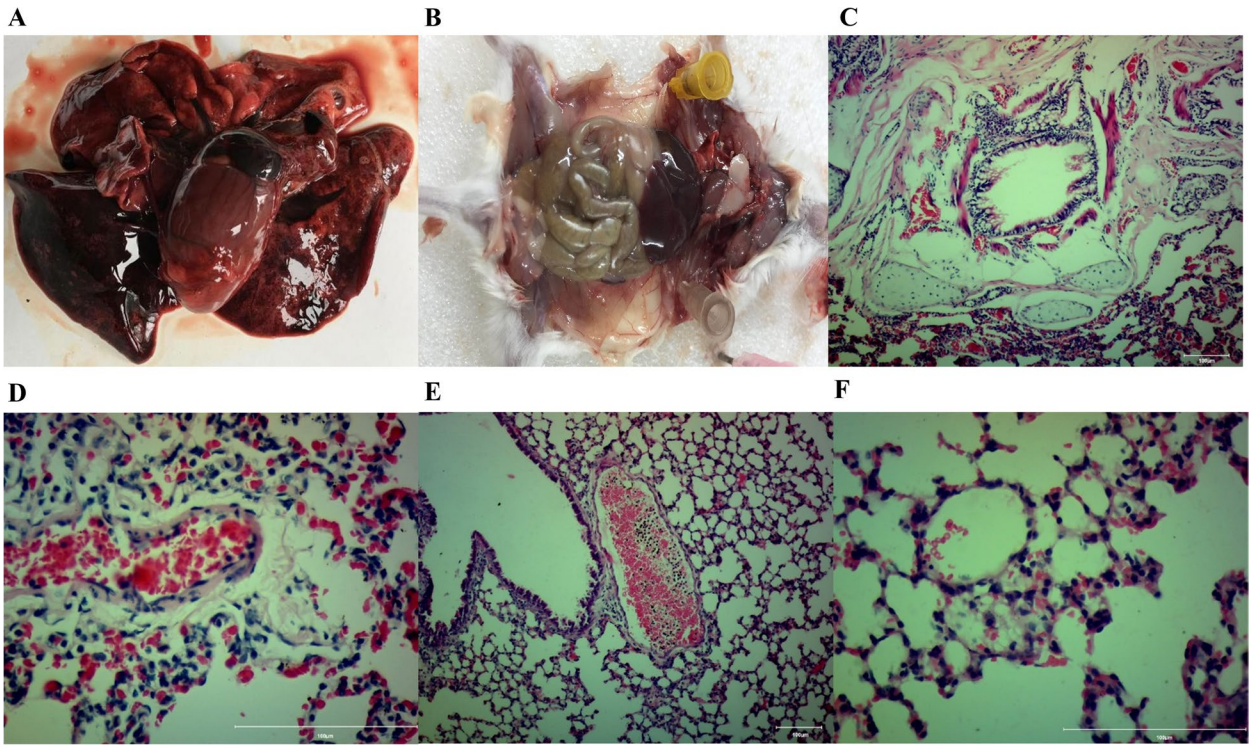

Fig. 6 Clinical signs and pathological lesions in minks and mice infected with L1. **A** Mink displayed large amounts of congestion and consolidation in the lungs. The lobe was enlarged with black-red discoloration due to its engorgement with blood. **B** Mouse lung showed discrete, multifocal areas of lesion. **C** Photomicrograph of a section of lung from mink infected with L1. Slurry exudation. Alveolar structure is incomplete. Hematoxylin–eosin staining; bar = 100 μm. **D** Photomicrograph of a section of lung from mink infected with L1. Alveolar wall capillary with perivascular edema, mural necrosis. H&E staining; bar = 100 μm. **E** Photomicrograph of a section of lung from mouse infected with L1. Alveolar space is diffusely distended due to the accumulation of blood and inflammatory cell infiltrates. H&E staining; bar = 40 μm. Slurry exudation. Alveolar structure is incomplete. H&E staining; bar = 100 μm. **F** Photomicrograph of a section of lung from mink in the experimental group. Alveoli show fibrin, inflammatory cell infiltrates, and extensive necrosis of the alveolar walls. Slurry exudation. H&E staining; bar = 100 μm

HP is one of the main fatal diseases of mink, and the important agents of HP are *P. aeruginosa* and *K. pneumoniae* [8, 51] Notably, *P. aeruginosa* was not detected in clinical samples during this outbreak, suggesting a shift in the dominant etiologic agent. In this study, we isolated ExPEC strain L1 from the lungs of HP-affected minks in Heilongjiang Province, and the involvement of other pathogens was ruled out. This finding aligns with recent reports of ExPEC involvement in mink HP cases in northern China [18, 19]. Molecular characterization revealed that L1 belongs to phylogenetic group B2 and sequence type ST131—lineages typically associated with severe extraintestinal infections in humans, and having higher morbidity and mortality than other sequence types [52–54]. Compared to strain 13, a previously reported ST131 isolate from mink [19]. L1 shared several virulence factors, including *mat*, *fyuA*, *ibeA*, and *cnf1*, but exhibited broader antimicrobial resistance, including resistance to cephalosporins, β-lactam combinations, quinolones, and macrolides. These features suggest that L1 may represent a more pathogenic and resistant variant of ST131. Unlike strain 13 (ST131-O25 serogroup), L1 was identified as belonging to serogroup O121—rare among mink isolates. The emergence of this serogroup-ST combination in mink raises concerns regarding potential zoonotic transmission and highlights the importance of monitoring animal reservoirs for atypical ExPEC variants. Although serogroup O121 ST131 strains have not previously been reported in HP-affected mink, they are known to cause foodborne outbreaks and severe infections in humans [55–62] Outbreaks of serogroup O121 have been traced to contaminated flour [63, 64], raw milk, and Gouda-like cheese [65], fresh or frozen produce, and dairy and beef products [66, 67]. Reports indicate that the consumption of meat from infected food animals may lead to infections [68, 69], and growing evidence suggests that humans carrying ST131 are a primary source of such infections, which occasionally spill over into animals [70]. Therefore, the clinical situation observed on this mink farm underscores the importance of thoroughly screening all sources of meat.

Given the rapid spread of disease on the farm and the co-housing of animals, a common infection source is likely. We speculate that contaminated water, feed, or contact with infected personnel may have introduced the pathogen [71]. This suggests that future studies should focus on investigating possible zoonotic transmission routes and environmental sources of ExPEC, such as contaminated feed and handling practices. This underscores the importance of comprehensive outbreak investigations and strict hygiene management in mink farms. Although our experimental infections via intraperitoneal and intramuscular injection fulfilled Koch's postulates, future work should explore natural transmission routes such as aerosol or intranasal exposure.

To our knowledge, this is the first study to confirm through animal challenge tests that ExPEC can be an etiological agent of HP in mink. A major limitation of this study is the restricted sample size. Although HP outbreaks have been reported across multiple mink farms, only one farm was investigated due to logistical constraints during the outbreak period. As a result, our analysis was limited to a single isolate, which may not fully capture the genetic and phenotypic diversity of ExPEC strains circulating among mink populations. Given the apparent epidemic nature of HP, future studies should involve broader epidemiological surveys across multiple farms and regions. This would enable a more comprehensive assessment of the prevalence, antimicrobial resistance patterns, and genomic variability of ExPEC associated with mink HP. Such data are essential to better understand the dynamics of ExPEC transmission and to evaluate the potential for zoonotic spread. Furthermore, expanding this investigation would help elucidate the mechanisms driving ExPEC persistence in mink populations and inform strategies for outbreak prevention and control. These efforts are particularly critical in the absence of vaccines and amid rising antimicrobial resistance.

Conclusions

Mink deaths caused by HP have occurred on different mink farms during our continuous monitoring in northern China. To the best of our knowledge, this is the first report of the pathogenicity testing of HP-associated ExPEC and the confirmation that ExPEC is a causative agent of mink HP, based on Koch's postulates. The results of this study indicate that multidrug-resistant isolate L1 belongs to ExPEC clonal group O121–B2–ST131, encodes abundant virulence factors, and is highly pathogenic to mink. This research also provides a basis for the development of control measures for the prevention and treatment of HP in mink.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04817-6.

Supplementary Material 1: Table S1. Primers for virulence genes.

Supplementary Material 2.

Supplementary Material 3.

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Authors' contributions

YL, JG and LZ: conceptualization, methodology, investigation, writing – original draft. FW, YY and DM: methodology. SW, XW, JY,ZG: data analysis, software. HC, JG, LZ: Project administration, Supervision, Writing – review & editing. All authors contributed to the article and approved the submitted version.

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Data availability

The original contributions presented in the study can be found online: https://www.jianguoyun.com/p/DfaZaPAQrfCuDBifsLYFIAA. DNA sequences generated in this study were submitted to GenBank (NCBI) following accession numbers: PP190255 and PP236910.

Declarations

Ethics approval and consent to participate

The Ethical Committee of the Institute approved all scientific experiments. All applicable international and national guidelines for the care and use of animals in experiments were followed and approved by the Institutional Committee of Northeast Agricultural University (NEAUEC20200338, 3 April 2020). We also obtained permission from the farmers to use the disease animals in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Mok CKP, Qin K. Mink infection with influenza A viruses: an ignored intermediate host? One Health Adv. 2023;1(1):5.
2. Barua A, Grot N, Plawski A. The basis of mink susceptibility to SARS-CoV-2 infection. J Appl Genet. 2022;63(3):543–55.
3. Ren J, Liu W, Sun N, Zhang P, Yin M, Guo L, Zhang H, Cheng S. Isolation and pathogenicity analysis of mink orthoreoviruses. Transbound Emerg Dis. 2022;69(2):623–31.

4. Vahedi SM, Salek Ardestani S, Banabazi MH, Clark F. Epidemiology, pathogenesis, and diagnosis of Aleutian disease caused by Aleutian mink disease virus: A literature review with a perspective of genomic breeding for disease control in American mink (Neogale vison). Virus Res. 2023;336:199208.
5. Kwok KTT, de Rooij MMT, Sinartio FF, Smit LAM, Koopmans MPG, Phan MVT. Genome Sequence of a Minacovirus Strain from a Farmed Mink in The Netherlands. Microbiol Resour Announc. 2021;10(8):e01451.
6. Bai X, Liu SG, Zhao JJ, Cheng YN, Zhang HL, Hu B, Zhang L, Shi QM, Zhang ZQ, Wu TL, et al. Epidemiology and molecular characterization of the antimicrobial resistance of Pseudomonas aeruginosa in Chinese mink infected by hemorrhagic pneumonia. Can J Vet Res-Rev Can Rech Vet. 2019;83(2):122–32.
7. Zhao YD, Guo LL, Li J, Fang BH, Huang XH. Molecular epidemiology, antimicrobial susceptibility, and pulsed-field gel electrophoresis genotyping of Pseudomonas aeruginosa isolates from mink. Can J Vet Res-Rev Can Rech Vet. 2018;82(4):256–63.
8. Jian-Li W, Yuan-Yuan S, Shou-Yu G, Fei-Fei D, Jia-Yu Y, Xue-Hua W, Yong-Feng Z, Shi-Jin J, Zhi-Jing X. Serotype and virulence genes of Klebsiella pneumoniae isolated from mink and its pathogenesis in mice and mink. Sci Rep. 2017;7(1):17291.
9. Salomonsen CM, Boye M, Hoiby N, Jensen TH, Hammer AS. Comparison of histological lesions in mink with acute hemorrhagic pneumonia associated with Pseudomonas aeruginosa or Escherichia coli. Can J Vet Res-Rev Can Rech Vet. 2013;77(3):199–204.
10. De Carli S, Ikuta N, Lehmann FKM, da Silveira VP, de Melo PG, Fonseca ASK, Lunge VR. Virulence gene content in Escherichia coli isolates from poultry flocks with clinical signs of colibacillosis in Brazil. Poult Sci. 2015;94(11):2635–40.
11. Tibbetts RJ, White DG, Dyer NW, Giddings CW, Nolan LK. Characterization of Escherichia coli isolates incriminated in colisepticaemia in mink. Vet Res Commun. 2003;27(5):341–57.
12. Derakhshandeh A, Eraghi V, Boroojeni AM, Niaki MA, Zare S, Naziri Z. Virulence factors, antibiotic resistance genes and genetic relatedness of commensal Escherichia coli isolates from dogs and their owners. Microb Pathog. 2018;116:241–5.
13. Tan C, Tang X, Zhang X, Ding Y, Zhao Z, Wu B, Cai X, Liu Z, He Q, Chen H. Serotypes and virulence genes of extraintestinal pathogenic Escherichia coli isolates from diseased pigs in China. Vet J. 2012;192(3):483–8.
14. Rimoldi GM, Moeller RB Jr. Escherichia fergusonii Associated with Pneumonia in a Beef Cow. J Vet Med. 2013;2013:829532.
15. Handt LK, Stoffregen DA, Prescott JS, Pouch WJ, Ngai DT, Anderson CA, Gatto NT, DebRoy C, Fairbrother JM, Motzel SL, et al. Clinical and microbiologic characterization of hemorrhagic pneumonia due to extraintestinal pathogenic Escherichia coli in four young dogs. Comp Med. 2003;53(6):663–70.
16. Sura R, Van Kruiningen HJ, DebRoy C, Hinckley LS, Greenberg KJ, Gordon Z, French RA. Extraintestinal pathogenic Escherichia coli-induced acute necrotizing pneumonia in cats. Zoonoses Public Health. 2007;54(8):307–13.
17. Highland MA, Byrne BA, Debroy C, Samitz EM, Peterson TS, Oslund KL. Extraintestinal pathogenic Escherichia coli-induced pneumonia in three kittens and fecal prevalence in a clinically healthy cohort population. J Vet Diagn Invest. 2009;21(5):609–15.
18. Qiu J, Jiang Z, Ju Z, Zhao X, Yang J, Guo H, Sun S. Molecular and Phenotypic Characteristics of Escherichia coli Isolates from Farmed Minks in Zhucheng. China Biomed Res Int. 2019;2019:3917841.
19. Yu Y, Hu B, Fan H, Zhang H, Lian S, Li H, Li S, Yan X, Wang S, Bai X. Molecular Epidemiology of Extraintestinal Pathogenic Escherichia coli Causing Hemorrhagic Pneumonia in Mink in Northern China. Front Cell Infect Microbiol. 2021;11:781068.
20. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, Smith KE. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic Escherichia coli from retail chicken products. Antimicrob Agents Chemother. 2003;47(7):2161–8.
21. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol. 2000;66(10):4555–8.
22. Wirth T, Falush D, Lan RT, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, et al. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol. 2006;60(5):1136–51.
23. Paddock Z, Shi XR, Bai JF, Nagaraja TG. Applicability of a multiplex PCR to detect O26, O45, O103, O111, O121, O145, and O157 serogroups of Escherichia coli in cattle feces. Vet Microbiol. 2012;156(3–4):381–8.
24. DebRoy C, Roberts E, Kundrat J, Davis MA, Briggs CE, Fratamico PM. Detection of Escherichia coli serogroups O26 and O113 by PCR amplification of the wzx and wzy genes. Appl Environ Microbiol. 2004;70(3):1830–2.
25. Shao J, Li M, Jia Q, Lu YQ, Wang PG. Sequence of Escherichia coli O128 antigen biosynthesis cluster and functional identification of an α-1,2-fucosyltransferase. FEBS Lett. 2003;553(1–2):99–103.
26. Golubeva IV, Andronova NI, Kiseleva BS, Ulisko IN, Nauman G. Serologic groups of O-antigens of E. coli isolated from urinary tract infections within the boundaries of the USSR and East Germany. Zh Mikrobiol Epidemiol Immunobiol. 1981;7:75–8.
27. Wang SH, Meng QM, Dai JJ, Han XG, Han Y, Ding C, Liu HW, Yu SQ. Development of an Allele-Specific PCR Assay for Simultaneous Sero-Typing of Avian Pathogenic Escherichia coli Predominant O1, O2, O18 and O78 Strains. PLoS One. 2014;9(5):6.
28. Pitout JD. Extraintestinal pathogenic Escherichia coli: a combination of virulence with antibiotic resistance. Front Microbiol. 2012;3:9.
29. Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Nolan LK. Characterizing the APEC pathotype. Vet Res. 2005;36(2):241–56.
30. Johnson JR, Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000;181(1):261–72.
31. Bessone FA, Bessone G, Marini S, Conde MB, Alustiza FE, Zielinski G. Presence and characterization of Escherichia coli virulence genes isolated from diseased pigs in the central region of Argentina. Veterinary world. 2017;10(8):939–45.
32. Ahmed W, Tucker J, Bettelheim KA, Neller R, Katouli M. Detection of virulence genes in Escherichia coli of an existing metabolic fingerprint database to predict the sources of pathogenic E. coli in surface waters. Water research. 2007;41(16):3785–91.
33. Shams Z, Tahamtan Y, Pourbakhsh A, Hosseiny MH, Kargar M, Hayati M. Detection of enterotoxigenic K99 (F5) and F41 from fecal sample of calves by molecular and serological methods. Comp Clin Pathol. 2012;21(4):475–8.
34. Schubert S, Rakin A, Karch H, Carniel E, Heesemann J. Prevalence of the "high-pathogenicity island" of Yersinia species among Escherichia coli strains that are pathogenic to humans. Infect Immun. 1998;66(2):480–5.
35. Ewers C, Li GW, Wilking H, Kiessling S, Alt K, Antáo EM, Laturnus C, Diehl I, Glodde S, Homeier T, et al. Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: How closely related are they? Int J Med Microbiol. 2007;297(3):163–76.
36. Tóth I, Hérault F, Beutin L, Oswald E. Production of cytolethal distending toxins by pathogenic Escherichia coli strains isolated from human and animal sources: establishment of the existence of a new cdt variant (Type IV). J Clin Microbiol. 2003;41(9):4285–91.
37. Matsumura Y, Pitout JDD, Peirano G, DeVinney R, Noguchi T, Yamamoto M, Gomi R, Matsuda T, Nakano S, Nagao M, et al. Rapid Identification of Different Escherichia coli Sequence Type 131 Clades. Antimicrob Agents Chemother. 2017;61(8):9.
38. Wang XL, Wang JQ, Hao HF, Qiu L, Liu HJ, Chen SL, Dang RY, Yang ZQ. Pathogenic providencia alcalifaciens strain that causes fatal hemorrhagic pneumonia in piglets. Curr Microbiol. 2014;68(3):278–84.
39. Vogeleer P, Tremblay YDN, Jubelin G, Jacques M, Harel J. Biofilm-forming abilities of shiga toxin-producing Escherichia coli isolates associated with human infections. Appl Environ Microbiol. 2015;82(5):1448–58.
40. Li D, Wyrsch ER, Elankumaran P, Dolejska M, Marenda MS, Browning GF, Bushell RN, McKinnon J, Chowdhury PR, Hitchick N, et al. Genomic comparisons of Escherichia coli ST131 from Australia. Microb Genom. 2021;7(12):000721.
41. Saidenberg ABS, Stegger M, Price LB, Johannesen TB, Aziz M, Cunha MPV, Moreno AM, Knöbl T. mcr-Positive Escherichia coli ST131-H22 from Poultry in Brazil. Emerg Infect Dis. 2020;26(8):1951–4.
42. Dewasmika W, Dissanayake DRA, Neelawala N, Dissanayake BGN, Prasada DVP, Francis VR, Octavia S, Lan R. High occurrence of ST131 among extraintestinal pathogenic Escherichia coli in humans and dogs in Sri Lanka. Zoonoses Public Health. 2025;72(2):200–6.
43. Birgy A, Levy C, Nicolas-Chanoine MH, Cointe A, Hobson CA, Magnan M, Bechet S, Bidet P, Cohen R, Bonacorsi S. Independent Host Factors and

Bacterial Genetic Determinants of the Emergence and Dominance of Escherichia coli Sequence Type 131 CTX-M-27 in a Community Pediatric Cohort Study. Antimicrob Agents Chemother. 2019;63(7):e00382.
44. Sura R, Van Kruiningen HJ, DebRoy C, Hinckley LS, Greenberg KJ, Gordon Z, French RA. Extraintestinal pathogenic Escherichia coli induced acute necrotizing pneumonia in cats. Zoonoses Public Health. 2007;54(8):307–13.
45. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal Escherichia coli. Nat Rev Microbiol. 2010;8(3):207–17.
46. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes Infect. 2003;5(5):449–56.
47. Smith JL, Fratamico PM, Gunther NW. Extraintestinal pathogenic Escherichia coli. Foodborne Pathog Dis. 2007;4(2):134–63.
48. Devine DA, Robinson L, Roberts AP. Occurrence of K1, K5 and O antigens in Escherichia coli isolates from patients with urinary tract infections or bacteraemia. J Med Microbiol. 1989;30(4):295–9.
49. Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, Boileau P. Neonatal bacterial meningitis: 444 cases in 7 years. Pediatr Infect Dis J. 2011;30(3):212–7.
50. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123–40.
51. Zhao YD, Guo LL, Li J, Fang B, Huang XF. Molecular epidemiology, antimicrobial susceptibility, and pulsed-field gel electrophoresis genotyping of Pseudomonas aeruginosa isolates from mink. Can J Vet Res. 2018;82(4):256–63.
52. Shaik S, Ranjan A, Tiwari SK, Hussain A, Nandanwar N, Kumar N, Jadhav S, Semmler T, Baddam R, Islam MA, et al. Comparative Genomic Analysis of Globally Dominant ST131 Clone with Other Epidemiologically Successful Extraintestinal Pathogenic Escherichia coli (ExPEC) Lineages. mBio. 2017;8(5):e01596-17.
53. White RT, Bull MJ, Barker CR, Arnott JM, Wootton M, Jones LS, Howe RA, Morgan M, Ashcroft MM, Forde BM, et al. Genomic epidemiology reveals geographical clustering of multidrug-resistant Escherichia coli ST131 associated with bacteraemia in Wales. Nat Commun. 2024;15(1):1371.
54. Hassuna NA, Rabea EM, Mahdi WKM, Abdelraheem WM. Biofilm formation and antimicrobial resistance pattern of uropathogenic E. coli ST131 isolated from children with malignant tumors. J Antibiot (Tokyo). 2024;77:324.
55. Kikuchi K, Lee K, Ueno H, Tomari K, Kobori S, Kaetsu A, Matsui M, Suzuki S, Sekizuka T, Kuroda M, et al. Enterohaemorrhagic Escherichia coli O121:H19 acquired an extended-spectrum β-lactamase gene during the development of an outbreak in two nurseries. Microb Genomics. 2019;5(7):11.
56. Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin-producing Escherichia coli. Clin Infect Dis. 2006;43(12):1587–95.
57. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, Park YJ, Lavigne JP, Pitout J, Johnson JR. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;61(2):273–81.
58. Nicolas-Chanoine MH, Bertrand X, Madec JY. Escherichia coli ST131, an intriguing clonal group. Clin Microbiol Rev. 2014;27(3):543–74.
59. Banerjee R, Johnson JR. A new clone sweeps clean: the enigmatic emergence of Escherichia coli sequence type 131. Antimicrob Agents Chemother. 2014;58(9):4997–5004.
60. Brolund A, Edquist PJ, Mäkitalo B, Olsson-Liljequist B, Söderblom T, Wisell KT, Giske CG. Epidemiology of extended-spectrum β-lactamase-producing Escherichia coli in Sweden 2007–2011. Clin Microbiol Infect. 2014;20(6):O344-352.
61. Mathers AJ, Peirano G, Pitout JD. Escherichia coli ST131: The quintessential example of an international multiresistant high-risk clone. Adv Appl Microbiol. 2015;90:109–54.
62. Rogers BA, Sidjabat HE, Paterson DL. Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011;66(1):1–14.
63. Morton V, Cheng JM, Sharma D, Kearney A. An outbreak of shiga toxin-producingEscherichia coli O121 infections associated with flour - Canada, 2016–2017. MMWR-Morb Mortal Wkly Rep. 2017;66(26):705–6.
64. Crowe SJ, Bottichio L, Shade LN, Whitney BM, Corral N, Melius B, Arends KD, Donovan D, Stone J, Allen K, et al. Shiga toxin-producing E. coli infections associated with flour. N Engl J Med. 2017;377(21):2036–43.
65. Boyd E, Trmcic A, Taylor M, Shyng S, Hasselback P, Man S, Tchao C, Stone J, Janz L, Hoang L, et al. Escherichia coli O121 outbreak associated with raw milk Gouda-like cheese in British Columbia, Canada, 2018. Can Commun Dis Rep. 2021;47(2):11–6.
66. Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H, Raynaud S, Thevenot D, Condron R, De Reu K, Govaris A, et al. Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. Int J Food Microbiol. 2013;162(2):190–212.
67. Bai XN, Wang H, Xin YQ, Wei RJ, Tang XY, Zhao AL, Sun H, Zhang W, Wang Y, Xu YM, et al. Prevalence and characteristics of Shiga toxin-producing Escherichia coli isolated from retail raw meats in China. Int J Food Microbiol. 2015;200:31–8.
68. Hedican EB, Medus C, Besser JM, Juni BA, Koziol B, Taylor C, Smith KE. Characteristics of O157 versus non-O157 Shiga toxin-producing Escherichia coli infections in Minnesota, 2000–2006. Clin Infect Dis. 2009;49(3):358–64.
69. Norman KN, Strockbine NA, Bono JL. Association of nucleotide polymorphisms within the O-antigen gene cluster of Escherichia coli O26, O45, O103, O111, O121, and O145 with serogroups and genetic subtypes. Appl Environ Microbiol. 2012;78(18):6689–703.
70. Lavigne JP, Vergunst AC, Goret L, Sotto A, Combescure C, Blanco J, O'Callaghan D, Nicolas-Chanoine MH. Virulence potential and genomic mapping of the worldwide clone Escherichia coli ST131. PLoS One. 2012;7(3):10.
71. Liu CM, Aziz M, Park DE, Wu Z, Stegger M, Li M, Wang Y, Schmidlin K, Johnson TJ, Koch BJ, et al. Using source-associated mobile genetic elements to identify zoonotic extraintestinal E. coli infections. One Health (Amsterdam, Netherlands). 2023;16:100518.

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