Original Article

Characteristics of one *Escherichia coli* strain isolated from the sick red panda excreting mucus-like faeces

Dongjie Cai[1*†] Lanjing Ma[1†] Xingxin Fu[2] Bin Tian[3] David Ranucci[4]

Abstract

The purpose of this study is to explore the main pathogens that caused one red panda to excrete mucus-containing faeces continuously. The anal swabs of sick red panda were collected for bacterial isolation, biochemical analysis, 16SrRNA sequencing identification, antimicrobial susceptibility testing, and research on pathogenicity in Mice. An *Escherichia coli* (*E. coli*) strain was isolated and determined. This strain was resistant to ampicillin, penicillin G, ceftiofur, and highly sensitive to levofloxacin, imipenem, azithromycin, kanamycin, and compound sulfamethoxazole. All mice died within 8-14 hours after injected intraperitoneally with 6×10^8 CFU/mL *E. coli*. Histopathology showed that the lungs, livers, kidneys, and spleens of mice killed by *E. coli* showed different degrees of congestion and hemorrhage. The *E. coli* isolated from the red panda is multidrug-resistant and highly pathogenic for mice. This study can provide references for drug use and enhance understanding of *E. coli*-induced diarrhea in red pandas.

Keywords: red panda, *Escherichia coli*, antimicrobial resistance

[1]*College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China*
[2]*The Second Affiliated Hospital of Chongqing Medical University, Chongqing 408599, China*
[3]*Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China*
[4]*Department of Veterinary, University of Perugia, Perugia 06126, Italy*
†*Dongjie Cai and Lanjing Ma contributed equally to this work*
***Correspondence:** *dongjie_cai@sicau.edu.cn* (D. Cai)
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Introduction

Escherichia coli is one of the primary intestinal commensal organisms found in humans and warm-blooded animals, and is widely disseminated in the environment. However, most strains have no pathogenic effect; some strains may become pathogenic through acquiring pathogenic factors, leading to infections in humans and animals. *E. coli* infections can occur in the poultry and pig industry (Tansawai *et al.*, 2019; Adorján *et al.*, 2021), and domestic animals (Wieczorek and Osek, 2020; Remfry *et al.*, 2021), as well as wild animals such as golden monkeys (Tanabe *et al.*, 2019) and giant pandas (Guo *et al.*, 2015; Nsofor *et al.*, 2021). The clinical symptoms caused by *E. coli* infection vary with the pathogenicity of the strains. Hemolytic *E. coli* can cause bloody stools, dehydration, and electrolyte disturbances (Gomes *et al.*, 2016). *E. coli* can cause neonatal septicemia in the United States (Cole *et al.*, 2017). Clinically, diarrhea is the most common symptom of *E. coli* disease in humans and animals (Croxen *et al.*, 2013); infantile meningitis occasionally occurs (Nowrouzian *et al.*, 2019; Riley, 2020). The *E. coli* epidemic has brought huge economic losses to the livestock industry (Nsofor and Iroegbu, 2012) and also poses a threat to the life and health of humans and wild animals.

Red pandas (*Ailurus fulgens*) are second-level key protected wildlife with unique ecological and ornamental values in China. The International Union for the Conservation of Nature (IUCN) classified the red pandas as an endangered species. Though the number of captives is increasing, the number of deaths caused by viruses, bacteria, nutrition, and trauma is also increasing. Therefore, the prevention and control of diseases have become a priority in the conservation of captive pandas. Bacterial infection is one of the most important health threats to captive populations in the breeding and conservation of red pandas (Liu *et al.*, 2020; Liu *et al.*, 2022; Ji *et al.*, 2022). To date, studies on the characteristics and antimicrobial resistance of intestinal *E. coli* isolates in red pandas are scarce. Therefore, there is an urgent need to expand the research data related to bacterial infections in red pandas.

In April 2021, two red pandas in one of the zoos in Sichuan Province developed clinical symptoms of depression, loss of appetite, and continuous discharge of mucus-like feces. The results of Canine Distemper Virus (CDV) and Canine Parvovirus (CPV) tests were negative. Therefore, bacterial infection was suspected. The swabs from one red panda were collected for bacterial isolation and identification. As a result, a strain of *E. coli* was isolated, and it was tested by biochemical tests, 16SrRNA genetic analysis, antimicrobial resistance, and pathogenicity tests in mice. This study can provide a reference for the diagnosis and clinical treatment of *E. coli* infection in red pandas.

Materials and Methods

One of the sick red pandas' anal swabs was collected from the zoo, then the swabs were immersed in an inoculation loop, inoculated in MacConkey agar and blood agar (Hangzhou Microbial Reagent Co., Hangzhou, China). Then these media were placed in a 37°C incubator for 18 h. Colony morphology was observed, and individual colonies were selected and further purified by successive line inoculations in the medium. Individual colonies were selected for Gram-stained microscopic examination. After initial identification and purification, individual colonies were inoculated with LB broth medium, incubated for 18-24 hours at 37°C, sterilized with 50% glycerol, and stored in an ultra-low temperature refrigerator.

On a larminar flow, individual colonies picked with an inoculating loop were added to galactose, maltose, fructose, arginine dihydrogenase, citric acid, sorbitol, mannitol, glucose peptone, malonic acid, rhamnose, peptone water (indigo substrate), Voges-Proskauer (VP) experiment, mannose, inositol, arabinose, phenylalanine, hydrogen sulfide, semi-solid agar and ornithine biochemical identification tubes (Hangzhou Microbiological Reagent Co., Hangzhou, China) respectively, which were placed in the incubator at 37°C for 12-24 h. The results were determined by reference to the instruction manual of the biochemical tubes and the Bergey's Manual of Systematics of Archaea and Bacteria (Whitman, 2015).

The 16SrRNA was amplified by PCR using purified bacterial sap as a template with an estimated amplified fragment length of 1500 bp, and the amplification universal primer (Sangon Biotech, Shanghai, China) sequences were: F：AGAGTTTGATCCTGGCTCAG ；R：TACGGCTACCTTGTTACGACTT (Clarridge, 2004). The PCR reaction product was electrophoresed in 1% agarose gel, the result was observed in a gel imaging system, and the PCR product with 1500 bp bands was sent to Chengdu Tsingke Biological Co. for sequencing. The sequenced gene sequences were analyzed by NCBI for sequence homology, and the 16SrRNA gene sequences of the published GenBank strains were selected to construct a phylogenetic tree. The phylogenetic tree was constructed by using the Neighbor-Joining method with MEGA-X software (Persada *et al.*, 2021) to analyze the relationship of homology.

The isolated strain was tested for drug sensitivity by the Kirby-Bauer method. The appropriate amount of bacterial solution was taken on the surface of Mueller-Hinton (MH) agar medium (Hangzhou Microbiological Reagent Co., Hangzhou, China) and incubated for 16 h at 37°C. The bacterial solution was diluted to 0.5 McSweeney's standard with sterile saline, evenly coated and pasted with drug-sensitive paper sheets (Hangzhou Microbiological Reagent Co.), incubated upside down for 16-18 h in a constant temperature incubator at 37°C, and then removed. The diameter of the inhibition circle was observed and measured with vernier calipers. The incubation time and the measurement were referred to the CLSI M100-Ed35 2025 standard (CLSI, 2025). The measurement results were based on the size of the inhibition circle as an indicator of susceptibility and resistance.

Ten 4- to 5-week-old Kunming mice (Dashuo Laboratory Animal Co., Chengdu, China) weighing 18-20 g of SPF grade were selected to determine the pathogenicity of the isolated *E. coli* strain. Mice were randomly divided into control and experimental groups. The bacterial inoculum was prepared by

growing the isolate in LB broth at 37°C to mid-log phase, harvesting by centrifugation, and resuspending in sterile saline to a concentration of 6×10^8 CFU/mL. Each mouse in the experimental group was injected intraperitoneally with 0.2 mL of this bacterial suspension. Each mouse in the control group was injected with an equal amount of sterile saline. All mice were monitored for mortality and clinical signs such as ruffled fur, lethargy, and dyspnea for 14 h post-inoculation. Mice that did not die after the test were euthanized using sodium phenobarbital for sampling according to American Veterinary Medical Association guidelines (Wang *et al*., 2020; Wang *et al*., 2022). Mouse organs were photographed and collected aseptically post-mortem and fixed in 4% paraformaldehyde (Chengdu Kolon Chemical Co., Chengdu, China) for routine processing and embedded in paraffin. Paraffin-embedded tissue samples were sectioned (5 μm) and stained with hematoxylin-eosin (H&E, Beyotime, Beijing, China) stain. Organ histopathology of all mice was documented by two pathologists who were blinded to treatments. The authors declare that all animal procedures were conducted following the guidelines in the documents approved by the Animal Ethics Committee of Sichuan Agricultural University (Approved permit number SYXK 2019-187).

Result and Discussion

The pre-enriched culture of the positive red panda anal swab sample was cultured on blood agar and MacConkey medium, yielding round, raised, smooth, moist red colonies (1-2 mm in diameter) after 24 hours (Fig. 1A). This aligns with *E. coli*'s typical lactose-fermenting phenotype on MacConkey medium, a key trait distinguishing it from non-lactose-fermenting Gram-negative bacteria (Buchanan, 1984), as red colonies result from lactose fermentation—a conserved marker for preliminary *E. coli* identification. Gram staining revealed non-spore-forming, flagellated, pink Gram-negative rods (individually or paired, short blunt ends; Fig. 1B), consistent with *E. coli*'s structural characteristics in Enterobacteriaceae (Buchanan, 1984).

Biochemical tests (Table 1) showed the isolate fermented glucose, lactose, and six other sugars but not raffinose or inositol; it was positive for malonic acid and lysine decarboxylase, negative for citrate and VP reaction, and produced hydrogen sulfide. These profiles match standard *E. coli* traits (Buchanan, 1984), excluding Salmonella and Klebsiella, leading to tentative identification. The combination of colonial morphology, Gram staining, and biochemical tests remains reliable for preliminary identification, especially in resource-limited settings.

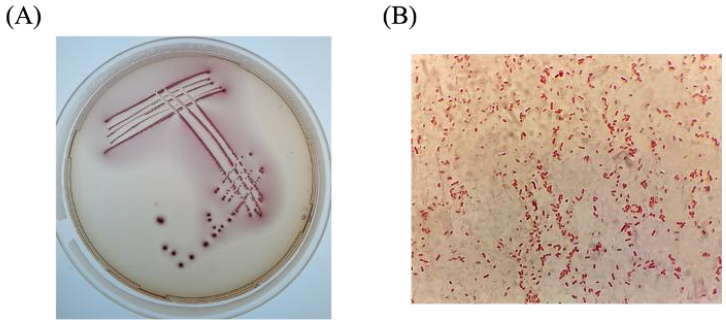


Figure 1 Morphological Identification of Isolates the isolated strain. (A): Culture morphology results of the isolated strain in MacConkey medium. (B): Gram staining results of the isolated strain (400×).

Table 1 Biochemical test results of *E. coli* 20210421.

Substrates	Results	Substrates	Results
Glucose	+	Malonate	+
Rhamnose	+	Citrate	—
Xylose	+	Nitrate reduction	—
Lactose	+	Lysine decarboxylase	+
Arabinose	+	Phenylalanine	—
Fructose	+	Inositol	—
Raffinose	—	Sorbitol	—
Maltose	+	Hydrogen sulfide	—
Mannose	+	VP reaction	—

Genomic DNA extraction and 16S rRNA gene amplification yielded a ~1500 bp band (Fig. 2A), consistent with the expected size. BLAST and phylogenetic analysis (Fig. 2B) clustered the isolate with *E. coli*, confirming species identity via this gold standard method (Woese *et al*., 1990), which avoids phenotypic overlap-induced misidentification critical for subsequent studies.

Antimicrobial susceptibility testing (Table 2) showed *E. coli* CD20210421 was resistant to ampicillin, ceftiofur, and five other antibiotics, intermediate to streptomycin and enrofloxacin, and sensitive to kanamycin, levofloxacin, and three others—exhibiting multidrug resistance (MDR) to β-lactams, tetracyclines, and other key classes. This MDR phenotype is a growing concern in zoonotic pathogens (Moore *et al*., 2021), as cross-species resistant gene transmission threatens human and animal medicine. Results were interpreted via CLSI guidelines (CLSI, 2025) due to no red panda-specific breakpoints, a common wildlife microbiology challenge.

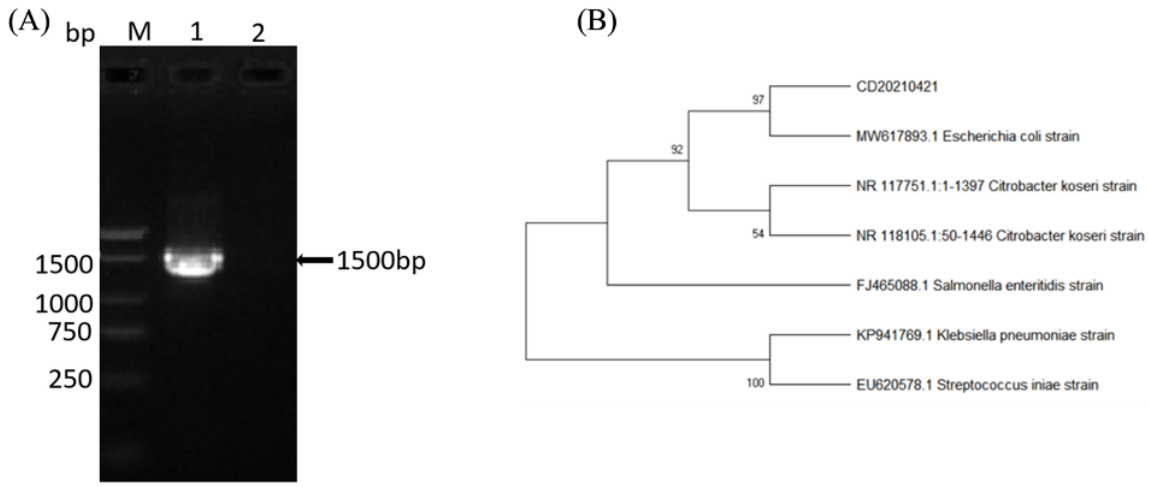


Figure 2 The 16SrRNA gene amplification result and phylogenetic trees of the isolated strain. (A): The 16SrRNA gene amplification result of the isolated strain. M, DNA molecular quality standard; 1, 16SrRNA amplification product; 2, Negative control. (B): Phylogenetic trees constructed by the neighbor-joining method based on the 16SrRNA gene of *E. coli*. The *E. coli* isolate was named CD20210421. The other 6 reference strains were retrieved from the GenBank database with accession numbers.

Table 2 Results of drug sensitivity test.

Category	Drug	Judgment standard of bacteriostatic circle diameter			Result	Sensitivity
	Ampicillin	≤13	14～16	≥17	6	R
β- Lactamides	Penicillin	≤28	-	≥29	6	R
	Cefotiofur	≤17	18～20	≥21	8	R
Aminoglycosides	Kanamycin	≤13	14～17	≥18	18	S
Macrolides	Streptomycin	≤11	12～14	≥15	12	I
Tetracyclines	Azithromycin	≤12	-	≥13	18	S
Chloramphenicols	Timicosin	≤12	13～14	≥15	12	R
Lincomamides	Tetracycline	≤12	13～14	≥15	8	R
Quinolones	Florfenicol	≤12	13～17	≥18	8	R
Sulfonamides	Lincomycin	≤14	15～20	≥21	6	R
Carbapenems	Levofloxacin	≤16	17～20	≥21	22	S
Aminoglycosides	Enrofloxacin	≤16	17～20	≥21	18	I
Macrolides	Compound sulfamethoxazole	≤10	11～15	≥16	16	S
Tetracyclines	Imipenem	≤19	20～22	≥23	26	S

Note: R-resistance; I- moderate sensitivity; S-highly sensitive

Intraperitoneal *E. coli* injection induced depression, erect fur, and eyelid discharge in mice within 3 hours, 12 hours post-infection, and eyelid and anal fur adhesion occurred. All infected mice died within 8-14 hours (100% mortality), contrasting with controls (no deaths), indicating high virulence (Moore *et al*., 2021). Necropsy revealed multi-organ congestion, intestinal distension, and wall thinning (Fig. 3A). Histopathology showed multi-organ congestion/hemorrhage (Fig. 3B), with brain tissue isolation confirming blood-brain barrier penetration—consistent with highly pathogenic ExPEC (Moore *et al*., 2021). Given the zoo's captive red panda setting, potential *E. coli* transmission sources may include contaminated bamboo, unsterilized drinking water, or cross-contamination via shared cleaning tools. Core prevention measures should involve regular microbiological testing of feed and water, plus enhanced disinfection of enclosures and feeding equipment. Additionally, strict caretaker hand hygiene and quarterly pathogen surveillance in the captive population can block transmission pathways.

This study identified a MDR, highly pathogenic *E. coli* from the red panda via integrated methods. Findings highlight the need for susceptibility testing before antibiotic use and strengthened captive hygiene to mitigate zoonotic risks, providing references for red panda health management. It is key to note that a single *E. coli* strain was isolated from a red panda in this study, which limits the generalizability of the findings. Further studies involving more isolates from multiple animals and locations are needed to gain a comprehensive understanding of *E. coli* epidemiology and resistance patterns in red pandas.

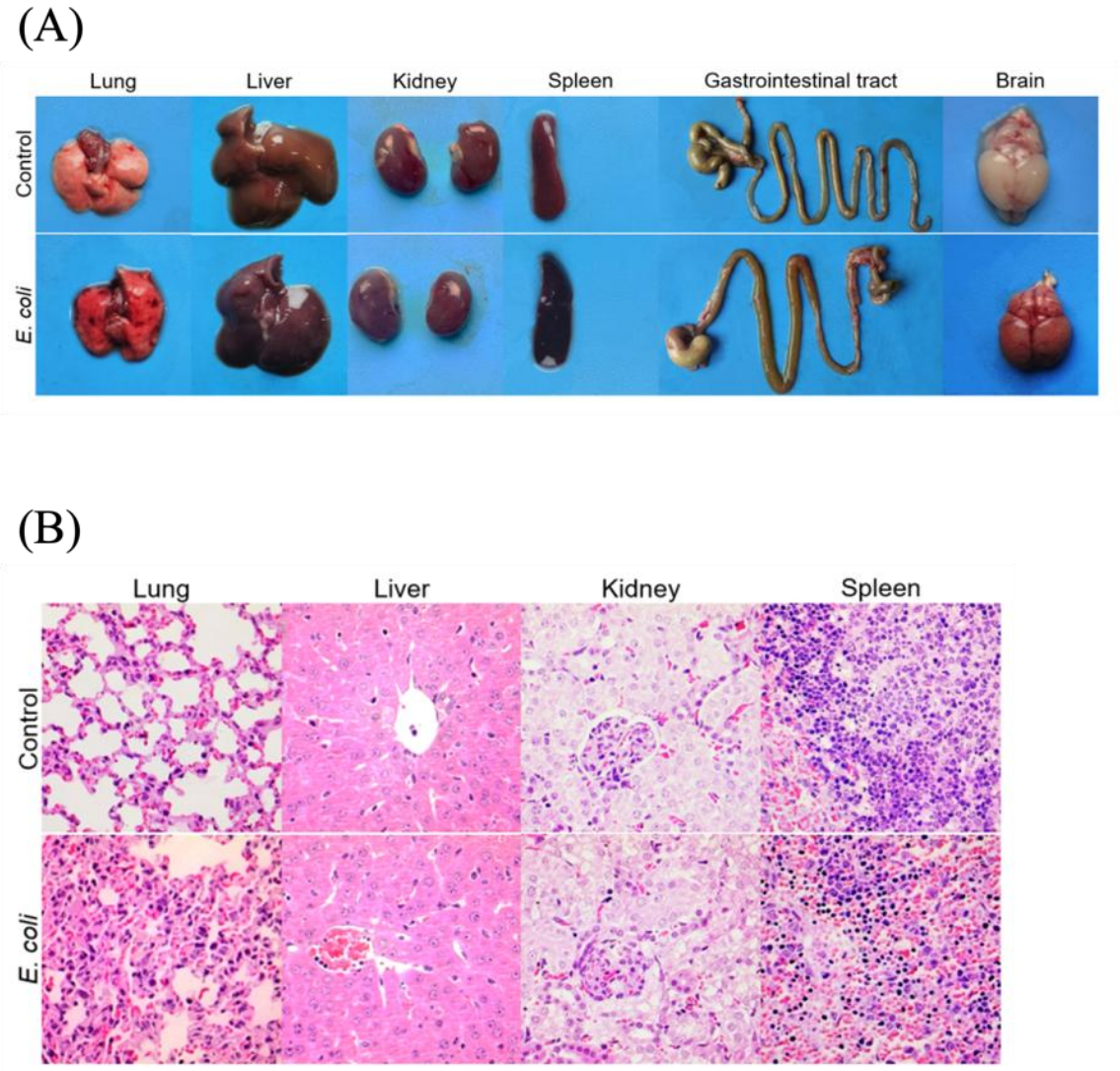


Figure 3 Results of pathological examination of mice. (A) The images show the morphological appearance of the lungs, liver, kidneys, spleen, gastrointestinal tract, and brain in mice from the normal control group and the *E. coli*-infected group. All mice died within 14 hours of the *E. coli* group. The mice in the control group were euthanized. The lungs, spleens, and kidneys of all groups were collected. (B): Representative photomicrographs of hematoxylin and eosin–stained lung, spleen, and kidney sections of mice (400×). All mice died within 14 hours of the *E. coli* group. The mice in the control group were sacrificed at the end of the observation period. The lungs, spleens, and kidneys of all groups were collected and fixed in 4% paraformaldehyde. Paraffin-embedded samples of the tissues were then sectioned (5 μm) and stained with hematoxylin and eosin (H&E).

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