BMC Microbiology

RESEARCH

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Genomic features, antimicrobial resistance and pathogenicity assessment of *Escherichia coli* serotype O177:H51 strain JS01 isolated from a diseased chicken

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Abstract

Background Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), remains one of the most significant bacterial diseases threatening global poultry production and causing substantial economic losses. The dominant serotypes of APEC regional diversity, complicating prevention and control efforts. Given the pathogen's importance, this study focused on the microbiological identification of an *E. coli* strain (JS01) isolated from diseased chickens on a poultry farm in China. Further analyses were conducted to characterize its antimicrobial resistance (AMR) phenotype and pathogenicity. To elucidate its multi-drug resistance and pathogenic mechanisms, whole-genome sequencing (WGS) of JS01 was performed, followed by functional gene annotation, sequence typing, and serotype analysis. Additionally, the genetic evolutionary characteristics of JS01 were investigated through comparative genomics and phylogenetic tree analysis.

Results Strain JS01 was identified as an ST155 *E. coli* of O177:H51 serotype, exhibiting multi-drug resistant and strong virulence. WGS revealed that JS01's genome consists of one chromosome and three plasmids, comprising 4,670 coding DNA sequences with a total length 5,089,394 bp. The genome harbored 64 AMR genes and 177 virulence factor (VF) genes. The AMR genes were primarily associated with defense mechanisms conferring antibiotic resistance, while the VF genes were linked to adhesins, motility, and effector systems. Comparative genomic analysis indicated that JS01 is closely related to GCA_900636075.1, ASM1038v1 and ASM3229062v1, which were isolated from different hosts.

Conclusion This study isolated and identified the O177: H51 serotype *E. coli* strain JS01 from chickens. The findings of this study provide valuable insights into the serotype distribution, antibiotic resistance patterns, and virulence characteristics of APEC isolates from diseased chickens in China. These results lay a foundation for future research to more accurately assess the impact of colibacillosis on the poultry industry and to develop targeted control strategies.

Keywords *Escherichia coli*, Antimicrobial resistance, Virulence factor, WGS, Chicken

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Introduction

Escherichia coli is widely distributed in nature, primarily inhabiting the intestines of humans and animals [1]. Due to its role in maintaining intestinal flora balance, biological antagonism, and vitamin synthesis, it was once considered a normal component of the gut microbiota [2]. However, by the mid-twentieth century, certain serotypes of *E. coli* were identified as pathogenic to humans and animals, leading to their classification as pathogenic *E. coli* [3]. Based on pathogenicity, pathogenic *E. coli* are divided into 11 types: Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Enteroadhesive *E. coli* (EAEC), Attaching and effacing *E. coli* (AEEC), Diffusely adherent *E. coli* (DAEC), Septicaemic *E. coli* (SEPEC), Avian pathogenic *E. coli* (APEC), and Uropathogenic *E. coli* (UPEC) [4, 5]. These classifications are not absolute and often overlap or evolve. For instance, the U.S. CDC classification excludes AEEC, and its STEC can also refer to EHEC and VTEC [6]. APEC, a term primarily used in veterinary medicine, is classified based on the host origin of the isolate. However, due to limited research, the pathogenic type of many APEC isolates remains unclear [7]. To date, approximately half of the over 200 O:H serotypes of *E. coli* are pathogenic to poultry [8, 9]. With the rapid growth of large-scale intensive poultry farming, the impact of pathogenic *E. coli* on humans and animals has become increasingly severe.

APEC is a Gram-negative enterobacterium that causes a range of diseases in poultry, including perihepatitis, pericarditis, airsacculitis, salpingitis, egg peritonitis, coli granuloma, cellulitis, omphalitis, and osteomyelitis [10, 11]. These infections lead to reduced meat and egg production [12], making APEC a significant pathogen associated with economic losses in global poultry production [10, 13]. For example, in China, *E. coli* infections cause annual economic losses of approximately 300 million RMB in the poultry industry [14]. Similarly, Norton [15] reported that the U.S. broiler industry loses about 50 million USD annually due to APEC-induced cellulitis, while in Denmark, *E. coli*-related peritonitis results in annual revenue losses of 3.3 million Euros in the layer industry [16]. Consequently, developing effective prevention and intervention strategies for poultry colibacillosis has become a global priority for veterinary researchers.

Despite the identification of various risk factors, such as immunological immaturity in chickens and stress-induced colibacillosis, controlling the disease remains challenging [17]. This difficulty stems from two main reasons. First, the diversity of APEC virulence genes complicates understanding their pathogenicity [17, 18]. Like other pathogenic *E. coli*, APEC's virulence potential is determined by specific virulence factors (VFs), including fimbriae, toxins, adhesins, siderophores, capsules, invasins, and hemolysins. These VFs enable the bacteria to evade host defenses, colonize critical anatomical sites, and trigger harmful inflammatory response, ultimately leading to disease [1, 19]. Additionally, the existence of numerous *E. coli* serotypes—comprising over 188 O groups and 56 H groups [20, 21]—and the regional variation in dominant serotypes, coupled with continuous strain evolution, result in lack or limited cross-protection between strains [22]. Although various vaccines, such as inactivated, live attenuated, and subunit vaccines, have been developed, none have demonstrated consistent and broad protection against APEC [23]. Moreover, vaccines effective against one serogroup ofen fail to protect against heterologous serogroups [24]. Second, while antibiotics have historically been effective in preventing and treating colibacillosis, their unscientific use—such as overuse, abuse, low-dose growth promotion—has led to the emergence of multi-drug resistant (MDR) strains of *E. coli*. Antimicrobial resistance (AMR) in *E. coli* has been extensively documented worldwide, with studies revealing significant regional variations in resistance spectrum among bacterial isolates [25–27]. Such geographic heterogeneity in AMR patterns complicates the implementation of standardized antibiotic-based strategies for preventing and controlling colibacillosis in livestock and poultry production systems. Although prior studies have demonstrated associations between APEC virulence traits and their repertoire of VF genes [28, 29], potential pathogenic characteristics linked to APEC strains necessitate further investigation into the roles of VF genes, MDR properties, and their interactions with particular hosts [17].

The APEC genome encodes a variety of VF genes and AMR genes, which vary across hosts and regions, making its pathogenesis and drug resistance mechanisms poorly understood [17]. Advances in genomic sequencing technologies over the past two decades have enabled comprehensive analysis of bacterial genomes, facilitating the identification of serotypes, virulence factors, and drug-resistant determinants [30]. Therefore, through whole-genome sequencing (WGS) and genomic analysis, the genotypes as well as serotypes, VFs and resistance coding genes of APEC strains from different regions and different hosts can be characterized quickly and accurately. This approach is crucial for elucidating APEC's pathogenicity, drug resistance and host specificity.

In this study, an APEC strain, JS01, was isolated and identified from diseased chickens in western Anhui Province, China. Its antibiotic resistance and pathogenicity were evaluated. Furthermore, the whole-genome of the JS01 was sequenced, and its functional genes were annotated to elucidate the mechanisms underlying its

antibiotic resistance and virulence phenotypes. This study contributes to an understanding the transmission of AMR genes and VF genes and the pathogenesis of APEC in poultry farms in China.

Materials and methods

Experimental animals

Diseased chickens were obtained from Huainan Mahuang (an endemic chicken breed of China) chicken farm, located in western Anhui Province, China. The diseased chickens exhibited disheveled feathers, lethargy, isolation from the flock, refusal to feed, and loose grayish-white and green watery stools. Despite receiving a two-day treatment with drinking water containing 10% doxycycline soluble powder, the chickens showed no improvement and were promptly transported to the Veterinary Clinical Diagnosis Laboratory of West Anhui University for further diagnosis. The chickens on the farm had been immunized according to the standard immunization procedures for Huainan Mahuang chickens and had received complete vaccinations. SPF C57BL/6 J mice aged 4–5 weeks were purchased from Skobes Biotechnology Co., Ltd (Henan, China; permit No: SCXK(YU) 2020–0005).

Ethics statement

The study protocol was approved by the Ethics Committee of West Anhui University (permit No. 2023-E(r)−004) for studies involving animals. Written consent was obtained from the farm handlers prior to sampling. All animal experimentation procedures adhered to the Declaration of Helsinki and relevant policies in China. Clinical trial number is not applicable in this study.

Pathological autopsy, sample collection and bacterial isolation

Pathological autopsies were performed on dead chickens. Sick chickens were euthanized using anesthesia by administering 100 mg/kg of Pentobarbital Sodium intravenously, followed by 0.5 mL/kg of 10% Potassium Chloride once unconscious. The autopsies were conducted on a sterile workbench (SW-CJ-2FD, Suzhou Purification Equipment Co., LTD.), and characteristic fibrinous pericarditis and other pathological changes were observed and recorded. Blood samples from infected chickens were collected for Avian influenza (AI) virus and Newcastle disease (ND) virus detection using AI Ag colloidal gold rapid detection cards and ND Ag colloidal gold rapid detection cards (Sanuolikang Biotechnology (Beijing) Co., LTD.) respectively. The results were negative for both viruses. Based on the clinical symptoms and pathological changes, a highly suspected *E. coli* infection was identified, prompting further isolation and identification of pathogenic bacteria. Liver tissues were aseptically collected from deceased chickens for bacteria isolation. The liver samples were streaked onto nutrient agar (NA, HopeBio™, China) plates and incubated at 37℃ for 24 h. A single colony was picked using an inoculation loop and streaked onto NA plates for purification. The purified colonies were then streaked onto MacConkey (MAC) agar (HopeBio™) and Eosin Methylene Blue (EMB) agar (HopeBio™) plates and incubated at 37℃ for 18 h. Colony characteristics on MAC and EMB were observed and recorded. A colony with a characteristic green metallic sheen on EMB was labeled as JS01 strain and used for Gram staining under a light microscope (BX53, Olympus) and further experimentation.

Biochemical identification of the JS01 strain

The JS01 strain was identified using the VITEK 2 Compact automatic microbial identification analyzer (BioMerieux, France). A small test tube attached to the analyzer was filled with 5 mL of sterile water containing 0.45% NaCl (pH 7.0). The purified JS01 strain was picked using an inoculation loop and placed into the test tube, and the bacterial solution was mixed by gentle shaking. Once the bacterial suspension reached a concentration of 0.5 ~ 0.63 McFarland (McF) on the turbidimeter, it was placed on the reaction rack. A GN identification card was inserted into the reaction tank corresponding to the test tube, and the reaction rack was placed in the analyzer. Biochemical identification of the JS01 strain (47 biochemical reactions) was conducted according to the manufacturer's instructions, with *E. coli* ATCC25922 (Beijing Zhongke Quality Inspection Biotechnology Co., Ltd., China) used as a control strain.

Antibiotic susceptibility testing of the JS01 strain

Antibiotic susceptibility of the JS01 strain was tested using the modified Kirby-bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) recommendations [31]. The antibiotics tested included Amikacin (AMK), Ceftriaxone (CTR), cefuroxime (CXM), cefazolin (CZ), Cefalexin (CN), Ampicillin (AMP), lincomycin (MY), vancomycin (VAN), streptomycin (STR), minocycline (MI), erythromycin (ERY), Ciprofloxacin (CIP), azithromycin (AZI), oxycycline (DO), levofloxacin (LEV), penicillin (PEN), gentamicin (GEN), kanamycin (KAN), florfenicol (FFC), Polymyxin B (PB), selectrin (SXT), chloramphenicol (CHL), clindamycin (CC), and imipenem (IPM), totaling 24 antibiotics. These antibiotics were selected based on their widespread use in poultry feed and for treating avian colibacillosis and other bacterial infections [32, 33]. All antibiotic discs were sourced from BKMAM™ (Changde Beekman Biotechnology Co., LTD., China). Briefly, the JS01 strain was

evenly coated on the NA plates, and 24 abtibiotic discs were evenly placed on the plates. The plates were incubated at 37℃ for 18 h, and the diameters of the inhibition zones were measured in millimetres. The results were classified as susceptible (S), intermediate (I), or resistant (R) to determine the antibiotic resistance of the isolated bacteria [1]. *E. coli* ATCC25922 served as the quality control strain.

Pathogenicity test of the JS01 strain

To assess the potential pathogenicity of the JS01 strain, a miouse challenge experiments was conducted. Pathogenic *E. coli* ATCC11775 (standard strain, Beijing Zhongke Quality Inspection Biotechnology Co., Ltd.) was uded as the infection control, and a blank control group was also established. The JS01 strain and *E. coli* ATCC11775 were inoculated in Tryptic Soy Broth (TSB, HopeBio™, China) and incubated overnight at 37℃ in a shaking incubator to the logarithmic phase. The bacterial solution was centrifuged at 12,000 r/min for 5 min, and the supernatant was discarded. The pellet was washed twice with sterilized water and resuspended in sterilized normal saline (0.9% NaCL) to achieve a bacterial a concentration of 1.5×10^8 CFU/mL. Twenty-four SPF mice were randomly assigned to three groups: the JS01 strain challenge group (EJCG), the *E. coli* ATCC11775 infection control group (EACG) and the blank control group (BCG), with 8 mice in each group. The mice were acclimated to laboratory conditions for one week and fed normally before the experiment. Mice in the EJCG, EACG, and BCG groups were intraperitoneally injected with 0.3 mL of JS01 strain suspension, *E. coli* ATCC11775 suspension, and sterilized normal saline, respectively. The mice were monitored for mortality, abnormal behaviors and clinical symptoms for 7 consecutive days. Pathogens from dead mice in the EJCG were isolated and identified, and compared with the JS01 strain. After the experiment, all mice were humanely euthanized through anesthesia. Anesthesia was induced using 4–5% isoflurane, followed by an intraperitoneal injection of Pentobarbital Sodium (100 mg/kg body weight) once unconsciousness was confirmed.

Genomic DNA extraction, sequencing and gene function annotation analysis of the JS01 strain

Genomic DNA of the JS01 strain was extracted using a bacterial DNA kit (D3350-01, Omega Bio-Tek, USA) following the manufacturer's instructions. The extracted DNA was purified using Ampure XP beads (Bechman, USA), and DNA concentration and purity were determined using the Qubit 3.0 Fluorometer (Life Technologies, USA) and the NanoDrop One spectrophotometer (NanoDrop Technologies, USA), respectively. The SQK-LSK110 kit (Oxford Nanopore Technologies, UK) and VAHTS® Universal Plus DNA Library Prep Kit for Illumina V2 (Vazyme, China) were used for library preparation for the 1D genomic DNA and small fragment respectively. After passing quality inspection, the genomic DNA was sequenced using a combination of second-generation sequencing on an Illumina platform (NovaSeq 6000, USA) and third-generation sequencing on a Nanopore sequencing platform (Oxford Nanopore Technologies, UK).

The raw data of Nanopore sequencing was converted into FASTQ format after base calling through GUPPY (Version: 5.0.16), and then the raw data were filtered (qualitf ≧ 7, length ≧ 1,600 bp) and obtained a total of 1,286,173,777 bp clean data. The raw data from illumina sequencing were filtered using fastp (Version: 0.23.2), yielding 1,752,025,326 bp of clean data. The filtered reads were assembled using Unicycler (Version: 0.5.0) [34]. Coding genes were predicted using Prokka (Version:1.14.6) [35], and gene function annotation was performed using UniProt (https://www.uniprot.org/), Pfam (https://pfam.xfam.org/), Refseq (https://www.ncbi.nlm.nih.gov/refseq/), the Non-Redundant Protein Sequence Database (NR) (https://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/), Tigrfam (http://tigrfams.jcvi.org/cgi-bin/index.cgi), Pathogen Host Interactions (PHI) (http://www.phi-base.org/), Gene Ontology (GO) [36], Kyoto Encyclopedia of Genes and Genomes (KEGG) [37], Clusters of Orthologous Groups (COG) [38], Virulence Factor Database (VFDB) [39], and the Comprehensive Antibiotic Resistance Database (CARD) [40]. Additionally, utilizing the Achtman scheme, WGS data were employed to predict in multi-locus sequence type (MLST), and the MLST software (Version: 2.23.0) was used to identify JS01 strain by MLST (identity: 95%, coverage: 80%); the serotype was determined by submitting WGS to the SerotypeFinder website (https://cge.food.dtu.dk/services/SerotypeFinder/); genomic islands (GIs) present in the genome were predicted using IslandPath (Version: 1.0.6) [41]. The WGS of JS01 strain was conducted by Wuhan Benagen Tech Solutions Company Limited in China. The whole-genome sequences of JS01 are accessible in GenBank with accession numbers CP148986-CP148989.

Comparative genomics and phylogenetic analysis

A total of 17 genome sequences, including 14 *E. coli* and 3 *Salmonella enterica* reference strains, were obtained from the NCBI for comparative genomics analysis (Supplementary Table S1). Pangenome analysis of 18 genomes (including JS01 strain) was performed using Roary software (https://github.com/sanger-pathogens/Roary). Genes common to all genomes were defined as core genes, while dispensable genes were shared by two or

more but not all strains, and unique genes were identified in only one strain [42]. All coding sequences (CDSs) from 18 strains were used for core and pan-genome analyses, functional annotation, clustering based on core and pan genes. To analyze the phylogeny of the JS01 strain, Orthofinder (Version: 2.5.5) was used to cluster the core genes, followed by multiple sequence alignment using Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/). A phylogenetic tree was constructed using RAxML (Version: 8.2.12) with the maximum-likelihood (ML) approach.

Results

Pathological autopsy observations

Pathological autopsy revealed fibrinous pericarditis in the hearts of dead chickens (Fig. 1A). The liver (Fig. 1B) and spleen (Fig. 1C) exhibited swelling with grayish-white necrotic spots on their surfaces. The adenogastric papilla was swollen withour hemorrhage, and no bleeding was observed at the junction of the adenogastric and muscular stomach (Fig. 1D). Additionally, cecal tonsil hemorrhage was noted (Fig. 1E). Based on these pathological changes and clinical symptoms (see Sect. 2.1), a bacterial infection was suspected.

Isolation and purification culture of pathogenic bacteria

A round milky-white colony with varying sizes, smooth edges, a central bulge, and a moist, opaque appearance was isolated from liver tissue samples of diseased chickens (Fig. 2A). A single colony was streaked onto a NA plate for purification, and the purified colonies exhibited characteristics consistent with the isolated strain (Fig. 2B). On MAC agar and EMB agar, the purified isolates formed pink colonies (Fig. 2C) and purple-black colonies with a green metallic sheen (Fig. 2D), respectively, and the morphology of the colonies were round with smooth edges, a slightly raised center, and a moist surface. The isolated pathogenic bacterium was designaed as strain JS01. Based on these cultural characteristics, JS01 was preliminarily identified as *E. coli*.

Gram staining and microscopic examination of JS01 strain

As shown in Fig. 2E, Gram staining revealed that JS01 colonies appeared purple-red, with bluntly rounded ends, occurring singly or in pairs. Microscopic examination identified JS01 as Gram-negative short bacilli.

Biochemical identification of JS01 strain

The biochemical identification results of JS01 strain are summarized in Supplementary Table S2. JS01 was capable of fermenting and utilizing SAC, dMNE, dMAL, dTRE, OFF, dSOR, dMAN and dGLU. It tested positive for O129R, ODC, LDC, BGAL, CMT, BGUR and AGAL, while all other reactions were negative. The only difference from *E. coli* ATCC25922 was the negative SUCT reaction. Using VITEK 2 Compact automated microbiological analyzer, JS01 was identified as *E. coli* with 99.0% confidence, confirming it as *E. coli*, and labeling it JS01 APEC.

Antibiotic sensitivity of JS01 APEC

JS01 APEC was susceptible to 11 antibiotics, including AMK, CTR, CXM, CZ, STR, CIP, LEV, GEN, KAN, PB and IPM, and showed intermediate sensitivity to CN, SXT, and MI (Supplementary Fig. S1, Table 1). However, JF01 APEC exhibited resistance to ten antibiotics: AMP, MY, VAN, ERY, AZI, DO, EN, FFC, CHL, and CC. Specifically, AMP and EN are β-lactams, MY and CC are lincosamides, FFC and CHL are amide alcohols, ERY and AZI are macrolides, VAN is a polypeptide, and DO

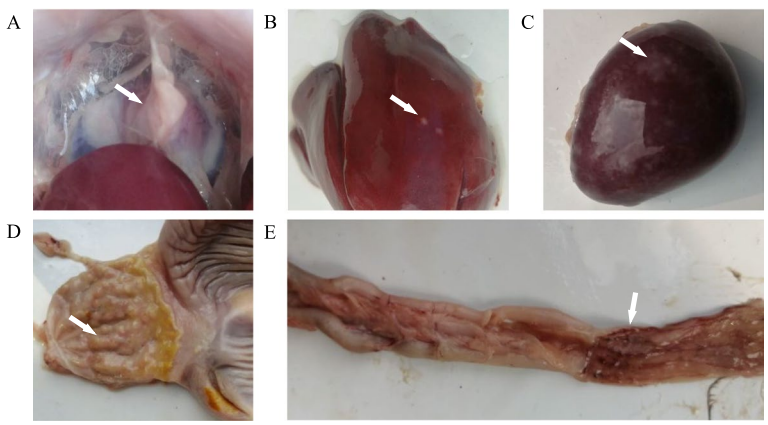

Fig. 1 Pathological autopsy observations. **A** Fibrinous pericarditis (indicated by arrow, same below). **B** Liver with slight swelling and grayish-white necrotic spots. **C** Spleen with swelling and grayish-white necrotic lesions. **D** Swelling of the adenogastric papilla. **E** Cecal tonsil hemorrhage

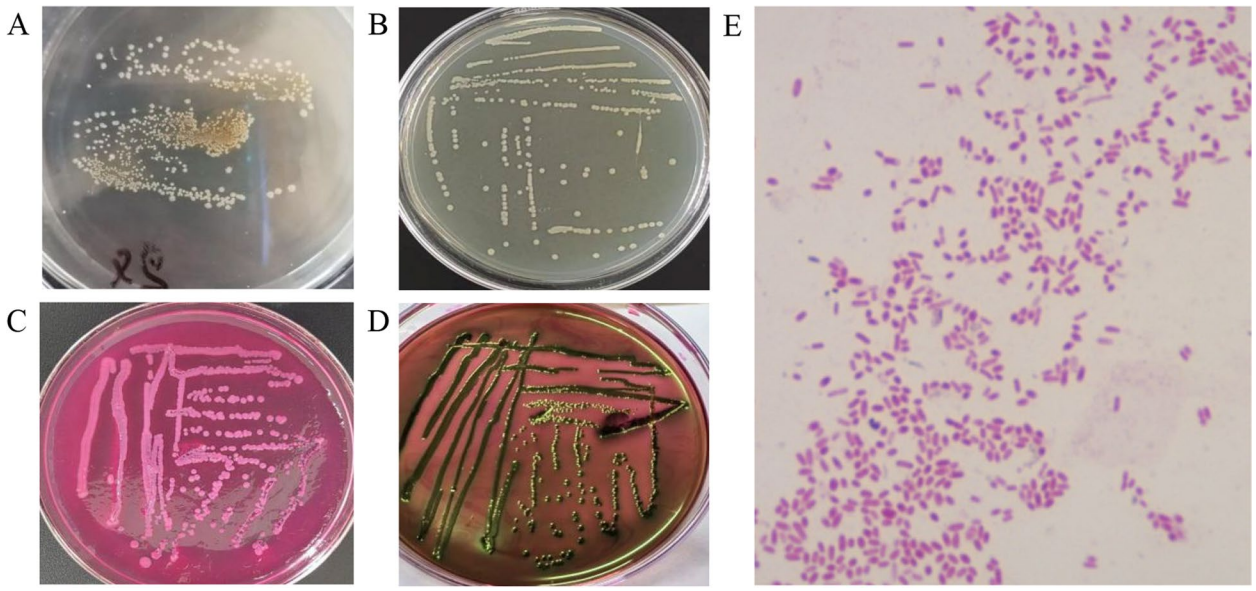

Fig. 2 Isolation and purification of pathogenic bacteria. **A** Pathogenic bacteria isolated from liver tissue samples of diseased chickens. **B** Purification culture of the pathogen. **C** Colony characteristics on MAC agar. **D** Colony characteristics on EMB agar. **E** Gram staining of JS01 strain under microscopy (magnification: × 1000)

Table 1 Drug resistance phenotype detection results of JF01 APEC

Antibiotic	Drug content (μg/tablet)	Bacteriostatic diameter/mm	Susceptibility	Criteria for drug susceptibility tests / mm		
				R	I	S
CZ	30	21	S	≤ 14	15 ~ 17	≥ 18
CN	30	18	I	≤ 13	14 ~ 20	≥ 21
AMP	10	0	R	≤ 13	14 ~ 16	≥ 17
AMK	30	22	S	≤ 14	15 ~ 16	≥ 17
CTR	30	30	S	≤ 14	15 ~ 17	≥ 18
CXM	30	21	S	≤ 14	15 ~ 17	≥ 18
SXT	25	14	I	≤ 10	11 ~ 15	≥ 16
PB	300(IU)	14	S	≤ 8	9 ~ 11	≥ 12
CHL	30	7	R	≤ 12	13 ~ 17	≥ 18
CC	2	0	R	≤ 14	15 ~ 20	≥ 21
IPM	10	30	S	≤ 13	14 ~ 15	≥ 16
FFC	30	0	R	≤ 15	16 ~ 20	≥ 21
LEV	5	28	S	≤ 13	14 ~ 16	≥ 17
DO	30	10	R	≤ 10	11 ~ 13	≥ 14
AZI	15	8	R	≤ 13	14 ~ 17	≥ 18
KAN	30	22	S	≤ 13	14 ~ 17	≥ 18
GEN	10	20	S	≤ 12	13 ~ 14	≥ 15
PEN	10(U)	7	R	≤ 13	14 ~ 16	≥ 17
CIP	5	26	S	≤ 15	16 ~ 20	≥ 21
ERY	15	7	R	≤ 13	14 ~ 22	≥ 23
MY	2	0	R	≤ 14	15 ~ 20	≥ 21
VAN	30	0	R	≤ 15	16 ~ 20	≥ 21
STR	10	17	S	≤ 11	12 ~ 14	≥ 15
MI	30	16	I	≤ 14	15 ~ 18	≥ 19

S Susceptible, *I* Intermediate, *R* Resistant

is tetracycline. This indicates that JF01 APEC has developed MDR. In this study, the farm where the outbreak occurred showed no therapeutic effect after using 10% DO soluble powder in drinking water two days post-onset, primarily due to JS01 APEC's the resistance to DO. Therefore, selecting appropriate antibiotics based on susceptibility is crucial for effective treatment in veterinary practice.

Pathogenicity of JS01 APEC in mice

Experimental infection with JS01 APEC at a dose of 0.3×10^8 CFU successfully demonstrated its pathogenicity in mice. Infected mice exhibited lethargy, reduced movement, and anorexia. As shown in Fig. 3, mice in the EJCG group began dying on the second day post-infection, with all mice succumbing within 72 h, resulting in a 0% survival rate. Mice in EACG group showed similar clinical symptoms, with 6 out of 8 mice dying by the 5th day, resulting in a 25% survival rate. In contrast, the BCG group had a 100% survival rate, significantly higher than the infected groups.

Pathogen isolation, Gram staining, and biochemical identification (via VITEK 2 Compact) of dead mice in the EJCG group confirmed that the cause of death was JS01 APEC (Supplementary Fig. S2). In summary, JS01 APEC is highly pathogenic to mice, more so than *E. coli* ATCC11775 standard strain.

Whole-genome sequencing and analysis

The JS01 strain genome was 5,089,394 bp in size (Table 2), consisting of a circular chromosome (4,815,411 bp, 50.75% GC content) and three plasmids: Plasmid 1 (145,399 bp, 50.98% GC), Plasmid 2 (91,910 bp, 47.97% GC), and Plasmid 3 (36,674 bp, 41.17% GC). The genome harbored 5,107 genes, including 347 RNA-coding genes (92 tRNA, 22 rRNA (5S = 8, 16S = 7, 23S = 7), 1 tmRNA, and 232 MiscRNA), 4,760 protein-coding genes (CDS), and 1,605 pseudogenes. Chromosome and plasmid information were depicted separately in a genome circle map (Fig. 4).

For a comprehensive function analysis of the JS01 strain genome, a total of 4,760 genes were annotated using multiple databases, including Tigerfam, RefSeq, Pfam, NR, UniProt, GO, COG, KEGG, KEGG Pathway, and CAZY (Supplementary Fig. S3 and Table S3). Among these, 4,758 (99.96%), 4,282 (89.96%), 4,746 (99.71%) and 2,667 (56.03%) genes were successfully annotated in the UniProt, Pfam, RefSeq and Tigerfam databases, respectively. GO annotations provided functional insights into the predicted genes (Fig. 5A), with 3,794 genes annotated, representing 79.71% of all coding genes. These genes were classified into three primary GO categories: within the molecular function category, 404 genes were associated with ATP binding, 393 with DNA binding, and 341 with metal ion binding; in the cellular component category, 867 genes were linked to integral component of membrane, 711 to plasma membrane, and 341 to cytoplasm; in the biological process category, 126 genes were involved in transcription regulation, 76 in transmembrane transport, and 65 in translation. Additionally, a significant number of genes were associated with specific activities: 147 with DNA-binding transcription factor activity, 115 with transmembrane transporter activity, and

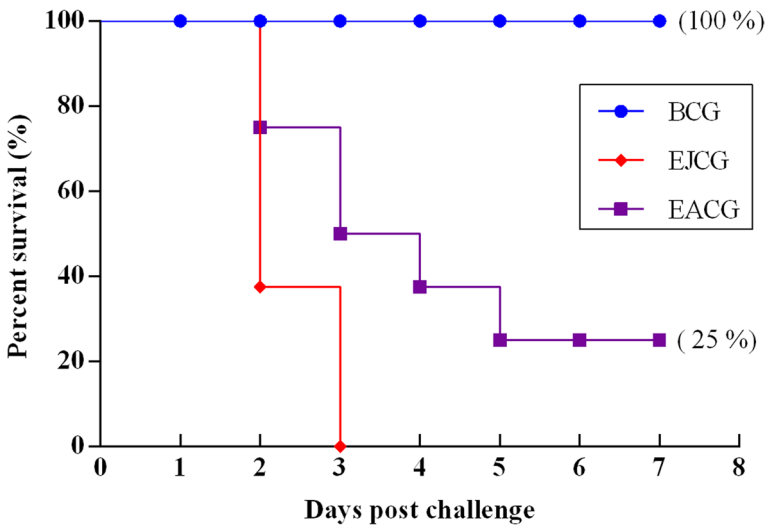

Fig. 3 Pathogenicity test of JS01 APEC in mice. Survival curve of mice in each group post-challenge. EJCG, EACG, and BCG group mice were intraperitoneally injected with 0.3 mL of 1.5×10^8 CFU/mL JS01 bacterial suspension, 0.3 mL of $1.5\times10.^8$ CFU/mL *E. coli* ATCC11775 suspension, and 0.3 mL of sterile saline, respectively. Survival rates were recorded for 7 days ($n = 8$)

Table 2 Genome characteristics of isolated JS01 APEC

Type		Length (bp)	GC content (%)	Plasmid name
Genome	chromosome	4,815,411	50.75	—
	Plasmid1	145,399	50.98	pGSH8M-2–1
	Plasmid2	91,910	47.97	pATCC10729_02
	Plasmid3	36,674	41.17	unnamed
	Number	Total length	Average length	Percentage of genome(%)
Gene	5,107	4,517,750	885	88.77
CDS	4,760	4,449,855	935	87.43
tRNA	92	7,225	79	0.14
23S rRNA	7	20,321	2,903	0.4
16S rRNA	7	10,766	1,538	0.21
5S rRNA	8	888	111	0.02
tmRNA	1	363	363	0.01
MiscRNA	232	28,332	122	0.56
Pseudo gene	1,605	356,698	222.24	7.01

58 with oxidoreductase activity. The COG database predicted 3,953 genes (83.05% of all coding genes) and annotated 4,586 functional features, of which 42.67% were related to metabolism. These included 292 genes nvolved in energy production and conversion, 422 in amino acid transport and metabolism, 112 in nucleotide transport and metabolism, 432 in carbohydrate transport and metabolism, and 201 in coenzyme transport and metabolism. Furthermore, 139 genes were associated with lipid transport and metabolism, 292 with inorganic ion transport and metabolism, and 67 with secondary metabolites biosynthesis, transport and catabolism (Fig. 5B). In the KEGG pathway database, 1,838 genes (38.61% of coding genes) were annotated across five functional categories: environmental information processing, cellular processes, metabolism, genetic information processing, and organismal systems. Among these, metabolism had the highest number of annotated genes (2,536 features), with 12 KEGG pathway entries primarily involved in global and overview maps, amino acid metabolism, carbohydrate metabolism, metabolism of cofactors and vitamins, and energy and nucleotide metabolism. This indicates that the JS01 strain possesses diverse metabolic pathways and robust adaptive capabilities to varying environmental conditions (Fig. 5C). The JS01 strain also harbored 92 genes encoding carbohydrate-active enzymes (CAZY), which were classified into five functional categories (Fig. 5D): glycoside hydrolases (GHs, 46 genes), glycosyl transferases (GTs, 31 genes), carbohydrate esterases (CEs, 9 genes), auxiliary activities (AAs, 3 genes) and carbohydrate-binding modules (CBMs, 3 genes). Notably, no genes related to polysaccharide lyases (Pls) were annotated. The study of carbohydrate-active enzymes provides valuable insights into the biological functions of carbohydrates in various cellular processes.

The annotation results from the NR database revealed that the species classification of JS01 was predominantly associated with *E. coli* and the family *Enterobacteriaceae*, further corroborating the identification of JS01 as a pathogenic strain of *E. coli* (Fig. 5E).

The Pfam database, a comprehensive collection of protein domain families, serves as a Homologous Protein Family database and encompasses over 16,000 protein families [43]. Based on Pfam domain annotations, the annotated genes in JS01 were statistically summarized, and the top 20 domains with the highest annotation cunts were visualized, as depicted in Fig. 5F. These domains were primarily associated with the major facilitator superfamily, ABC transporters, bacterial regulatory helix-turn-helix proteins, binding-protein-dependent transport system inner membrane components, the LysR substrate-binding domain, the response regulator receiver domain, fimbrial proteins, bacterial regulatory proteins, the GntR family, the integrase core domain, the 4Fe-4S binding domain, the EamA-like transporter family, the 4Fe-4S dicluster domain, amino acid permeases, the methyltransferase domain, periplasmic binding proteins, the sugar-binding domain of LacI family, and histidine kinase-, DNA gyrase B-, and HSP90-like ATPase domains. Domains can appear in various combinations within different proteins, contributing to the diversity of protein structures and functions. Identifying these domains is crucial for elucidating the functional roles of proteins.

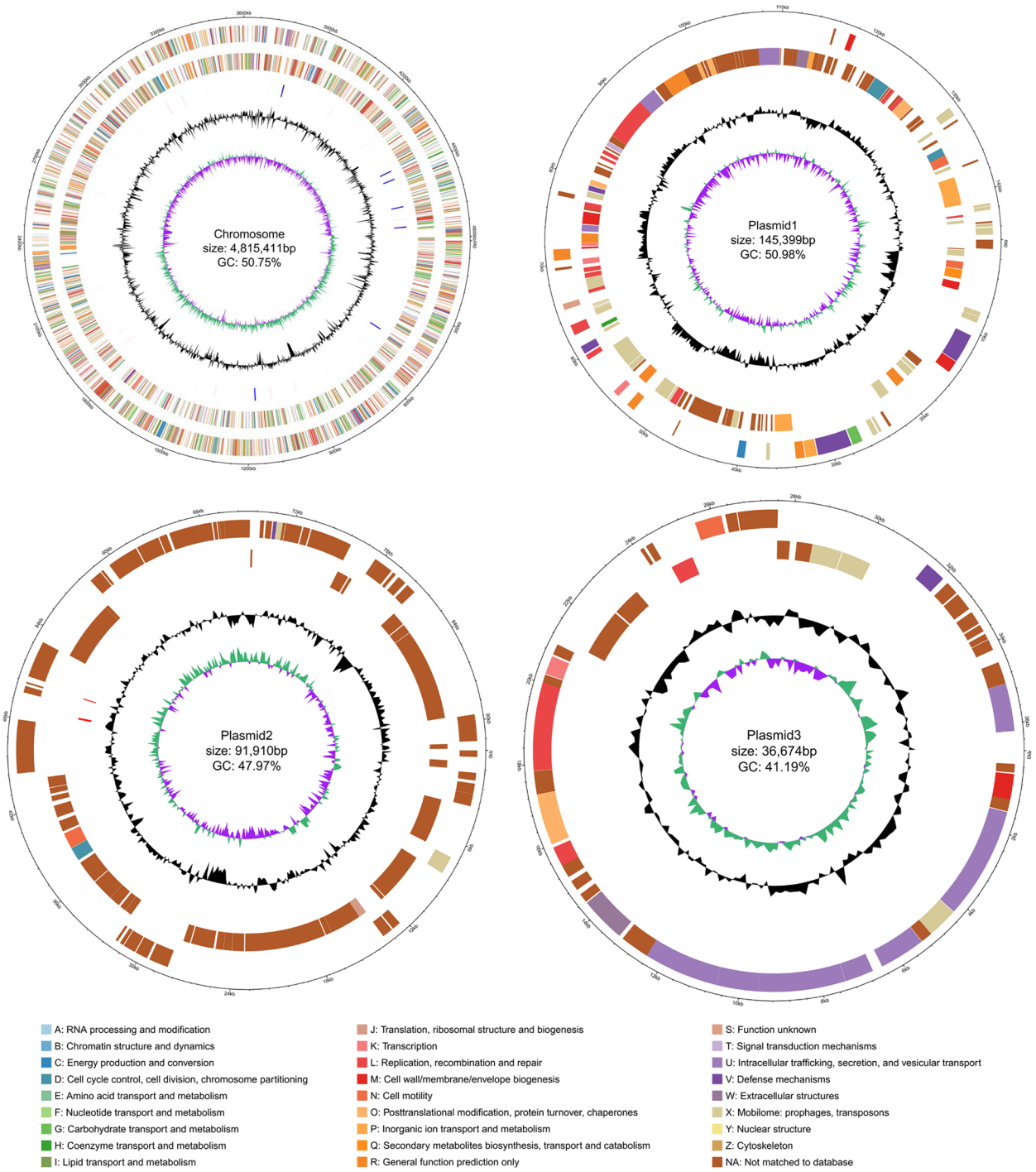

Fig. 4 Circular genome representation of JS01 APEC. From outer to inner: The peripheral circle represents the genome with sizes marked in Mb; the second and third circles show genes on the positive and negative strands, respectively, with different colors representing COG functional categories; the fourth circle indicates rRNA (blue) and tRNA (red); the fifth circle shows the GC content curve; the sixth circle represents the GC skew curve, with green indicating G > C, and purple indicating G < C

In the annotation results of the PHI database (Fig. 5G), 260 genes were associated with reduced virulence, 119 genes with unaffected pathogenicity, 17 genes with increased virulence (hypervirulence), and 9 genes with lose of pathogenicity. Additionally, 8 genes were identified as effectors (plant avirulence

Fig. 5 Annotation of genes in the JS01 strain genome. **A** GO functional annotation, showing the distribution of genes across the three main GO categories: biological process, cellular component, and molecular function. **B** COG functional annotation. **C** KEGG pathway annotation. **D** CAZY database annotation. **E** Distribution of top 10 species based on NR database annotations. **F** sStatistics of Pfam domains. **G** PHI annotation

determinants), 3 as lethal genes, and 72 as mixed outcome genes. The 17 hypervirulence-associated genes [*Hns*, *Lon*, *EF-P*, *PmrA*, *RcsA*, *MAM_(YPK_1779)*, *Ndk*, *Hns*, *BcsA*, *QseB*, *RaxP_(cysD)*, *YmoA*, *GroEL*, *RaxQ*, *KatE*, *RsmS_(ECA1172)*, *PA0336_(RppH)*; Supplementary Table S4] exhibited diverse functions, including roles in capsular polysaccharide biosynthesis, protein translation extension factor, effect regulatory protein of two-component regulatory system, biofilm formation, transcription regulatory factor, and other processes.

Table 3 Resistance Genes of APEC strain JS01

AMR Gene Family Category	Drug Class	Related gene	Number
ABC antibiotic efflux pump	nitroimidazole	*msbA*	1
	peptide	*YojI*	1
MFS antibiotic efflux pump	fluoroquinolone	*emrA*, *emrB*, *emrR*, *mdtH*,	4
	tetracycline	*emrY*, *tet(A)*(2), *tetC*(*2*)	5
	nucleoside, acridine dye, disinfecting agents and intercalating dyes	*mdtN*, *mdtO*(2), *mdtP*	4
	Macrolide, aminoglycoside, cephalosporin, tetracycline, peptide, rifamycin	*KpnE*, *KpnF*	2
	fosfomycin	*mdtG*	1
	Fluoroquinolone, lincosamide, nucleoside, acridine dye, phenicol, disinfecting agents and intercalating dyes	*mdtM*	1
	phenicol	*floR*	1
	Tetracycline, benzalkonium chloride, rhodamine	*mdfA*	1
	tetracycline	*emrK*	1
RND antibiotic efflux pump	Aminoglycoside, aminocoumarin	*baeR*, *baeS*, *cpxA*	3
	aminoglycoside	*acrD*, *mdtA*, *mdtB*, *mdtC*	4
	Macrolide, fluoroquinolone, penam	*CRP*, *mdtE*, *mdtF*, *gadX*	4
	Fluoroquinolone, diaminopyrimidine, phenicol	*rsmA*	1
	Fluoroquinolone, cephalosporin, cephamycin, penam	*AcrE*, *AcrF*	2
	Fluoroquinolone, cephalosporin, glycylcycline, cephamycin, penam, tetracycline, rifamycin, phenicol, triclosan	*AcrS*	1
	Fluoroquinolone, cephalosporin, glycylcycline, penam, tetracycline, rifamycin, phenicol, triclosan	*acrB*, *acrA*, *acrR*, *marR*	4
SMR antibiotic efflux pump	macrolide	*emrE*	1
ABC, MFS, RND antibiotic efflux pump	Macrolide, fluoroquinolone, aminoglycoside, carbapenem, cephalosporin, glycylcycline, cephamycin, penam, tetracycline, peptide, aminocoumarin, rifamycin, phenicol, triclosan, penem	*TolC*	1
	Fluoroquinolone, cephalosporin, glycylcycline, penam, tetracycline, rifamycin, phenicol, triclosan	*soxR*, *soxS*	2
MFS, RND antibiotic efflux pump	Macrolide, fluoroquinolone, penam; tetracycline	*evgA*, *evgS*	2
	Macrolide, fluoroquinolone, cephalosporin, cephamycin, penam, tetracycline	*H-NS*	1

Table 3 (continued)

AMR Gene Family Category		Drug Class	Related gene	Number
RND antibiotic efflux pump; General Bacterial Porin with reduced permeability to beta-lactams		Fluoroquinolone, monobactam, carbapenem, cephalosporin, glycylcycline, cephamycin, penam, tetracycline, rifamycin, phenicol, triclosan, penem	*marA*	1
β-lactamase	AmpC-type	Cephalosporin, penam	*ampC1*, *ampH*	2
	EC-type	Cephalosporin	*EC-14*	1
	TEM-type	monobactam; cephalosporin; penam; penem	*TEM-135*	1
	Penicillin-binding protein mutations conferring resistance	Cephalosporin, cephamycin, penam	*PBP3*	1
Pmr phosphoethanolamine transferase		peptide antibiotic	*eptA*, *PmrF*, *ugd*	3
kdpDE		aminoglycoside	*kdpE*	1
Elfamycin resistant EF-Tu		elfamycin antibiotic	*EF-Tu* (2)	2
Antibiotic-resistant GlpT		fosfomycin	*GlpT*	1
Undecaprenyl pyrophosphate related proteins		peptide	*bacA*	1
Qnr		fluoroquinolone	*QnrS1*	1
Trimethoprim resistant dihydrofolate reductase dfr		diaminopyrimidine	*dfrA14*	1

The number in () indicates the number of coding genes annotated. The following table is the same

Analysis of drug resistance genes and virulence genes

In this study, 64 AMR genes, 177 VF genes, 12 GIs, and 4 plasmid replicons (repB) were annotated in the JS01 strain (Tables 3 and 4, Supplementary Table S5, Fig. 6B). The GIs, designated as GI1 to GI12, were distributed as follows: GI1 to GI10 were located on the chromosome, GI11 on plasmid 2, and GI12 on plasmid 3. Among these, GI2, GI4 and GI9 contained multiple virulence genes, most of which were associated with secretion systems (Fig. 6A, Table 4). Additionally, the three plasmids carried multiple AMR genes, VF genes and genes related to the effector delivery system (Fig. 6B).

As shown in Table 3, the 64 resistance genes were primarily classified into 12 AMR gene families, including ATP-binding cassette (ABC) antibiotic efflux pumps, β-lactamases, Pmr phosphoethanolamine transferases, Major facilitator superfamily (MFS) antibiotic efflux pumps, Antibiotic-resistant GlpT, Elfamycin-resistant EF-Tu, Small multidrug resistance (SMR) antibiotic efflux pumps, Resistance-nodulation-cell division (RND) antibiotic efflux pumps, kdpDE, Undecaprenyl pyrophosphate-related proteins, Quinolone resistance protein (Qnr), and Trimethoprim-esistant dihydrofolate reductases. Four major antibiotic efflux pump systems were identified: ABC efflux pumps (*msbA*, *YojI*), MFS efflux pumps (*emrA*, *emrB*, *emrR*, *mdtH*, *mdtN*, *mdtO*, *mdtP*, *emrY*, *tet(A)*, *tetC*, *KpnE*, *KpnF*, *mdtG*, *mdtM*, *floR*, *mdfA*, *emrK*), RND efflux pumps (*baeR*, *baeS*, *cpxA*, *acrD*, *mdtA*, *mdtB*, *mdtC*, *CRP*, *mdtE*, *mdtF*, *gadX*, *rsmA*, *AcrE*, *AcrF*, *AcrS*, *acrB*, *acrA*, *acrR*, *marR*), SMR efflux pumps (*emrE*). The drug resistance determinants associated with specific antibiotics included β-lactam resistance genes (*ampC1*, *ampH*, *EC-14*, *TEM-135*, *PBP3*, *marA*, *soxS*), tetracycline resistance genes (*emrY*, *tet(A)*, *tetC*, *mdfA*, *emrK*, *KpnE*, *KpnF*, *AcrS*, *acrB*, *acrA*, *acrR*, *marR*, *TolC*, *soxR*, *soxS*, *marA*, *evgA*, *evgS*, *H-NS*), peptide antibiotic resistance genes (*eptA*, *PmrF*, *ugd*, *bacA*, *TolC*, *YojI*, *KpnE*, *KpnF*), macrolide resistance genes (*emrE*, *KpnE*, *KpnF*, *CRP*, *mdtE*, *mdtF*, *gadX*, *TolC*, *evgA*, *evgS*, *H-NS*), and other resistance genes with diverse mechanisms. Notably, genes such as *TolC*, *soxR*, *soxS*, *marA*, *evgA*, *evgS* and *H-NS* belonged to multiple AMR gene families and exhibited various resistance mechanisms. Among these, *tetC*, *floR*, *tet(A)*, *QnrS1*, *dfrA14* and *TEM-135* were located on plasmid 1, while no AMR genes were detected on plasmid 2 and plasmid 3. The primary resistance mechanisms of these genes included antibiotic efflux, inactivation, target replacement or alteration, and decreased antibiotic permeability.

As shown in Table 4, the virulence genes were primarily classified into 10 categories: adhesins, effector delivery systems, invasion factors, antimicrobial activity/competitive advantage factors, motility-related factors, immune modulation factors, exotoxins, nutritional/metabolic factors, biofilm formation and regulatory factors. Among these, adhesins comprised the largest number of virulence genes, with 12 virulence factors and 79 associated genes. This was followed by effector

Table 4 Virulence Genes of APEC strain JS01

VF category	VF_name	Related gene	Number
Adhesins	ECP	*ykgK/ecpR*, *yagZ/ecpA*, *yagY/ecpB*, *yagX/ecpC*, *yagW/ecpD*, *yagV/ecpE*	6
	Hemorrhagic E.coli pilus	*hcpA*, *hcpB*, *hcpC*	3
	Curli fibers	*csgA*, *csgB*, *csgC*, *csgD*, *csgE*, *csgF*, *csgG*	7
	E.coli laminin-binding fimbriae	*elfA*, *elfC*, *elfD*, *elfG*,	4
	Adhesive fimbriae	*cfaA*, *cfaB*, *cfaC*, *cfaD/cfaE*	4
	F9 fimbriae	*fimA*, *Z_RS10335*, *ydeQ*	3
	Type 1 fimbriae	*fimA*, *fimB*, *fimC*, *fimD*, *fimD*, *fimE*, *fimF*, *fimF*, *fimG*, *fimH*, *fimI*, *fimG*	12
	Stg fimbriae	*stgA*, *stgC*, *stgB*, *stgD*	4
	AIDA-I type	*ehaB*, *ehaA*, *aatA*	3
	UpaG adhesin, trimeric AT	*upaG/ehaG*	1
	EaeH	*eaeH*	1
	Z1307	*ompA*	1
Effector delivery system	TTSS secreted effectors	*espR1*, *espL4*, *espX5*, *espL1*(2), *espX1*	6
	T6SS-II	*clpV*	1
	T6SS	*hcp1/tssD1*, *rhs/PAAR*	2
	Gsp	*gspC*, *gspD*, *gspE*, *gspF*, *gspI*, *gspL*, *yghD*	7
	T2SS	*gspG*, *gspH*, *gspK*,	3
	ETT2	*ECs_3712(2)*, *ECs_3715*, *ECs_3719*, *etrA*, *ygeG*, *ygeH*, *epaQ*, *epaO*, *escR*, *eprS*	11
	EHS	*Z_RS01240*, *Z_RS01215*, *aec16*, *tssF*	4
	ACE T6SS	*aec27/clpV*, *aec23*, *aec26*, *aec25*, *aec15*, *aec22*, *aec24*, *aec28*, *aec17*, *aec18*, *aec19*, *aec29*	12
	T4SS (plasmid 2 encode)	*virB*	1
Motility	Peritrichous flagella	*fliA*, *fliD*, *fliE*, *fliF*, *fliG*, *fliH*, *fliI*, *fliJ*, *fliK*, *fliL*, *fliM*, *fliN*, *fliO*, *fliP*, *fliQ*, *fliR*, *fliS*, *fliT*, *fliZ*, *flgA*, *flgB*, *flgC*, *flgD*, *flgE*, *flgF*, *flgG*, *flgH*, *flgI*, *flgJ*, *flgK*, *flgL*, *flgN*, *flhA*, *flhB*, *flhC*, *flhD*, *flhe*, *cheA*, *cheB*, *cheR*, *cheW*, *cheY*, *cheZ*, *motA*, *motB*, *tar*, *flk*	47
Biofilm	Cah, AIDA-I type	*cah*, *cah*	2
Antimicrobial activity/Competitive advantage	AcrAB	*acrB*	1
Exotoxin	Hemolysin/cytolysin A	*hlyE/clyA*	1
Immune modulation	Capsule	*gndA*	1
Invasion	Ibes	*ibeB*, *ibeC*	2
	TraJ	*AAA92657*	1
Nutritional/Metabolic factor	Allantion utilization	*allB*	1
	Enterobactin synthesis and transport	*entA*, *entB*, *entC*, *entD*, *entE*, *entF*, *entS*, *fepA*, *fepB*, *fepC*, *fepD*, *fepE*, *fepG*, *fes*	14
	Salmochelin siderophore	*iroB*, *iroC*, *iroD*, *iroE*, *iroN*, *sitA*, *sitB*, *sitC*, *sitD*	9
Regulation	RcsAB	*rcsB*	1
	PhoPQ	*phoP*	1

delivery systems, which included TTSS secreted effectors, T6SS-II, T6SS, Gsp, T2SS, ETT2, EHS, ACE T6SS and T4SS, encompassing 9 virulence factors and 47 virulence genes. Peritrichous flagella-related virulence factors also represented a significant category, with 47 associated virulence genes. Additionally, the JS01 strain harbored three plasmids, with plasmid 1 containing 10 virulence genes and 4 secretion system-related genes, while plasmids 2 carried one gene related to T4SS (Fig. 6B), and all three plasmids had multiple T3SS coding genes. These virulence factors are likely to play critical roles in the invasion and infection processes of JS01 as an APEC. Previous studies have demonstrated that adhesins, peritrichous flagella-related virulence factors and effector delivery systems are essential for *E. coli* invasion and infection [3, 44].

MLST typing and serotype analysis

MLST analysis classified strain JS01 as sequence ST155 (Supplementary Table S6). Serotyping via SerotypeFinder databases (version 1.0.0) further identified the strain as serotype O177:H51 (Supplementary Table S7).

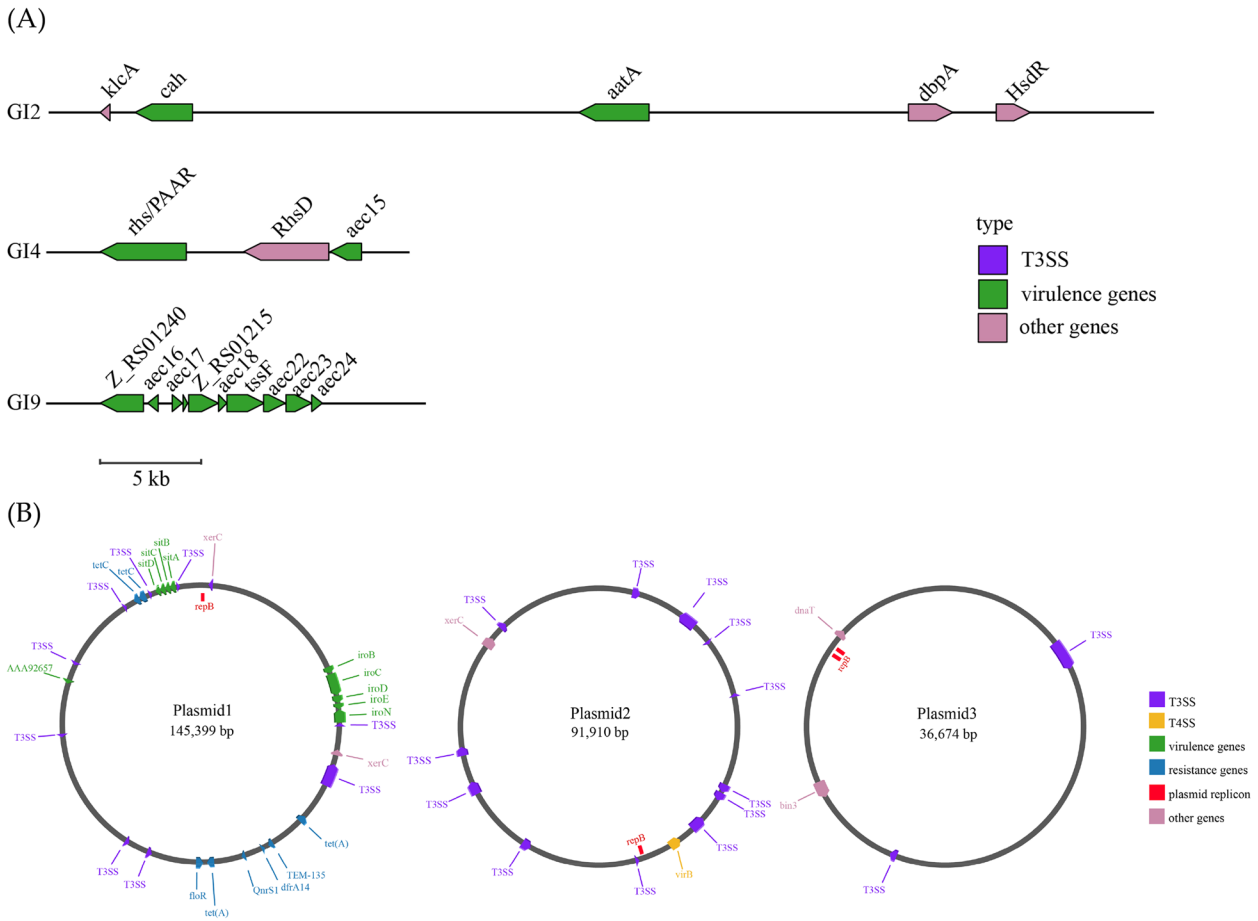

Fig. 6 Distribution of resistance and virulence genes on the JS01 strain plasmids and genomic islands. **A** Genomic islands GI2, GI4 and GI9. **B** Distribution of drug resistance and virulence genes on JS01 plasmids

Comparative genomics analysis

The core and pan-genomic analysis revealed that the core genome of the JS01 strain and 17 reference strains comprised of 676 genes, while the distribution of unique genes across these strains ranged from 48 to 866 (Fig. 7A). Based on the COG database analysis, core genes and dispensable genes were enriched into 21 and 24 functional subcategories, respectively, with metabolism being the primary function for both types of genes. Among the 676 core genes, 81 were associated with amino acid transport and metabolism, 63 with energy production and conversion, 60 with carbohydrate transport and metabolism, 39 with nucleotide transport and metabolism, 30 with coenzyme transport and metabolism, 33 with inorganic ion transport and metabolism, 26 with lipid transport and metabolism, and 6 with secondary metabolite biosynthesis, transport, and catabolism. Additionally, 122 genes were assigned to translation, ribosomal structure, and biogenesis, representing the largest proportion (Fig. 7B). For dispensable genes, 958 were involved in carbohydrate transport and metabolism, 812 in amino acid transport and metabolism, 644 in inorganic ion transport and metabolism, 436 in coenzyme transport and metabolism, 272 in lipid transport and metabolism, 160 in nucleotide transport and metabolism. Furthmore, 1,381 genes were associated with the mobilome, including prophages and transposons, which accounted for the largest proportion (Fig. 7C). COG functional annotation was also performed on the unique genes of 18 strains, resulting in 1,888 annotated unique genes. In the JS01 strain, unique genes were primarily enriched in mobilome (including prophages and transposons), intracellular trafficking, cell motility, translation, ribosomal structure and biogenesis, secretion and vesicular transport, as well as energy production and conversion. The ASM3229062v1 strain exhibited the highest number of unique genes, totaling 317, with 67 involved in mobilome, specifically prophages and transposons. In contrast, the ASM197378v2 strain had only 9 unique genes, including 4 associated with the transcription, 1 with mobilome, 1 with carbohydrate

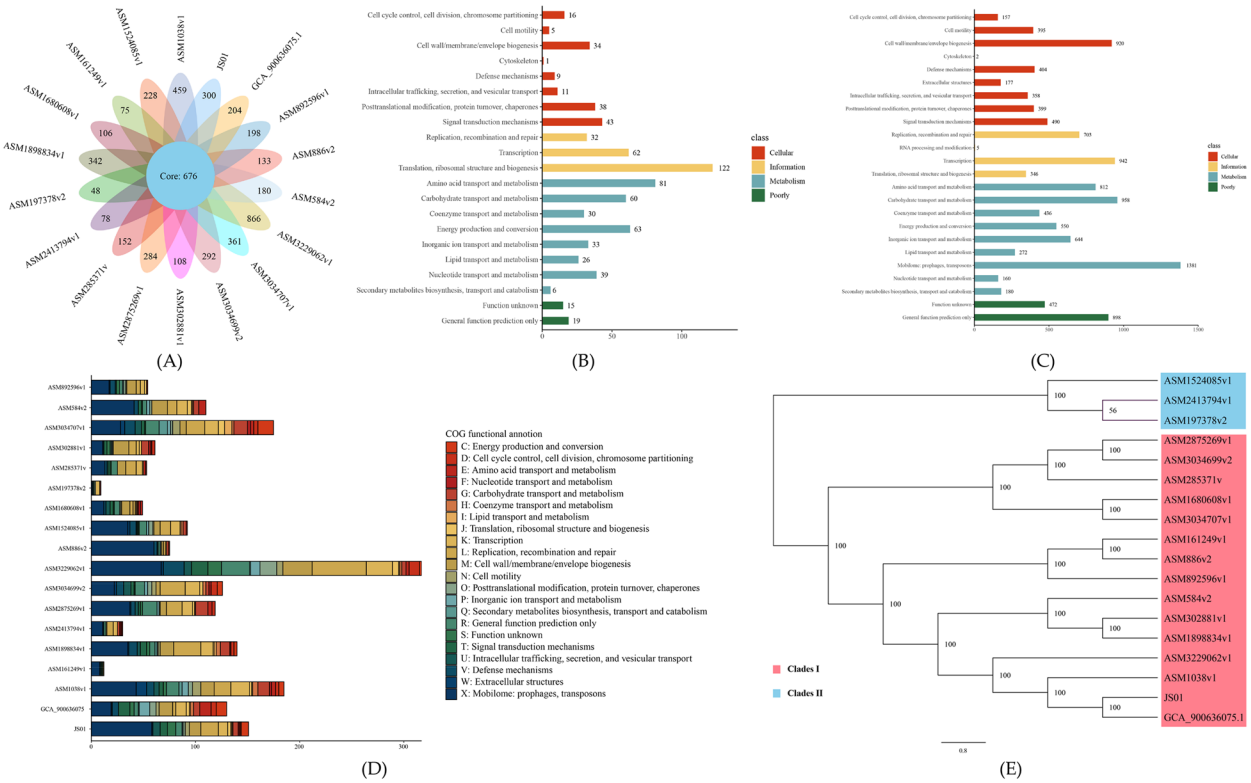

Fig. 7 Comparative analysis of core and pan genes. **A** Venn diagram of the pan genes set. **B** COG functional annotation of core genes. **C** COG functional annotation of dispensable genes. **D** COG functional annotation of unique genes. **E** Maximum-likelihood phylogenetic tree based on the core genes results of 18 isolates

transport and metabolism, 1 with intracellular trafficking, secretion, and vesicular transport, 1 with posttranslational modification, protein turnover, chaperones, and 1 with general function prediction only (Fig. 7D).

To investigate the evolutionary relationships of the JS01 strain, a phylogenetic tree was reconstructed using core gene analysis of 18 bacterial isolates through the RAxML tool (Fig. 7E). The phylogenetic analysis showed that these 18 strains formed two primary clades: Clade I and Clade II. Clade I comprised 15 *E. coli* strains forming a distinct monophyletic group, within which the JS01 strain demonstrated close phylogenetic clustering with isolates GCA_900636075.1, ASM1038v1 and ASM3229062v1. Notably, these four *E. coli* strains, while isolated from distinct continental regions (Supplementary Table S1), exhibited remarkable genomic conservation. Among them, the hosts of JS01, ASM1038v1 and ASM3229062v1 are chicken, *Homo sapiens* and pig, respectively, the host origin of GCA_900636075.1 remains undocumented. This phylogenetic convergence suggests an evolutionary association between APEC-associated *E. coli* strains and those isolated from *Homo sapiens* or pig. Despite their diverse host origins, these strains demonstrated conserved core genome architecture and limited genomic divergence, providing genomic evidence for their cross-host transmission capabilities [33]. In contrast, Clade II consisted of three *Salmonella enterica* strains seving as the out-group, forming a separate phylogenetic lineage distinct from the *E. coli* cluster..

Discussion

Pathogenic *E. coli* is a common pathogen responsible for avian colibacillosis, a disease caused by diverse strains of APEC that impacts poultry flocks globally, including in China [17, 45]. Most APEC strains belong to the phylogenetic group linked with extraintestinal pathogenic E. coli (ExPEC), which can cause a spectrum of diseases in poultry, ranging from localized to systemic infections. Previous studies have reported APEC infections in various animals, including chickens, fish [4], turkeys [13], wild birds [25], pigs [46], cattle [47], and others, with over 200 serotypes identified due to extensiv serological diversity [9]. Common APEC serogroups isolated from poultry include O1, O2, O8, O35, O78, and others [48]. In this study, the JS01 strain was identified as serotype O177:H51. Literature searches via ScienceDirect (https://www.sciencedirect.com/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and CNKI (http://www.cnki.net)

revealed that *E. coli* O177 antigen has only been reported in cattle faeces isolates [49]. To our knowledge, this is the first report of an O177: H51 serotype APEC strain isolated from broiler chickens in China.

As we all know, establishing an effective vaccination program continues to be a top priority in preventing and controlling *E. coli* diseases in domestic poultry [24]. However, due to the diversity of APEC serotypes and lack of cross-protective immunity among them, the prevention and control of APEC is difficult. The rapid expansion of commercialized and intensive poultry industries, particularly in developing countries like China, had led to extensive utilization of antibiotics for both disease prevention and growth enhancement. This practice has significantly exacerbated the persistent issue of excessive and inappropriate antibiotic use in food animal production. Consequently, it has contributed to the global challenge of AMR and the rising emergence of MDR pathogens [50]. In our research, we evaluated the sensitivity of JS01 to 24 antibiotics. While demonstrating sensitivity to 11 antibiotics, JS01 exhibited resistance to 10 antibiotics across 5 classes: β-lactams (AMP, PEN), lincosamides (MY, CC), amide alcohols (FFC, CHL), macrolides (ERY, AZI), glycopeptides (VAN), and tetracyclines (DO). This MDR profile of JS01 is, consistent with previous reports [1, 33]. The global prevalence of MDR in *E. coli* has been documented across diverse geographical regions, such as Pakistan [1], Canada [27], Bangladesh [51], China [52], Korea [53], and Czech [54]. The emergence of AMR and MDR in APEC represents a significant public health challenge. Numerous studies have demonstrated that APEC isolates from both food-producing animals and humans clinical samples frequently exhibit resistance to commonly used antibiotics like SXT, AMP, VAN, ERY, DO, CHL, and PEN [24, 46, 55]. Our finding with JS01, showing limited susceptibility to these antibiotics, further emphasize the severity of AMR in APEC among food-producing animals. Notably, despite several studies have reported the prevalence of PB resistantce in *E. coli* [56–58], the JS01 strain in our study remained susceptible to PB, and no Polymyxin resistance-associated genes (such as *mcr-1*, *pmrAB*, *phoPQ and mgrB*) were annotated in this isolate.

Recent decades, scientists have identified a significant number of AMR genes in drug-resistant strains. Generally, the existence of AMR-specific genes corresponding to particular antibiotic classes correlates with the observed AMR pattern. In this study, analysis of the CARD revealed that the JS01 strain of APCE harbors a diverse array of AMR genes (Table 3), consistent with its high-level MDR phenotype. JS01 was found to contain multiple β-lactamase (*bla*) genes, including TEM-type (*TEM-135*), AmpC-type (*ampC1, ampH*), EC-type (*EC-14*) and penicillin-binding protein mutations conferring resistance (*PBP3*). These genes conferred resistance to AMP and PEN in JS01 strain. The *bla* genes are known to mediate resistance to β-lactam antibiotics by hydrolyzing their β-lactam rings, rendering these antibiotics inactive [59, 60]. The overexpression of both intrinsic and acquired efflux pumps has been identified as a key factor in bacterial MDR [59]. The primary efflux pump in the JS01 strain was identified as the MFS, comprising genes such as *tet(A)*, *emrY*, *emrK*, *KpnE*, and *KpnF*, which encode secondary active transporter proteins responsible for effluxing various antibiotics with different structures [61]. The *tet(A)*, *emrY* and *emrK* genes are primarily associated with tetracycline resistance, while *KpnE* and *KpnF* genes impact resistance to macrolides, tetracyclines, peptides, and rifamycins. Additionally, the annotated *emrE* gene, belonging to SMR gene family, encodes a protein that enhances resistance to macrolide antibiotics such as AZI and ERY. These AMR genes represent potential targets for therapeutic interventions. Furthermore, JS01 was annotated with numerous other AMR gene types, such as aminoglycoside resistance genes (*kdpE, baeR*, *baeS*, *cpxA*, *mdtA, mdtB, mdtC*, etc.) and fluoroquinolone resistance genes (*emrA*, *emrB*, *emrR*, *mdtH*, *rsmA*, *AcrE*, *AcrF*, etc.). Interestingly, despite the presence of these genes, JS01 exhibited sensitivity to both aminoglycoside (tested antibiotics: AMK, STR, GEN, KAN) and fluoroquinolone (CIP, LEV). This suggests that the presence of specific AMR genes does not always directly correlate with the observed AMR phenotype [24], potentially due to factors such as gene mutations leading to non-expression. The underlying mechanisms warrant further investigation.

The VFs of *E. coli* are predominantly encoded on both chromosome and plasmids, enabling *E. coli* to evade host immune defenses and successfully infect, proliferate, and spread across diverse host environments. In this report, we identified and functionally annotated the virulence genes present in strain JS01. According to the VFDB, these genes primarily play roles in transmission and infection processes, encompassing adhesins, motility, toxins, virulence regulation, metabolism and secretion. The interaction between bacteria and host cells begins with the adhesion process, a critical prerequisite for bacterial colonization and subsequent invasion. In this process, adhesion-related VFs such as Type 1 fimbriae (T1F), *E. coli* common pilus (ECP), Curli fibers, UpaG, EaeH in the JS01 genome may play significant roles. T1F, encoded by *fim* operon consisting of *fimABCDEFGHI* genes, is one of the most extensively studied adhesion factors [62, 63]. Within the cluster of Fim proteins, FimA (a subunit encoded by *FimA*) is the main structural component of pili, while FimH, a lectin-like protein, is directly responsible for T1F binding properties. These proteins may influence *E. coli* host specificity and infection outcomes

[63, 64]. The ECP fimbrial operon is ubiquitous and conserved in *E. coli*. The six-gene (*ykgK/ecpR, yagZ/ecpA, yagY/ecpB, yagV/ecpE, yagX/ecpC, yagW/ecpD*)-encoded ECP fimbria were predicted to exist in strain JS01, which is necessary for temperature-dependent biofilm formation on abiotic surfaces [65]. Curli fibers, encoded by the *csgABCDEFG* genes, are functional amyloids found in the biofilm extracellular matrix of many bacterial phyla [66] and are essential for adhesion [67]. UpaG, derived from *E. coli*, is a trimeric autotransporter (AT) adhesin that facilitates aggregation, biofilm development, and binding to a range of extracellular matrix (ECM) proteins. Members of the trimeric AT adhesin family are important VFs for pathogenic *E. coli* and mediate adherence to eukaryotic cells and ECM proteins [68]. EaeH, a highly conserved chromosomally encoded intimin, is a member of the bacterial adhesion molecule family. It interacts with intestinal epithelial cell surfaces, aiding bacterial adhesion, facilitating the delivery of heat-labile toxins, and promoting colonization within the small intestine [69]. In addition to these adhesion-related VFs, other specific VFs were detected in strain JS01, including TTSS, T6SS, Gsp, ETT2 and T4SS, which are associated with effector delivery system; Ibes and TraJ, which are associated with invasion; and hemolysin/cytolysin A, which is associated with exotoxins. These predicted VFs likely work synergistically to contribute to the pathogenicity of the JS01 strain. The high pathogenicity of JS01 was confirmed by challenge tests in mice, and its pathogenesis needs further exploration.

In recent years, WGS technology has emerged as a powerful tool for studying pathogens. Genomic analysis, leveraging WGS, provides comprehensive gene data on pathogens and enables precise identification of serotypes, virulence and AMR determinants. In this study, WGS-based serotyping and MSLT of the JS01 strain were performed using open access tools. Strain JS01 was confirmed to belong to ST151 and serotype O177:H51 *E. coli*, circumventing the time and costs associated with conventional techniques and enabling rapid and accurate serotype determination [30]. This study represents the first isolation of an O177: H51 APEC strain from chicken farms worldwide, which is crucial for detecting outbreaks, epidemiological surveillance, taxonomic differentiation of *E. coli*, and clonal and evolutionary studies. Additionally, WGS can predict AMR by detecting resistance genes, which aligns accurately with resistance phenotypes determined by the Kirby-Bauer disk diffusion method [17, 25, 59]. This was consistent with the results of our study. WGS also enables the prediction of VFs by identifying VF genes, providing insights into the virulence and pathogenic mechanisms of the pathogen. Previous studies have demonstrated that the comprehensive information provided by WGS enhances the surveillance of pathogens transmitted across various hosts. Genomic data can be used to trace the origin of epidemics, delineate their progression, idengtify mutations in VF and AMR genes, and understand the evolutionary dynamics of strains, significantly accelerating tracking studies [30, 70]. As early as 2015, WGS was employed as a promising tool for the surveillance and control of human tuberculosis [71]. Compared to traditional microbial and molecular biology methodologies, WGS offers faster, more cost-effective, and precise monitoring outcomes.

Conclusion

To summarize, a study was conducted on an MDR APEC strain named JS01, isolated from a chicken farm in western Anhui Province, China. The research involved resistance testing, mouse pathogenicity assessments, WGS, and comparative genomics analysis.The results revealed JS01's resistance to multiple antibiotics, including β-lactams, lincosamides, amide alcohols, macrolides, polypeptides, and tetracyclines. It also showed the strain's pathogenicity, virulence factors, MLST type (ST155), and serotype (O177:H51). JS01carries various virulence and resistance genes, indicating its high pathogenic potential. Additionally, the study analyzed JS01's genetic evolution using phylogenetic trees based on core genes. The findings suggested potential transmission of APEC strains among different hosts, such as chickens, pigs, food, cattle, humans, and other animals. This highlights the prevalence of APEC, which was not only diverse but also tightly linked to various hosts. Overall, this research provides crucial insights into APEC'S AMR mechanisms genomic features, diversity, and epidemiological characteristics, These findings could help develop new strategies for preventing and treating APEC infections.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03925-5.

Supplementary Material 1. Supplementary Table S1-S7 and Figure S1-S3.

Acknowledgements

We are deeply grateful to the Anhui Provincial Department of Education and West Anhui University for funding the research project. In addition, we are grateful to the poultry farm owners for giving access to the samples. We would also like to thank the Veterinary Clinical Diagnosis Laboratory of West Anhui University for their support during the research. We also thank the editor and anonymous reviewers for their constructive comments that help to improve the quality of this paper.

Clinical trial number

Not applicable.

Authors' contributions

L.X. and S.B.: conceptualization, data curation, investigation, methodology, writing— original draft; G.W., J.C. and D.Q.: data curation, methodology,

software, visualization; N.C.: methodology, writing—review and editing; L.X.: funding acquisition, supervision. All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by Natural Science Research Key Project of Universities in Anhui Province (grant number No. KJ2021A0956), Anhui Scientific Research and Innovation Team of Quality Evaluation and Improvement of Traditional Chinese Medicine (grant number No. 2022AH010090), Anhui Provincial Quality Engineering Project of Higher Education (grant number No. 2022ZYBJ108), Platform Collaborative Innovation Project of Anhui Province (grant number No. 0041122066) and Teaching Research Quality Project of West Anhui University (Project No. WXXY2022007).

Data availability
All data generated or analyzed during this study are included in this published article. The nucleotide sequence of the chromosome and plasmids of E. coli isolate JS01 have been deposited in GenBank under accession number CP148986–CP148989.

Declarations

Ethics approval and consent to participate
Ethical approval was granted from the Ethics Committee of Western Anhui University (permit No. 2023-E(r)-004) for studies involving animals. In addition, written consent was taken from the farm handlers before sampling.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 10 December 2024 Accepted: 21 March 2025
Published online: 04 April 2025

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