# Isolation and Drug Sensitivity Analysis of Diarrhea Pathogens in a Duck Farm, Chongqing

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**Abstract:** In order to investigate the cause of diarrhea death of young ducks in a duck farm in Tongliang, Chongqing. The pathogen was isolated and identified in various organs, blood and abdominal effusion of sick ducks, and the isolated bacteria were identified by routine bacteriological identification, 16SrDNA sequence molecular and animal pathogenicity tests, and drug resistance was analyzed by drug sensitivity test and drug resistance gene detection. The results showed that plague, Newcastle disease and Tambusu virus of duck were not detected in clinical samples, but two strains of bacteria were isolated from the organs of infected ducks, which were identified as *Bacillus cereus* (SPL1507) and *Escherichia coli* (SPL1508), respectively. Both strains of bacteria were lethal to mice, and strain SPL1508 had strong resistance. The relationship between resistance genes and phenotypes was explored through drug susceptibility test combined with drug resistance gene detection. The relationship between drug resistance genes and phenotypes was explored through drug sensitivity test combined with drug resistance gene detection. β-lactam resistance genes CTX-M gene, sulfamides sul1 and sul2 gene, and quinolone Aac(6')-Ib-cr gene were all detected, which was consistent with the results of drug sensitivity test in this study. The results provide reference for clinical disease prevention and treatment.

## 1. Introduction

*Bacillus cereus* (*B. cereus*) is a common and hazardous foodborne pathogen widely found in various foods[1]. As a highly pathogenic bacterium, it ranks third among the common foodborne pathogens in China[2] and second only to *Staphylococcus aureus* in France[3]. *B. cereus* causes diarrhea and vomiting, which are two different types of gastrointestinal illnesses caused by food contamination[4]. Diarrheal syndrome is caused by an intolerant enterotoxin, while vomiting syndrome is caused by a highly heat-resistant gluten toxin[5]. Therefore, the pathogen has a great threat to the animal husbandry industry and human health and safety.

*Escherichia coli* (*E. coli*) is an important zoonotic pathogen[6]. In domestic animals, especially young animals are particularly susceptible, and cause the growth retardation of sick animals, low production capacity, and even death, which brings serious losses to animal husbandry[7]. Duck colibacillosis is an acute septicemic infectious disease caused by pathogenic *E. coli*, which is an opportunistic pathogen and exists in the environment for many years[8]. In the daily production management of farms, various harmful environmental factors such as dietary nutrition level, stress factors, and poor ventilation can cause primary and secondary infection of *E. coli*, which is a great harm to the industry[9].

In this study, pathological dissection of diseased ducks was performed, and the organs with serious pathological changes, blood and abdominal fluid were collected for pathogen isolation and identification. Then drug sensitivity test and animal pathogenicity test were carried out on the isolated bacteria to explore the main etiology and clinical sensitive drugs causing the disease of ducks, and to provide scientific basis for the prevention and control of the disease.

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## 2. Materials and Methods

### 2.1 Experimental Animals and EthicsStatement

All mouse use was approved by the experimental animal ethics committee of Southwest University (Chongqing, China) (Permit No: IACUC-20211020-09). Cherry Valley duckling and Kunming mice (female, 7-8-week-old) were purchased from the Hunan SJA Laboratory Animal Co.,Ltd (Hunan, China). Total 20 Cherry Valley duckling,and 40 Kunming mice were used in this study. Mice and duckling were raised in a special culture system that was individually ventilated and free of pathogens (temperature at 20-30 ˚C, relative humidity at 50-60 %, and lighting cycle at 12 h/day) with free food and water.

### 2.2 Acquisition of Pathological Tissue Samples

Twelve Cherry Valley duckling with typical symptoms of different weeks of age were selected from a duck farm in Tongliang and sent to the laboratory for testing. Pathological dissection was performed on the sick ducks submitted for examination. Aseptically, one sample of heart, liver, spleen, lung, kidney, abdominal effusion and blood were taken, numbered 1-7, and the tissue samples were respectively stained by Gram and Wright‘s for microscopic examination.

### 2.3 Processing of Tissue Samples

In a sterile environment, the obtained visceral tissue samples were cut up and put into 2 mL centrifuge tubes, and 1 mL PBS buffer with pH 7.2 was added to grind in a homogenizer. Then it was repeatedly frozen and thawed with liquid nitrogen for three times, centrifuge for 5 minutes at 10000 r/min, absorb the supernatant and add penicillin and streptomycin double resistance solution to the final concentration of 1000 U/mL, and then the supernatant was filtered by 0.22μm filter membrane and stored at -80 ℃.

### 2.4 Virus Detection

Filtrate was extracted from ultra-low temperature refrigerator, viral DNA and RNA were extracted by viral genome extraction kit, RNA was reverse-transcribed into cDNA by primers, and samples were stored at -20℃. Duck plague virus (DPV), Duck tembusu virus (DTMUV) and Duck newcastle disease virus (DNDV) were detected by PCR and qRT-PCR. The primer sequence is shown in Table 1.

Table 1 Primers for detection of duck virus.

| Virus | Primers | Primer sequence | Sizes(bp) | Annealing temperature |
|---|---|---|---|---|
| DPV | WF1 | GGACAGCGTACCACAGAT | 498 | 50℃ |
| | WR1 | ACAAATCCCAAGCGTAG | | |
| DPV | WF2 | GACAATAACAACAATGAGACGCAACA | 3100 | 58.6℃ |
| | WR2 | CCACGGCATTATTTTCATCAGTCTTC | | |
| DTMUV | TF1 | GCCACGGAATTAGCGGTTGT | 500 | 62℃ |
| | TR1 | TAATCCTCCATCTCAGCGGTGTAG | | |
| DTMUV | TF2 | GTGGATGGATTCGACCAAAG | 1880 | 53.18℃ |
| | TR2 | CCCACATGTTGTGCTCGAGCC | | |
| DNDV | XF1 | ATGGGCTCCAGACCTTCTACCAG | 1662 | 56.71℃ |
| | XR1 | TTGTAGTGGCCCTCATCTGATCGA | | |
| DNDV | XF2 | AACAGAGAATCCGTAAGTTAC | 483 | 53.12℃ |
| | XR2 | TGGCATCTTCGCTAACAGCAA | | |

### 2.5 Isolation and Morphological Observation of Pathogenic Bacteria

The samples were inoculated with ordinary, Martin and rabbit blood Martin AGAR medium and cultured at 37 ℃ for 18-24 h. The morphological differences of colonies were observed and classified. After isolation and purification, the pure culture of bacteria were taken and stained by Gram and Wright’s respectively. The morphology and size of the isolated bacteria were observed by

ordinary optical microscope.

### 2.6 Biochemical Identification

According to colony morphology and bacterial staining characteristics, the two isolates were named SPL1507 and SPL1508 respectively, and were inoculated into various biochemical identification tubes and cultured at 37 ℃ for 24 h.

### 2.7 PCR Amplification and Sequencing of 16S rDNA

1.5mL cultured bacterial solution was taken and total DNA was extracted by TianGen bacterial DNA extraction kit. PCR amplification was performed with universal primers of the bacterial 16S rDNA gene, and the primer sequences were shown in Table 2. Total reaction system (50μL): 2×TaqMix 25μL, upper and downstream primers 2μL, DNA template 2μL, dd$H_2O$ 19μL. Reaction conditions: predenaturation at 95 ℃ for 5 min, denatured at 95 ℃ for 30 s, annealed at 56 ℃ for 30 s, extended at 72 ℃ for 90 s, a total of 30 cycles, extend at 72 ℃ for 7 min. The amplified products were detected by 1.0% agar-gel electrophoresis, and the recovered products were sequenced by Sangong Biotech (Shanghai) Co., LTD.

Table 2 Universal primers for 16S rDNA gene

| Primer number | Primer sequence | Sizes(bp) | Annealing temperature |
|---|---|---|---|
| 16S-F | AGAGTTTGATCCTGGCTCAG | 1450 | 60℃ |
| 16S-R | GGTTACCTTGTTACGACTT | | |

### 2.8 Homology Analysis of 16S rDNA Sequence

DNAStar and MegaX software were used to compare the 16S rDNA sequences of the isolates with those in the NCBI database, and the phylogenetic tree was constructed, the tree construction method was NJ and Bootstrap was set to 1000.

### 2.9 Pathogenicity Test in Mice

The mice were divided into two groups, with experimental group (n=10) and control group (n=10) in each group. The liquid culture of isolated bacteria SPL1507 and SPL1508 were injected intraperitoneally at the dose of 0.1 mL/ mouse (the bacterial concentration was $1.76\times10^9$ CFU and $1.53\times10^9$ CFU, respectively), and the sterile liquid medium was injected intraperitoneally in the control group. After inoculation, each group was fed in isolation, and the clinical manifestations of mice were observed every 6 hours for 1 week. The mice with symptoms and death in each group were dissected and observed, and the bacteria in the diseased organs were isolated and identified.

### 2.10 Duckling Infection Test

The ducklings were divided into two groups, with experimental group (n=5) and control group (n=5) in each group. The identified SPL1507 and SPL1508 liquid cultures were injected intramuscularily at 0.5 mL/ ducklings (bacterial concentration was $1.76\times10^9$ CFU and $1.53\times10^9$ CFU, respectively), and the ducklings in the control group were injected intramuscularily with sterile liquid medium, and then fed separately. Bacteria were isolated and identified from dead ducklings.

### 2.11 Drug Sensitivity Detection

The sensitivity of isolated pathogens to drugs was determined by disk diffusion test (κ-B method) on Martin AGAR medium containing 10% horse serum. According to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing [10], bacteria were divided into sensitive (S), intermediate (I) and resistant (R) according to the size of antibacterial zone.

### 2.12 Detection of Drug Resistance Gene in SPL1508

In view of the fact that all the *E. coli* strains isolated in this study were resistant to commonly

used clinical antibiotics, in order to study the relationship between bacterial drug resistance phenotype and drug resistance genes, this study designed specific primers of related drug resistance genes (Table 3). Using SPL1508 bacterial DNA as a template, PCR amplification was performed on its drug resistance genes. The total PCR amplification system (20 μL): 2×TaqMix 10 μL, upper and downstream primers 1 μL, DNA template 1 μL, dd$H_2O$ 7 μL, reaction parameters are shown in Table 4. The amplified products were detected by 1.0% agarose gel electrophoresis and the results were observed.

Table 3 PCR amplification primer sequence of *E. coli* drug resistance gene

| Drug resistance gene number | Primer sequence (5 '→ 3') | Sizes(bp) | Annealing temperature |
|---|---|---|---|
| CTX-MF | AAGGCGTTTTGACAGACTATTCAT | 950bp | 56.65℃ |
| CTX-MR | CCGTTTCCGCTATTACAAACC | | 53.12℃ |
| TEM-F | TGAGTATTCAACATTTCCGTGTCG | 860bp | 56.67℃ |
| TEM-R | TTACCAATGCTTAATCAGTGAGGC | | 56.67℃ |
| SHV-F | TGACGGTCGGCGAACTCT | 450bp | 58℃ |
| SHV-R | GGGTATCCCGCAGATAAATCAC | | 54.43℃ |
| sul1-F | ATGGTGACGGTGTTCGGCAT | 840bp | 62℃ |
| sul1-R | CTAGGCATGATCTAACCCTCGGT | | 55.63℃ |
| sul2-F | AGCCCCCATGAATAAATCGCTC | 816bp | 54.43℃ |
| sul2-R | ATTCTTGCGGTTTCTTTCAGCG | | 54.41℃ |
| sul3-F | ATGAGCAAGATTTTTGGAATCGT | 792bp | 55.56℃ |
| sul3-R | CTAACCTAGGGCTTTGGATATTTTC | | 57.66℃ |
| Aac (6')-Ib-cr-F | TTGCGATGCTCTATGAGTGGCTA | 482bp | 55.61℃ |
| Aac(6')-Ib-cr-R | CTCGAATGCCTGGCGTGTTT | | 62℃ |
| qnrA-F | TTGCCAGGCACAGATCTTGAC | 582bp | 53.14℃ |
| qnrA-R | AAGAGGATTTCTCACGCCAGG | | 53.14℃ |
| qnrB-F | TGGGMATHGAAATTCGCCACTG | 268bp | 54.41℃ |
| qnrB-R | AGTTTGCYGYYCGCCAGTCGAA | | 54.43℃ |
| qnrS-F | CTGCAAGTTCATTGAACAGGGTG | 430bp | 55.61℃ |
| qnrS-R | TCTAAACCGTCGAGTTCGGCG | | 53.16℃ |

Table 4 PCR reaction parameters of drug resistance gene in *E. coli*

| Target gene | PCR operating parameters |
|---|---|
| CTX-M | (95℃, 5min)+{(95℃, 1min)+(56℃, 1min)+(72℃, 90s)}×35+(72℃, 7min) |
| TEM | (95℃, 5min)+{(95℃, 1min)+(56℃, 1min)+(72℃, 90s)}×35+(72℃, 7min) |
| SHV | (95℃, 5min)+{(95℃, 30s)+(56℃, 30s)+(72℃, 1min)}×35+(72℃, 7min) |
| sul1 | (95℃, 5min)+{(95℃, 1min)+(59℃, 1min)+(72℃, 90s)}×35+(72℃, 7min) |
| sul2 | (95℃, 5min)+{(95℃, 1min)+(55℃, 1min)+(72℃, 90s)}×35+(72℃, 7min) |
| sul3 | (95℃, 5min)+{95℃, 1min)+(57℃, 1min)+(72℃, 50s)}×35+(72℃, 7min) |
| Aac(6')-Ib-cr | (95℃, 5min)+{(95℃, 30s)+(57℃, 30s)+(72℃, 30s)}×35+(72℃, 7min) |
| qnrA | (95℃, 5min)+{(95℃, 30s)+(55℃, 30s)+(72℃, 30s)}×35+(72℃, 7min) |
| qnrB | (95℃, 5min)+{(95℃, 30s)+(55℃, 30s)+(72℃, 30s)}×35+(72℃, 7min) |
| qnrS | (95℃, 5min)+{(95℃, 30s)+(54℃, 30s)+(72℃, 30s)}×35+(72℃, 7min) |

## 3. Results

### 3.1 The Results of the Virus Detection

The DNA and cDNA of the samples were amplified by PCR and qRT-PCR to detect duck plague virus, duck Newcastle disease virus and duck Tambusu virus. The results showed that no positive samples were detected.

### 3.2 Results of Isolation of Pathogenic Bacteria

The two bacteria were isolated after the tissue samples were inoculated on ordinary, Martin and rabbit blood Martin medium plate at 37 ℃ for 24 h. One is fast growing, showing a milky white and smooth surface of the round colony, the colony edges are neat, slightly metallic luster, translucent, slightly raised. The other was incubated at 37 ℃ for 24 h and grew a round or nearly round white colony with a diameter of 5-7 mm, soft texture, no pigment and rough surface, which showed hemolysis on the rabbit blood medium plate (Fig. 1).

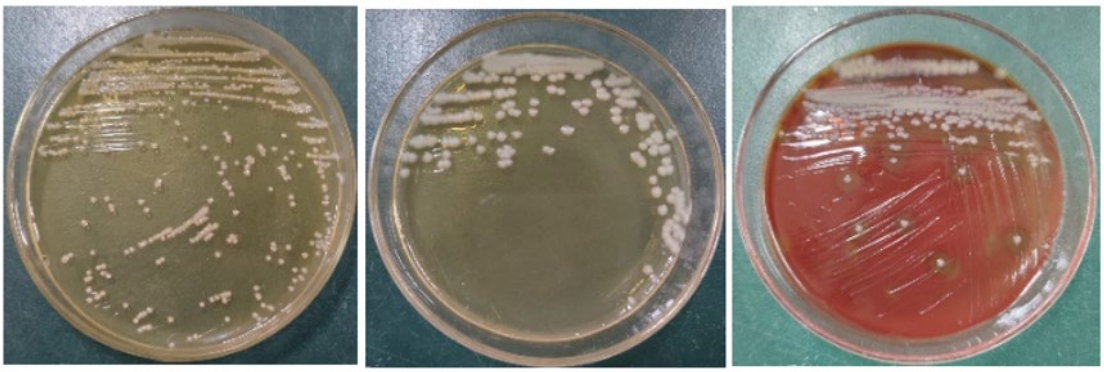
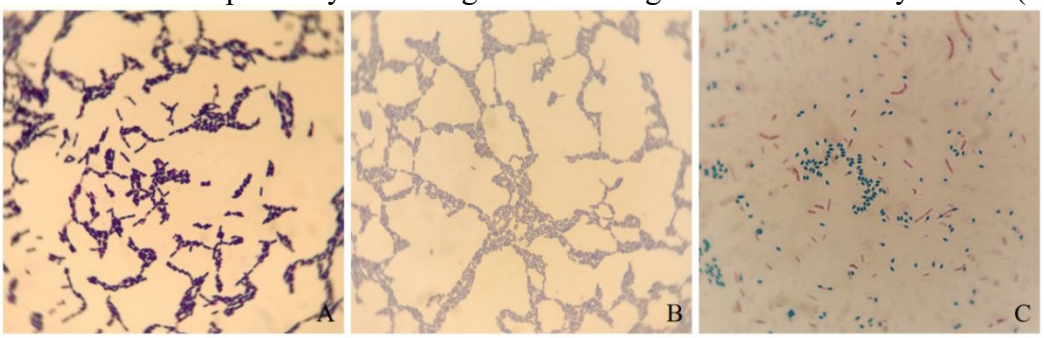



Figure 1 Colony morphology of two strains on different agar plate media.

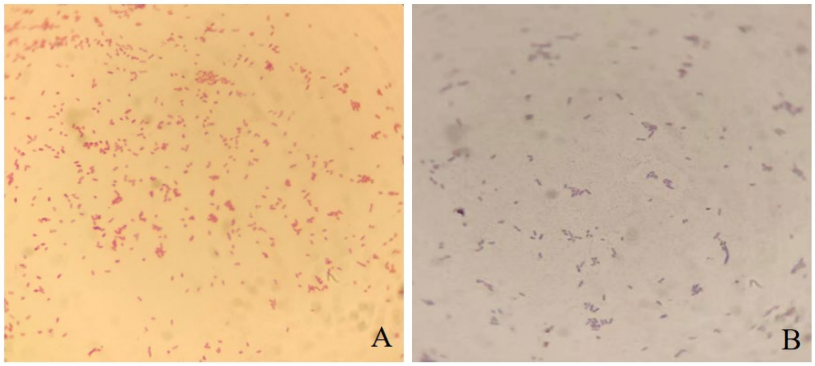
### 3.3 Results of Bacterial Staining Microscopy

Two strains of bacteria were isolated by Gram staining and Wright's staining, respectively. Under the microscope, one was observed to be short bacillus, with blunt round ends and gram negative, scattered in distribution. The other thallus is rod-shaped, terminal square, long chain, gram positive, spores can be seen by spore staining (green for spores, red for thallus). The pathogens were named SPL1507 and SPL1508 respectively according to the naming order of laboratory isolates(Fig. 2).




A: Gram staining of SPL1507 (1000×); B: Wright's staining of SPL1507 (1000×); C: Spore staining of SPL1507 strain (1000×).

Figure 2 Microscopic results of SPL1507.




A: Gram staining of SPL1508 (1000×); B: Wright's staining of SPL1508 (1000×).

Figure 3 Microscopic results of SPL1508.

### 3.4 Results of Biochemical Identification

The biochemical experiment results of isolated strains are shown in Table 5. According to the results of microscopic examination, colony morphology and biochemical experiments, SPL1507 belonged to *Bacillus cereus* and SPL1508 belonged to *Escherichia coli*.

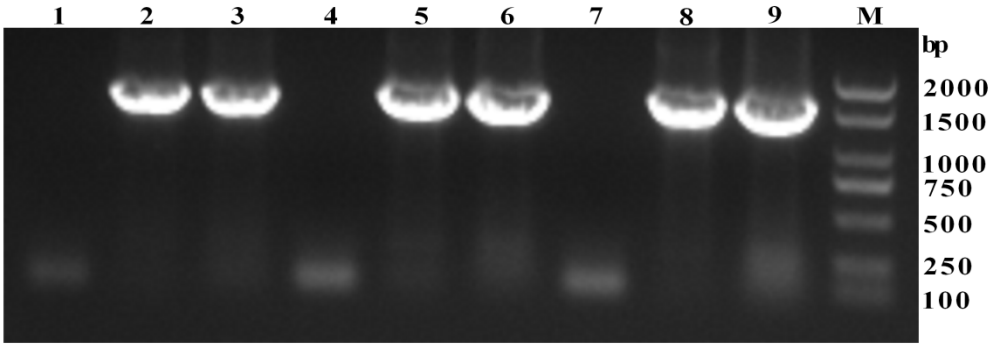
Table 5 Physiological and biochemical characteristics of isolated strains

| Items | Results | |
|---|---|---|
| | SPL1507 | SPL1508 |
| Hydrogen Sulfide | — | — |
| Phenylalanine | — | — |
| Gluconate | — | + |
| Peptone Water | — | + |
| MR | + | + |
| VP | + | — |
| Citrate | + | — |
| Urea | — | — |
| Semi Solid Agar | + | + |
| Glucose | — | + |
| Lysine | + | + |
| Ornithine | + | + |
| Raffinose | — | — |
| Sorbitol | — | + |
| Adonitol | — | — |
| Peroxidase | + | + |
| Nitrate reduction | + | + |
| Hemolysis | + | — |

Note: " + " positive, " − " negative

### 3.5 Results of 16S rDNA PCR Amplification and Sequencing Analysis

Total DNA of pure culture bacteria SPL1507 and SPL1508 was used as template for PCR amplification using bacterial 16SrDNA universal primers. After 1% agarose gel electrophoresis, the amplified fragment size was 1450 bp, as shown in Fig. 3.




M: DL2000 DNA marker ; 1,4,7: Negative; 2,3: Test strain;
5,6:SPL1507 and SPL1508 strains isolated from pathogenicity test mice;
8,9:SPL1507 and SPL1508 strains isolated from ducks were reinfected.

Figure 4 Electropherogram of 16S rDNA amplification results.

The amplified product sequencing results were compared by Blast on NCBI, and sequences with high homology were downloaded and the homology analysis was performed between the sequenced columns and the reference sequences by DNAStar software, as shown in Fig. 5 and 6. The results showed that SPL1507 (0001) had the highest homology with 98.9% of B. cereus NPK1110

(MN691533), while it had low homology with other strains of *Bacillus*. Therefore, strain SPL1507 (0001) could be identified as *B. cereus*. SPL1508 (0002) has the highest homology (98.2%) with *E. coli* AH01 (CP055251), and has a low homology with other *Escherichia* strains, so SPL1508 (0002) is *E. coli*.

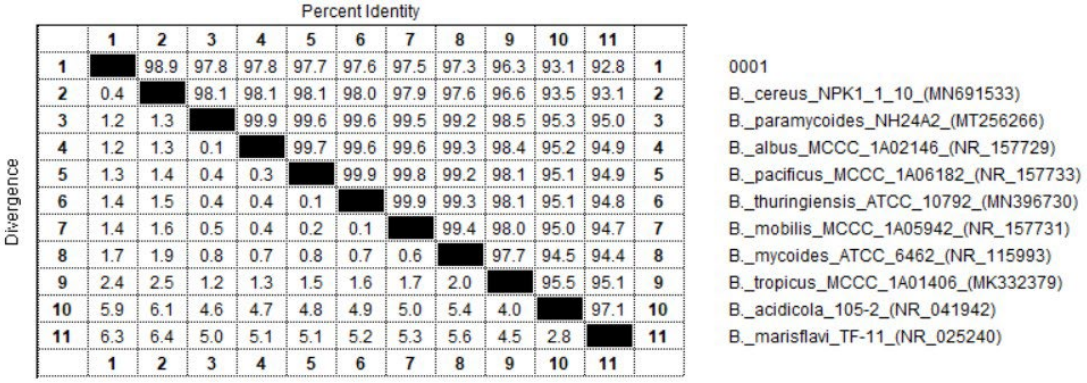



Figure 5 SPL1507 was homologous with reference strains at home and abroad.

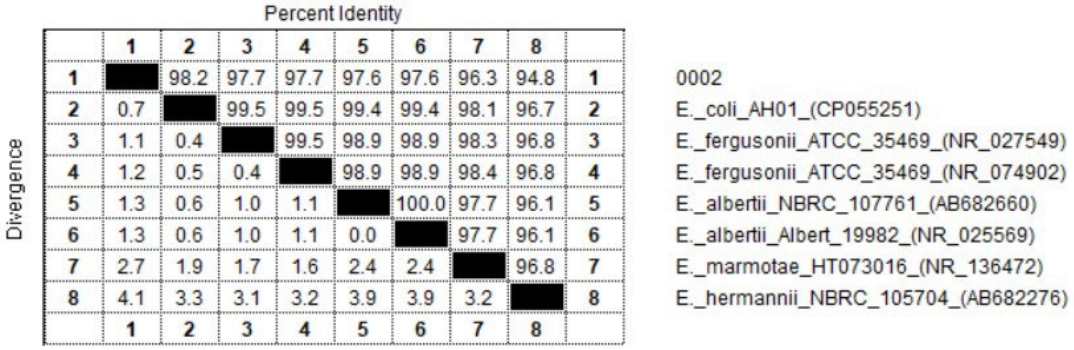



Figure 6 SPL1508 was homologous with reference strains at home and abroad.

According to the results of the evolutionary tree (Fig. 7 and 8), SPL1507 (0001) belongs to the genus Bacillus, which has the highest homology with B. cereus NPK111 10 (MN691533) and is clustered in the same branch. The SPL1508 (0002) sample belongs to the genus Escherichia and is clustered in the branch as E. coli AH01 (CP055251).

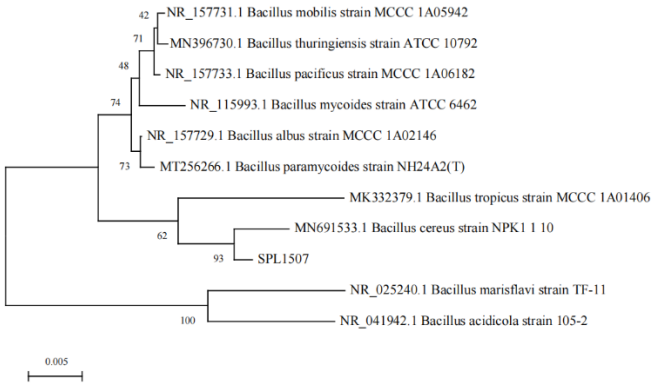



Figure 7 Phylogenetic tree of SPL1507 16S rDNA.

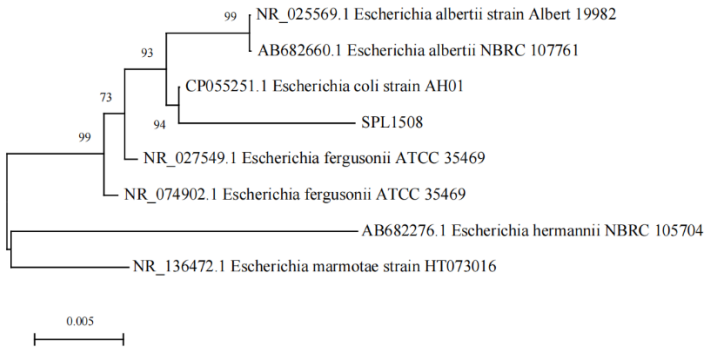



Figure 8 Phylogenetic tree of SPL1508 16S rDNA.

### 3.6 Results of pathogenicity test in mice

The experimental rats were artificially infected with isolated bacteria and observed every 2 hours. After 4 hours, the experimental group showed depression, slow movement, shortness of breath, disarranged hair, standing motionless with closed eyes, crouching and huddling. Strain SPL1507 died at the earliest 6 h, and all died within 48 h. Strain SPL1508 group began to die at the earliest 4 h, and all died after 24 h. The control group had no obvious clinical symptoms and all survived (Table 6). Sterile pathological autopsy was performed on the dead mice of the experimental group to collect disease materials. Consistent strains were isolated from the heart blood, liver, kidney and lung of the mice, and the colony morphology, cell shape, 16SrDNA PCR results and basic physicochemical characteristics were consistent with the isolated strains. No bacteria could be isolated from the corresponding organs of control mice.

Table 6 Pathogenicity of isolated strains in mice

| Strain | Experimental group (numbers of deaths / numbers of experiments) | Mortality rate | Time of death | Control group (numbers of deaths/ numbers of experiments) |
|---|---|---|---|---|
| SPL1507 | 10/10 | 100% | 1d | 0/10 |
| SPL1508 | 10/10 | 100% | 1-2d | 0/10 |

### 3.7 Results of Infection Test in Ducklings

After infection with the isolated strain, 24 hours later, all ducklings showed depression, lethargy, loss of appetite and even abandonment, huddling up, discharging light green stool and other phenomena. After 144 h, all the ducklings in the SPL1508 group died, and after 168 h, 2 ducks in the SPL1507 group died, and no obvious pathological phenomenon was found in the control group. The dead ducks of the experimental group were subjected to aseptic pathological autopsy to collect disease materials. Consistent strains were isolated from the heart, liver and kidney of the ducks. The colony morphology, cell shape and 16S rDNA PCR results were consistent with those of the infected isolates. No bacteria could be isolated from the corresponding organs of the control group.

Table 7 Experimental study on regression infection of isolated strains in ducklings

| Strain | Experimental group (numbers of deaths / numbers of experiments) | Mortality rate | Time of death | Control group (numbers of deaths/ numbers of experiments) |
|---|---|---|---|---|
| SPL1507 | 2/5 | 40% | 3-7d | 0/5 |
| SPL1508 | 55 | 100% | 3-7d | 0/5 |

### 3.8 Results of Drug Sensitivity

The sensitivity test results of the two isolates to more than 20 commonly used antibiotics are shown in Table 8. It can be seen from the table that the isolated bacteria had certain resistance to most of the commonly used antibiotics in clinic, and the isolated strain SPL1507 was sensitive to cephalexin, cefradine, amikacin, gentamicin, kanamycin, neomycin, midecamycin, norfloxacin,

ciprofloxacin and vancomycin. SPL1508 is only sensitive to gentamicin.

Table 8 Drug sensitivity of isolated strains

| Drug name | Dose(μg) | SPL1507 | SPL1508 |
|---|---|---|---|
| Penicillin | 30 | R(0.8) | R(0.0) |
| Oxacillin | 1 | R(0.0) | R(0.0) |
| Ampicillin | 10 | R(0.8) | R(0.0) |
| Carbenicillin | 100 | R(1.0) | R(0.0) |
| Pipracil | 100 | R(1.5) | R(1.2) |
| Cefalexin | 30 | S(1.9) | R(0.0) |
| Cefazolin | 30 | R(1.3) | R(0.0) |
| Cefradine | 30 | S(2.3) | R(0.0) |
| Cefuroxime | 30 | R(0.0) | R(0.0) |
| Ceftazidime | 30 | R(0.0) | R(1.4) |
| Ceftriaxone | 30 | R(1.0) | R(1.1) |
| Cefoperazone | 75 | I(1.7) | R(1.2) |
| Amikacin | 30 | S(1.9) | I(1.5) |
| Gentamicin | 10 | S(1.7) | S(1.7) |
| Kanamycin | 30 | S(1.9) | R(1.3) |
| Neomycin | 30 | S(1.7) | R(1.2) |
| Tetracycline | 30 | R(1.3) | R(0.0) |
| Doxycycline | 30 | I(1.5) | R(0.0) |
| Minocycline | 30 | R(1.2) | I(1.5) |
| Erythromycin | 15 | I(1.8) | R(0.0) |
| Midecamycin | 30 | S(1.9) | R(0.0) |
| Norfloxacin | 10 | S(1.9) | R(0.9) |
| Ciprofloxacin | 5 | S(2.1) | R(1.1) |
| Vancomycin | 30 | S(1.4) | R(0.0) |
| Polymyxin B | 300IU | R(0.0) | R(1.1) |
| Compound Sulfamethoxazole | 3.75/1.25 | R(0.0) | R(0.0) |
| Furazolidone | 300 | R(1.3) | R(1.1) |
| Clindamycin | 2 | I(1.8) | R(0.0) |

Note: S(sensitive), I(intermediate), R(resistant). The value in parentheses is the diameter of the zone of inhibition(mm).

### 3.9 Detection Results of Drug Resistance Gene in *E. coli* SPL1508

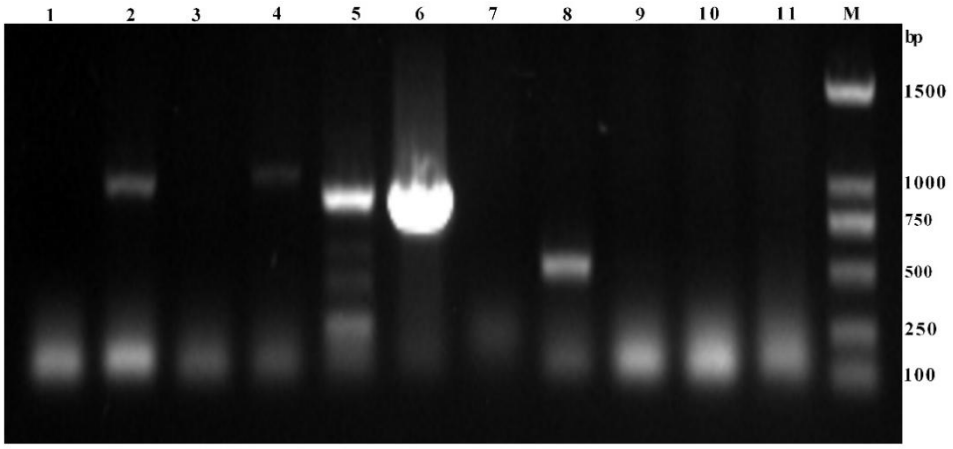



Figure 9 PCR amplification of drug resistance gene of SPL1508.

In order to verify the relationship between the drug-resistance phenotype and drug-resistance genes of E. coli SPL1508, this study designed 10 drug-resistance genes of E. coli in three categories

for verification, namely β-lactam (CTX-M, TEM, SHV), sulfamides (sul1, sul2, sul3), and quinolones (Aac(6')-Ib-cr, qnrA, qnrB, qnrS). The isolated genomic DNA of strain SPL1508 was used as the template for amplification of the above drug resistance genes, and the results were shown in Fig.9. Among them, CTX-M gene, sul1 and sul2 genes, and Aac(6')-Ib-cr gene were all detected to be consistent with the drug resistance phenotype.

## 4. Discussion

China is the largest country in the production and consumption of meat ducks, and the demand for livestock and poultry products is also increasing year by year[11]. In the process of development of duck breeding industry, although the overall breeding mode is changing to intensive production, the overall management level and breeding environment are still relatively extensive, and the biosecurity measures are not perfect, and ducks in farms are prone to cross infection [12]. In this study, two strains of bacteria were isolated from the organs of sick ducks and identified as E. coli and B. cereus through morphological characteristics observation and molecular biology techniques. In the comparative analysis of their homology, SPL1507 was B. cereus and SPL1508 was E. coli. The above two kinds of bacteria are the main pathogens causing the large-scale diarrhea death of ducks. Through the later pathogenicity test, it was found that the pathogenicity of B. cereus isolated in this time was much higher than that of E. coli in the mouse model, but E. coli showed stronger pathogenicity than B. cereus in the infection model of ducks. It is speculated that different strains have different host tropism, and the specific mechanism needs to be studied later.

After investigation, the duck farm since the onset of the disease has been a high dose of multiple types of antibiotics to feed the treatment program, but the disease has not been alleviated, resulting in the duck farm pathogenic bacteria resistant to most antibiotics. The results of laboratory drug susceptibility test showed that SPL1507 was sensitive to cephalexin, cefradine, amikacin, gentamicin, kanamycin, neomycin, midecamycin, norfloxacin, ciprofloxacin and vancomycin. SPL1508 is only sensitive to gentamicin and is basically resistant to common clinical antibiotics. At the same time, three types of 10 kinds of drug resistance genes were detected for the isolated high drug resistance E. coli strains, and the coincidence rate of drug resistance phenotype was high. The drug susceptibility test showed that drug resistance genes mediated the occurrence of drug resistance phenotype. By co-screening drug resistance genes and phenotypes, sensitive drugs could be predicted, providing reliable theoretical guidance for clinical drug use, and reducing the generation of multi-drug resistant strains. According to the above experimental results, this study formulated a reasonable control plan for the farm, using gentamicin and amikacin respectively to treat the sick ducks, and finally the epidemic was effectively controlled.

In recent years, the problem of bacterial resistance has aroused great attention around the world [13], so it is particularly important to improve the monitoring methods of bacterial resistance and establish comprehensive and accurate guidelines on antibiotic use. At present, κ-B method and various drug sensitivity analysis systems are common methods for detecting bacterial resistance in laboratories[14]. κ-B method has been widely used due to its advantages such as simple operation and easy observation of results[15], but low sensitivity and easy contamination. Therefore, PCR detection based on drug resistance genes came into being, which can support the results of drug susceptibility test to improve the speed and accuracy of the experiment[16]. In this study, conventional biochemical experiments and molecular biological identification methods were combined to explore the pathogenicity and drug sensitivity of the two isolated strains, quickly and accurately judge the cause, and drugs with good inhibitory effect against the pathogen were screened through drug sensitivity tests, providing data support for the prevention and treatment of diarrhea disease in ducks. It also provides a reliable basis for clinical treatment and diagnosis.

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