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Drug Resistance Detection and Class I integron Gene Cassette Analysis of Sika Deer Derived *Escherichia coli*

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HZ, YW, WL: Sample collection. RW, HZ, YW and BG: Drug resistance phenotype detection. HZ and WS: Integron detection. HZ: Prepared first draft. YW: Article revision. BG: Multi-drug resistance data analysis. WS: Sequence analysis. DW: Analysis of integrons-gene cassettes. YZ: Designed the research route, provided guidance on research content and methods, analyzed data, revised the manuscript.

Key words
Sika deer, *E. coli*, Drug resistance, Integration, Gene cassettes

ABSTRACT

The current study shows that the resistance of *Escherichia coli* is closely related to the integron gene cassette. To evaluate the drug resistance and integron carriage of *E. coli* isolated from Sika deer in 4 deer farms in Heilongjiang Province, antibiotic resistance phenotypes and the presence of various types of integrons were investigated. The test strains were collected from fresh feces of Sika deer from four different farms in Heilongjiang Province. Among them, 130 trains of *E. coli* originating from Sika deer were successfully isolated and purified. The test strains were tested for drug sensitivity of 12 different antimicrobial drugs by the KB disc diffusion method. Then PCR was used to detect E. coli three types of integrase genes (*intI1, intI2 and intI3*) and three sequencing analysis of the class I integron gene cassette. Susceptibility test results show that the test strains in this experiment was resistant to ten drugs. The detection rate of class I integrons was 33.85%, while class II integrons and class III integrons were not detected. The detection rate of class I integron-gene cassette as 26.15%. The sequence analysis showed that strains carried different integrator-gene cassette: dfrA1-aadA1 and dfrA27.In this study, the resistance level of *E. coli* obtained from Sika deer was found to be relatively low. Class I integrons as identified, which carried relatively limited kinds of gene cassettes. The experimental results demonstrated a strong correlation between class I integrons and drug resistance of *E. coli*. Therefore, investigating class I integrons provide a valuable guide to studying the pread and the expression of resistance genes and thus finding effective measures to prevent bacterial resistance.

INTRODUCTION

Deer species are one of the most economically valuable animal groups. In China, there has been a steady increase in demand for deer products, making them one of the major economic animals (Wu *et al*., 2023). *Escherichia coli* is a Gram-negative bacterium that is widely present in the gastrointestinal tract of humans and animals. It is a facultative pathogen that lacks pathogenicity under normal conditions. However, when the immune function of the host is compromised, *E. coli* can cause various intestinal and extraintestinal diseases (Yin and Wan, 2019). As a typical strain to detect drug resistance, its drug resistance can reflect the current situation of drug resistance, and it is also the source and reservoir of drug resistance genes (Laopiem *et al*., 2025). The increasing changes in antibiotic resistance of *E. coli*, the simultaneous emergence of multi-drug resistance, and the rapid horizontal transmission of antibiotic resistance in *E. coli* have been widely concerned. The spread of drug resistance in *E. coli* mainly depends on the resistance genes in its body, which is usually caused by the rapid spread of resistance genes carried by plasmids, transposons and integrons. Integrons have attracted much attention in the study of the mechanism of drug resistance genes in *E. coli*. Integron is a kind of genetic recombination system that can be inherited, which can specifically bind to and capture drug resistance genes and be integrated into its own genome, and then complete the expression of drug resistance genes under the catalysis of integrase (Chang *et al*., 2007). In this study, analysis of the antibiotic resistance profile and analysis of gene cassettes carried by

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class I integrons in Sika deer *E. coli* strains isolated from four deer farms were conducted to provide a database and research direction for controlling multidrug resistance in Sika deer derived *E. coli* strains.

MATERIAL AND METHODS

Strains

E. coli quality control strain (ATCC25922), purchased from the Chinese Veterinary Drug Administration. *E. coli* 130 strains for testing,isolated from four Sika deer farms.

Drug-sensitive test

The WHO-recommended Kirby-Bauer diffusion method was used to detect the resistance of 130 strains of *E. coli* of Sika deer origin to 12 drugs. 12 drug-sensitive tablets were purchased from Hangzhou Tianhe Microbial Reagent Co., LTD. The overnight culture was evenly applied to the surface of the M-H agar medium and then incubated at 37℃ for 18-24 h. The diameter of the inhibition circle was measured and the susceptible or resistant profile was determined based on the standard range of the American Clinical and Laboratory Standards Institute (CLSI, 2021).

DNA extraction

The boiling method was used for DNA extraction (Dolejska *et al*., 2007). Two colonies of pure isolated bacteria were put into a tube containing 100 μL of double-distilled water. Tubes were heated to 100 °C for 10 min and then centrifuged. The supernatant containing DNA was stored at −20 °C.

Identification of integrase genes

Integrases are site-specific recombinases in which *E. coli* can integrate a free gene cassette associated with drug resistance into its genome, catalyzed by an integrase. Each class of integron has a corresponding integrase, such as class I integrase (IntI1), class II integrase (IntI2), and class III integrase (IntI3).The 130 isolated *E. coli* strains were tested for the integron genes.The primer sequences for the drug resistance genes and integrons were designed according to references (Ren *et al*., 2013; Li *et al*., 2013) and their sequence information is shown in Table I. IntI1, intI2, and intI3 were amplified with primers with amplicon sizes of 280 bp, 288 bp, and 600 bp, respectively. PCR amplification system (25μL): 10×buffer 2. 5 μL, dNTP 2.5 mM, rTaqase1075 U/μL, 2μLbacterial DNA,0.5μL (stock solution 10 μmol/L) each of upstream and downstream primers, made up to 25 μL with deionized water. The PCR amplification program for intI1 and intI2 was as follows: pre-denaturation at 94 ℃ for 3 min, denaturation at 94 ℃ for 1 min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min, 35cycles, and final extension at 72℃ for 10 min. The procedure for PCR amplification of intI3 was: pre-denaturation at 94℃ for 12 min, denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 30s, extension at 72 ℃ for 1 min, 30 cycles, and final extension at 72 ℃ for 8 min. Among the 130 *E. coli* strains tested, the rate of detection of the integrase gene was calculated based on the number of intI1, intI2, and intI3 integrase gene-positive samples detected in all strains.

Table I. Primer information.

Gene name	Primer sequences (5`→3`)	Annealing temp.	Length of output
IntI1	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	55.0 ℃	280 bp
IntI2	F:TTGCGAGTATCCATAACCTG R:TTACCTGCACTGGATTAAGC	55.0 ℃	288bp
IntI3	F:AGTGGGTGGCGAATGAGTG R:TGTTCTTGTATCGGCAGGTG	60.0 ℃	600bp
Class I integrons	F: GGCATCCAAGCAGCAAG R: AAGCAGACTTGACCTGA	55.4℃	Variable

Class I gene cassette detection and sequencing analysis

130 *E. coli* strains were tested for integron-gene cassette and negative control experiments. Specific primers for the variable region of class I integron were designed according to reference (Sun *et al*., 2022). The primer information is shown in Table I. PCR amplification system (25μL): 10×buffer 2.5 μL, dNTP 2.5 mM, rTaqase 5 U/μL, 2 μL bacterial DNA, 0.5 μL (stock solution 10 μmol/L) each of upstream and downstream primers, made up to 25 μL with deionized water. The procedure of PCR amplification was: pre-denaturation at 94 ℃ for 5 min; denaturation at 94℃ for 30 s, annealing at 55.4 ℃ for 30 s, extension at 72 ℃ for 1 min, 30 cycles. The final elongation was 10 min at 72 ℃. Electrophoresis was performed in TAE buffer using 1% agarose gel; then the gel was stained and observed. The detection rate was calculated according to the observation results.

The positive PCR product DNA fragment was recovered, ligated to pMD-18T vector, and cloned into recipient cells *E. coli* DH5α. The receptor cells were cultured on LB solid medium containing ampicillin for 12 h, then single colonies were picked and cultured on LB liquid medium containing ampicillin for 12 h, finally, the plasmids were extracted and sent to Harbin Comate Biosciences Co. Ltd. for sequencing. The sequences obtained were analyzed by EditSeq software and compared to the National Centerfor Biotechnology Information

(NCBI) database.

RESULT AND DISCUSSIN

Drug resistance in E. coli

The results of the drug sensitivity tests of Sika deer *E. coli* drugs are shown in Figure 1. The drug resistance of 130 strains in this study was generally low, and the drug resistance of aztreonam and gentamicin was 0. The proportion of strains resistant to the remaining ten drugs was not more than 15%.Resistant rate from high to low in turn to ciprofloxacin (13.08%), norfloxacin (11.54%), ofloxacin (10.77%), tetracycline (8.46%), ampicillin (6.15%), amikacin (4.62%), compound sulfamethoxazole(3.85%), kanamycin (2.31%), chloramphenicol(1.54%), Ceftazidime (0.77%).

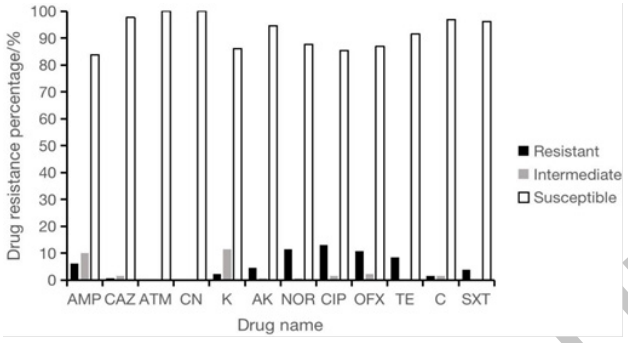

Fig. 1. Results of susceptibility test for 12 antibiotics of *E. coli* derived from Sika deer sources: AMP, Ampicillin; CAZ, ceftazidime; ATM, aztreonam; CN, gentamicin, K, kanamycin, AK, amikacin, NOR, norfloxacin, CIP, ciprofloxacin; OFX, ofloxacin, TE, tetracyclines; C,. chloramphenicol, SXT, compound sulfamethoxazole.

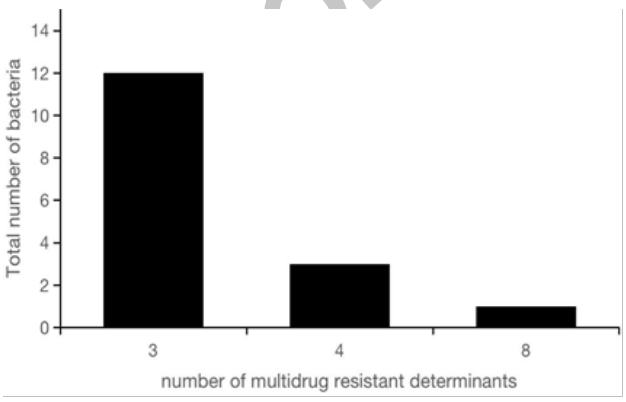

Fig. 2. Results of multidrug resistance tests for Sika deer-derived *E. coli*.

The results of the multi-drug resistance test for *E. coli* in Sika deer are shown in Figure 2. In this study, only 12.31% of the strains were multi-drug resistant.

Table II shows the antimicrobial resistance class pattern distribution for 130 multidrug-resistant *E. coli* isolates from the Sika deer.

Table II. Antimicrobial resistance class pattern distribution for 130 multidrug-resistant *E. coli* isolates from Sika deer.

Antimicrobial resistance class pattern	No. of isolates for each pattern	Total n=130 (%)
NOR-CIP-OFX	12	9.23
AMP-CIP-TE-SXT	1	0.77
AK-NOR-CIP-OFX	1	0.77
AMP-CAZ-TE-C	1	0.77
AMP-AK-NOR-CIP-OFX-TE-C-SXT	1	0.77

Integrase genes in E. coli

Among 130 strains of *E. coli* of Sika deer origin, the number of positive samples for integrase gene intI1 was 44 positive, with a detection rate of 33.85%. The results of the PCR assay are shown in Figure 3A. The size of the amplified PCR product is 280bp, which was in line with the expected size. The integrase gene intI2 was not detected in the 130 Sika deer-derived E. coli strains. The integrase gene intI3 was not detected in any of the 130 Sika deer-derived *E. coli* strains.

Class I integron-gene cassette

Among the tested *E. coli* isolates of Sika deer origin, bands of 2 different fragment lengths were detected in the PCR products of the class I integrons positive samples. The number of class I integrons positive samples was 34 out of 130 strains of *E. coli* of Sika deer origin, with a statistical positivity rate of 26.15% .

As expected, the PCR products amplified were 1586 bp, 715 bp and 153 bp in size. PCR amplification results are shown in Figure 3B.

The sequencing results of class I integrons were compared and analyzed, and the homology with class I integrons in the GenBank database was fully matched, and the combination mode of class I integron-gene cassette obtained was dfrA1-aadA1 and dfrA27. The sizes of class I integron-gene cassette fragments were 1586 bp and 715 bp. The sample quantity is 3 and 15, respectively (Table III).

The strains resistant to three or more antibiotics were defined as multi-drug resistant bacteria, and the number of multi-drug resistant bacteria in this experiment was 16. Among them, triple drug resistance accounted for the

largest proportion, the number was 2.

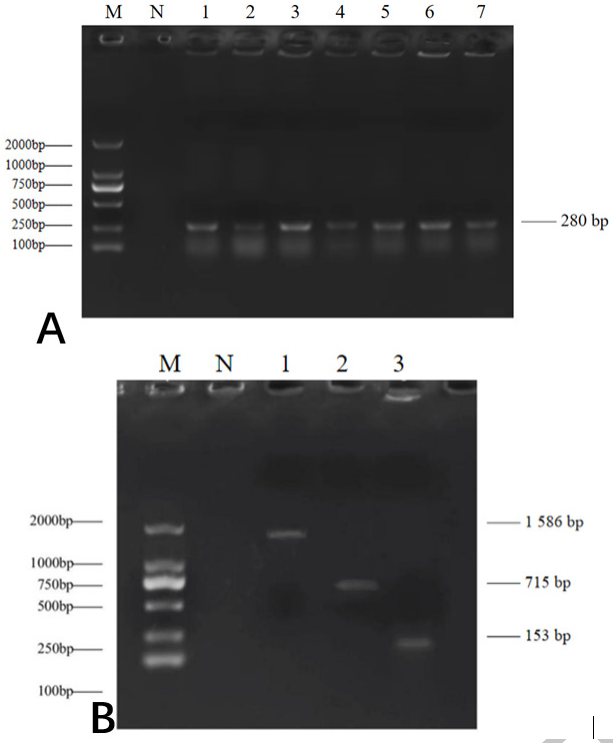

Fig. 3. PCR results for integrase gene intI1 (A), and class I integrons gene (B).
M, DL2000 Marker; N, Negative control; Lanes in A 1-7, Integrase gene intI1 in different individuals B, 3, The class I integrons gene in different individuals.

Table III. The gene cassettes carried by integrons.

Category	Gene cassette	Fragment size	No. of resistant strains
1	dfrA1-aadA1	1586bp	3
2	dfrA27	715bp	15
3	empty integron	153bp	16

The above results showed that the resistance rate of *E. coli* from Sika deer was generally low in these four farms, and multi-drug resistance was not common. There may be two reasons for this. First, China has banned the addition of antibiotics to feed in 2020, so Sika deer will not be added antibiotics to their daily feed, which greatly reduces the possibility of large-scale growth of drug-resistant bacteria. Second: Although the farming pattern of farm-raised sika deer is similar to the domestic ruminants (e.g., cattle, sheep); there are still some uniqueness in its farming production (Li *et al*., 2013). Sika deer breeding mode has a certain uniqueness, the main purpose of breeding sika deer in our country is to obtain deer antler, rather than meat production, the feeding cycle is usually longer than two years, so we will not apply antibiotics to improve growth performance, unless the disease outbreak will be treated with antibiotics, the normal growth environment of sika deer is not exposed to antibiotics. This also prevents outbreaks of resistant bacteria.

As shown in Figure 3, neither class II nor class III integrons were detected. In contrast, 33.85% (n= 44/130) of the assays were detected positive for the presence of intI1, which is a marker for class I integrons. The number of class I integrons positive samples was 17 out of 130 strains of *E. coli* of Sika deer origin, with a statistical positivity rate of 26.15% (n=34/130).

Integrons were first identified because of their central role in assembling and disseminating antibiotic resistance genes in commensal and pathogenic bacteria (Ghaly *et al*., 2021). Integrons are potentially mobile-end genetic elements capable of capturing and spreading specific exogenous gene cassettes, often located on transposons, and have been found to be located at sites of gene segment-specific incorporation and excision (Tadesse *et al*., 2012). The integron consists of three important parts, including an integrase gene (intI), which defines a site-specific recombinase, and an attI site, which is detected by the integrase and acts as a receptor. Integrase detects and acts as a acceptor for the gene cassette, as well as a promoter region (PC) (Koeleman *et al*., 2001). As a recombinant system, it can excision, integrate and express a variety of drug resistance genes, which is extremely important for the capture and spread of drug resistance genes. Based on the results of this assay, a comparative analysis of the GenBank nucleic acid sequence database revealed that the gene cassette arrays of class I integrons that emerged from this assay were in the form of dfrA1-aadA1 and dfrA27. In the experiment, the cassette structure was relatively simple, and only two bands with lengths of 1586bp and 715bp appeared. This suggests that the horizontal transmission of resistance in *E. coli* is closely related to the integron system. The proteins encoded by gene cassettes can rapidly produce drug resistance between bacteria. Among various mechanisms that are involved in the dissemination of ARGs, integrons play a vital role (Laopiem *et al*., 2025). Relatively few gene cassettes were detected in this experiment, which may be one of the reasons for the low rate of drug resistance in the results of drug susceptibility.

It is worth mentioning that there are empty integrons in the experimental strains, with a probability of 12.3% (n=16/130). In previous studies and reports, a large number of empty integrons were found to be widespread. In past studies and reports, empty integron was found in a large

number of Gram-negative bacteria, which may be related to drug resistance genes of bacteria and the selection pressure of antibiotics (Tadesse *et al*., 2012). Empty integrons have the ability to capture resistance genes from the environment and pose a potential threat to the growth of resistant bacteria.

Antibiotics were once misused by the feeding industry, but this haseased since China banned them in feed in 2020. The number of drug-resistant *E. coli* was significantly reduced. However, the integron system still has potential threats in the capture of drug resistance genes, and it can not be ignored for drug resistance detection. We should be aware of the harm of misuse of antibiotics, strictly abide by rules and regulations, and improve preventive measures. In the environment of the farm, veterinary staff should use drugs rationally, strictly limit the use of antibiotics, avoid single drug and substandard drug dosage. New antimicrobial drugs, lysozyme preparations, herbal preparations, and microecological preparations should be developed, and therapeutic strategies to replace antimicrobial drugs should be actively sought in the future to address the problem of resistance from a new perspective (Zhu *et al*., 2021). Reduce the possibility of drug resistant bacteria outbreak, and do everything possible to ensure the safety of human public health.

DECLARATIONS

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Declaration of ethical approval

The author declares that the research content of the article meets ethical requirements.

Data availability statement

All data generated or analyzed during this study are included in this article.

Declaration of randomized controlled trials

This study does not apply to randomized controlled trials.

Generative AI and AI-assisted technology statement

The authors declare that no genrative AI was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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