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SHORT COMMUNICATION

Drug Resistance and Genetic Relatedness of *Escherichia coli* from Mink in Northeast China

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ABSTRACT

To analyze the drug resistance and Pulsed Field Gel Electrophoresis (PFGE) typing of *Escherichia coli* (*E. coli*) from mink, this study collected healthy mink feces from four farms in Northeast China (Liaoning Province, Jilin Province and Heilongjiang Province) from 2016 to 2019. 126 *E. coli* strains ranging from 155 mink feces were identified by laboratory isolation and culture and 16S rDNA PCR and sequencing analysis. All strains were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method and PFGE to determine their genomic relatedness. The results showed that 126 strains had different rates of resistance to 15 antimicrobials. They were highly resistant to ampicillin, tetracycline, doxycycline, chloramphenicol and cotrimoxazole, with resistance rates above 60%; they were more susceptible to amikacin, amitriptyline, doxycycline, gentamicin and imipenem, with sensitivity rates above 60%. 26 randomly selected highly resistant strains had different PFGE typing, and 14 of them had homology coefficients lower than 50%. These results suggest that mink-derived *E. coli* in the Northeast China have antimicrobial resistance and high genetic diversity.

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INTRODUCTION

Generally, *E. coli* is parasitic in the intestinal tract of humans and warm-blooded animals and is excreted and dispersed with feces. Therefore, *E. coli* is frequently used as a detection indicator of direct or indirect fecal contamination in environmental hygiene and food sanitation (Lu *et al*., 2007).

As a key furbearing region in China, antibiotic drugs play an important role in the prevention and treatment of mink diseases caused by E. coli in the Northeast (Cantas *et al*., 2013). The long-term irrational use of antibiotics is the main reason for the development of drug-resistant bacteria, which form resistance to self-protection in order to adapt to the environment and antibiotic pressure (Bélanger *et al*., 2011). Bacterial resistance of human and animal origin, resulted from improper use of antibacterial drugs, has become an important public health problem, and it is of great practical significance to establish a sound bacterial resistance monitoring system in China.

Pulsed Field Gel Electrophoresis (PFGE), which traces strains by comparing the similarity of DNA fragments, is known as the "gold standard" for bacterial typing (Healy *et al*., 2005). In this study, PFGE typing was carried out based on the detection of drug resistance of *E. coli* derived from mink, which provided basic data for the study of drug resistance.

MATERIALS AND METHODS

Isolates and identification of *E. coli*: A total of 155 healthy mink feces were collected from four farms in Northeast China (Liaoning Province, Jilin Province and Heilongjiang Province) between 2016 and 2019. The quality control strain ATCC25922 *E. coli* and the PFGE standard strain ATCC H9812 were kept in this laboratory.

Isolation and culture of bacteria: The freshly collected stool samples were seeded onto McConkey medium and incubated at 37°C for 12 hours, then the pink colonies were collected and inoculated on Erythromelan medium and incubated at 37°C for 12 hours. Finally, the purple-black colonies with metallic luster were picked and placed on LB solid medium at 37°C for 12 to 16 hours for purification of *E. coli*.

16S rDNA based identification of *E. coli*: To further confirm the identification results, *E. coli* conserved gene primers 16S rDNA were designed (F:5'-

AGAGTTTGATCATGGCTCAG-3', R:5'-GGACTACCA GGGTATCTAAT-3 '). The primer sequences were synthesized by Shanghai Yingjun Biotechnology Co., Ltd. and the target fragment length was 1503 bp. The PCR products were sent to Jilin Kumi Biotechnology Co., Ltd. for gene sequencing, and the sequencing return results were compared with the *E. coli* sequences registered in the GenBank database.

Antimicrobial susceptibility testing*:* The susceptibility of 126 isolates to 15 antibiotics was tested by the Bauer–Kirby disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021). The following drugs were used: ampicillin (AMP，10ug/ tablets), ceftazidime (CAZ,30ug/ tablets), amoxicillin (AMC,20ug/ tablets), imipenem (IPM,30ug/ tablets), aztreonam (ATM,30ug/ tablets), gentamicin (CN,10ug/ tablets), kanamycin (K,30/ tablets), amikacin (AK,30ug/ tablets), norfloxacin (NOR,10ug/ tablets), ciprofloxacin (CIP,5ug/ tablets), ofloxacin (OFX,5ug/ tablets), tetracycline (TE10ug/ tablets), doxycycline (DO,10ug/ tablets), chloramphenicol (C,30ug/ tablets), trimesulf (SXT,1.25/23.75ug/ tablets). *E. coli* strain ATCC25922 were used for quality control and the diameter of the inhibition ring was measured by vernier calipers. The susceptibility was classified into Susceptible (S), Intermediate (I) and Resistance (R) according to CLSI and phenotypes of isolates were analyzed.

Pulsed Field Gel Electrophoresis (PFGE)*:* Twenty-six strains of *E. coli* with high drug resistance were randomly selected for PFGE typing analysis. The *E. coli* was inoculated on M-H medium for 16 h at 37℃, followed by picking appropriate colonies with TE buffer to an optical density of 1.3-1.4 at 610 nm. Small gels were prepared by mixing the bacterial suspension with 1 % SeaKem Gold in a 1:1 ratio by volume. After that, 2 mg/mL of Proteinase K was added and mixed thoroughly, added to the gel molds and left to stand for 20 min at room temperature. The solidified gels were placed in 5mL of cell lysis solution and 5μL of proteinase K in a 54℃ water bath shaker for 2h. After lysis, the gels were washed three times with ddH2O and TE buffer repeatedly for 15 min, respectively. Subsequently, the reaction was carried out with 5μL of restriction endonuclease Xba I in a water bath at 37℃ for 3 h. After addition of samples, electrophoresis was carried out in 14℃ for 19 h. At the end of the reaction, the samples were stained with Gene green stain for 30 min and decolorized with ddH20 for 30 min, and the images were read under UV light and photographed. Based on the images, the phylogenetic tree was drawn with Bionumerics 3.0 analysis software to analyze the homology among strains.

RESULTS

Bacterial isolation and identification: The 155 healthy mink fecal samples were isolated and cultured, and *E. coli* was seen as pink round, neatly edged, smooth and moist surface colonies on McConkey medium; dark purple, metallic shiny colonies with moist and smooth surface and larger colonies on Erythromelan medium; white, moist and smooth colonies on LB solid medium. Combined with the result that the 16S rDNA had 99%~100% sequence homology with *E. coli* in GenBank database, 126 *E. coli* isolates of mink origin were finally obtained, and they were all mixed in 30% glycerol and stored in -20℃ refrigerator.

Analysis of antimicrobial resistance: The results of 126 *E. coli* isolates drug sensitivity tests from Heilongjiang, Jilin and Liaoning provinces are shown in Fig. 1. The isolates were more than 60% resistant to ampicillin, tetracycline and cotrimoxazole, and more than 60% sensitive to aminoglutethimide, amikacin and gentamicin. After pooling the drug resistance data of these isolates, the results are shown in Fig. 1. The resistance rates of mink-derived *E. coli* to five antimicrobial drugs, namely ampicillin, tetracycline, cotrimoxazole, chloramphenicol and doxycycline, were higher in the range of 61.9% to 88.89%, with the highest resistance rates of 88.89% for ampicillin and tetracycline; the resistance rates to six antibacterial drugs of ciprofloxacin, norfloxacin, ofloxacin, amoxicillin, ceftazidime and kanamycin were not different from each other in 41.27%~55.56%; the resistance rates to gentamicin, imipenem, aminotrans, amikacin 4 antibacterial drugs was lower in 2.38%~26.98%, among which the lowest resistance rate to amikacin was 2.38%.

The results of multiple resistance ratio of 126 *E. coli* strains of mink origin to the 15 antimicrobial drugs tested are shown in Fig. 2 where all strains were resistant, distributed from 1 to 13 resistance.

A total of 115 isolates were resistant to three or more clinical antimicrobial drugs, accounting for 91.27% (115/126) of all strains. Among them, there were 17 strains that were resistant to 9 clinical antimicrobial drugs at the most, accounting for 13.49% (17/126) of all strains. The strains that were resistant to 13 antimicrobial drugs at the least were only 2 strains, accounting for 1.59% (2/126).

Analysis of PFGE: Based on the result of drug sensitivity tests, 26 highly resistant *E. coli* strains of mink origin were randomly selected for PFGE typing. According to the PFGE criteria recommended by Talon, strains with band similarity greater than 85% can be identified as clonal strains, while strains with band similarity less than 50% can be considered epidemiologically unrelated (Talon *et al*., 1996). All 26 strains of mink-derived *E. coli* were able to be typed by Xba I , indicating that all 26 strains of *E. coli* had typing ability. The PFGE typing results and corresponding resistance profiles are shown in Fig. 3. The number of DNA fragments obtained by enzymatic cleavage for each strain ranged from 14 to 20, but none of the strains had a homology coefficient of more than 85%, and all strains had different PFGE typing, indicating the complexity of the strain genotypes. The homology coefficients between 9M, 30S, 12E, 22S, 21S, 32M, 16S, 21E, 17E, 23E, 6E and 30E strains ranged from 50% to 85%, indicating that they were from the same genus. The remaining 14 strains of mink origin had homology coefficients below 50% and were considered epidemiologically unrelated. And strains numbered 17S, 12E and 21E, 18S and 16S had the same resistance profile.

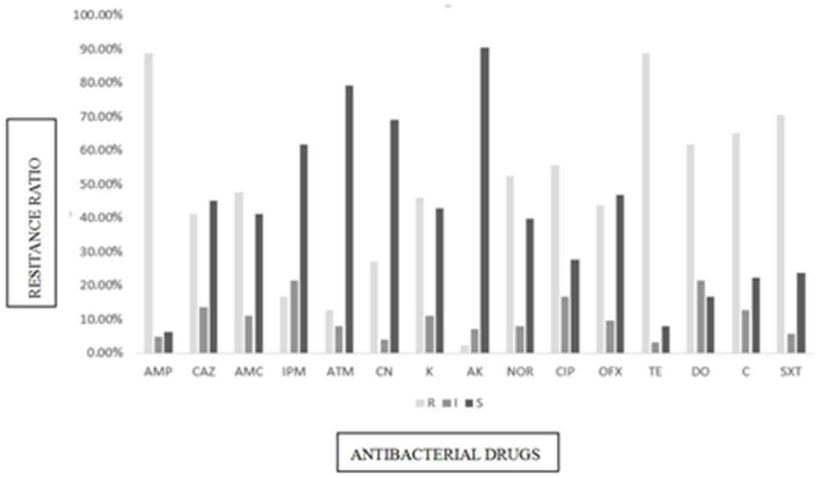

Fig. 1: The quantity and proportion of resistant strains of *Escherichia coli* from mink in Northeast China.

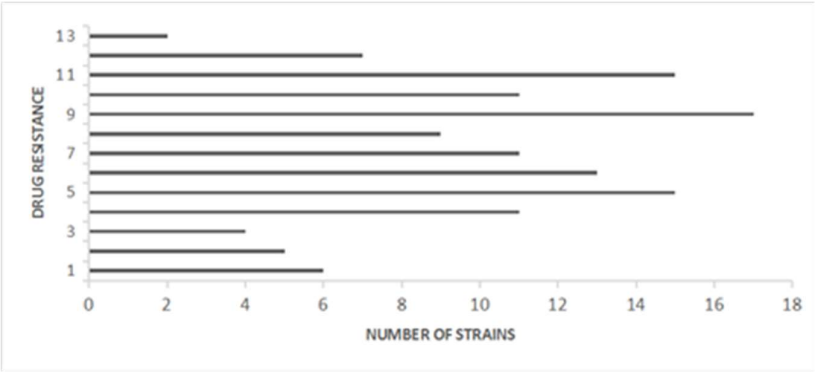

Fig. 2: The number of multiple drug-resistant strains of 126 *Escherichia coli* from mink.

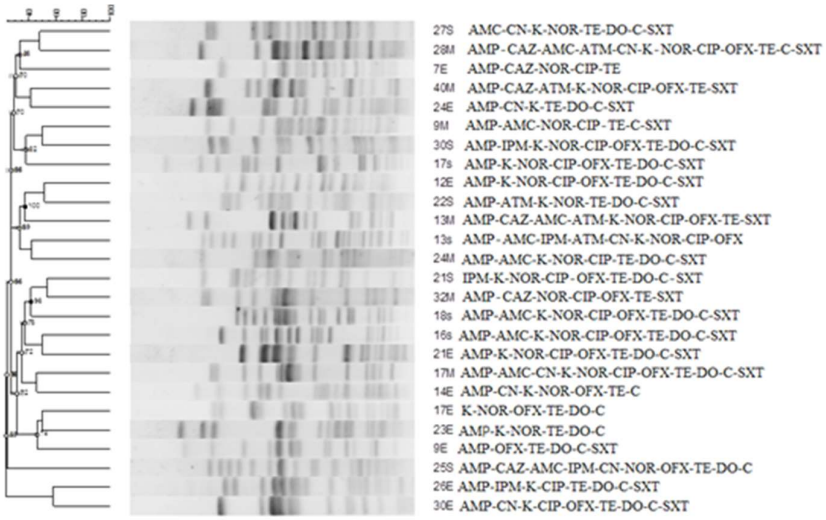

Fig. 3: PFGE results and drug resistance spectrum.

DISCUSSION

In the mink industry, *E. coli* is one of the major diseases that affect farmed mink, and each year, the occurrence of *E. coli*-related diseases causes significant economic losses directly or indirectly to the mink industry. Therefore, in the farming industry, understanding and monitoring the current status and changing trend of drug resistance of mink-derived *E. coli* is of practical significance to guide the rational clinical use of drugs and control the spread of drug resistance.

In this study, the Kirby-Bauer paper diffusion method was used to detect the drug resistance phenotypes of 126 mink-derived *E. coli* strains. From the result of drug sensitivity testing, drug resistance existed in mink-derived *E. coli* in Northeastern China, and the resistance rates of

ampicillin, tetracycline, doxycycline, chloramphenicol and compound trimoxazole were greater than 60%. However, the strains still maintained high susceptibility to some antimicrobial drugs, 90.48% for amikacin, 79.37% for amytriptyline, 69.05% for gentamicin and 61.90% for imipenem. E. coli of fox origin isolated from Northeast China by An Fuyu et al. were more than 60% resistant to aminopenicillin, cefothiophene, kanamycin, tetracycline, chloramphenicol, doxycycline and compound trimoxazole and more than 70% susceptible to amikacin, imipenem, polymyxinB and aztreonam (An *et al.,* 2019). The resistance rates of deer-derived E. coli isolated from Northeast China by Fan Junwen for sulfamethoxazole, ampicillin, tetracycline, and chloramphenicol were at more than 60% (Fan *et al.,* 2008).Overall comparing the above results with the present experimental results, although there are deviations in individual data, which may be related to the actual situation of different farms and differences in different species, it can still be seen that the drug resistance situation in the Northeast is basically the same. In public health safety, it provides a data basis for preventing bacterial diseases and guiding veterinary clinical drug use.

The result of drug sensitivity tests showed that the 115 strains were resistant to three or more antimicrobial drugs. Among them, 9 drug-resistant strains were the most abundant with 17 strains, accounting for 13.49% (17/126) and 5 drug-resistant and 11 drug-resistant strains each had 15 strains, accounting for 11.90% (15/126) each. The number of resistant strains was concentrated between 4 and 11 resistant strains in a bell-shaped distribution, indicating the presence of drug resistance in *E. coli* of mink origin, which poses a problem for veterinary clinical use. The multi-drug resistance rate in this study was 91. 27%, which was higher than the 85.48% detected by Jiang Zhiyu *et al*. for mink-derived *E. coli* in Zhucheng, Shandong (Jiang *et al*., 2019), but lower than the 100% detected by Liu Boxing et al. for mink-derived *E. coli* in Hebei (Liu *et al*., 2020). Although the three sets of data were not identical, they all showed the same high rate of multi-drug resistance, which can be reasonably suspected to be related to the general irregular management and drug abuse in mink farming in China.

In this study, 26 strains of mink-derived *E. coli* with high drug resistance were typed using PFGE technique. The test result showed that all 26 strains of mink-derived E. coli had the ability to be typed into 26 different PFGE typing, indicating that the isolates were genetically different and had complex genetic diversity. 14 strains of mink-derived *E. coli* were below 50% similarity and considered epidemiologically unrelated, and the remaining 12 strains of mink-derived E. coli 9M and 30S, 12E and 22S, 21S and 32M, 16S and 21E, 17E and 23E, and 26E and 30E had homology coefficients between 50% and 85%, meaning that these *E. coli* were from the same genus. *E. coli* numbered 17E, 23E, 26E and 30E were isolates from the same mink farm, indicating that there was horizontal transmission between *E. coli* in this farm, but their ability to influence the transmission was not strong. In contrast, *E. coli* numbered 9M, 30S, 12E, 22S, 21S, 32M, 16S and 21E were from different mink farms, yet possessed some homology, showing that there was cross-farm transmission of the strains. PFGE is the "gold-standard" for bacterial typing; it has demonstrated superiority and staying power compared to other methods which were developed at the same time. Protocol harmonisation across laboratories has led to its wide usage for phylogenetic studies, infection control, outbreak investigation and also for food safety surveillance.

Conclusions: The results of susceptibility testing and PFGE typing showed that the epidemiology of strains with the same resistance spectrum was not correlated, and the resistance profiles of the same genus were different. Therefore, it is necessary to establish a more detailed and complete surveillance system for the typing of resistant bacteria.

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Declaration of Competing Interest: The authors declare no conflict of interest with this research.

Authors' Contribution: Y Xue and X Li conceived and designed the study. X Li done the amplification and sequencing of antimicrobial resistance genes. X Zhu done Pulsed-Field Gel Electrophoresis. X Li collected the clinical samples. Y Xue, X Li and X Zhu analyzed the data. X Zhu and X Li wrote the article.

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