Genotypic Diversity and Antimicrobial Resistance of Escherichia Coli Isolated From Pigs in Hubei, China

Xiaoyue Li  
Huazhong Agricultural University

Zewen Liu  
Hubei Academy of Agricultural Sciences

Ningning Wang  
Huazhong Agricultural University

Wei Liu  
Hubei Academy of Agricultural Sciences

Ting Gao  
Hubei Academy of Agricultural Sciences

Danna Zhou  
Hubei Academy of Agricultural Sciences

Keli Yang  
Hubei Academy of Agricultural Sciences

Rui Guo  
Hubei Academy of Agricultural Sciences

Wan Liang  
Hubei Academy of Agricultural Sciences

Weicheng Bei  
Huazhong Agricultural University

Fangyan Yuan (fangyanyuan12@163.com)  
Hubei Academy of Agricultural Sciences  
https://orcid.org/0000-0002-9548-8234

Yongxiang Tian  
Hubei Academy of Agricultural Sciences

Research

Keywords: Escherichia coli, Antibiotic resistance, MLST

Posted Date: October 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-942081/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background

Intestinal infections with *Escherichia coli* (*E. coli*) are mostly occur in piglets between 1~10 days old, which major lead to diarrhea and edema in newborn piglets. These diseases caused by *E. coli* can increase mortality, morbidity and growth delay of piglets, which are responsible for economic losses. In this study was to investigate the prevalence of antibiotic resistance, transmission mechanisms, and molecular epidemiology of *E. coli* strains isolated from pig farms in Hubei province. Furthermore, clonal and genetic diversity of isolates were identified.

Results

A total of 29 *E. coli* isolates were obtained from fecal of weaned piglets from Hubei province. The *E. coli* isolates in different regions demonstrate different genetic diversity. Multilocus sequence typing (MLST) presented that ST165 was the common sequence type, accounting for 27.6% of all *E. coli* isolates, followed by ST744, ST1081, ST101 and ST10. All of the isolates were resistant to the tested antibiotics to vary degrees, and more than 80% of *E. coli* isolates presented high resistance rates to ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin. There was one *E. coli* strain that was resistant to the fifteen antimicrobial agents tested. Overall, most of the isolates were conferring resistance to 5-7 antimicrobial agents tested.

Conclusions

Our study reported *E. coli* isolates with high antimicrobial resistance and explores the genetic diversity of *E. coli* isolated from swine-origin. From the results obtained it can be concluded that these isolates present high prevalent multi-drug resistance. These data provide a greater understanding of the genetic diversity and antimicrobial resistance of *E. coli*.

Highlights

- Most of *E. coli* isolates advent high prevalent multi-drug resistance
- All of the isolates were resistant to the tested antibiotics to vary degrees, and more than 80% of *E. coli* isolates presented high resistance rates to ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin.
- These *E. coli* isolates in this study presented ampicillin, sulfaisoxazole and tetracycline of several old drugs with a high resistance rate (100%).
- The *E. coli* isolates in different regions demonstrate different genetic diversity

Introduction

*Escherichia coli* (*E. coli*) is one of the major commensal bacteria that normally operates in the digestive tract of normal humans and warm-blooded animals [1,2,3]. Intestinal infections with *E. coli* principally occur in piglets between and 1 ~ 10 days old, which major lead to diarrhea and edema in newborn piglets [4]. *E. coli* can be classified as commensal *E. coli* and pathogenic *E. coli* based on the differences in pathogenicity [5]. Pathogenic *E. coli* includes at six major categories on the basis of the virulence mechanisms and prospective progression to infection: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), adherent invasive *E. coli* (AIEC) [6]. Furthermore, several types of *E. coli* have been causing infections in humans, as a public health problem, which has been influencing all aspects of life, causing severe economic losses to the world [7].

Several studies of epidemiological studies on *E. coli* have been initiated in parts of China. In Northeastern China, a survey showed that the separation rate of *E. coli* isolated from pig fecal samples reached 88% [8]. An investigation on pig farms in Henan province found that the positive rate of *E. coli* was 70.74%, of which the commonly sequenced types (STs) 10 and 101 were identified [9]. However, data on genetic diversity and antimicrobial resistance of *E. coli* is still restricted in the Hubei province of central China.

In the last few decades, antimicrobial resistance has emerged as one of the considerable global threats to human health [10]. The enhancing antimicrobial resistance of Gram-negative bacteria isolated from animals, which can be transmission from animal to human via the food chain and the environment [11]. In subsequent years, antibiotic resistance has expanded with the application of antibiotics in piglets. In Great Britain, the antimicrobial resistance of *E. coli* isolates from pigs seems higher than that of *E. coli* isolates from cattle and sheep [12]. Therefore, it is important to comprehend the antibiotic resistance of pathogenic *E. coli* in pig farms in China. From 2011 to 2012, a survey of drug resistance in pigs demonstrated that the great majority of swine-origin *E. coli* isolates resistance to tetracycline (79.57%), trimethoprim-sulphamethoxazole (73.12%) and kanamycin (55.91%) [13]. A survey in Guangdong Province showed that a total of 333 *E. coli* isolates were acquired from pig farms from 2013 to 2016, which were all multidrug-resistant strains [14]. Multidrug-resistance Gram-negative bacterial clinical isolates are responsible for high rates globally [15] and comprise a challenge for animal treatment.
Multilocus sequence typing (MLST) has been used to study the evolution and epidemiology of a number of bacterial pathogens. It has become the method of choice for typing epidemiologically important strains. MLST is a standard molecular subtyping technique that identifies the genetic relatedness of strains and determines the strains with high discriminatory power\(^{[16]}\). Investigating the trend characterization of epidemic strains will help us to better understand epidemiology.

This present study was carried out to investigate the prevalence and characteristics of \textit{E. coli}, the MLST genotypes and antibiotic resistance of \textit{E. coli} isolates were examined, collected from 2018 to 2019 in Hubei province of China. These findings provide information and implication for safeguarding and commanding the occurrence of diseases in future studies.

**Methods**

**Isolation of \textit{Escherichia coli}**

From 2018 to 2019, a total of 29 \textit{E. coli} isolates were collected from pig fecal samples in Hubei province. These strains isolated by MacConkey agar incubated at 37°C and further identified by PCR test as previously described\(^{[17]}\) with some slight modifications. Total DNA was available by boiling the lysis of isolated colonies for 10 min in distilled water. The samples were then centrifuged at 12,000×rpm/min for 5 min and the supernatant was transferred into a new 1.5 mL tube to be used as a template and applied for the following reactions.

**Antibiotic resistance profiles**

According to the guidelines of the Clinical and Laboratory Standards Institute\(^{[18]}\), the confirmed \textit{E. coli} was identified for antimicrobial susceptibility. \textit{E. coli} isolates were examination for susceptibility to antimicrobial drugs utilizing a disk diffusion assay. All samples were analyzed for the presence of resistant bacteria. A total of 18 antimicrobials were tested, comprising cefuroxime (CXM), ceftriaxone (CRO), cephalothin (CEP), cefotaxime (CTX), ampicillin (AMP), amoxicillin (AMX), lincomycin (MY), doxycycline (DOC), tetracycline (TEC), kanamycin (KMC), gentamicin (GEN), amikacin (AMK), ciprofloxacin (CIP), enoxacin (ENO), lomefloxacin (LOM), azithromycin (AZM), sulfafurazole (SFN). Inoculated plates were incubated at 37 °C for 24 h, subsequently the diameters (in mm) of the inhibition zone were measured. Based on the minimal inhibitory concentration determined for each drug, the isolates were classified as “susceptible”, “intermediate”, or “resistant”. The \textit{E. coli} strain ATCC 25922 was utilized for quality control. Multidrug-resistant (MDR) of an isolate was designated as clinical resistance to at least one agent in three or more antimicrobial categories\(^{[19]}\).

**MLST and Phylogenetic tree**

The multilocus sequence typing (MLST) was executed on 29 \textit{E. coli} isolates according to the \textit{E. coli} MLST database guidelines (http://enterobase.warwick.ac.uk/), accreting to the protocols published on the web site. Briefly, the seven house-keeping genes adk, fumC, gyrB, icd, mdh, purA and recA were amplified employing a PCR protocol, and the amplicons sequenced utilizing the amplication primers. Investigated individual gene sequences and allocated an allelic profile number in line with the MLST database. Sequence type (STs) and clone complexes (CCs) designations of each strain were comprised of seven alleles. The calculated tree of the \textit{E. coli} resistant isolates was constructed by applying the UPGMA cluster analysis based on seven housekeeping gene sequences.

**Result**

**Isolation of \textit{Escherichia coli}**

As shown in Table 1, \textit{E. coli} isolates were obtained from fecal of weaned piglets from Hubei province. 29 strains of \textit{E. coli} were separated from positive samples and further identified by PCR tests as \textit{E. coli}. There are 3 strains of \textit{E. coli} isolated from Suizhou city, 6 strains of \textit{E. coli} isolated from Xiangyang city, 15 strains of \textit{E. coli} isolated from Wuhan city and 5 strains of \textit{E. coli} isolated from Yichang city.

| Sources | Sampling site (City) | No. of positive samples |
|---------|----------------------|-------------------------|
| Fecal   | Suizhou              | 3                       |
|         | Xiangyang            | 6                       |
|         | Wuhan                | 15                      |
|         | Yichang              | 5                       |
|         | **Total**            | **29**                  |

Table 1: The prevalence of \textit{E. coli} in pig farms in Hubei province.
Antimicrobial Susceptibility profile of Escherichia coli isolates

As shown in Table 2, E. coli in different regions has separate antibiotic resistance. All of the E. coli isolates showed resistance to lincosamides, tetracyclines, and sulfonamide. As shown in the Fig. 1, a high rate of resistance to ampicillin, lincomycin, doxycycline, tetracycline, sulfasoxazole, amoxicillin and gentamicin has also been observed for the isolates, among which 100.00% of the E. coli was resistant to ampicillin, lincomycin, doxycycline, tetracycline, sulfasoxazole; 93.10% of the E. coli was resistant to amoxicillin; and 58.62% of the E. coli was resistant to gentamicin. The isolates demonstrated a relatively low rate of resistance to cefuroxime (37.93% of the E. coli), cephalothin (37.93% of the E. coli), kanamycin (37.93% of the E. coli), enoxacin (34.48% of the E. coli), ciprooxacin (20.69% of the E. coli), lomeoxacin (20.69% of the E. coli), azithromycin (20.69% of the E. coli), amikacin (17.24% of the E. coli), ceftriaxone (17.24% of the E. coli) and cefotaxime (3.45% of the E. coli).

Table 2

Antimicrobial resistance of E. coli isolates with different regions

| Classes       | Members | Suizhou (n=3) | Xiangyang (n=6) | Wuhan (n=15) | Yichang (n=5) |
|---------------|---------|---------------|-----------------|--------------|---------------|
|               |         | No. of resistant isolates | Resistance rates (%) | No. of resistant isolates | Resistance rates (%) | No. of resistant isolates | Resistance rates (%) | No. of resistant isolates | Resistance rates (%) | No. of resistant isolates | Resistance rates (%) |
| β-lactams     | cefuroxime | 2 | 66.7 | 2 | 33.3 | 3 | 20.0 | 4 | 80.0 |
|               | ceftriaxone | 1 | 33.3 | 0 | 0.0 | 0 | 0.0 | 4 | 80.0 |
|               | cephalothin | 2 | 66.7 | 2 | 33.3 | 3 | 20.0 | 4 | 80.0 |
|               | cefotaxime | 1 | 33.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
|               | ampicillin | 3 | 100.0 | 6 | 100.0 | 15 | 100.0 | 5 | 100.0 |
|               | amoxicillin | 3 | 100.0 | 4 | 66.7 | 15 | 100.0 | 5 | 100.0 |
| lincosamides  | lincomycin | 3 | 100.0 | 6 | 100.0 | 15 | 100.0 | 5 | 100.0 |
| tetracyclines | doxycycline | 3 | 100.0 | 6 | 100.0 | 15 | 100.0 | 5 | 100.0 |
|               | tetracycline | 3 | 100.0 | 6 | 100.0 | 15 | 100.0 | 5 | 100.0 |
| aminoglycosides | kanamycin | 3 | 100.0 | 3 | 50.0 | 1 | 6.7 | 4 | 80.0 |
|               | gentamicin | 1 | 33.3 | 3 | 50.0 | 8 | 53.3 | 5 | 100.0 |
|               | amikacin | 0 | 0.0 | 0 | 0.0 | 1 | 6.7 | 4 | 80.0 |
| quinolones    | ciprofloxacin | 3 | 100.0 | 1 | 16.7 | 0 | 0.0 | 2 | 40.0 |
|               | enoxacin | 3 | 100.0 | 2 | 33.3 | 0 | 0.0 | 5 | 100.0 |
|               | lomeoxacin | 3 | 100.0 | 1 | 16.7 | 0 | 0.0 | 2 | 40.0 |
| macrolides    | azithromycin | 3 | 100.0 | 3 | 50.0 | 0 | 0.0 | 0 | 0.0 |
| sulfonamides  | sulfasoxazole | 3 | 100.0 | 6 | 100.0 | 15 | 100.0 | 5 | 100.0 |

As shown in the Fig. 2, all of the isolates were resistant to test antibiotics to varying degrees, and 100% of the isolates were resistant to more than six drug classes. There was one E. coli strain that was resistant to the fifteen antimicrobial agents tested. Overall, most of the isolates were conferring resistance to 5–7 antimicrobial agents tested. The most frequent multidrug resistance pattern was resistance to ampicillin, amoxicillin, lincomycin, doxycycline, tetracycline, gentamicin and sulfasoxazole, which covered 8 isolates.

Sequence Types of Escherichia coli

The genetic diversity of these E. coli isolates was analyzed with MLST, and the details of MLST results have been listed in Table 3. A total of 29 E. coli isolates were analyzed utilizing MLST, the identification of 12 sequence types (STs). Sequence type 165 (8/29, 27.6%) was the most frequent ST, followed by ST744 (4/29, 13.8%), ST1081 (3/29, 10.3%) and ST101 (3/29, 10.3%). Each of the following STs accounted for 6.9% (2/29): ST10, ST46 and ST533. The other strains were individually classified into 5 different STs (ST410, ST617, ST1990, ST3744 and ST533) (Table 3).
from 2014 to 2015, isolates exhibited resistance to tetracycline (50%), sulfamethoxazole (49%), ampicillin (26%), gentamicin (17%), ciprofloxacin.

In 2011, Danish scholar Agers raised the ST10 from human infections in China. In this study, the most frequent ST was ST10 (2/29, 6.9%), followed by ST744 (8/171, 4.7%), ST101 (7/171, 4.1%), ST165 (5/171, 2.9%). In Yang’s study, the common ST was ST10 (22/171, 12.9%), followed by ST101 (7/53, 13.21%). The application of MLST in isolates better comprehending the genetic diversity of these E. coli isolates. In this study, the most frequent ST was ST10, followed by ST744, ST101, ST165 and ST515, and then the other strains were individually classified into 5 different STs.

Discussion

Escherichia coli is one of the main pathogenic bacteria that impact the production and growth of pigs in pig farms. It is associated with gastrointestinal diseases such as diarrhea, edema disease, and systemic infections such as septicemia and polyserositis. These diseases caused by E. coli can increase mortality, morbidity and growth delays of piglets, which are responsible for economic losses. This study analyzed the prevalence, genetic diversity and antibiotic resistance of disease, which may help us to improve methods of prevention and treatment.

The distribution of swine-origin E. coli in pigs differs between countries and regions. From 2002 to 2008, the prevalence of E. coli isolated from pork chop samples was 44% in the United States. However, various incidence rates have also been reported in different regions of China. From 2003 to 2009, the prevalence of E. coli isolates from pig farms was 77.78% in China. From 2013 to 2016, the positive rates of E. coli between farm 1 and farm 2 were 40.25% and 59.75% in Guangdong province. Between 2016 and 2017, a survey indicated that the separation rate of E. coli isolated from pig fecal swabs reached 88% in northeastern China, including Heilongjiang, Jilin and Liaoning. In this study, we collected samples from the fecal of weaned piglets, and a total of 29 strains of E. coli were isolated from 4 cities in Hubei province.

Diarrhea in weaned piglets driven by E. coli remains a principal cause of economic losses for the pig industry. This commonly seeks antimicrobial drug treatment, which is considerable to cure pathogen animals. In 2011, Danish scholar Agerse et al. study found that 32% of isolates have multidrug resistance, mainly concentrated on ampicillin (27%) and tetracycline (29%). In 2012, Tadesse et al. tested 1729 isolates of E. coli antibiotic susceptibility varied from different sources, the resistance rate of E. coli increased from 7.2–63.6% but the most common resistance to tetracycline and sulfonamides.

Total of 131 E. coli isolates were obtained from the pigs presenting from diarrhea in Switzerland between 2016 and 2017, isolates exhibited resistance to tetracycline (50%), sulfamethoxazole (49%), ampicillin (26%), gentamicin (17%), ciprofloxacin.

Table 3

| ST type | Allele Profile | Clone Complex | No. of Isolates |
|---------|----------------|---------------|----------------|
| ST10    | 10,11,4,8,8,8,2 | CC-10         | 2              |
| ST46    | 8,7,1,8,8,8,6   | CC-46         | 2              |
| ST101   | 43,41,15,18,11,7,6 | CC-101       | 3              |
| ST165   | 10,27,5,10,12,8,2 | CC-165       | 8              |
| ST410   | 6,4,12,1,20,18,7 | CC-23         | 1              |
| ST515   | 57,11,1,109,7,8,2 | –             | 2              |
| ST617   | 10,11,4,8,8,13,73 | CC-10         | 1              |
| ST744   | 10,11,135,8,8,8,2 | –             | 4              |
| ST1081  | 6,4,5,18,11,8,2  | –             | 3              |
| ST1990  | 6,4,5,1,20,12,7  | –             | 1              |
| ST3744  | 10,11,135,8,8,8,2 | –             | 1              |
| ST5334  | 10,11,1,8,8,8,2  | –             | 1              |

Note. aAllele number for adK, fumC, gyrB, icD, mdH, purA, and recA, respectively (one for each ST). bClone complex.
However, this caused a rise in the employment of various antimicrobial agents, such as lincosamides, tetracyclines and sulfonamides, which may expand antimicrobial resistance.

In this study, *E. coli* isolates results of the antimicrobial susceptibility tests presented that the most prevalent antibiotic resistance was to lincosamides, tetracyclines, sulfonamides. More than 80% of *E. coli* isolates presented high resistance rates to ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin. All of the isolates were resistant to test antibiotics to vary degrees, and Most of *E. coli* isolates advent high prevalent multi-drug resistance. There was one *E. coli* strain that was resistant to the fifteen antimicrobial agents tested. Overall, more than half of the isolates were conferring resistance to 8–15 antimicrobial agents tested. The most frequent multidrug resistance pattern was resistance to ampicillin, amoxicillin, lincomycin, doxycycline, tetracycline, gentamicin and sulfaisoxazole, which covered 8 isolates. In some other studies in China, Jiang et al. revealed that *E. coli* isolates had high rates of resistance to ampicillin (99.5%), tetracycline (93.4%) and amoxicillin (65.1%). Resistance to cephalosporins, quinolones, and aminoglycodies was also quite prevalent. Meng et al. study results showed that the great majority of *E. coli* isolates resistance to tetracycline (79.57%), trimethoprim-sulfamethoxazole (73.12%) and kanamycin in China (55.91%) [13]. However, *E. coli* isolates showed the highest resistance to sulfamethoxazole (61.6%), followed by tetracycline (61.2%), ampicillin (48.2%) and kanamycin (22.4%) in Sichuan province between 2012 and 2013 [28]. *E. coli* were isolated from pig farms from seven provinces that the resistance rate to ampicillin was 81.44%, 94.37% to tetracycline and 88.36% to sulfaisoxazole [29]. These findings provide important information and implications for the application of antibiotics in future studies.

**Conclusions**

In this study, a high antimicrobial resistance and the genotypic diversity of *E. coli* were observed isolated from swine-origin in Hubei province. From the results obtained it can be concluded that these isolates present high prevalent multi-drug resistance. These data provide a greater understanding of the genetic diversity and antimicrobial resistance of *E. coli*.

**Abbreviations**

MLST: multilocus sequence typing

STs: sequence types

CCs: clonal complexes.

**Declarations**

**Author contribution**

The experiments were performed mainly by ZL, XL, NW and WL, and some experiments were performed with the help of TG and DZ, XL, ZL, RG, WL and WB performed the data analysis. The study was designed by FY and YT. All authors read and approved the final manuscript.

**Author details**

1. **Key Laboratory of Prevention and Control Agents for Animal Bacteriosis(Ministry of Agriculture and Rural Affairs), Institute of Animal Husbandry and Veterinary, Hubei Academy of Agricultural Sciences, Wuhan, 430064, China**

2. **Hubei Provincial Key Laboratory of Animal Pathogenic Microbiology, Institute of Animal and Veterinary, Hubei Academy of Agricultural Sciences, Wuhan, 430064, China**

3. **State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China**

4. **Cooperative Innovation Center of Sustainable Pig Production, Wuhan, 430070, China**

**Acknowledgements**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**
The authors declare that all data supporting the findings of this study are available within the article.

**Consent for publication**

Not applicable

**Ethics approval and consent to participate**

The experiments were approved by the Ethics Committee of Hubei Academy of Agricultural Sciences according to Hubei Province Laboratory Animal Management Regulations—2005.

**Funding**

This work was supported by grants from the National Key R&D Program of China (2017YFD0500605); Technical Innovation Project of Hubei Province (2020ABA016); the Natural Science Foundation of China (31672560) and Natural Science Foundation of Hubei Province (2018CFA045).

**Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**References**

1. Marques C, Belas A, Franco A, Aboim C, Gama LT, Pomba C. Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. J Antimicrob Chemother 2018;73:377-84.

2. Fairbrother JM, Nadeau E, Gyles CL. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev 2005;6:17-39.

3. <Characterization of Antibiotic Resistance Genes and Virulence Factors in Faecal Enterococci of Wild Animals in Portugal.pdf>.

4. <Different kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic Escherichia coli strain or an F18 verotoxigenic Escherichia coli strain.pdf>.

5. Alteri CJ, Mobley HL. Escherichia coli physiology and metabolism dictates adaptation to diverse host microenvironments. Curr Opin Microbiol 2012;15:3-9.

6. Pasqua M, Michelacci V, Di Martino ML, Tozzozi R, Grossi M, Colonna B, et al. The Intriguing Evolutionary Journey of Enteroinvasive E. coli (EIEC) toward Pathogenicity. Front Microbiol 2017;8:2390.

7. Yang SC, Lin CH, Aljuffali IA, Fang JY. Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. Arch Microbiol 2017;199:811-25.

8. Cheng P, Yang Y, Cao S, Liu H, Li X, Sun J, et al. Prevalence and Characteristic of Swine-Origin mcr-1-Positive Escherichia coli in Northeastern China. Front Microbiol 2021;12:712707.

9. Hu Z, Peng Z, Zhang X, Li Z, Jia C, Li X, et al. Prevalence and Molecular Characterization of Antimicrobial-Resistant Escherichia coli in Pig Farms, Slaughterhouses, and Terminal Markets in Henan Province of China. Foodborne Pathog Dis 2021.

10. <The antimicrobial resistance containment and surveillance approach — a public health tool.pdf>.

11. Umpierrez A, Bado I, Oliver M, Acquistapace S, Etcheverria A, Padola NL, et al. Zoonotic Potential and Antibiotic Resistance of Escherichia coli in Neonatal Calves in Uruguay. Microbes Environ 2017;32:275-82.

12. Enne VI, Cassar C, Sprigings K, Woodward MJ, Bennett PM. A high prevalence of antimicrobial resistant Escherichia coli isolated from pigs and a low prevalence of antimicrobial resistant E. coli from cattle and sheep in Great Britain at slaughter. FEMS Microbiol Lett 2008;278:193-9.

13. <Characterization of Shiga toxin-producing Escherichia coli isolated from healthy pigs in China.pdf>.

14. Zhang Y, Sun YH, Wang JY, Chang MX, Zhao QY, Jiang HX. A Novel Structure Harboring blaCTX-M-27 on IncF Plasmids in Escherichia coli Isolated from Swine in China. Antibiotics (Basel) 2021;10.

15. De Waele JJ, Boelens J, Leroux-Roels I. Multidrug-resistant bacteria in ICU: fact or myth. Curr Opin Anaesthesiol 2020;33:156-61.
Yun KW, Kim DS, Kim W, Lim IS. Molecular typing of uropathogenic Escherichia coli isolated from Korean children with urinary tract infection. Korean J Pediatr 2015;58:20-7.

Yang GY, Guo L, Su JH, Zhu YH, Jiao LG, Wang JF. Frequency of Diarrheagenic Virulence Genes and Characteristics in Escherichia coli Isolates from Pigs with Diarrhea in China. Microorganisms 2019;7.

<Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.pdf>.

Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria, an international expert proposal for interim standard definitions for acquired resistance.pdf>.

Zhao S, Blickenstaff K, Bodeis-Jones S, Gaines SA, Tong E, McDermott PF. Comparison of the prevalences and antimicrobial resistances of Escherichia coli isolates from different retail meats in the United States, 2002 to 2008. Appl Environ Microbiol 2012;78:1701-7.

Zhao QY, Li W, Cai RM, Lu YW, Zhang Y, Cai P, et al. Mobilization of Tn1721-like structure harboring blaCTX-M-27 between P1-like bacteriophage in Salmonella and plasmids in Escherichia coli in China. Vet Microbiol 2021;253:108944.

Peng Z, Liang W, Hu Z, Li X, Guo R, Hua L, et al. O-serogroups, virulence genes, antimicrobial susceptibility, and MLST genotypes of Shiga toxin-producing Escherichia coli from swine and cattle in Central China. BMC Vet Res 2019;15:427.

Yu T, He T, Yao H, Zhang JB, Li XN, Zhang RM, et al. Prevalence of 16S rRNA Methylase Gene rmtB Among Escherichia coli Isolated from Bovine Mastitis in Ningxia, China. Foodborne Pathog Dis 2015;12:770-7.

<DANMAP 2012 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.pdf>.

Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, et al. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. Emerg Infect Dis 2012;18:741-9.

Brand P, Gobeli S, Perreten V. Pathotyping and antibiotic resistance of porcine enterovirulent Escherichia coli strains from Switzerland (2014-2015). Schweiz Arch Tierheilkd 2017;159:373-80.

Jiang HX, Lu DH, Chen ZL, Wang XM, Chen JR, Liu YH, et al. High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China. Vet J 2011;187:99-103.

Zhang A, He X, Meng Y, Guo L, Long M, Yu H, et al. Antibiotic and Disinfectant Resistance of Escherichia coli Isolated from Retail Meats in Sichuan, China. Microb Drug Resist 2016;22:80-7.

Zhang P, Shen Z, Zhang C, Song L, Wang B, Shang J, et al. Surveillance of antimicrobial resistance among Escherichia coli from chicken and swine, China, 2008-2015. Vet Microbiol 2017;203:49-55.

Figures
Figure 1

Antimicrobial resistance of E. coli isolates. The resistance rate of E. coli isolates to 17 agents. CXM, cefuroxime; CRO, ceftriaxone; CEP, cephalothin; CTX, cefotaxime; AMP, ampicillin; AMX, amoxicillin; MY, lincomycin; DOC, doxycycline; TEC, tetracycline; KMC, kanamycin; CEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; ENO, enoxacin; LOM, lomefloxacin; AZM, azithromycin; SFN, sulfafurazole.
Figure 2
Antimicrobial resistance of E. coli isolates. Multidrug resistance of E. coli isolates to 17 agents.

Figure 3
Dendrogram of multilocus sequence typing (MLST) profiles among the 29 E. coli isolates. UA means unallocated in E. coli.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.pdf