

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

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## Research

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# 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, sulfisoxazole 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 <sup>[1,2,3]</sup>. 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 <sup>[4]</sup>. *E. coli* can be classified as commensal *E. coli* and pathogenic *E. coli* based on the differences in pathogenicity <sup>[5]</sup>. 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) <sup>[6]</sup>. 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 <sup>[7]</sup>.

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% <sup>[8]</sup>. 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 <sup>[9]</sup>. 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 <sup>[10]</sup>. 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 <sup>[11]</sup>. 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 <sup>[12]</sup>. 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-sulfamethoxazole (73.12%) and kanamycin (55.91%) <sup>[13]</sup>. 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 <sup>[14]</sup>. Multidrug-resistance Gram-negative bacterial clinical isolates are responsible for high rates globally <sup>[15]</sup> 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<sup>[16]</sup>. 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 *E. coli*, the MLST genotypes and antibiotic resistance of *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 *Escherichia coli*

From 2018 to 2019, a total of 29 *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<sup>[17]</sup> 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<sup>[18]</sup>, the confirmed *E. coli* was identified for antimicrobial susceptibility. *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 *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<sup>[19]</sup>.

## MLST and Phylogenetic tree

The multilocus sequence typing (MLST) was executed on 29 *E. coli* isolates according to the *E. coli* MLST database guidelines (<http://enterobase.warwick.ac.uk/>

species/ecoli/allele st search), 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 amplification 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 *E. coli* resistant isolates was constructed by applying the UPGMA cluster analysis based on seven housekeeping gene sequences.

## Result

### Isolation of *Escherichia coli*

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

Table 1  
The prevalence of *E. coli* in pig farms in Hubei province

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

## 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, sulfaisoxazole, 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, sulfaisoxazole; 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*), ciprofloxacin (20.69% of the *E. coli*), lomefloxacin (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 (%)
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
	lomefloxacin	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 vary 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 sulfaisoxazole, 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 ST515. The other strains were individually classified into 5 different STs (ST410, ST617, ST1990, ST3744 and ST533) (Table 3).

Table 3  
Diversity profiles of *E. coli* isolates based on MLST

ST type	Allele Profile <sup>a</sup>	Clone Complex <sup>b</sup>	No. of Isolates
ST10	10,11,4,8,8,2	CC-10	2
ST46	8,7,1,8,8,6	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. <sup>a</sup>Allele number for adK, fumC, gyrB, icD, mdH, purA, and recA, respectively (one for each ST). <sup>b</sup>Clone complex.

The identification of 12 ST types belonged to 5 clone complexes and unassigned clone complexes. To further analyzing STs utilized the UPGMA cluster analysis (Fig. 3). 29 identified STs were classified into three major groups. Group 1 covered a great majority of STs, containing 24 isolates, belonging to the CC-10, CC-46, CC-165 and unassigned clone complexes. CC-10 contained 2 of our STs (ST10 and ST617). Group 2 included 3 isolates that only belonged to one CC-101. There are 2 isolates included CC-23 and unassigned clone complexes constitute Group 3.

## 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 [2]. 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 [20]. 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 [21]. 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 [14]. 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 [8]. 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.

The application of MLST in *E. coli* isolates better comprehending the genetic diversity of these *E. coli* isolates. In this study, the most frequent ST was ST165, followed by ST744, ST1081, ST101, ST10, ST46 and ST515, and then the other strains were individually classified into 5 different STs. It reveals that there is diversity in ST types in Hubei province. In some other surveys, ST101 (7/53, 13.21%) [22] and ST101 (3/32, 9.4%) [14] were common STs, these are similar to our result ST101 (3/29, 10.3%). In Yang's study, the common ST was ST10 (22/171, 12.9%), followed by ST744 (8/171, 4.7%), ST101 (7/171, 4.1%), ST165 (5/171, 2.9%) [17]. Recently, *E. coli* ST10 has not only been presented by animals in China but also raised the ST10 from human infections in China [23]. Zhang et al. showed that the most prevalent ST was ST10 (16/32, 50%) [14]. However, ST10 (2/29, 6.9 %) of the result in this study seem to conflict with those from most previous studies. The potential reason for these results is the limitation of the data and region.

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 multi-drug resistance, mainly concentrated on ampicillin (27%) and tetracycline (29%) [24]. 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, streptomycin and sulfonamides [25]. Total of 131 *E. coli* isolates were obtained from the pigs presenting from diarrhea in Switzerland from 2014 to 2015, isolates exhibited resistance to tetracycline (50%), sulfamethoxazole (49%), ampicillin (26%), gentamicin (17%), ciprofloxacin

(8%)<sup>[26]</sup>. 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 aminoglycosides was also quite prevalent<sup>[27]</sup>. 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%)<sup>[13]</sup>. 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<sup>[28]</sup>. *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<sup>[29]</sup>. 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.

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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.

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### Publisher's Note

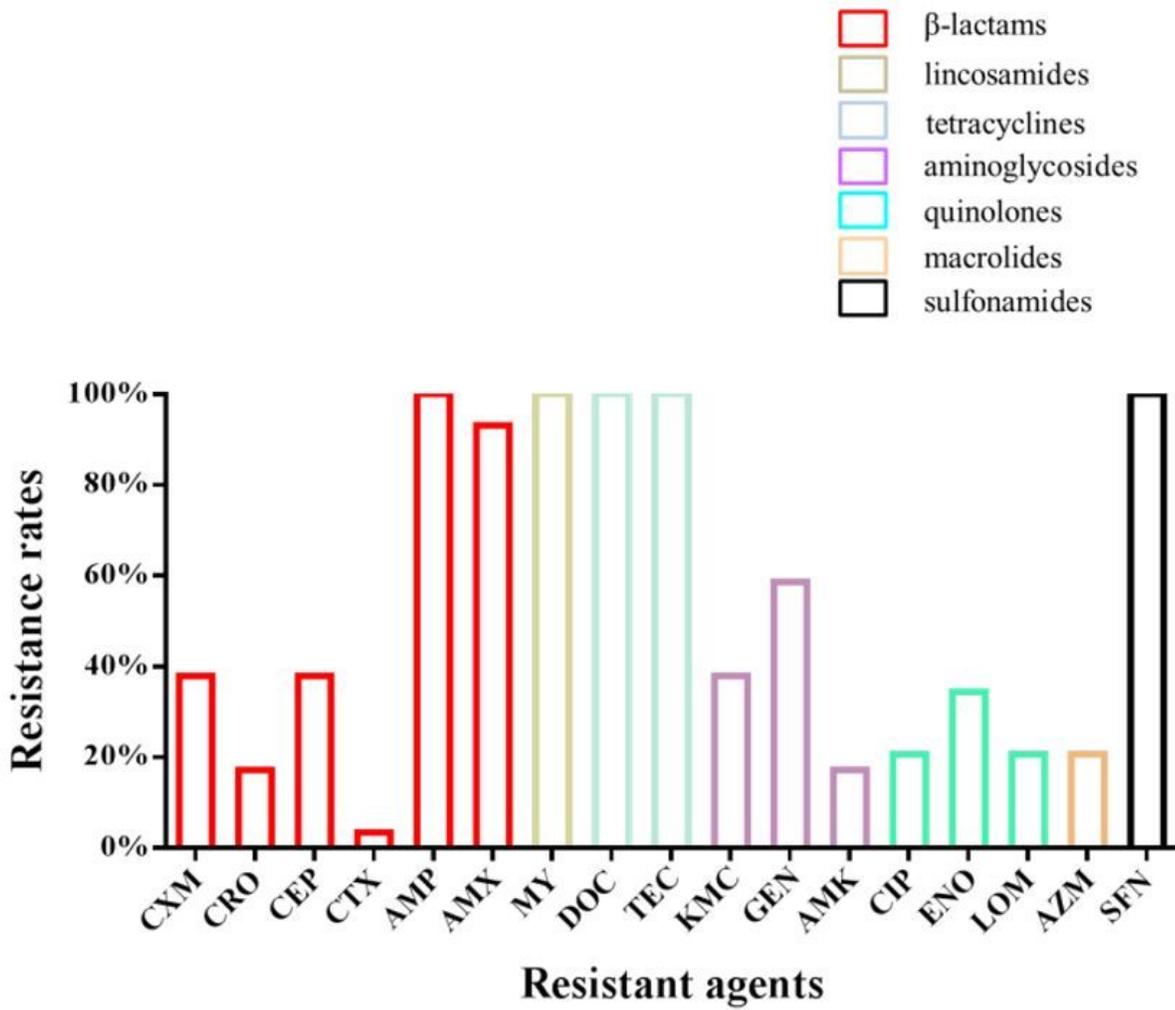
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## Figures



**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; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; ENO, enoxacin; LOM, lomefloxacin; AZM, azithromycin; SFN, sulfafurazole.

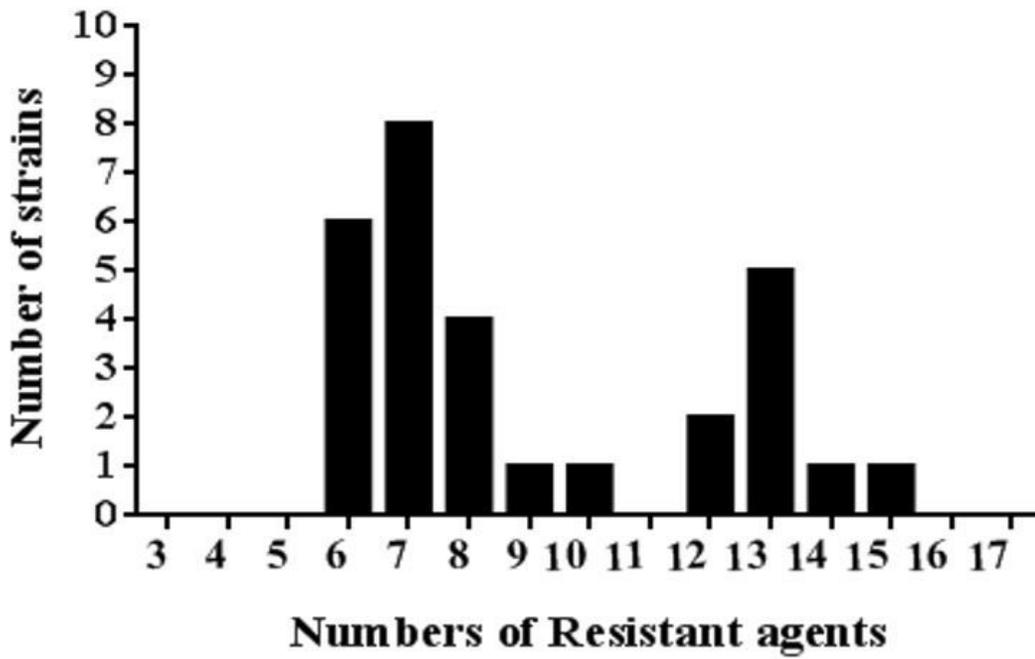


Figure 2

Antimicrobial resistance of *E. coli* isolates. Multidrug resistance of *E. coli* isolates to 17 agents.

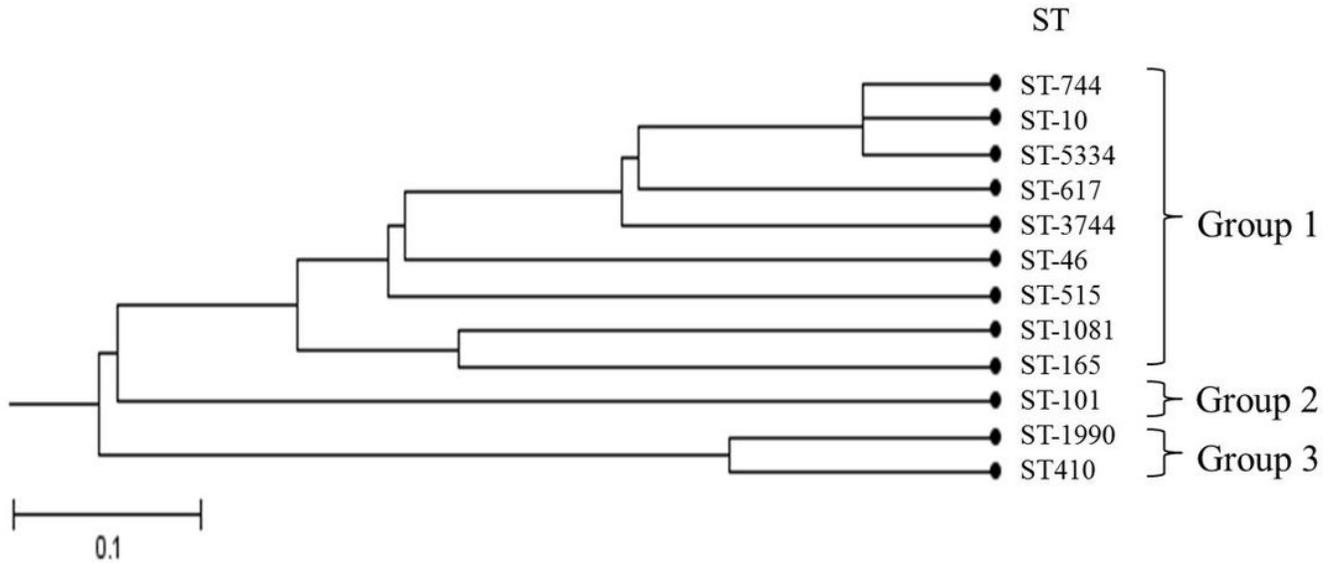


Figure 3

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](#)