

1 **Genotypic diversity and antimicrobial resistance of *Escherichia coli* isolated from**
2 **pigs in Hubei, China**

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19
20 **Highlights**

- 21
22 ● Most of *E. coli* isolates advent high prevalent multi-drug resistance
23 ● All of the isolates were resistant to the tested antibiotics to vary degrees, and
24 more than 80% of *E. coli* isolates presented high resistance rates to ampicillin,
25 lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin.
26 ● These *E. coli* isolates in this study presented ampicillin, sulfisoxazole and
27 tetracycline of several old drugs with a high resistance rate (100%).
28 ● The *E. coli* isolates in different regions demonstrate different genetic diversity
29

30 **Abstract**

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

38 **Results:** A total of 29 *E. coli* isolates were obtained from fecal of weaned piglets from
39 Hubei province. The *E. coli* isolates in different regions demonstrate different genetic
40 diversity. Multilocus sequence typing (MLST) presented that ST165 was the common

41 sequence type, accounting for 27.6% of all *E. coli* isolates, followed by ST744,
42 ST1081, ST101 and ST10. All of the isolates were resistant to the tested antibiotics to
43 vary degrees, and more than 80% of *E. coli* isolates presented high resistance rates to
44 ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin.
45 There was one *E. coli* strain that was resistant to the fifteen antimicrobial agents tested.
46 Overall, most of the isolates were conferring resistance to 5-7 antimicrobial agents
47 tested.

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

53 **Keywords:** *Escherichia coli*, Antibiotic resistance, MLST

54 **Introduction**

55 *Escherichia coli* (*E. coli*) is one of the major commensal bacteria that normally
56 operates in the digestive tract of normal humans and warm-blooded animals ^[1, 2, 3].
57 Intestinal infections with *E. coli* principally occur in piglets between and 1~10 days
58 old, which major lead to diarrhea and edema in newborn piglets ^[4]. *E. coli* can be
59 classified as commensal *E. coli* and pathogenic *E. coli* based on the differences in
60 pathogenicity ^[5]. Pathogenic *E. coli* includes at six major categories on the basis of
61 the virulence mechanisms and prospective progression to infection: enteropathogenic
62 *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), Shiga
63 toxin-producing *E. coli* (STEC), diffusely adherent *E. coli* (DAEC), enteroaggregative
64 *E. coli* (EAEC), adherent invasive *E. coli* (AIEC) ^[6]. Furthermore, several types of *E.*
65 *coli* have been causing infections in humans, as a public health problem, which has
66 been influencing all aspects of life, causing severe economic losses to the world ^[7].
67 Several studies of epidemiological studies on *E. coli* have been initiated in parts of
68 China. In Northeastern China, a survey showed that the separation rate of *E. coli*
69 isolated from pig fecal samples reached 88% ^[8]. An investigation on pig farms in

70 Henan province found that the positive rate of *E. coli* was 70.74%, of which the
71 commonly sequenced types (STs) 10 and 101 were identified ^[9]. However, data on
72 genetic diversity and antimicrobial resistance of *E. coli* is still restricted in the Hubei
73 province of central China.

74 In the last few decades, antimicrobial resistance has emerged as one of the
75 considerable global threats to human health ^[10]. The enhancing antimicrobial
76 resistance of Gram-negative bacteria isolated from animals, which can be
77 transmission from animal to human via the food chain and the environment ^[11]. In
78 subsequent years, antibiotic resistance has expanded with the application of antibiotics
79 in piglets. In Great Britain, the antimicrobial resistance of *E. coli* isolates from pigs
80 seems higher than that of *E. coli* isolates from cattle and sheep ^[12]. Therefore, it is
81 important to comprehend the antibiotic resistance of pathogenic *E. coli* in pig farms in
82 China. From 2011 to 2012, a survey of drug resistance in pigs demonstrated that the
83 great majority of swine-origin *E. coli* isolates resistance to tetracycline (79.57%),
84 trimethoprim-sulfamethoxazole (73.12%) and kanamycin (55.91%) ^[13]. A survey in
85 Guangdong Province showed that a total of 333 *E. coli* isolates were acquired from
86 pig farms from 2013 to 2016, which were all multidrug-resistant strains ^[14].
87 Multidrug-resistance Gram-negative bacterial clinical isolates are responsible for high
88 rates globally ^[15] and comprise a challenge for animal treatment.

89 Multilocus sequence typing (MLST) has been used to study the evolution and
90 epidemiology of a number of bacterial pathogens. It has become the method of choice
91 for typing epidemiologically important strains. MLST is a standard molecular
92 subtyping technique that identifies the genetic relatedness of strains and determines
93 the strains with high discriminatory power ^[16]. Investigating the trend characterization
94 of epidemic strains will help us to better understand epidemiology.

95 This present study was carried out to investigate the prevalence and characteristics of
96 *E. coli*, the MLST genotypes and antibiotic resistance of *E. coli* isolates were
97 examined, collected from 2018 to 2019 in Hubei province of China. These findings
98 provide information and implication for safeguarding and commanding the occurrence

99 of diseases in future studies.

100 **Methods**

101 **Isolation of *Escherichia coli***

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

109 **Antibiotic resistance profiles**

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

125 **MLST and Phylogenetic tree**

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

128 species/ecoli/allele st search), accreting to the protocols published on the web site.
129 Briefly, the seven house-keeping genes *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*
130 were amplified employing a PCR protocol, and the amplicons sequenced utilizing the
131 amplification primers. Investigated individual gene sequences and allocated an allelic
132 profile number in line with the MLST database. Sequence type (STs) and clone
133 complexes (CCs) designations of each strain were comprised of seven alleles. The
134 calculated tree of the *E. coli* resistant isolates was constructed by applying the
135 UPGMA cluster analysis based on seven housekeeping gene sequences.

136 **Result**

137 **Isolation of *Escherichia coli***

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

143 **Antimicrobial Susceptibility profile of *Escherichia coli* isolates**

144 As shown in Table 2, *E. coli* in different regions has separate antibiotic resistance. All
145 of the *E. coli* isolates showed resistance to lincosamides, tetracyclines, and
146 sulfonamide. As shown in the Fig. 1, a high rate of resistance to ampicillin,
147 lincomycin, doxycycline, tetracycline, sulfaisoxazole, amoxicillin and gentamicin has
148 also been observed for the isolates, among which 100.00% of the *E. coli* was resistant
149 to ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole; 93.10% of the *E.*
150 *coli* was resistant to amoxicillin; and 58.62% of the *E. coli* was resistant to gentamicin.
151 The isolates demonstrated a relatively low rate of resistance to cefuroxime (37.93% of
152 the *E. coli*), cephalothin (37.93% of the *E. coli*), kanamycin (37.93% of the *E. coli*),
153 enoxacin (34.48% of the *E. coli*), ciprofloxacin (20.69% of the *E. coli*), lomefloxacin
154 (20.69% of the *E. coli*), azithromycin (20.69% of the *E. coli*), amikacin (17.24% of
155 the *E. coli*), ceftriaxone (17.24% of the *E. coli*) and cefotaxime (3.45% of the *E. coli*).
156 As shown in the Fig. 2, all of the isolates were resistant to test antibiotics to vary

157 degrees, and 100% of the isolates were resistant to more than six drug classes. There
158 was one *E. coli* strain that was resistant to the fifteen antimicrobial agents tested.
159 Overall, most of the isolates were conferring resistance to 5-7 antimicrobial agents
160 tested. The most frequent multidrug resistance pattern was resistance to ampicillin,
161 amoxicillin, lincomycin, doxycycline, tetracycline, gentamicin and sulfaisoxazole,
162 which covered 8 isolates.

163 **Sequence Types of *Escherichia coli***

164 The genetic diversity of these *E. coli* isolates was analyzed with MLST, and the
165 details of MLST results have been listed in Table 3. A total of 29 *E. coli* isolates were
166 analyzed utilizing MLST, the identification of 12 sequence types (STs). Sequence type
167 165 (8/29, 27.6%) was the most frequent ST, followed by ST744 (4/29, 13.8%),
168 ST1081 (3/29, 10.3%) and ST101 (3/29, 10.3%). Each of the following STs accounted
169 for 6.9 % (2/29): ST10, ST46 and ST515. The other strains were individually
170 classified into 5 different STs (ST410, ST617, ST1990, ST3744 and ST533) (Table 3).
171 The identification of 12 ST types belonged to 5 clone complexes and unassigned
172 clone complexes. To further analyzing STs utilized the UPGMA cluster analysis (Fig.
173 3). 29 identified STs were classified into three major groups. Group 1 covered a great
174 majority of STs, containing 24 isolates, belonging to the CC-10, CC-46, CC-165 and
175 unassigned clone complexes. CC-10 contained 2 of our STs (ST10 and ST617). Group
176 2 included 3 isolates that only belonged to one CC-101. There are 2 isolates included
177 CC-23 and unassigned clone complexes constitute Group 3.

178 **Discussion**

179 *Escherichia coli* is one of the main pathogenic bacteria that impact the production and
180 growth of pigs in pig farms. It is associated with gastrointestinal diseases such as
181 diarrhea, edema disease, and systemic infections such as septicemia and polyserositis
182 ^[2]. These diseases caused by *E. coli* can increase mortality, morbidity and growth
183 delays of piglets, which are responsible for economic losses. This study analyzed the
184 prevalence, genetic diversity and antibiotic resistance of disease, which may help us
185 to improve methods of prevention and treatment.

186 The distribution of swine-origin *E. coli* in pigs differs between countries and regions.
187 From 2002 to 2008, the prevalence of *E. coli* isolated from pork chop samples was 44%
188 in the United States ^[20]. However, various incidence rates have also been reported in
189 different regions of China. From 2003 to 2009, the prevalence of *E. coli* isolates from
190 pig farms was 77.78% in China ^[21]. From 2013 to 2016, the positive rates of *E. coli*
191 between farm 1 and farm 2 were 40.25% and 59.75% in Guangdong province ^[14].
192 Between 2016 and 2017, a survey indicated that the separation rate of *E. coli* isolated
193 from pig fecal swabs reached 88% in northeastern China, including Heilongjiang, Jilin
194 and Liaoning ^[8]. In this study, we collected samples from the fecal of weaned piglets,
195 and a total of 29 strains of *E. coli* were isolated from 4 cities in Hubei province.

196 The application of MLST in *E. coli* isolates better comprehending the genetic
197 diversity of these *E. coli* isolates. In this study, the most frequent ST was ST165,
198 followed by ST744, ST1081, ST101, ST10, ST46 and ST515, and then the other
199 strains were individually classified into 5 different STs. It reveals that there is
200 diversity in ST types in Hubei province. In some other surveys, ST101 (7/53, 13.21%)
201 ^[22] and ST101 (3/32, 9.4%) ^[14] were common STs, these are similar to our result
202 ST101 (3/29, 10.3%). In Yang's study, the common ST was ST10 (22/171, 12.9%),
203 followed by ST744 (8/171, 4.7%), ST101 (7/171, 4.1%), ST165 (5/171, 2.9%) ^[17].
204 Recently, *E. coli* ST10 has not only been presented by animals in China but also
205 raised the ST10 from human infections in China ^[23]. Zhang et al. showed that the most
206 prevalent ST was ST10 (16/32, 50%) ^[14]. However, ST10 (2/29, 6.9 %) of the result in
207 this study seem to conflict with those from most previous studies. The potential
208 reason for these results is the limitation of the data and region.

209 Diarrhea in weaned piglets driven by *E. coli* remains a principal cause of economic
210 losses for the pig industry. This commonly seeks antimicrobial drug treatment, which
211 is considerable to cure pathogen animals. In 2011, Danish scholar Agerse et al. study
212 found that 32% of isolates have multi-drug resistance, mainly concentrated on
213 ampicillin (27%) and tetracycline (29%) ^[24]. In 2012, Tadesse et al. tested 1729
214 isolates of *E. coli* antibiotic susceptibility varied from different sources, the resistance

215 rate of *E. coli* increased from 7.2% to 63.6% but the most common resistance to
216 tetracycline, streptomycin and sulfonamides ^[25]. Total of 131 *E. coli* isolates were
217 obtained from the pigs presenting from diarrhea in Switzerland from 2014 to 2015,
218 isolates exhibited resistance to tetracycline (50%), sulfamethoxazole (49%),
219 ampicillin (26%), gentamicin (17%), ciprofloxacin (8%) ^[26]. However, this caused a
220 rise in the employment of various antimicrobial agents, such as lincosamides,
221 tetracyclines and sulfonamides, which may expand antimicrobial resistance.

222 In this study, *E. coli* isolates results of the antimicrobial susceptibility tests presented
223 that the most prevalent antibiotic resistance was to lincosamides, tetracyclines,
224 sulfonamides. More than 80% of *E. coli* isolates presented high resistance rates to
225 ampicillin, lincomycin, doxycycline, tetracycline, sulfaisoxazole and ampicillin. All of
226 the isolates were resistant to test antibiotics to vary degrees, and Most of *E. coli*
227 isolates advent high prevalent multi-drug resistance. There was one *E. coli* strain that
228 was resistant to the fifteen antimicrobial agents tested. Overall, more than half of the
229 isolates were conferring resistance to 8-15 antimicrobial agents tested. The most
230 frequent multidrug resistance pattern was resistance to ampicillin, amoxicillin,
231 lincomycin, doxycycline, tetracycline, gentamicin and sulfaisoxazole, which covered
232 8 isolates. In some other studies in China, Jiang et al. revealed that *E. coli* isolates had
233 high rates of resistance to ampicillin (99.5%), tetracycline (93.4%) and amoxicillin
234 (65.1%). Resistance to cephalosporins, quinolones, and aminoglycosides was also
235 quite prevalent ^[27]. Meng et al. study results showed that the great majority of *E. coli*
236 isolates resistance to tetracycline (79.57%), trimethoprim-sulfamethoxazole (73.12%)
237 and kanamycin in China (55.91%) ^[13]. However, *E. coli* isolates showed the highest
238 resistance to sulfamethoxazole (61.6%), followed by tetracycline (61.2%), ampicillin
239 (48.2%) and kanamycin (22.4%) in Sichuan province between 2012 and 2013 ^[28]. *E.*
240 *coli* were isolated from pig farms from seven provinces that the resistance rate to
241 ampicillin was 81.44%, 94.37% to tetracycline and 88.36% to sulfaisoxazole ^[29].

242 These findings provide important information and implications for the application of
243 antibiotics in future studies.

244 **Conclusions**

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

250

251 **Abbreviations**

252 MLST: multilocus sequence typing; STs: sequence types; CCs: clonal complexes.

253

254 **Author' contribution**

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

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273

274 **Competing interests**

275 The authors declare that they have no competing interests.

276

277 **Availability of data and materials**

278 The authors declare that all data supporting the findings of this study are available within the
279 article.

280

281 **Consent for publication**

282 Not applicable

283

284 **Ethics approval and consent to participate**

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

287

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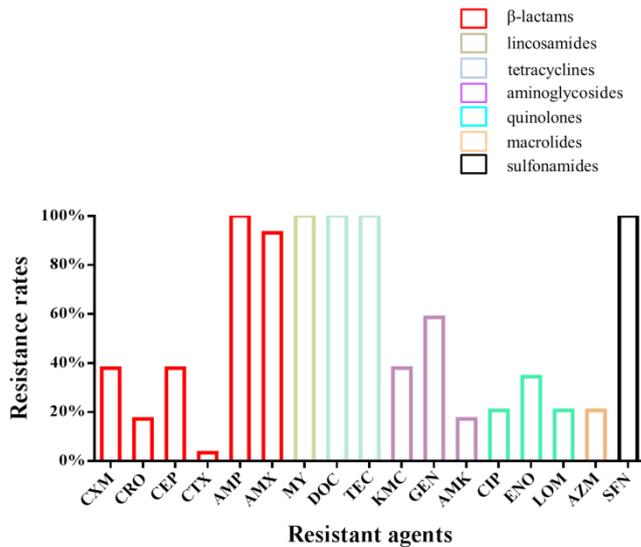
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371

372 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

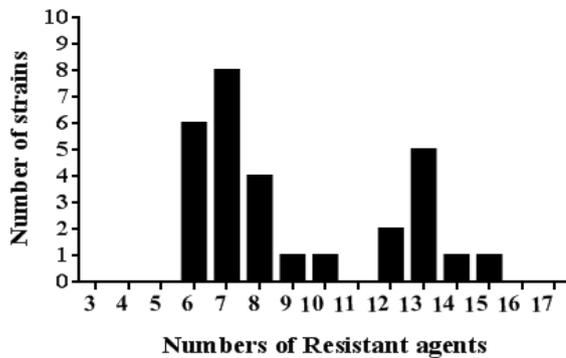
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374

375 Figure 1 Antimicrobial resistance of *E. coli* isolates. The resistance rate of *E. coli* isolates to 17 agents.
 376 CXM, cefuroxime; CRO, ceftriaxone; CEP, cephalothin; CTX, cefotaxime; AMP, ampicillin; AMX,
 377 amoxicillin; MY, lincomycin; DOC, doxycycline; TEC, tetracycline; KMC, kanamycin; CEN,
 378 gentamicin; AMK, amikacin; CIP, ciprofloxacin; ENO, enoxacin; LOM, lomefloxacin; AZM,
 379 azithromycin; SFN, sulfafurazole.

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382 Figure 2 Antimicrobial resistance of *E. coli* isolates. Multidrug resistance of *E. coli* isolates to 17
 383 agents.

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388 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 (%)						
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

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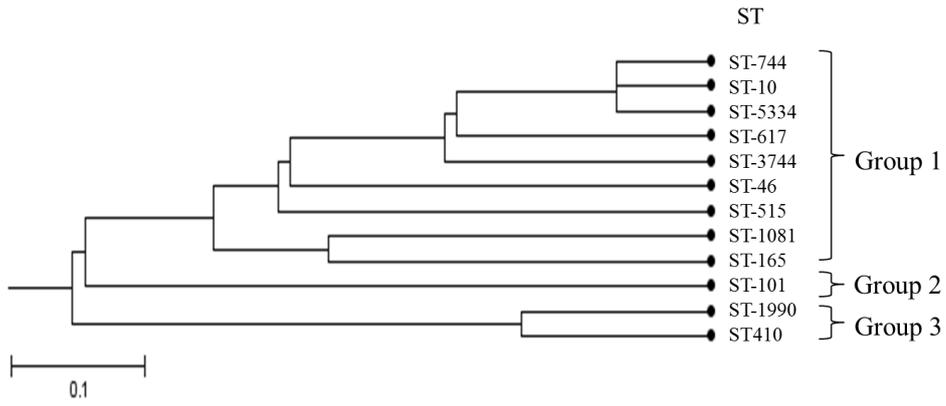
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391 Table 3 Diversity profiles of *E. coli* isolates based on MLST

ST type	Allele Profile ^a	Clone Complex ^b	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

392 Note. ^aAllele number for adK, fumC, gyrB, icD, mdH, purA, and recA, respectively (one for each393 ST). ^bClone complex.

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

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