World Rabbit Sci. 2022, 30: 131-140
doi:10.4995/wrs.2022.15273
WRSA, UPV, 2003

# GENETIC DIVERSITY AND VIRULENCE GENE PROFILES OF *ESCHERICHIA COLI* FROM DIARRHOEAL RABBITS IN SICHUAN PROVINCE, CHINA

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Abstract: *Escherichia coli* (*E. coli*) can cause diarrhoea in a wide range of hosts. Moreover, some strains with high virulence and drug resistance pose a serious threat to public health and livestock products. Diarrhoea caused by *E. coli* outbreaks in rabbitries result in serious economic losses. The aim of this study was to investigate the distribution of virulence genes and molecular genetic characteristics of *E. coli* from diarrhoeal rabbits in the main rearing areas of Sichuan province, China in 2015-2017. In total, 39 *E. coli* isolates were identified and undivided divided into 17 sequence types by multilocus sequence typing (MLST) and grouped in 22 clusters by pulsed-field gel electrophoresis. Polymerase chain reaction tests detected 6 virulence genes: *eae* (41.0%), *ler* (41.0%), *ral* (33.3%), *afr2* (10.3%), *irp2* (15.4%) and *astA* (7.7%) of the tested 17 virulence genes identifying 16 enteropathogenic *E. coli* (EPEC) isolates. The main sequence types U328, ST328 and ST20 carried rabbit EPEC associated virulence genes (*eae*, *ler*, *ral* and *afr2*). The results showed that the distribution of virulence genes varied by year and area; genotype had major types in local rearing areas but was of high diversity in Sichuan province.

Key Words: *Escherichia coli*, genotyping, rabbit, virulence gene profiles, MLST, PFGE.

## INTRODUCTION

*Escherichia coli* (*E. coli*) is a widely distributed bacteria in the environment, animals and humans. *E. coli* strains can be roughly divided into 3 categories: commensal strains, intestinal pathogenic (enteric or diarrhoeagenic) strains, and extraintestinal pathogenic strains (Russo and Johnson, 2000). Moreover, six types of diarrhoea-related *E. coli* are: Shiga toxin-producing *Escherichia coli* (STEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enterotoxigenic *Escherichia coli* (ETEC), Enteropathogenic *Escherichia coli* (EPEC), Enteroinvasive *Escherichia coli* (EIEC) and Enteroaggregative *Escherichia. coli* (EAEC) (Gomes *et al*., 2016). Diarrhoeagenic *Escherichia coli* (DEC) has been found in surface water used to irrigate food products (Canizalez-Roman *et al*., 2019) and from cow's milk, cheese and dairy cattle farm environments (Rios *et al*., 2019). It has also been found in pigs (Yang *et al*., 2019), cattle, poultry, diarrhoeic patients (Parvej *et al*., 2020) and so on. This means that DEC exists not only in the environment, but is also pathogenic for several animal species and for humans.

Diarrhoeagenic *E. coli* in rabbits is an infectious disease primarily due to enteropathogenic *E. coli* (EPEC) characterised with high morbidity and mortality (Pohl *et al*., 1993; Blanco *et al*., 1997; Milon *et al*., 1999; Dow *et al*., 2005; Solans *et al*., 2019). EPEC was identified by testing the *eae* gene (Yang and Chai, 2004). It is more common in suckling and weaning rabbits, frequently with poor therapeutic responses and a high fatality rate. The disease can also be

Correspondence: Y. Geng, *gengyisicau@126.com*. Received *March 2021* - Accepted *March 2022*.
https://doi.org/10.4995/wrs.2022.15273

Cite as: Yang Y., Wang S., Geng Y., Liu H., Qin Z., Feng Y., Yang Z., Lai W. 2022. Genetic diversity and virulence gene profiles of Escherichia coli from diarrhoeal rabbits in Sichuan province, China. *World Rabbit Sci., 30: 131-140. https://doi.org/10.4995/wrs.2022.15273*

secondary to coccidiosis and other diseases (Liu and Ning, 2006). In 2018, the output value of the rabbit industry reached about $4.3 billion, accounting for 1.01% of the output value of animal husbandry, higher than that of 2017 (0.86%) in China (Wu and Qin, 2019). Intensive rabbitries are mainly concentrated in Sichuan, Chongqing and other south-western regions, as well as Shandong and Henan provinces. In 2016, Sichuan ranked the first place in column quantity of rabbits (43.75%) (Wu *et al.*, 2019). Intensive breeding and rearing can easily lead to large-scale disease epidemics (Solans *et al.*, 2019). High mortality rate seriously affects the breeding of rabbits in rabbitries. Therefore, the distribution of virulence gene profiles and genotype of rabbit diarrhoeagenic *E. coli* has attracted our attention. In this study, samples were taken from diarrhoeal rabbits in five rearing areas (Chengdu, Deyang, Yaan, Zigong and Mianyang) in Sichuan province, China, which were the main rabbit breeding areas. The areas were representative, to explore the distribution of virulence genes and genotypes of rabbit *E. coli* in Sichuan province, China. The aim of this study was to investigate the virulence genes of *E. coli* strains isolated from diarrhoeal rabbits in the main rearing areas of Sichuan by using published polymerase chain reaction (PCR) systems. Moreover, multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were also used to investigate the molecular epidemic characteristics of rabbit *E. coli*.

## MATERIALS AND METHODS

### *Bacterial sampling and identification*

Diarrhoeal growing rabbits (>35 d old) (main types: meat-producing rabbits and wool-producing rabbits) were randomly selected from rabbitries (intensive type and the number of livestock on hand more than 1000 rabbits) in each region (Chengdu, Deyang, Yaan, Zigong and Mianyang) in Sichuan province from 2015 to 2017. The anal swab samples were streaked on the surfaces of MacConkey agar (MAC) and cultured at 37°C for 16-18 h. The Lactose-positive colony on MAC was cultured on the Eosin Methylene Blue agar (EMB) at 37°C for 16-18 h. EMB cultured positive colonies were differentiated by a species-specific PCR. The species-specific PCR forward primer was 5'-ATCAACCGAGATTCCCCCAGT-3' and reverse primer was 5'-TCACTATCGGTCAGTCAGGAG-3'. The size of product amplified was 232 bp (Riffon *et al.*, 2001). Sequences were analysed using Basic Local Alignment Search Tool (BLAST).

### *Multilocus sequence typing and phylogenetic analysis*

MLST was carried out by amplifying and sequencing 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) as previously described (Wirth *et al.*, 2006). PCR amplification followed the protocol detailed at EnteroBase website (http://enterobase.warwick.ac.uk/species/index/ecoli). The PCR products were sequenced by TIANGEN Biotech Inc. Beijing, then the sequences were contrasted with the Warwick data to determine the allele numbers and sequence types.

The 7 housekeeping gene sequences were concatenated to generate a 3423 bp DNA sequence for phylogenetic analysis. The phylogenetic tree was built by MEGA 6.0 software with maximum likelihood method, General Time Reversible model and a bootstrapping of 1000 replications. SplitsTree4 software with Neighbour-Joining (NJ) method was used to construct a dendrogram for visualisation of evolutionary relationship between different types of strains.

### *Pulsed-field gel electrophoresis analysis*

The experiment was carried out according to *E. coli* non-O157 PFGE standard procedure described by PulseNet (http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). DNA was digested with 50U *Xba I*/sample (Thermo Fisher Scientific Co., Ltd, China) in agarose plug. Each sample was loaded in 1% Seakem Gold agarose gel (Bio-Rad Laboratories. Beijing, China) for electrophoresis by CHEF Mapper® XA (Bio-Rad Laboratories. Beijing, China). Comparison of PFGE profiles was done with Quantity One v.4.62 software. The dendrogram was constructed by the unweighted pair group method using average linkages (UPGMA) method and simples which band similarity ≥85% were clustered in one group.

### *Virulence genes determination*

All 39 strains were tested for virulence genes by conventional PCR: for the presence of enterotoxin genes (LT, $ST_a$, $ST_b$ and *astA*), Shiga toxin genes ($stx_1$ and $stx_2$), invasive factor genes (*lpaH*), EAEC-associated virulence genes (*aggR*, *aap*, *aggA* and *aaf*), LEE pathogenicity island genes (*eae* and *ler*), HPI gene (*irp2*) and rabbit fimbrial adhesin genes (*afr1*, *afr2* and *ral*). Published primers were used to amplify all virulence genes (Table 1). Each virulence gene fragment sequence amplified by PCR was compared with the data from the National Center of Biotechnology Information (NCBI): https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Table 1: Primers used for detection of *E. coli* virulence genes by PCR amplification.

| Virulence gene | Primer and sequence (5'-3') | Annealing Temperature (°C) | Amplicon size (bp) | Reference |
|---|---|---|---|---|
| $ST_a$ | F-TCCGTGAAACAACATGACGG | 55-57 | 244 | (Ojeniyi *et al.*, 1994) |
| | R-ATAACATCCAGCACAGGCAG | | | |
| $ST_b$ | F-GCCTATGCATCTACACAATC | 53-55 | 279 | (Ojeniyi *et al.*, 1994) |
| | R-TGAGAAATGGACAATGTCCG | | | |
| LT | F-GCACACGGAGCTCCTCAGTC | 61-63 | 218 | (Vidal et al., 2004) |
| | R-TCCTTCATCCTTTCAATGGCTTT | | | |
| *astA* | F-CCATCAACACAGTATATCCGA | 55 | 111 | (Yamamoto and Nakazawa, 1997) |
| | R-GGTCGCGAGTGACGGCTTTGT | | | |
| $stx_1$ | F-CAGTTAATGTGGTGGCGAAGG | 64 | 348 | (Cebula *et al.*, 1995) |
| | R-CACCAGACAATGTAACCGCTG | | | |
| $stx_2$ | F-ATCCTATTCCCGGGAGTTTACG | 64 | 584 | (Cebula *et al.*, 1995) |
| | R-GCGTCATCGTATACACAGGAGC | | | |
| *lpaH* | F-GTTCCTTGACCGCCTTTCCGATACCGTC | 50-52 | 619 | (Sethabutr *et al.*, 1993) |
| | R-GCCGGTCAGCCACCCTCTGAGAGTAC | | | |
| *aggR* | F-CTAATTGTACAATCGATGTA | 55 | 457 | (Cerna *et al.*, 2003) |
| | R-AGAGTCCATCTCTTTGATAAG | | | |
| *eae* | F-CCAGCAGCCAGGCTTCGTCA | 57 | 833 | (Yang *et al.*, 2006) |
| | R-AATCTTCTGCGTACTGTGTTC | | | |
| *ler* | F-CGCACACAACAAGCCCATAC | 58 | 195 | (Zhang *et al.*, 2015) |
| | R-GATGAGTTCCGGCGAGCAA | | | |
| *irp2* | F-AAGGATTCGCTGTTACCGGAC | 60 | 280 | (Cheng *et al.*, 2006) |
| | R-TCGTCGGGCAGCGTTTCTTCT | | | |
| *afr1* | F-TACCGTTACTGCGAAGACCT | 60 | 280 | (Dow *et al.*, 2005) |
| | R-CGTGCTGTTAATCGCCACTA | | | |
| *afr2* | F-AAGTTAGGGGACGCCATTAC | 57 | 518 | (Dow *et al.*, 2005) |
| | R-CCAGGACTTATTCTGACCAG | | | |
| *ral* | F-GATCTTTGGCAGTGGACAC | 58 | 577 | (Dow *et al.*, 2005) |
| | R-CGGCAACAGTTCCTTTTGAA | | | |
| *aap* | F-CTTGGGTATCAGCCTGAATG | 55 | 310 | (Cerna *et al.*, 2003) |
| | R-AACCCATTCGGTTAGAGCAC | | | |
| *aggA* | F-GCTAACGCTGCGTTAGAAAGACC | 60 | 352 | (Gomes *et al.*, 2016) |
| | R-GGAGTATCATTCTATATTCGCC | | | |
| *aaf* | F-GACAACCGCAACGCTGCGCTG | 60 | 301 | (Piva *et al.*, 2003) |
| | R-GATAGCCGGTGTAATTGAGCC | | | |

### *Statistical analysis*

Significance was established by Fisher's exact test with SPSS 19.0 software. The threshold for statistical significance was *P*-values of <0.05.

## RESULTS

### *MLST analysis and phylogenetic relationships of the rabbit E. coli isolates*

In total, 39 *E. coli* isolates were identified (Table 2), which belonged to 17 sequence types. 11 (U328, U316, U1196, and U218) of 39 isolates could not be referred to the existing sequence types in Warwick database, but had their allele numbers (Table 3). The most frequent sequence type was U328 (n=8, 20.5%) from Chengdu in 2017, followed by ST162 from Deyang, Yaan, and Zigong in 2015-2016 (n=6, 15.4%).

A SplitsTree graph showed the relative distances between sequence types. Most sequence types were classified in one branch alone, such as ST328 and U328, ST1196 and U1196 etc. (Figure 1), except for a few sequence types. The genetic relationship between ST328 and U328 was quite close, but they did not belong to the same area and the same year. Furthermore, ST162 was gathered alone into one branch; they were all from the same year but not the same area.

### *Diversity of PFGE genotypes*

As shown in the PFGE clustering tree (Figure 2), samples with 85% similarity to the electropherogram were clustered into one class. PFGE divided 39 strains of *E. coli* into 22 types and the samples showed high diversity. The largest cluster population, XVIII, had 5 strains of the same type: XB1, XB2, XB4, XB5 and XB6. Types I, IX, X, XVI and XXII each had three strains. Types II, IV and XIX each had two strains. The remaining 13 samples each contained one type.

The strains of type I, IX, XVI, XVIII and XXII were each obtained from the same area, respectively. Among them, 50% (5/10) of the strains in 2017, Chengdu were XVIII type and 67% (6/9) strains in 2017, Deyang were I and XXII. Furthermore, X and XIX which appeared in two areas (Zigong, Deyang; Yaan, Zigong) were more prevalent. The genotypes were different in Chengdu and Deyang in 2015-2016 and 2017 and the types are most abundant in Deyang.

### *Distribution of virulence genes*

Seventeen virulence genes were tested. Six virulence genes were positive: 41.0% for *eae*, 41.0% for *ler*, 33.3% for *ral*, 15.4% for *irp2*, 7.7% for *astA,* while none of the other 11 genes (*aggR*, *aap*, *aaf*, *aggA*, *lpaH*, $ST_a$, $ST_b$, LT, $stx_1$, $stx_2$, *afr1*) were detected. The 16 (41.0%) *eae*$^+$ strains were designated as EPEC. All EPEC strains harboured *ler* gene (Figure 2). Moreover, one rabbit fimbrial adhesin gene (*afr2* or *ral*) was also distributed in the majority of EPEC strains (15/ 16, 93.75%). Both *afr2* and *ral* gene was detected in strain XB2. 8 strains (20.5%) harboured *irp2* or/ and *astA* genes. None of the virulence genes were detected in the remaining 15 strains (38.4%).

Table 2: Origin of *E. coli* strains isolated from diarrhoeal rabbits in parts of Sichuan Province.

| Strain | Year | Region |
|---|---|---|
| XA1, XA2, XA3, XB1, XB2, XB3, XB4, XB5, XB6, XB7 | 2017 | Chengdu |
| DA1, DA2, DA3, DA4, DA5, DA6, DA7, DA8, DB1 | 2017 | Deyang |
| XJ1, XJ2, XJ3, XJ4 | 2015-2016 | Chengdu |
| DY1, DY2, DY3, DY4, DY5, DY6 | 2015-2016 | Deyang |
| YA1, YA2, YA3, YA4 | 2015-2016 | Yaan |
| ZG1, ZG2, ZG3, ZG4, ZG5 | 2015-2016 | Zigong |
| MY1 | 2015-2016 | Mianyang |

**Table 3:** Multilocus sequence typing sequence types and allele number of 39 *E. coli* isolates from diarrhoeal rabbits in Sichuan Province.

| | Allele number | | | | | | | | Chengdu | Chengdu | Deyang | Deyang | Yaan | Zigong | Mianyang |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ST (ST Complex) | *adk* | *fumC* | *gyrB* | *icd* | *mdh* | *purA* | *recA* | Number of isolates(n) | 2015-2016 | 2017 | 2015-2016 | 2017 | 2015-2016 | 2015-2016 | 2015-2016 |
| U328* | 9 | 23 | 81 | 18 | 11 | 8 | 8 | 8(XA1;XA2;XB1-XB6) | | 8 | | | | | |
| 162(469) | 9 | 65 | 5 | 1 | 9 | 13 | 6 | 6(DY1;YA1/2/4;ZG1/2) | | | 1 | | 3 | 2 | |
| 328(278) | 9 | 23 | 81 | 18 | 11 | 8 | 6 | 4(DA1/3/4;MY1) | | | | 3 | | | 1 |
| 380 | 6 | 11 | 5 | 18 | 11 | 8 | 6 | 3(DA2/7/8) | | | | 3 | | | |
| 20(20) | 6 | 4 | 3 | 18 | 7 | 7 | 6 | 3(DY4-6) | | | 3 | | | | |
| 1125 | 6 | 4 | 15 | 18 | 24 | 26 | 7 | 2(YA3;ZG3) | | | | | 1 | 1 | |
| 155(155) | 6 | 4 | 14 | 16 | 24 | 8 | 14 | 2(XJ2/4) | 2 | | | | | | |
| 200(40) | 6 | 4 | 5 | 26 | 7 | 8 | 14 | 2(DY3;XJ1) | 1 | | 1 | | | | |
| 1463 | 6 | 95 | 4 | 222 | 7 | 7 | 7 | 1(XJ3) | 1 | | | | | | |
| U316* | 6 | 23 | 81 | 18 | 11 | 8 | 8 | 1(XA3) | | 1 | | | | | |
| 2144 | 6 | 6 | 15 | 56 | 11 | 26 | 6 | 1(DA6) | | | | 1 | | | |
| U1196* | 6 | 6 | 33 | 26 | 11 | 26 | 2 | 1(ZG5) | | | | | | 1 | |
| 1196 | 6 | 6 | 33 | 26 | 11 | 8 | 2 | 1(DA5) | | | | 1 | | | |
| 224 | 6 | 4 | 33 | 16 | 11 | 8 | 6 | 1(DB1) | | | | 1 | | | |
| 10(10) | 10 | 11 | 4 | 8 | 8 | 8 | 2 | 1(DY2) | | | 1 | | | | |
| 3107 | 10 | 11 | 5 | 8 | 7 | 219 | 2 | 1(ZG4) | | | | | | 1 | |
| U218* | 10 | 11 | 22 | 12 | 8 | 8 | 2 | 1(XB7) | | 1 | | | | | |

Total:17 types, 39 strains

* The sequence type of ST number was not obtained in Warwick database

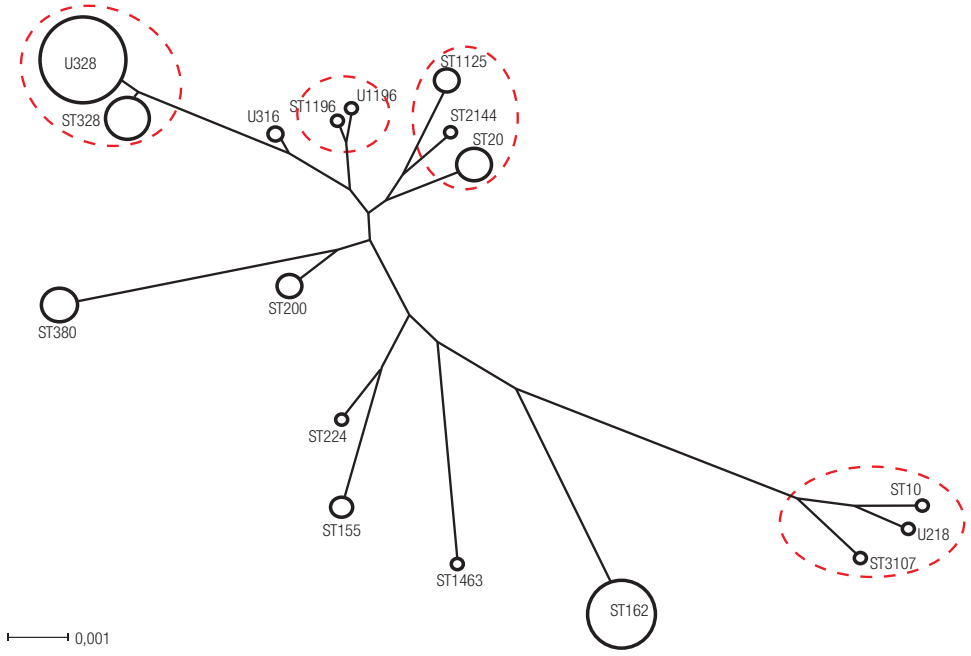


Figure 1: Genetic evolution profile of 39 *E. coli* strains isolated from diarrhoeal rabbits in parts of Sichuan Province constructed by SplitsTree. Remark: The circle size represented the number of the sequence type in the sample. The red dotted line surrounded represents the sequence of similar genetic relationships.

The frequencies and association of 6 virulence genes with sequence types are summarised in Figure 2. Virulence genes distribution in disparate years and regions were different. In 2015-2016 and 2017 in Chengdu, Deyang (Table 4), statistically highly significant difference was in the presence of *ral* and *irp2*: *ral* was only identified in 2017 ($P<0.01$), while *irp2* was only identified in 2015-2016 ($P<0.01$). In 2015-2016 (Table 5), there was a statistically significant difference in the presence of *eae*, *ler*, and *ral* from five different regions.

Based on an 85% cut-off-value, 22 different PFGE clusters (I-XXII) were identified in the 39 *E. coli* isolates (Figure 2) and roughly showed a high degree of consistency with MLST. Four sequence types of U328, ST20, ST162 and ST200 corresponded to incomplete aggregation of PFGE spectrum, indicating that the resolution of PFGE typing is slightly higher than that of MLST.

The prevalence of virulence genes among different MLST sequence types suggested some regularity (Figure 2). The distribution of EPEC strains from Deyang (2015-2016), Deyang (2017) and Chengdu (2017) seemed to be characteristic. There was only one MLST type of EPEC strain in each area (except XA3-U316 strain) and each MLST

Table 4: Differences of virulence genes distribution of rabbit *E. coli* between 2015-2016 and 2017 from Chengdu and Deyang.

| The year of Chengdu and Deyang | Number of strains | *eae* | *ler* | *afr2* | *astA* | *ral* | *irp2* |
|---|---|---|---|---|---|---|---|
| 2017 | 19 | 12 | 12 | 1 | 1 | 12 | 0 |
| | | 63.2% | 63.2% | 5.3% | 5.3% | 63.2% | |
| 2015-2016 | 10 | 3 | 3 | 3 | 1 | 0 | 4 |
| | | 30.0% | 30.0% | 30.0% | 10.0% | | 40.0% |
| Total | 29 | | | | | | |
| *P* | | 0.128 | 0.128 | 0.105 | 1 | 0.001 | 0.009 |

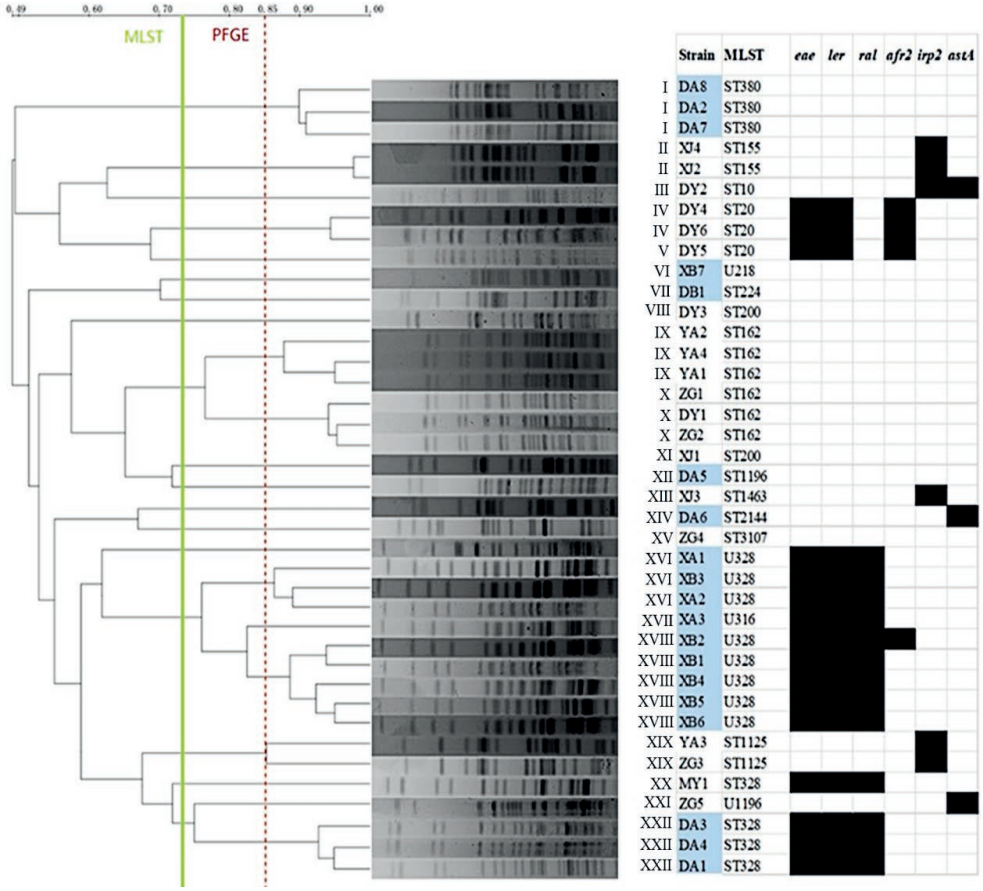


Figure 2: Genotypes and virulence genes distribution of 39 *E. coli* isolated from diarrhoeal rabbits in Sichuan Province.

Remark: From left to right, they were the clustering tree (pulsed-field gel electrophoresis [PFGE] clustering tree: red dotted line; Multilocus sequence typing (MLST) clustering tree: approximate green solid line), PFGE profiles, the number of PFGE types (marked with I-XXII), strain names (the names of the strains related to the areas where the *E. coli* isolated from; blue background marked: strains from 2017; white background marked: strains from 2015-2016), MLST sequence type number and the prevalence of virulence genes (*eae*, *ler*, *ral*, *afr2*, *irp2* and *astA*) (black square: the presence of virulence genes).

type only harboured one rabbit fimbrial adhesin gene (except XB2-U328 strain). The proportion of EPEC in *E. coli* from diarrhoea rabbits was high in each area (50.0% in 2015-2016, Deyang; 90.0% in 2017, Chengdu; 33.3% in 2017, Deyang).

Chengdu (2015-2016) contained three different sequence types (ST115, ST200 and ST1463) and the type ST1125 also existed in Yaan and Zigong. 6 strains of ST162 could distribute in three areas (Yaan, Zigong and Deyang), but none of virulence genes were detected. There was an obvious corresponding relationship between rabbit EPEC associated virulence genes (*eae*, *ler*, *afr2* and *ral*) and MLST sequence types (ST20, ST328 and U328) in the research. There did not seem to be an association between sequence types and *irp2* positive strains.

## DISCUSSION

In China, the usual typing method for rabbit *E. coli* is O serotyping (Wang *et al.*, 2000), but in recent years, PFGE and multilocus sequence typing (MLST) has become the common molecular typing method used for suspected pathogenic

Table 5: Differences of virulence genes distribution of rabbit *E. coli* among different regions in 2015-2016.

| The region of 2015-2016 | Number of strains | *eae* | *ler* | *afr2* | *astA* | *ral* | *irp2* |
|---|---|---|---|---|---|---|---|
| Chengdu | 4 | 0 | 0 | 0 | 0 | 0 | 3(75.0%) |
| Deyang | 6 | 3(50%) | 3(50%) | 3(50%) | 1(16.7%) | 0 | 1(16.7%) |
| Yaan | 4 | 0 | 0 | 0 | 0 | 0 | 1(25.0%) |
| Zigong | 5 | 0 | 0 | 0 | 1(20.0%) | 0 | 1(20.0%) |
| Mianyang | 1 | 1(100%) | 1(100%) | 0 | 0 | 1(100%) | 0 |
| Total | 20 | | | | | | |
| *P* | | 0.027 | 0.027 | 0.115 | 1 | 0.05 | 0.39 |

*E. coli*. MLST is usually applied to characterise bacterial isolates based on the sequence of several housekeeping genes and is established for long-term global epidemiology (Rajkhowa *et al.*, 2010). PFGE has been used in the investigation and control of food-borne infection outbreaks for decades due to the demonstration of reproducibility, high discriminatory power and good epidemiological concordance (Nadon *et al.*, 2017). In short, PFGE and MLST are more feasible to type and compare various *E. coli* strains.

EAEC has not been proven to be present in animals (Cassar *et al.*, 2004). Regarding EPEC, the first Chinese report on rabbit EPEC was on *E. coli* from diarrhoeal weaned rabbits (Yang and Chai, 2004) without characterisation of strains for virulence genes or by MLST or PFGE. The EPEC-ST328 and EPEC-ST20 strains, which were frequent here, were also recorded by the Warwick database as originating from diarrhoeic rabbits and hares. The relationship between U328-EPEC and ST328-EPEC was close and the number of two sequence types occupied a considerable percentage.

High-pathogenicity Island (HPI) mediates biosynthesis and uptake of the siderophore yersiniabactin and a mouse-lethal phenotype. Its core area is composed of *irp2*, *irpl* and *fyuA* genes, among which *irp2* can be used as the detection marker of HPI virulence island (Rakin *et al.*, 1999). It was first described in pathogenic *Yersinia* strains (Karch *et al.*, 1999). HPI was also widely distributed in human pathogenic members of the family of Enterobacteriaceae, above all in extraintestinal pathogenic *Escherichia coli* (ExPEC). It has demonstrated a highly significant correlation of a functional HPI and extraintestinal virulence in *E. coli*. Moreover, using a mouse infection model, it also has showed that the HPI contributes to the virulence of ExPEC (Schubert *et al.*, 2002). In this research, HPI virulence gene *irp2* was first detected in diarrhoeic rabbit *E. coli* in 2015-2016 (6/20, 30.0%). Thus, the role of HPI virulence gene *irp2* in rabbit diarrhoeal *E. coli* is unclear.

No virulence genes were detected in 14 strains of *E. coli* (14/39). Since no animal regression test has been conducted, further confirmation is needed to determine whether they cause diarrhoea. As for *E. coli* with virulence genes (25/39), statistically significant differences were observed in the prevalence of *ral* ($P$=0.001) and *irp2* ($P$=0.009) genes between strains in 2015-2016 and 2017 from Chengdu and Deyang, respectively. Besides the prevalence of *eae* ($P$=0.027), *ler* ($P$=0.027) and *ral* ($P$=0.050) genes among strains from Chengdu, Deyang, Yaan, Zigong and Mianyang in 2015-2016 were significantly different, respectively. Distribution of virulence genes of *E. coli* was diverse on the whole and had major types in local rearing areas, while there were multiple genotypes in some areas.

Distribution of virulence gene profiles was significantly correlated with MLST sequence types in UPEC (uropathogenic *E. coli*). Frequently occurring sequence types contained closely related virulence genotypes and the similarity of the virulence genotype of each strain within the sequence type was up to 65%-100% (Gibreel *et al.*, 2012). Wirth *et al.* used the global MLST database to analyse the genetic relationship between pathogenic *E. coli* and found that specific pathogen types (EHEC, EPEC, EIEC, K1 and *Shigella*) have arisen independently and repeatedly in several lineages. These data indicate the correlation between virulence gene profiles and *E. coli* genotypes. On the basis of this correlation, we may predict the pathogenicity of some *E. coli* bacteria by genotype. The relationship between MLST sequence types and virulence have been analysed in this research and it was found that rabbit EPEC associated virulence genes *eae*, *ler*, *afr2* and *ral* have a corresponding relationship with U328, ST328 and ST20.

## CONCLUSION

The 39 *E. coli* strains from diarrhoeal rabbits in Sichuan were divided into 17 sequence types by MLST and grouped in 22 clusters by PFGE. The most frequent genotypes were MLST-U328 and PFGE-XVIII, respectively. The results showed there was a high genetic diversity of *E. coli* from rabbits in Sichuan province, but the genotype had major types in local rearing areas. There were 6 kinds of virulence genes (*eae*, *ler*, *ral*, *afr2*, *irp2* and *astA*) detected in these *E. coli* isolates and the virulence gene profiles varied in time and areas. The 16 EPEC isolates were identified. The main sequence types U328, ST328 and ST20 carried rabbit EPEC associated virulence genes (*eae*, *ler*, *ral* and *afr2*), suggesting that the virulence genes appeared to be related to specific sequence types of rabbit EPEC.

Contribution: Yang Yuqing and Wang Shizhen contributes equally to this work.

Conflict of interests: The authors have declared that there are no competing interests.

Acknowledgement: This work was supported by Sichuan Technology Support Planning (2016NZ0002).

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