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Serotype Identification and Detection of Related Genes of HPI Pathogenic Island of *Escherichia coli* Isolated from Pigs in Hebei Province of China

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Abstract: A total of 54 samples including duodenum, small intestine contents, lymphonodi mesenterici and diarrhea feces were collected from pigs died of diarrhea in Hebei Province of China in 2009~2011. These samples were examined for the presence of *E. coli* and serotype identification. High pathogenicity island was detected from *E. coli* isolates. The isolation and identification of O serotype of *E. coli* were conducted by common method, *fyuA* and *irp2* genes were detected using PCR. The 54 *E. coli* strains referred to ten serotypes, O38 was the dominant serotype whose proportion was 51.5% (17/33). The positive rate of *fyuA* gene was 24.4% (11/45), *irp2* was 42.2% (19/45), *irp2* and *fruA* was 13.3% (6/45).

Key words: *E. coli* from pigs, O serotype, high pathogenicity island, gene, O38

INTRODUCTION

As the increasing density of piglets and rearing scale, swine colibacillosis is becoming the important disease including diarrhea of newborn piglets (or yellow and white scour of newborn piglets), weaned piglet diarrhea and/or Edema disease of pigs (Fairbrother, 1999).

Pathogenicity island is the virulence gene cluster in the bacterial chromosome whose molecular weight is >30 kb usually, there is significant difference in G+C mol% and using password between bacterial and host bacterial. PAI is adjacent to the site of tRNA and phage integration site. There are often equidirectional repeat sequences in both sides, inserted sequence occasionally. PAI carries potentially removable components and has instability. PAI exists in some pathogenic bacterium and may be related to virulence evolution of pathogens newly discovered bacteria. HPI pathogenicity island was firstly discovered in the *Yersinia* genus. It is named high pathogenicity island because it is closely related to the mouse lethal phenotypes of *Yersinia* genus (Carniel *et al*., 1996; Mokracka *et al*., 2004). HPI is a large chromosomal segments determining the levels of virulence or pathogenicity of Yersinia and contains the genes and regulatory genes of coding the synthesis and uptake of ironophore and Yersini abactin (Ybt). HPI has the function of regulation and iron uptake is the essential genetic unit of expressing the murine lethal phenotypes (Shen, 2003; Chen *et al*., 2004, 2006). *irp2* and *fyuA* genes were the main structural gene in the core area of HPI. *irp2* gene can be the detection sign of HPI (Gao *et al*., 1999; Cheng *et al*., 2006). Now the exist of HPI virulence island in diarrhea of enteropathogenic *E. coli* from human, cattle, rabbit is confirmed and it is closely related to pathogenicity (Jores *et al*., 2001; Sperandio *et al*., 1998; Penteado *et al*., 2002; Carniel, 2001; Bach *et al*., 2000; Clermont *et al*., 2001). In order to illuminate the relation of O serotype and carrying HPI from piglets *E. coli* in Hebei Province, researchers selected 54 piglets *E. coli* strains from parts of Hebei Province, *HPI* genes including irp2 and fyuA were determined.

MATERIALS AND METHODS

The 54 samples including duodenum, small intestine contents, lymphonodi mesenterici and diarrhea feces were collected from pigs died of diarrhea in Qin Huangdao, Tangshan, Handan, Xingtai, ZhangJiakou, ShiJiazhuang Chengde, Hengshui and Langfang in 2009~2011. The materials were streaked on Mai Kangkai plate at 37°C for 18 h, picked the typical colony then streaked on Mai Kangkai plate at 37°C for 18 h, picked the single colony to cultivate purely then numbered and conserved. The reference strains for fyuA sequence were DQ273751, Z35104, Z35105, Z35107, Z35485, Z35486, Z35496, Z38064,

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Z38065 and Z35487 from Genbank while for irp2 sequence were AF091251, AP010953, CP000468, CP001855, FN554766, L18881, Z46919 and Z35456.

Biochemical experiment: All of the *E. coli* isolates were determined for biochemical index according to other reports (Gao *et al*., 1999).

Identification of O serotype: Picked the smooth colony and then inoculated on bevel tubule at 37°C for 24 h. The 2 mL 0.5% phenol physiological saline douched the bevel culture into the round bottom tube, autoclaving for 2 h in order to destroy K antigen. The 15 standard single factor serums were carried by glass plate agglutination reaction; the control was admixture of phenol physiological saline and high pressure antigen. If there was obvious agglutination within 0.5 min which showed positive or no agglutination showed negative.

Identification and sequences analysis of HPI: *E. coli* HPI genomic DNA was extracted from bacteria cells using Genome Extraction kit (Takara Blotechnology Dalian Co., Ltd.) according to the manufacturer's instructions. Two pairs of specific primer sets for PCR were designed from the reported conservative nucleotide sequences for HPI in Genbank. A 953 bp DNA section of fyuA was amplified from the genomic DNA with two primers PAF (5'-ACACGGCTT TAT CCT CTGGC-3') and PAR (5'-GGCATATTGACG ATTAACGAA-3'), another 301 bp DNA study of irp2 was amplified with two primers PEF (5'-AAGGATTCGCTGTTACCGGA-3') and PER (5'-TCGGCCA GGATGATT CGTCG-3') by PCR. Nucleotide sequences of these DNA sections were determined by Sangon Biological Engineering Technology and Service Co., Shanghai, China. The result was analyzed by DNA Sart. The total amplification volume was 25 uL including double PCR Buffer 12.5 uL, each primer was 0.5 uL, the DNA template 2 μL and Nuclease-Free Water 9.5 uL. PCR conditions consist of denaturation for 5 min at 94°C followed by 30 cycles of 94°C for 30 sec, 58°C for 50 sec, 72°C for 90 sec and then for 10 min at 72°C. The 5 uL PCR product was detected using 1% Agarose Gel Electrophoresis (AGE) finally.

RESULTS AND DISCUSSION

Biochemical test: The 54 *E. coli* strains were isolated from nine doubtful diarrhea of newborn piglets, weaned piglet diarrhea and/or edema disease of pigs in QinHuangdao, Tangshan, Handan, Xingtai, ZhangJiakou, ShiJiazhuang, Chengde, Hengshui and Langfang. They were consistent with *E. coli* by biochemical identification including glucose, lactose, maltose, sucrose, mannitol, adonitol, indole, M.R, V-P and utilization of citrate.

Serotype identification: Serotype identification was diagnosed by *E. coli* O antigen. The 33 (61.1%) *E. coli* strains were serotyped, 14 (25.9%) no serotypes and 7 (13%) self coagulation (Table 1).

Identification of *fyuA* and *irp2* gene: *fyuA* (953 bp) and *irp2* (301 bp) genes were detected by PCR. The result showed in Fig. 1 and 2. The positive rate of fyuA was 24.4% (11/45), referred to serotype O38, O107, O53 while irp2 was 42.2% (19/45), referred to O38, O24, O93, O107, O53, O78, *fyuA* and *irp2* gene was only 13.3% (6/45) referred to O38, O107, O53 (Table 2).

Sequence analysis of *fyuA* and *irp2* gene: The 7 *E. coli* strains with fyuA virulence island gene isolated from different areas and serotypes were selected, the determined gene sequences were retrieved by BLAST. The results showed that sequence comparisons showed nucleotide identities were >99.5% among the *fyuA* genes

Table 1: Result of serotype identification of 54 *E. coli* strains

Serotype	Number	Percentage
O38	17/33	51.10
O78	2/33	6.10
O107	2/33	6.16
O53	2/33	6.10
O24	2/33	6.10
O93	2/33	6.10
O4	1/33	3.00
O111	1/33	3.00
O11	1/33	3.00
O8	3/33	9.10
Untyped	14/54	25.90
Self coagulation	7/54	13.00

Table 2: Relevance of virulence island and O serotype of *E. coli* isolates

The virulence island	Count	Percentage	Serotype
fyuA+	11	24.4% (11/45)	O38, O107, O53
irp2+	19	42.2% (19/45)	O38, O24, O93, O107, O53, O78
fyuA+*irp2*+	6	13.3% (6/45)	O107, O53, O38

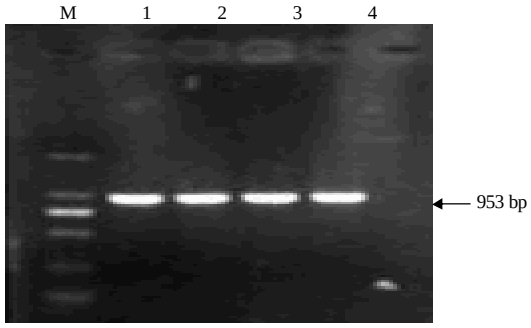

Fig. 1: PCR amplification result of *fyuA* gene; 1-4: PCR of fyuA amplified from *E. coli*; M: DS2000 maker

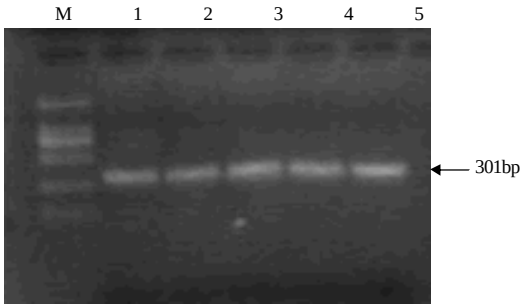

Fig. 2: PCR amplification result of *irp2* gene; 1-4: PCR of irp2 amplified from *E. coli*; M: DS2000 maker

of *E. coli* strains and they shared 92.1~100% identity to the sequences from reference *E. coli fyuA* genes from GenBank (DQ273751, Z35104, Z35105, Z35107, Z35485, Z35486, Z35496, Z38064, Z38065 and Z35487) the results were showed in Table 3. The phylogenetic trees constructed from the *fyuA* genes demonstrated that the 7 *E. coli* strains were clustered into two groups. Z35104, Z35496, Z35487, Z35485, Z35486 was clustered in group while Z38065, Z35105, Z35107, DQ273751, Z38064 and all provided strains were clustered in I group. All of the 7 *E. coli* strains [numbered 11 (O91), 12 (O107), 15 (O38), 21 (O53), 59 (O38), 152 (O38) and 155 (O38)] and those strains from Genbank Z38065, Z35105, Z35107, DQ273751, Z38064 have closer relations (Table 4).

Table 3: Sequences homologous analysis of *fyuA* gene from *E. coli*

Percent identity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	-	97.0	97.3	97.6	96.5	97.2	98.4	98.2	98.1	98.4	98.4	98.4	97.0	97.0	97.0	93.7	98.0
2	1.4	-	97.3	97.1	98.0	98.1	98.6	98.2	98.1	98.4	98.4	98.4	97.0	97.0	97.0	94.0	98.2
3	1.4	1.3	-	96.5	97.6	97.3	99.6	99.6	99.5	99.7	99.7	99.7	98.4	98.4	98.4	95.5	97.4
4	1.5	1.6	1.7	-	97.1	97.6	98.0	97.6	97.5	97.7	97.7	97.7	96.4	96.4	96.4	93.6	97.3
5	1.5	1.6	0.8	1.8	-	98.8	98.5	98.5	98.4	98.6	98.6	98.6	97.3	97.3	97.3	93.8	98.0
6	1.4	1.5	0.7	1.4	0.9	-	98.5	98.5	98.4	98.6	98.6	98.6	97.3	97.3	97.3	94.6	98.2
7	0.8	0.3	0.4	0.6	0.3	0.3	-	99.8	99.7	99.9	99.9	99.9	98.6	98.6	98.6	97.3	98.5
8	0.8	0.6	0.4	1.0	0.3	0.3	0.2	-	99.7	99.9	99.9	99.9	98.6	98.6	98.6	97.3	98.2
9	0.9	0.7	0.6	1.1	0.4	0.4	0.3	0.3	-	99.8	99.8	99.8	98.9	98.9	98.9	97.6	98.1
10	0.7	0.4	0.3	0.9	0.2	0.2	0.1	0.1	0.2	-	100.0	100.0	98.7	98.7	98.7	97.6	98.4
11	0.7	0.4	0.3	0.9	0.2	0.2	0.1	0.1	0.2	0.0	-	100.0	98.7	98.7	98.7	97.4	98.4
12	0.7	0.4	0.3	0.9	0.2	0.2	0.1	0.1	0.2	0.0	0.0	-	98.7	98.7	98.7	97.4	98.4
13	1.9	1.7	1.6	2.1	1.4	1.4	1.3	1.3	1.0	1.2	1.2	1.2	-	100.0	100.0	97.4	97.0
14	1.9	1.7	1.6	2.1	1.4	1.4	1.3	1.3	1.0	1.2	1.2	1.2	0.0	-	100.0	98.7	97.0
15	1.9	1.7	1.6	2.1	1.4	1.4	1.3	1.3	1.0	1.2	1.2	1.2	0.0	0.0	-	98.7	97.0
16	2.0	1.8	1.7	2.2	1.6	1.6	1.4	1.4	1.1	1.3	1.3	1.3	0.1	0.1	0.1	-	94.0
17	1.0	0.9	1.1	1.8	1.2	1.1	0.3	0.4	0.6	0.3	0.3	0.3	1.6	1.6	1.6	1.70	-

1 = (11).seq; 2 = (15).seq; 3 = (21).seq; 4 = (59).seq; 5 = (152).seq; 6 = (155).seq; 7 = Z38065 *E. coli*; 8 = DQ273751 *E. coli*; 9 = Z35104 *Y. pestis*; 10 = Z35105 *E. coli*; 11 = Z35107 *Y. pseu*; 12 = Z38064 *E. coli*; 13 = Z35496 *Y. enterocolitica*; 14 = Z35485 *Y. enterocolitica*; 15 = Z35486 *Y. enterocolitica*; 16 = Z35487 *Y. enterocolitica*; 17 = (12).seq

Table 4: Sequences homologous analysis of *irp2* gene from *E. coli*

Percent identity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	-	94.4	95.2	95.9	95.2	94.8	95.9	95.6	96.7	96.7	95.2	92.6	94.1	96.3	96.7	97.1	98.1	97.3	97.7	97.7	97.7	95.8	97.1	95.2
2	4.3	-	98.2	97.0	96.3	95.6	96.0	95.6	95.2	96.3	97.1	94.1	96.7	95.2	96.3	98.8	98.8	98.8	99.2	99.2	99.2	97.3	98.8	97.1
3	4.2	1.9	-	97.8	94.9	95.2	96.3	95.2	94.9	95.2	96.0	94.1	95.6	96.7	94.9	98.8	98.8	98.8	99.2	99.2	99.2	97.3	98.8	96.0
4	3.1	2.7	2.3	-	95.9	94.5	95.6	95.6	97.0	96.3	96.3	94.8	95.6	97.0	96.3	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	97.0
5	2.7	1.1	3.0	1.9	-	96.7	97.8	98.5	96.3	96.0	97.1	96.3	95.2	96.0	96.7	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	97.1
6	3.0	1.5	1.9	3.0	0.7	-	97.4	97.8	96.0	95.6	96.4	93.4	95.7	95.2	96.0	98.8	98.8	98.8	99.2	99.2	99.2	97.3	98.8	96.0
7	2.3	2.3	2.7	2.3	1.1	1.1	-	97.1	96.7	96.3	97.8	94.5	94.5	94.5	96.3	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	96.7
8	3.0	1.9	2.7	1.9	0.4	1.5	1.1	-	97.4	96.3	95.6	95.6	95.6	95.6	96.7	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	95.6
9	3.1	2.3	2.3	0.4	1.5	3.0	2.2	1.5	-	96.3	96.0	92.7	94.9	96.3	96.0	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	96.0
10	3.0	3.4	3.5	2.7	1.1	2.3	1.9	1.5	1.9	-	95.6	93.0	94.5	96.3	97.8	97.1	97.3	97.3	97.7	97.7	97.7	95.8	97.1	95.2
11	2.7	1.5	2.3	1.5	0.7	0.7	1.1	1.5	1.5	2.3	-	94.9	94.5	94.9	96.7	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	98.5
12	3.8	0.8	3.1	2.3	0.7	2.3	1.5	0.7	1.5	2.3	1.5	-	92.7	93.8	93.0	97.5	95.4	95.4	95.8	95.8	95.8	95.8	97.5	94.5
13	4.2	0.4	3.4	3.8	1.1	1.5	2.6	2.2	3.0	3.4	1.5	1.1	-	94.9	96.3	99.6	99.6	99.6	100.0	100.0	100.0	98.1	99.6	94.5
14	3.8	2.3	1.1	2.3	3.4	2.6	2.7	3.0	2.6	3.8	3.0	3.4	3.8	-	95.6	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	95.6
15	2.6	1.5	2.7	1.5	0.4	1.1	1.5	1.1	1.1	2.2	0.7	1.5	1.5	2.7	-	97.5	97.7	97.7	98.1	98.1	98.1	96.2	97.5	95.6
16	1.3	0.4	1.3	2.1	0.8	0.4	1.3	1.3	2.1	1.3	0.8	1.3	0.4	1.3	0.8	-	99.2	99.2	99.6	99.6	99.6	98.3	100.0	97.5
17	0.4	0.4	1.2	1.9	0.8	0.4	1.2	1.2	1.9	1.2	0.8	1.2	0.4	1.2	0.8	0.8	-	99.2	99.6	99.6	99.6	97.7	99.2	97.7
18	1.2	0.4	1.2	1.9	0.8	0.4	1.2	1.2	1.9	1.2	0.8	1.2	0.4	1.2	0.8	0.8	0.8	-	99.6	99.6	99.6	97.7	99.2	97.7
19	0.8	0.0	0.8	1.6	0.4	0.0	0.8	0.8	1.6	0.8	0.4	0.8	0.0	0.8	0.4	0.4	0.4	0.4	-	100.0	100.0	98.1	99.6	98.1
20	0.8	0.0	0.8	1.6	0.4	0.0	0.8	0.8	1.6	0.8	0.4	0.8	0.0	0.8	0.4	0.4	0.4	0.4	0.0	-	100.0	98.1	99.6	98.1
21	0.8	0.0	0.8	1.6	0.4	0.0	0.8	0.8	1.6	0.8	0.4	0.8	0.0	0.8	0.4	0.4	0.4	0.4	0.0	0.0	-	98.1	99.6	98.1
22	2.8	2.0	2.7	3.5	2.4	2.0	2.8	2.8	3.5	2.8	2.4	2.8	2.0	2.8	2.4	1.7	2.3	2.3	2.0	2.0	2.0	-	98.3	96.2
23	1.3	0.4	1.3	2.1	0.8	0.4	1.3	1.3	2.1	1.3	0.8	1.3	0.4	1.3	0.8	0.0	0.8	0.8	0.4	0.4	0.4	1.7	-	97.5
24	2.7	1.5	2.7	1.9	0.8	1.5	1.5	1.5	1.5	1.9	0.7	0.8	1.1	3.4	1.1	0.8	0.8	0.8	0.4	0.4	0.4	2.4	0.8	-

1 = (L25).seq; 2 = (L28).seq; 3 = (L40).seq; 4 = (15).seq; 5 = (155).seq; 6 = (21).seq; 7 = (158).seq; 8 = (153).seq; 9 = (166).seq; 10 (152); 11 = (161).seq; 12 = (163).seq; 13 = (12).seq; 14 = (31).seq; 15 = (135).seq; 16 = Z46919 *E. coli*.seq 17 = AF091251 Yersinia.seq; 18 = AP010953 *E. coli*.seq; 19 = CP000468 E. *coli*.seq; 20 = CP001855 E. *coli*.seq; 21 FN554766 E. *coli*.seq; 22 = L18881 YEPIRP2H.seq 23 = Z35456 E. *coli*.seq; 24 = (L30).seq

The 17 *E. coli* strains with irp2 virulence island gene isolated from different areas and serotypes were selected, the determined gene sequences were retrieved by BLAST. The results showed that sequence comparisons showed nucleotide identities were >99.6% among the *irp2* genes of *E. coli* strains and they shared 97.2~100% identity to the sequences from reference *E. coli irp2* genes from GenBank (AF091251, AP010953, CP000468, CP001855, FN554766, L18881, Z46919 and Z35456), the results were showed in Fig. 3. The phylogenetic trees constructed from the *irp2* genes demonstrated that the 17 *E. coli* strains were clustered into seven groups. The L18881 was clustered in group. The referenced strains AF091251, AP010953, CP000468, CP001855, FN554766, Z46919 and Z35456 were clustered in I-VII groups, respectively. The other provided strains [numbered 15(O38), 152(O38), 158(O38), L28(O11), L32(O93), 12(O107), L25(O38), 155(O38), 166(O38), L30(O38), 161(O38), 153(O38), 135(O8), 31(O78), 21(O107), L40(O38)] were clustered in I, III, IV, V, VII group (Fig. 4).

O serotype of *E. coli*: As the test showed that the 14 of 54 *E. coli* strains (25.9%) isolated from piglets in different areas were not serotyped and 7 of 54 (13%) were self coagulation. Other strains were clustered in ten serotypes: O38, O78, O107, O93, O53, O8, O24, O4, O11 and O111. The rate of typed strains was 61.1% while O38 was 31.5%, serotype O38 was the dominant serotype including 17 strains. The results were consistent with previous study by Liu *et al.* (2001). In which O141, O8, O2, O157, O1, O9 and O149 were isolated from Sichuan Province. In the survey conducted by Wang, O107, O101, O93,O139, O141

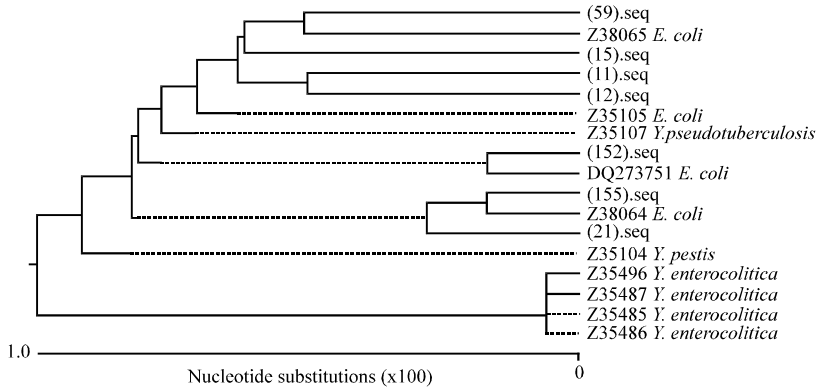

Fig. 3: The phylogenetic tree of nucleotide sequence of *fyuA* gene

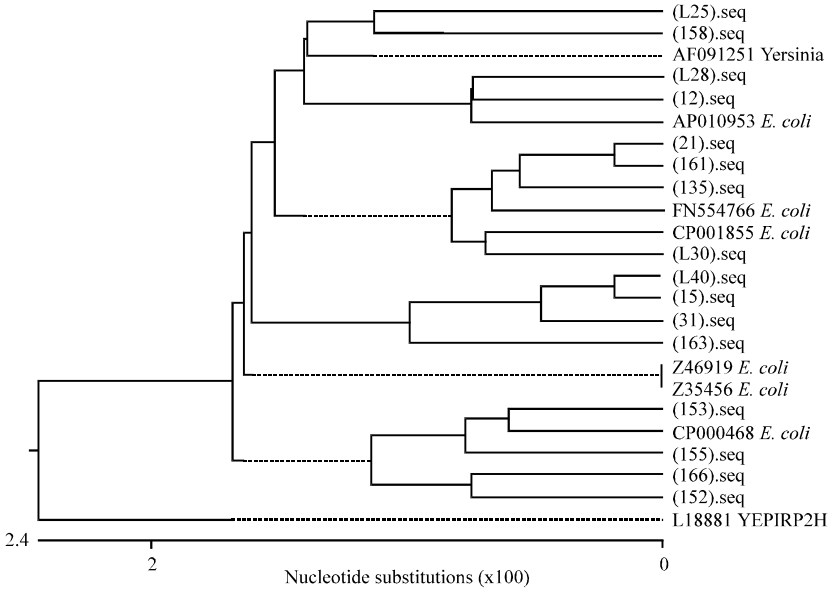

Fig. 4: The phylogenetic tree of nucleotide sequence of *irp2* genes

and O157 were major serotypes in Hubei Province while in Hubei, Henan and Jiangxi were O152, O54, O6, O9, O93 and O107 majorly, conducted by Liu mengyun; O101, O9, O138, O141, O86, O94 and O45 were dominant serotypes in Beijing by Zhang. Based on the above data, multiple serotypes exist in the same region. The local dominant serotype exists in most areas but some are alike, some are different in distinct places.

Pathogenicity island: The earlier study was conducted by Chen *et al.* (2004, 2006), 1007 *E. coli* strains from piglets were isolated in some areas of nine provinces including Jiangsu in which HPI strains (12.7%) carried Yersinia *HPI* genes. The O93 and O107 were common serotypes of piglets HPI *E. coli*. In the survey on *HPI* gene from piglets *E. coli* conducted by Gaosong, the positive rate of *fyuA* and *irp2* genes was 61.7% (95/154). Half of clinical piglets infected with HPI+ *E. coli* was conducted by Cheng *et al.* (2009). In this study, the *E. coli* strains from piglets in Hebei province carrying HPI *fyuA* and *irp2* genes have more serotypes. The isolating rate of fyuA was 24.4% (11/45), referred to serotype O38, O107 and O53; *irp2* was 42.2% (19/45), referred to serotype O38, O24, O93, O107, O53 and O78 while the isolating rate of *fyuA* and *irp2* was 13.3% (6/45), referred to serotype O107, O53 and O38. In view of numerous serotypes of HPI, there are more difficulties in the prevention and treatment of diseases. Although, it is proved that *fyuA* and *irp2* genes of HPI exist in pathogenic *E. coli* from piglets in this study but the function of HPI *E. coli* in diarrhea of newborn piglets is unknown which is further studied.

CONCLUSION

The O38 was the common serotype of *E. coli* from pigs, 13.3% strains were carried with Yersinia *HPI* genes (*irp2* and *fruA*).

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