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Prevalence and characterization of virulence genes in *Escherichia coli* isolated from piglets suffering post-weaning diarrhoea in Shandong Province, China

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Abstract

The present study was performed to investigate the prevalence and characteristics of virulence genes in *Escherichia coli* (*E. coli*) isolated from piglets suffering post-weaning diarrhoea (PWD) in Shandong Province, China. The standard bacteriological method was used to isolate and identify *E. coli*, and then multiplex polymerase chain reaction (mPCR) was performed to determine virulence genes in *E. coli*. Among the 300 isolates, 166 (55.3%) harboured at least one virulence gene. Among the 166 isolates, 155 (93.4%) contained toxin-related genes. For enterotoxin genes, EAST1 (58/166, 34.9%) and LT-I (45/166, 27.1%) were the most common, followed by STa (32/166, 19.3%) and STb (21/166, 12.7%); for pathogenicity island (PAI) genes, *irp2* (49/166, 29.5%) was the most dominant, followed by *eae* (48/166, 28.9%); for Shiga toxigenic *E. coli* (STEC)-associated toxin genes, Stx2e and *hlyA* genes were observed in 19 (19/166, 11.4%) and three strains (3/166, 1.8%) respectively. In addition, of the 166 isolates, 95 (95/166, 57.2%) contained adhesin genes, and AIDA-I (33/166, 19.9%) was the most common, followed by *paa* (27/166, 16.3%), F5 (K99) (20/166, 12.0%), F18 (15/166, 9.0%) and F41 (12/166, 7.2%). In summary, these findings demonstrated the prevalence and characteristics of virulence factors in *E. coli* isolates from piglets with PWD in Shandong Province of China, and the data may be useful for establishing preventive measures for post-weaning piglet diarrhoea.

KEYWORDS
adhesin gene, *Escherichia coli*, post-weaning diarrhoea, toxin gene, virulence factors

1 | INTRODUCTION

Pathogenic *Escherichia coli* (*E. coli*) can induce post-weaning diarrhoea (PWD) in piglets and can lead to significant economic losses in swine industry because of high morbidity, mortality, growth retardation and increased therapy costs (Alexa, Hamřík, Konstantinová, & Šrámková-Zajacová, 2011; Fairbrother & Nadeau, 2005; van Beers-Schreurs, Vellenga, Wensing, & Breukink, 1992). Numerous studies have showed that enterotoxigenic *E. coli* (ETEC) can cause piglet diarrhoea, which is primarily associated with the fact that ETEC can express fimbriae to mediate attachment to pig enterocytes and colonization; ETEC F4 (K88) and F18 are the leading cause of PWD of piglets, other fimbrial types, including F5 (K99), F6 (987P) and F41 are typical of neonatal diarrhoea (Kim, Kim, Hur, & Lee, 2010; Kwon et al., 2002; Vu-Khac et al., 2007; Zhang, Zhao, Ruesch, Omot, & Francis, 2007). In addition, ETEC can produce enterotoxins, including heat-labile (LT-I and LT-II)

and heat-stable (STa and STb), to destroy fluid homoeostasis in the intestine leading to diarrhoea (Liu et al., 2014; Luppi et al., 2016). Many investigations demonstrated that enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) is frequently detected among pathogenic *E. coli* isolates from humans and animals with diarrhoea (Liu et al., 2014; Toledo et al., 2012; Vu-Khac et al., 2007; Zhang et al., 2007).

Pathogenic *E. coli* can also produce other virulence factors, which are closely related with PWD cases in piglets. These virulence factors mainly included pathogenicity islands (PAI), Shiga toxigenic *E. coli* (STEC)-associated virulence factors and non-fimbrial adhesins (Liu et al., 2014). Specifically, the locus of enterocyte effacement (LEE) and high-pathogenicity island (HPI) are the significant PAIs and are frequently detected in enteropathogenic *E. coli* (EPEC) isolated from piglets with diarrhoea (Cheng, Sun, Xu, & Gao, 2006; Liu et al., 2014). The LEE encodes attaching and effacing factor (*eae*) and a transcriptional regulator, *ler* (Barba et al., 2005; Jores, Rumer, & Wieler, 2004), and HPI carries *irp2* gene, which is regarded as a specific detection marker for the HPI (Rakin, Noelting, Schubert, & Heesemann, 1999). STEC can cause oedema and diarrhoea in piglets due to expression of many kinds of virulence factors, such as Shiga toxins (Stx1, Stx2 and their variants), LEE, PAI, α-haemolysin (*hlyA*) and the STEC autoagglutinating adhesin (*saa*) (Gyles, 2007; Toledo et al., 2012). Additionally, adhesins involved in diffuse adherence (AIDA-I) and porcine attaching and effacing-associated (*paa*) factors were important non-fimbrial adhesins frequently found in *E. coli* strains isolated from piglets with diarrhoea (Liu et al., 2014; Zhang et al., 2007).

In general, *E. coli* isolated from piglets with diarrhoea carry different types of virulence factors, which can enhance pathogenicity of *E. coli* via synergistic effects. Furthermore, the detection frequency of these virulence factors is known to vary greatly over time (Kim et al., 2010; Liu et al., 2014; Toledo et al., 2012; Zhang et al., 2007). In Shandong Province of China, a large number of small-scale pig farms (10–50 sows per each farm, and semi-intensive management method), many of which frequently suffer economic losses due to PWD in piglets caused by pathogenic *E. coli*, however, little data are available about characteristics of virulence genes of *E. coli* isolated from piglets with PWD (Chen & He, 2017). Therefore, the present study was performed to characterize virulence genes of *E. coli* isolated from piglets with PWD in Shandong Province, China, with the aim to provide reference data for controlling PWD in piglets.

2 | MATERIALS AND METHODS

2.1 | Collection of samples

A cross-section sampling was carried out between 2016 and 2018. Rectal samples were obtained from weaned piglets (5–7 weeks old) with obvious symptoms of PWD on 36 pig farms (10–50 sows per each farm, semi-intensive management method and the pigs were kept in backyard farms) in Shandong Province, China. Clinical signs of piglets were diarrhoea, depression, dehydration and increased mortality (van Beers-Schreurs et al., 1992).

When clinical signs of piglets with PWD were observed by farmers, faecal samples were immediately collected within 24 hr.

Sampling was conducted using sterile cotton swabs. Collected samples were promptly transported on ice in a cool box to our microbiological laboratory within 24 hr for bacterial culture and isolation (Ikwap et al., 2014).

2.2 | Culture, isolation and identification of *E. coli*

Bacterial cultivation for *E. coli* was conducted based on previously published procedures (Ikwap et al., 2016). Briefly, each sample was directly cultured onto sterile MacConkey agar (Hopebiol, Qingdao, China) and incubated at 37°C overnight under aerobic condition. From each sample, 2–4 lactose-fermenting colonies with obvious morphological differences were selected and biochemically confirmed. The confirmed *E. coli* were stored at −20°C in Luria–Bertani (LB) broth containing 20% glycerol (Hopebiol, Qingdao, China) for DNA extraction.

2.3 | DNA extraction and multiplex PCR for virulence genes

The boiling method was used to extract total bacterial DNA of each *E. coli* strain (Levesque, Piche, Larose, & Roy, 1995). As previously described (Bertin, Martin, Girardeau, Pohl, & Contrepois, 1998; Cheng et al., 2006; Liu et al., 2014; Osman, Mustafa, Aly, & AbdElhamed, 2012; Paton & Paton, 1998, 2002; Vu-Khac et al., 2007; Zhang et al., 2007), multiplex polymerase chain reaction (mPCR) was used to determine 21 virulence genes encoding enterotoxins (LT-I, LT-II, STa, STb, and EAST1), fimbriae (K88, K99, 987P, F41, and F18), non-fimbrial adhesins (AIDA-I, *paa*, *eae*, and *saa*), Shiga toxins (Stx1, Stx2, and Stx2e), HPI and α-haemolysin (*hlyA*). The reaction volume (20μL) comprised 2μM of each primer (Table 1), 2 × Master Mix (Takara, Dalian, China) and 3μl of DNA template. mPCR conditions were as follows: 95°C denaturation (15 min), 94°C denaturation for 1 min, 58°C annealing for 1 min, 72°C extension for 1 min (30 cycles) and a final extension for 7 min at 72°C. The products were visualized in 2% agarose gels stained with ethidium bromide using electrophoresis. Positive and negative control bacteria used in the mPCR were displayed in the Table 2.

2.4 | Statistical analyses

The Chi-squared test was employed to analyse the data. When *P* values were less than 0.05, difference was considered statistically significant. Data analyses were performed using the Stata 11 software (StataCorp Lp).

3 | RESULTS

3.1 | *E. coli* isolates

A total of 300 *E. coli* isolates were obtained from 162 samples from different piglets with PWD. The results of mPCR showed that 166 *E.*

TABLE 1 Primers used in this study to amplify *E. coli* virulence factor genes

Virulence factors	Forward primers (5'–3')	Reverse primers (5'–3')	Size of product (base pairs)
LT-I	TAGAGACCGGTATTACAGAAATCTGA	TCATCCCGAATTCTGTTATATATGTC	282
LT-II	AGATATAATGATGGATATGTATC	TAACCCTCGAAATAAATCTC	300
STa	GGGTTGGCAATTTTTATTTCTGTA	ATTACAACAAAGTTCACAGCAGTA	183
STb	ATGTAAATACCTACAACGGGTGAT	TATTTGGGCGCCAAAGCATGCTCC	360
EAST1	ATGCCATCAACACAGTATATC	TCAGGTCGCGAGTGACGG	117
K88	GATGAAAAAGACTCTGATTGCA	GATTGCTACGTTCAGCGGAGCG	841
K99	CTGAAAAAAACACTGCTAGCTATT	CATATAAGTGACTAAGAAGGATGC	543
987P	GTTACTGCCAGTCTATGCCAAGTG	TCGGTGTACCTGCTGAACGAATAG	463
F41	GATGAAAAAGACTCTGATTGCA	TCTGAGGTCATCCCAATTGTGG	682
F18	ATGAAAAGACTAGTGTTTATTTCTT	TTACTTGTAAGTAACCGCGTAAGCC	520
AIDA-I	ACAGTATCATATGGAGCCA	TGTGCGCCAGAACTATTA	586
paa	CCATAAAGACAGCTTCAGTGAAAA	GTATTACTGGTACCACCACCATCA	162
eae	ATATCCGTTTTAATGGCTATCT	AATCTTCTGCGTACTGTGTTCA	425
ler	AACAAGCCCATACATTCAGC	GCCATCATCAGGCACATTAG	169
irp2	AAGGATTCGCTGTTACCGGAC	TCGTCGGGCAGCGTTTCTTCT	287
Stx1	ATTCGCTGAATGTCATTCGCT	ACGCTTCCCAGAATTGCATTA	664
Stx2	GAATGAAGAAGATGTTTATAGCGG	GGTTATGCCTCAGTCATTATTAA	281
Stx2e	GAATGAAGAAGATGTTTATAGCGG	TTTTATGGAACGTAGGTATTACC	454
saa	CGTGATGAACAGGCTATTGC	ATGGACATGCCTGTGGCAAC	119
hlyA	GCATCATCAAGCGTACGTTCC	AATGAGCCAAGCTGGTTAAGCT	533

TABLE 2 Positive and negative control bacteria used in the mPCR

Strain	Virulence factor	Source
C83903	K88, LT-I, STb, EAST1	CIVDC
C83920	K99, F41, STa	CIVDC
C83529	K99, STa	CIVDC
C83915	987P, STa	CIVDC
C83684	F18ab, Stx2e	CIVDC
CMCC44498	F18ab, Stx2e	CIVDC
TSMC152	LEE, *hlyA*	TSMC
TSMC129	EAST1, HPI, F17	TSMC
TSMC117	Stx1, LEE, HPI, EAST1, K88, CS31A	TSMC
TSMC02	HPI, F17, 987P	TSMC
TSMC09	HPI, AIDA-I	TSMC
TSMC107	HPI, *eae*	TSMC
TSMC122	Stx1, *irp2*, *saa*	TSMC
TSMC163	K88, *ler*	TSMC
TSMC103	AIDA-I	TSMC
TAMC108	*paa*	TSMC
TSMC212	None	TSMC

Abbreviations: CIVDC, China Institute of Veterinary Drug Control; TSMC, Taishan Medical University, China.

coli isolates contained at least one virulence gene (166/300, 55.3%), whereas no virulence gene was detected in the other 134 strains (134/300, 44.7%).

3.2 | Toxin-associated genes and category

Among the 166 isolates carrying virulence genes, 155 (155/166, 93.4%) were detected positive for toxin-related genes. In terms of enterotoxin genes, EAST1 (58/166, 34.9%) and LT-I (45/166, 27.1%) were the most common, followed by STa (32/166, 19.3%) and STb (21/166, 12.7%), but no LT-II gene was found in this study. In terms of PAI genes, *irp2* (49/166, 29.5%) was the most dominant, followed by *eae* (48/166, 28.9%), whereas no *ler* gene was detected. In terms of STEC-associated toxin genes, Stx2e and *hlyA* genes were respectively observed in 19 (19/166, 11.4%) and three strains (3/166, 1.8%), but no isolate carried the Stx1 or Stx2 genes (Table 3).

These 155 *E. coli* can be divided into seven categories: ETEC, EPEC, STEC, ETEC/EPEC, ETEC/EPEC/STEC, ETEC/STEC and STEC/EPEC, with 52 (52/166, 31.3%), 41 (41/166, 24.7%), 1 (1/166, 0.6%), 40 (40/166, 24.1%), 9 (9/166, 5.4%), 5 (5/166, 3.0%) and 7 (7/166, 4.2%) strains for each category respectively (Table 3).

3.3 | Fimbrial and non-fimbrial adhesin genes

Among the 166 isolates carrying virulence genes, 95 (95/166, 57.2%) contained fimbrial and non-fimbrial adhesin genes, and AIDA-I (33/166, 19.9%) was the most common, followed by *paa* (27/166, 16.3%), F5 (K99) (20/166, 12.0%), F18 (15/166, 9.0%), F41 (12/166, 7.2%), F6 (987P) (10/166, 6.0%) and F4 (K88) (10/166, 6.0%).

Noticeably, seven different combinations of adhesin factors were observed, including K88/*paa*, F6/F41, K99/*paa*, AIDA-I/*paa*,

TABLE 3 Distribution and category of *E. coli* isolates positive for toxin-associated genes

Category	Toxin gene	No. of isolates
ETEC	STa	1
	STa/LT-I	5
	STa/STb/LT-I	8
	LT-I/STa/STb/EAST1	8
	LT-I	1
	EAST1	29
		52 (52/166, 31.3%)
EPEC	*eae*	16
	irp2	25
		41 (41/166, 24.7%)
STEC	Stx2e	1
		1 (1/166, 0.6%)
ETEC/EPEC	STa/*eae*	2
	STa/LT-I/*eae*	5
	LT-I/*eae*	12
	irp2/EAST1	21
		40 (40/166, 24.1%)
ETEC/EPEC/STEC	STa/*irp2*/*hlyA*	3
	LT-I/Stx2e/*eae*	6
		9 (9/166, 5.4%)
ETEC/STEC	STb/Stx2e	5
STEC/EPEC	Stx2e/*eae*	7
None[a]		11(11/166, 6.6%)
Total		166

[a]Isolates negative for toxin-associated genes.

F18/F41, K99/F41 and K99/F18/F41. The most common combinations were AIDA-I/*paa* (eight isolates), K99/*paa* (seven isolates) and K99/F41(six isolates) (Table 4).

3.4 | Combination of fimbriae and enterotoxins

Among the 166 isolates carrying virulence genes, 20 (20/166, 12.0%) concurrently contained fimbriae and enterotoxins. Of the 20 isolates, 15 different combinations were observed, with K99/*paa*/EAST1 being the most predominant (four isolates), followed by F18/STa/LT-I (two isolates) (Table 5).

3.5 | Distribution and characteristics of virulence factors

Among the 166 isolates carrying virulence genes, 71 (71/166, 42.8%) did not contain any fimbrial or non-fimbrial adhesin genes. Of the 71 isolates, nine different combinations of toxin-associated genes were observed, with *irp2*/EAST1 being the most predominant (21 isolates) (Table 5).

TABLE 4 Distribution and characteristics of *E. coli* isolates positive for adhesin genes

Adhesin gene	No. of isolates (%)
K88	8 (4.8)
F6	8 (4.8)
K88/*paa*	2 (1.2)
F6/F41	2 (1.2)
K99/*paa*	7 (4.2)
paa	10 (6.0)
AIDA-I/*paa*	8 (4.8)
AIDA-I	25 (17.5)
F18	11 (6.6)
K99	4 (2.4)
F18/F41	1 (0.6)
K99/F41	6 (3.6)
K99/F18/F41	3 (1.8)
None[a]	71(42.8)
Total	166

[a]Isolates negative for adhesin genes.

For the most common adhesin factors AIDA-I and *paa*, the AIDA-I-positive strains frequently carried EAST1 (21/33) or *irp2* (12/33); the *paa*-positive strains also frequently contained EAST1 (12/27) and *irp2* (11/27). Of note, no toxin-associated genes were detected in seven K88-positive isolates and four *paa* positive isolates (Table 5).

There were 31 different combinations of colonization factors and toxin-associated genes in this study, and the most common profiles were AIDA-I/EAST1 (12 isolates), AIDA-I/*irp2* (8 isolates) and F6/*eae* (8 isolates) (Table 5). AIDA-I was significantly related with EAST1 and *irp2* ($p < .05$).

4 | DISCUSSION

During the past decades, the occurrence and spread of the PWD in piglets have posed an enormous economic loss to the development of swine breeding industry in mainland China (Cheng et al., 2006; Liu et al., 2014). Numerous studies have showed that ETEC are the most important agents of enteric colibacillosis in pig, and fimbrial adhesins are necessary in the pathogenetic mechanism (Do, Byun, & Lee, 2018; Toledo et al., 2012; Vu-Khac et al., 2007; Zhang et al., 2007). Of note, *E. coli* with fimbrial adhesins can be also detected in pigs without diarrhoea. Moredo et al. (2015) demonstrated that the percentage of ETEC-positive non-diarrhoeic pigs was 16.6% during the lactation period, 66% in the nursery phase and 17.3% in the finisher population.

In this study, F5 (K99) (12.0%), F18 (9.0%), F41 (7.2%), F6 (987P) (6.0%) and F4 (K88) (6.0%) were also frequently identified, which indicated that fimbriae were closely related with pathogenic *E. coli* from piglets with PWD.

TABLE 5 Distribution of virulence genes among 166 virulence-positive *E. coli* isolates in this study

Adhesin gene	toxin gene	No. of isolates
K88	ND[a]	7
K88	STa/*irp2*/*hlyA*	1
F6	*eae*	8
K88/*paa*	EAST1	1
K88/*paa*	*irp2*	1
F6/F41	STa/LT-I	2
K99/*paa*	EAST1	4
K99/*paa*	*irp2*	3
paa	ND	4
paa	EAST1	3
paa	*irp2*	3
AIDA-I/*paa*	EAST1	4
AIDA-I/*paa*	*irp2*	4
AIDA-I	LT-I/STa/STb/EAST1	5
AIDA-I	EAST1	12
AIDA-I	*irp2*	8
F18	*eae*	1
F18	STa	1
F18	LT-I	1
F18	STa/LT-I	2
F18	Stx2e/*eae*	4
F18	LT-I/Stx2e/*eae*	1
F18	STa/LT-I/*eae*	1
K99	*eae*	2
K99	STa/LT-I	1
K99	STa/STb/LT-I	1
F18/F41	Stx2e	1
K99/F41	*eae*	2
K99/F41	STa/*eae*	1
K99/F41	LT-I/*eae*	1
K99/F41	STb/Stx2e	1
K99/F41	STa/STb/LT-I	1
K99/F18/F41	Stx2e/*eae*	3
ND	*eae*	3
ND	EAST1	5
ND	*irp2*	6
ND	STa/*eae*	1
ND	*irp2*/EAST1	21
ND	LT-I/*eae*	11
ND	STb/Stx2e	4
ND	LT-I/Stx2e/*eae*	5
ND	STa/LT-I/*eae*	4
ND	STa/STb/LT-I	6
ND	STa/*irp2*/*hlyA*	2
ND	LT-I/STa/STb/EAST1	3
Total		166

[a]Isolates negative for adhesin or toxin genes.

F4 and F18 fimbriae have been frequently detected in weaned piglets in several countries such as Korea, Japan, Europe and United States (Do, Byun, & Lee, 2019; Kusumoto et al., 2016; Luppi et al., 2016; Zhang et al., 2007). It is interesting and surprising to observe such a high prevalence of genes enconding for fimbriae F5, F41, F6 in this study. The frequency of different virotypes (with different adhesins) is mainly mediated by the different specific receptors on porcine intestinal brush border epithelial cells (enterocytes). These receptors change from pigs depending on the age (Luppi, 2017) and infection with ETEC carrying the F5, F6 or F41 fimbriae, occurs in pigs less than 2 weeks of age (Nagy & Fekete, 2005). Another possible explanation is that in Shandong Province of China, inactivated vaccines for sows have been used widely, and these vaccines contain *E. coli* whole cells with F4 and F18 (Chen & He, 2017), which may have decreased the frequency of F4 and F18 and caused the emergence of new adhesin antigens such as F5, F6 and F41 (Do et al., 2019, 2006).

It has been reported that AIDA-I is related with ETEC strains isolated from weaned pigs with PWD, but its role in colibacillosis in pigs is needed to be elucidated (Luppi, 2017). Both AIDA-I (33/166, 19.9%) and *paa* (27/166, 16.3%) were commonly observed in this study. Similarly, the two non-fimbrial adhesin genes were also frequently identified in China and other countries. For example, the AIDA-I gene was detected in 27/104 (26.0%) pathogenic *E. coli* isolated from piglets with diarrhoea in Hessia, Germany (Niewerth et al., 2001). Both the AIDA-I and *paa* genes were detected in 79/304 (26.0%) and 133/304 (43.8%) pathogenic *E. coli* isolates from young pigs with diarrhoea in the United States respectively (Zhang et al., 2007). In the northeastern region of China, both the AIDA-I (34/206, 16.5%) and *paa* (50/206, 24.3%) were observed in the virulence gene-positive *E. coli* from suckling pigs with diarrhoea (Liu et al., 2014). Noticeably, seven K88-positive isolates and four *paa*-positive *E. coli* isolates in the study did not harbour any toxin-related genes. Similarly, *paa* was also detected in non-EPEC isolates (Ngeleka, Pritchard, Appleyard, Middleton, & Fairbrother, 2003). In addition, seven K88-positive *E.coli* isolates in the study did not carry any toxin-related genes, which should be further studied in animal experiments.

Noticeably, relatively high isolation rate of *E. coli* without any fimbrial or non-fimbrial adhesin genes (71/166, 42.8%) was observed in the present study, which was in agreement with the result conducted in the northeastern region of China (Liu et al., 2014). A previous animal experiment showed that if EPEC cannot complete attachment to epithelial cells via adhesins, young pigs would not develop diarrhoea (Sellwood, Gibbons, Jones, & Rutter, 1975), so the result should be further investigated and studied in animal experiments.

For enterotoxins detected in this study, the gene encoding the STa was frequently identified (32/166, 19.3%), which was consistent with the results conducted in Argentina (Paton & Paton, 1998), Korea (Kwon et al., 2002) and Vietnam (Do et al., 2006). Of note, the detection of LT-I (45/166, 27.1%) was more frequent than the STa in this study, which was similar with the findings reported in China and other countries (Liu et al., 2014; Madoroba et al., 2009; Vu-Khac et al., 2007).

In the present study, for the most common colonization factors AIDA-I and *paa*, the AIDA-I-positive strains also carried EAST1 (21/33) and *irp2* (12/33); the *paa*-positive strains also contained EAST1 (12/27) and *irp2* (11/27). Similar findings have been reported in numerous studies (Ngeleka et al., 2003; Pritchard, Ngeleka, & Middleton, 2004; Ravi et al., 2007).

In this study, 155 (155/166, 93.4%) isolates were detected positive for toxin-related genes. The 155 *E. coli* can be divided into seven combinations according to the presence of toxin-related genes: ETEC, EPEC, STEC, ETEC/EPEC, ETEC/EPEC/STEC, ETEC/STEC and STEC/EPEC (Vidal et al., 2005). Of note, 52 *E. coli* isolates belonged to ETEC (52/166, 31.3%). The result is similar to findings conducted in Jiangsu and Guangdong Provinces, China (Chen, Gao, Jiao, & Liu, 2004; Wang et al., 2010), indicating that ETEC may be an important pathogen leading to PWD in piglets in China.

Haemolytic activity of *E.coli* strains was not evaluated and the diagnosis of enteric colibacillosis was not conducted in this study, which are limitations of our study, however, these findings demonstrated the distribution and characteristics of virulence factors in *E. coli* isolates from piglets with PWD in Shandong Province of China, and the data may be useful for establishing preventive measures for post-weaning diarrhoea of piglets.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL STATEMENT

The sampling procedure was approved by the Animal Care and Use Committee of Taishan Medical University (Permit Number: TSMU201606018).

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