



DOI: 10.22144/ctu.jen.2019.017

## Antimicrobial resistance of *Escherichia coli* causing edema disease in post-weaning pigs in Vinh Long province

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### Article info.

Received 01 Oct 2018

Revised 11 Apr 2019

Accepted 30 Jul 2019

### Keywords

Antimicrobial resistance,  
*Escherichia coli*, edema dis-  
ease, piglet, Vinh Long

### ABSTRACT

The study was conducted to determine the antimicrobial resistance of *Escherichia coli* which causes edema disease in post-weaning pigs in Vinh Long province. The results showed that 150 out of 769 examined piglets were positive with *E. coli* causing edema disease (19.51%). Among the infected piglets, the mortality rate of the disease was 57.33% (86/150). The proportion of *E. coli* infection in weaned pigs was higher on 1-2 weeks post weaning (66.95%) compared to over two weeks of post weaning (33.05%). The proportion of *E. coli* isolated from piglets at households was significantly higher than that at the farms (61.02% vs 38.98% respectively). The most common symptoms of *E. coli* infection in piglets were swollen eyelids (100%), followed by swollen head (83.90%), and convulsions (82.20%). Moreover, the lesions including fluid accumulation in abdominal cavity (97.67%), hemorrhage in mesentery (96.51%) and small intestines (96.51%) were frequently detected in infected piglets. Positive rate of gene encoding virulent factor Stx2e in post-weaning pigs was quite high (42.37%). *E. coli* strains were highly resistant to Ampicillin (92%) and Bactrim (77%), followed by Streptomycin (68%) and Gentamycin (50%). The 117/118 strains were multi-resistant to 2-7 antibiotics with 66 different diversified and complicated types. *E. coli* was still sensitive to ceftazidime, cefuroxime, amikacin, and amoxicillin-clavulanic acid.

Cited as: Khai, L.T.L. and Lai, L.T.C., 2019. Antimicrobial resistance of *Escherichia coli* causing edema disease in post-weaning pigs in Vinh Long province. Can Tho University Journal of Science. 11(2): 1-8.

## 1 INTRODUCTION

Pig production is an important food industry providing high nutrient for humans. As pig production is developed more and more that increased breed demand, diversified and diseases occurred more complicated. The remaining large percentage of small pig farms leads to usual occurrence of diseases, e.g. edema disease caused by *Escherichia coli*.

*E. coli* strains producing Stx2e toxin which was important virulent caused edema disease in post-

weaning pigs. The most common strains of *E. coli* which caused edema disease with high morbidity and mortality rate were F4 (K88) and F18 (Zimmerman *et al.*, 2012). Nguyen Thi Thanh Hoa *et al.* (2010) showed that pigs in small scale farms in Phu Tho had edema disease in high morbidity (28.06%) and mortality rate (56.47%). Farmers used several antibiotics which were added to feed and drinking water for disease prevention. However, abuse of antibiotics in pig production might lead to antibiotic resistance. According to Uemura *et al.* (2004), increase of antibiotic resistance of *E. coli* causing edema disease was deserved well concern

and should be studied more. In Vietnam, the antibiotic resistance in animal production became a matter of social concern for human and animal health. Nguyen Thi Kim Lan (2003) reported that *E. coli* causing edema diseases in weaned pigs in Thai Nguyen province was resistant to ampicillin and cefuroxime. Vinh Long province has a big herd of swine. Edema disease caused by *E. coli* is commonly occurred in post-weaning pigs, but treatment was often ineffective and economic loss for farmers. Therefore, study on antimicrobial resistance of *E. coli* causing edema disease in post-weaning pigs was essential for animal productions. The study was conducted to determine the edema morbidity rate, virulent factor of Stx2e, common strains F4, F18 and antibiotic resistance of *E. coli* causing edema in post-weaning pigs in Vinh Long province.

## 2 MATERIALS AND METHODS

### 2.1 Materials

One hundred and eighteen feces samples and 86 mesenteric lymph nodes of post-weaning piglets (from 769 examined piglets) were collected from 12 farms and 21 households at four districts in Vinh Long province from August 2016 to February 2018.

The following antibiotic discs were used: amoxicillin/clavulanic acid (Ac) 20/10 µg, colistin (Co) 10 µg, gentamicin (Ge) 10 µg, amikacin (Ak) 30 µg, streptomycin (Sm) 10 µg, tetracycline (Te) 30 µg, doxycycline (Dx) 30 µg, ampicillin (Am) 10 µg, trimethoprim-sulfamethoxazole (bactrim) (Bt) 1.25/23.75 µg, ceftazidime (Cz) 30 µg, cefuroxime (Cu) 30 µg, and levofloxacin (Lv) 5 µg (Nam Khoa Co. Ltd, Vietnam).

Materials used for Polymerase chain reaction (PCR): Forward and reverse primers of F4, F18 and Stx2e (Integrated DNA Technologies, USA) (Table 1); PCR Kit, Go Taq® Green Master Mix, 2X (Promega, USA); 100 bp (Gel loading buffer - Invitrogen) (Promega, USA).

### 2.2 Methods

#### 2.2.1 Sampling methods

Sampling method was performed according to the TCVN 10782: 2015 (ISO 13307: 2013) (DAH, 1990). Twenty-five grams of feces samples and two mesenteric lymph nodes were collected from piglets having edema disease. In each farm and household, samples were collected from 2-3 piglets/litter from 2-3 herds then stored at cold condition.

#### 2.2.2 Isolation and identification of antimicrobial resistance of *E. coli*

*E. coli* was isolated according to Barrow and Feltham (2003) and TCVN 5155-90 (DAH, 2015). Disc diffusion method was performed to test antimicrobial resistance of *E. coli* (Bauer *et al.*, 1966). Zones of growth inhibition were evaluated in accordance with Clinical Laboratory Standards Institute standards (CLSI, 2016) when adequate reference values were available.

#### 2.2.3 Methods of F4, F18 *E. coli* strains identification and virulent gene Stx2e examination

##### DNA extraction

DNA of *E. coli* was extracted by heat shock as described by Cerna *et al.* (2003) and Botteldoorn *et al.* (2003). Process of PCR was done following the protocol of Promega Company, USA.

**Table 1: List of primers for detection of *E. coli* F4, F18 strains and virulent encoding gene Stx2e of *E. coli* causing edema disease in post-weaning pigs using in PCR**

Virulence	Primer sequence (5'–3')	Amplified product size (bp)	Reference
F4 ( <i>faeG</i> )	GAA TCT GTC CGA GAA TAT CA GTT GGT ACA GGT CTT AAT GG	499	Boerlin <i>et al.</i> , 2005
F18 ( <i>fedA</i> )	TGG TAA CGT ATC AGC AAC TA ACT TAC AGT GCT ATT CGA CG	313	Boerlin <i>et al.</i> , 2005
Stx2e	CCT TAA CTA AAA GGA ATA TA CTG GTG GTG TAT GAT TAA TA	230	Fratamico <i>et al.</i> , 2004

### 2.3 Statistical analysis

Chi-square test with statistical significance set at the 95% confidence level ( $p < 0.05$ ) was used for statistical comparisons of prevalence, Chi-square was used for comparison of proportions.

## 3 RESULTS AND DISCUSSIONS

### 3.1 Results of the survey on edema disease in post-weaning pigs in Vinh Long province

The results of the survey on edema disease in post-weaning pigs was shown in Table 2.

Out of 769 examined post-weaning pigs of 33 herds at 4 districts in Vinh Long province, 150 edema disease piglets were examined. The morbidity rate of edema disease piglets was 19.51%. Bertschinger *et al.* (1992) reported that the morbidity rate ranged from 30-40% and may reach to 80%. The morbidity rate of edema disease in post-weaning pigs in Vinh Long was lower than other studies. In the survey in Vinh Long province, the reason might be due to the instability of animal production, great reduced prices causing herds in the region decreased, farmer restricted to give feed to piglets. While edema disease often occurred in big, greedy piglets leading over protein intake which serve a good environment for *E. coli* to develop in intestine then cause edema disease (Carlton *et al.*, 2010). The morbidity rate of

edema disease in piglets in Vung Liem district (22.97%), was similar to Mang Thit (21.08%), Long Ho (18.41%) and Tra On district (14.29%). ( $P = 0.337$ ). This might be due to that the four districts are not far from each other, and pigs were traded across the province. The mortality rate was high (57.33%); it is similar to Nguyen Thi Kim Lan's (2003) study which reported that the edema mortality rate of piglets in Binh Dinh province was 61.44%. Zimmerman *et al.* (2012) suggested that the mortality rate ranged from 50% to 90% of edema disease in piglets. The mortality rate at 4 districts was very significant difference ( $P = 0.000$ ). Those differences might be due to different knowledge and diseases prevention procedures that affected to mortality rate in those areas.

**Table 2: Results of the survey on edema disease in post- weaning pigs in Vinh Long province**

Location (district)	Herd	No. of examined samples	Morbidity		Mortality	
			Number	(%)	Number	(%)
Tra On	12	161	23	14.29	12	52.17
Vung Liem	7	222	51	22.97	6	11.76
Long Ho	7	201	37	18.41	34	91.89
Mang Thit	7	185	39	21.08	34	87.18
			$P=0.337$		$P=0.000$	
Total	33	769	150	19.51	86	57.33

**3.2 Result of isolation of *E. coli* causing edema in post-weaning pigs in Vinh Long province**

The result of isolation of *E. coli* causing edema in post-weaning pigs was shown in Table 3, 4 and 5.

There were 118/118 feces samples and 86/86 lymph node samples from 118 edema disease piglets were positive with *E. coli* in ratio of 100%. These results contributed to specifying edema disease piglets

caused by *E. coli* with typical symptoms. According to Carlton *et al.* (2010), *E. coli* invaded to the epithelial cells of the intestinal wall by various adhesive factors, entered the mesenteric lymph nodes through the lymphatic system and secreted Stx2e toxin, then the toxin entered the blood stream and located in different organs. Therefore, the isolation rate of *E. coli* in colonic mesentery lymph nodes was 100%.

**Table 3: Result of isolation of *E. coli* causing edema in post-weaning pigs in Vinh Long province**

Location (district)	Feces samples			Mesenteric lymph nodes		
	No. of isolates	No. of positive	Rate (%)	No. of isolates	No. of positive	Rate (%)
Tra On	25	25	100	12	12	100
Vung Liem	25	25	100	6	6	100
Long Ho	34	34	100	34	34	100
Mang Thit	34	34	100	34	34	100
Total	118	118	100	86	86	100

The positive rate of *E. coli* isolation in post-weaning pigs about two weeks after weaning (66.95%) was higher than that over two weeks (33.05%), and there was statistically significant difference ( $P<0.01$ ). In the first stage of 1-2 weeks, weaned pigs might confront sudden changes such as temperature, humidity, barns, feed, separating or putting in herd, as well as immature digestion and immune systems. These factors created opportunities for pathogenic

agents to invade and cause diseases in piglets. Bertschinger *et al.* (1992) reported that the edema disease often occurred in piglets about two weeks after weaning and 4-12 weeks of age. Therefore, in the post-weaning period, farmers should early detect symptoms of edema disease. Moreover, they should ensure the proper nutrition, appropriate temperature, and regularly keep sanitary and disinfected barns.

**Table 4: Result of isolation of *E. coli* causing edema disease in post-weaning pigs in Vinh Long province by age**

After weaning (week)	Positive results	Rate (%)
1 – 2	79	66.95
> 2	39	33.05
		<i>P=0.000</i>
Total	118	100

**Table 5: Result of isolation of *E. coli* causing edema disease in post-weaning pigs in Vinh Long province by farm types**

Types	Examined herds	No. of positive samples	Rate (%)
Household	21	72	61.02
Farm	12	46	38.98
		<i>P= 0.001</i>	
Total	33	118	100

The morbidity rate of edema disease in post-weaning pigs in household with 61.02% were higher than that in farms (38.98%). There was statistically significant difference between two farm types ( $P<0.01$ ). In this survey, farmers in industrial farms had raising and caring techniques, and knowledge on disease prevention. Conversely, farmers in household had limited on technical rising and caring such as periodic vaccination, frequent barn. The risky factors lead to edema disease in piglets caused by *E. coli*. Thus, the farm types were significantly impacted to morbidity rate of edema disease in post-weaning pigs. Furthermore, susceptibility of piglets to edema disease depended on several factors, especially on genetic resistance, feeding, and immunity (Bertschinger and Gyles, 1994).

**Table 7: Typical lesions frequently occurred on edema disease caused by *E. coli* in post-weaning pigs in Vinh Long province**

Lesions	Frequency of occurrence	Rate (%)
Fluid accumulate in abdominal cavity	84	97.67
Small intestinal hemorrhage	83	96.51
Mesenteric hemorrhage	83	96.51
Mesenteric lymph nodes swelling	82	95.35
Fluid accumulation in chest cavity	80	93.02
Pericardia swelling	70	81.40
Stomach swelling and hemorrhage	62	72.09
		<i>P=0.000</i>

Of the 86 dead piglets caused by edema disease, the proportion of fluid accumulation in the abdominal cavity was highest with 97.67%, followed by small intestinal hemorrhage, mesenteric hemorrhage, mesenteric lymph nodes swelling, fluid accumulate in chest cavity, pericardium swelling, stomach swelling, and hemorrhage with 96.51%, 96.51%,

**3.3 Results of symptoms and lesions observation of edema disease caused by *E. coli* in post-weaning pigs in Vinh Long province**

The symptoms and lesions observation of edema disease caused by *E. coli* in post-weaning pigs were shown in Table 6 and 7.

**Table 6: Typical symptoms frequently occurred on edema disease caused by *E. coli* in post-weaning pigs in Vinh Long province**

Symptoms	Frequency of occurrence	Rate (%)
Swelling of the eyelids	118	100.00
Moved convulsions	99	83.90
Swelling of the head	97	82.20
Hoarse sound	85	72.03
Diarrhea	62	52.54
		<i>P=0.000</i>

There were typical symptoms in piglets infected with edema disease that were observed in Vinh Long province. Swelling of the eyelids was the most common typical symptoms frequently occurred in edema disease of post-weaning pigs (100%), followed by convulsions (83.90%), swelling of the head (82.20%), hoarse sound (72.03%), and the least common symptom was diarrhea (52.54%). There was statistically significant difference among the symptoms ( $P<0.01$ ). Similarly, Zimmerman *et al.* (2012) described edema occurrence in the head, eyelids, laryngeal swelling which causes hoarse sound, nervous signs as crush headlong into the wall, walking around and convulsion. According to Carlton *et al.* (2010), fever or/and diarrhea might not show in edema disease.

95.35%, 93.02%, 81.40% and 72.09%, respectively ( $P < 0,01$ ). These lesions were frequently observed in piglets infected with edema disease in Vinh Long province. Zimmerman *et al.* (2012) showed that the most obvious lesions were gastric hemorrhage, intestinal hemorrhage, fluid accumulated in chest cavity and abdomen, and brain edema.

### 3.4 Results of identification of F4, F18 strains and virulent encoding gene Stx2e of *E. coli* causing edema disease in post-weaning pigs in Vinh Long province

The F4, F18 strains and virulent gene Stx2e of *E. coli* causing edema disease in post-weaning pigs were identified in Table 8.

Strain F18 with the highest rate (59.32%) were identified in 70 samples, and the lowest were F4 with 20.34% (24/118 samples). The results showed that F18 was the most common strains causing edema disease in post-weaning pigs in Vinh Long province. Zimmerman *et al.* (2012) suggested that F18 was the dominant strain in edema disease piglets. Gene encoding virulent factor Stx2e of *E. coli* causing edema disease in post-weaning pigs was 42.37%.

**Table 8: Results of identification of F4, F18 strains and Stx2e gene of *E. coli* causing edema in post-weaning pigs in Vinh Long province (n=118)**

Places	Genes					
	F4		F18		Stx2e	
	No. of positive samples	Rate (%)	No. of positive samples	Rate (%)	No. of positive samples	Rate (%)
Tra On	8	33.33	16	22.86	4	3.39
Vung Liem	4	16.67	15	21.43	7	5.93
Long Ho	7	29.17	25	35.71	14	11.86
Mang Thit	5	20.83	14	20.00	25	21.19
		<i>P</i> =0.528		<i>P</i> =0.118		<i>P</i> = 0.000
Total	24	20.34	70	59.32	50	42.37

### 3.5 Results of antibiotics resistance of *E. coli* strains causing edema disease in post-weaning pigs in Vinh Long province

The result of antibiotics resistance of *E. coli* strains causing edema disease in post-weaning pigs was described in Table 9 and 10.

The prevalence of multidrug-resistant bacteria in poultry and pig production was a public health concern. These bacteria could be transmitted to humans via the food chain or direct contact (Docic and Bilkei, 2003). The result of this study showed that *E. coli* strains isolated from piglets infected with edema disease in Vinh Long province were highly resistant to ampicillin (92.37%), bactrim (77.12%), streptomycin (67.80%), and fairly resistant to gentamicin (50%). In this study, both of F4 and F18

The distribution of Stx2e gene was different among four districts with *P* = 0.000. It might be due to the numbers of dead piglets in Mang Thit and Long Ho was higher than those in Vung Liem and Tra On (Table 2). *E. coli* invaded to the epithelium of the intestinal wall by various adhesive factors then moved to the mesenteric lymph nodes through the lymphatic system and secreted Stx2e toxin (Carlton *et al.*, 2010). Thus, the rate of gene encoding virulent factor Stx2e from dead piglets was higher. In addition, Botteldoorn *et al.* (2003) showed that the Stx2e gene of *E. coli* strains isolated from edema disease piglets was 31%. This study showed the differences among geographic areas, farm types and samples.

strains showed high resistance to ampicillin (91.67%, 91.43%), moderate resistance to bactrim (70.83%, 75.71%), and streptomycin (66.67%, 64.29%), respectively. *E. coli* causing edema disease in Vinh Long was highly resistant to ampicillin and streptomycin because the two antibiotics were frequently used to treat animal diseases by farmers. Choi *et al.* (2002) reported that *E. coli* isolated from diarrhea and edema disease in post-weaning pigs in Korea were highly resistant to ampicillin (91.19%), tiamulin (91.19%), tylosin (90.70%), and trimethoprim/sulfamethoxazole (87.21%). The antibiotic resistance of *E. coli* in this study were higher than that in other studies by Okello *et al.* (2015) with ampicillin (41.00%), by Bessone *et al.* (2017) with ampicillin (61.90%), bactrim (41.37%), and gentamicin (9.52%). These results showed that antibiotic resistance was increased and diverse in various areas

**Table 9: Result of antibiotics resistance of E. coli F4, F18 strains causing edema disease in post weaning pigs in Vinh Long province**

Antibiotics	Total (n=118)		F4 (n=24)		F18 (n=70)	
	Resistance (%)	Susceptibility (%)	Resistance (%)	Susceptibility (%)	Resistance (%)	Susceptibility (%)
Ampicillin	92.37	7.63	91.67	8.33	91.43	8.57
Bactrim	77.12	22.88	70.83	29.17	75.71	24.29
Streptomycin	67.80	32.20	66.67	33.33	64.29	35.71
Gentamycin	50.00	50.00	54.17	45.83	47.14	52.86
Levofloxacin	47.46	52.54	45.83	54.17	38.57	61.43
Tetracycline	34.75	65.25	41.67	58.33	34.29	65.71
Colistin	31.36	68.64	33.33	66.67	30.00	70.00
Doxycycline	24.58	75.42	20.83	79.17	22.86	77.14
Ceftazidime	3.39	96.61	4.17	95.83	4.29	95.71
Cefuroxime	2.54	97.46	4.17	95.83	4.29	95.71
Amikacin	0.85	99.15	0	100.00	0	100.00
Amoxicillin-Clavulanic acid	0	100.00	0	100.00	0	100.00

**Table 10: Multidrug resistance of E. coli strains causing edema disease in post-weaning pigs in Vinh Long province**

No. of antibiotics	Phenotypes resistance	No. of multi-resistant types	No. of phenotypes / 117 strains tested	Rate (%)
2	Am+Bt	7	6	5.13
	Am+Ge		3	2.56
	Am+Lv		2	1.71
	Am+Sm		1	0.85
	Am+Sm		1	0.85
	Lv+Bt		1	0.85
	Sm+Lv		1	0.85
<i>Subtotal</i>			15	12.82
3	Am+Co+Bt	11	1	0.85
	Am+Co+Dx		1	0.85
	Am+Dx+Bt		1	0.85
	Am+Lv+Bt		4	3.42
	Am+Sm+Bt		7	5.98
	Am+Sm+Ge		3	2.56
	Am+Te+Bt		1	0.85
	Ge+Lv+Bt		1	0.85
	Lv+Te+Bt		1	0.85
	Sm+Ge+Bt		1	0.85
	Sm+Ge+Lv		1	0.85
<i>Subtotal</i>			22	18.80
4	Am+Co+Dx+Te	13	1	0.85
	Am+Ge+Dx+Lv		1	0.85
	Am+Ge+Lv+Bt		1	0.85
	Am+Sm+Co+Bt		2	1.71
	Am+Sm+Cz+Bt		1	0.85
	Am+Sm+Dx+Bt		1	0.85
	Am+Sm+Ge+Bt		7	5.98
	Am+Sm+Ge+Lv		3	2.56
	Am+Sm+Ge+Te		1	0.85
	Am+Sm+Lv+Bt		2	1.71
	Am+Sm+Lv+Te		1	0.85
	Am+Sm+Te+Bt		2	1.71

No. of antibiotics	Phenotypes resistance	No. of multi-resistant types	No. of phenotypes / 117 strains tested	Rate (%)
	Ge+Co+Dx+Te		1	0.85
<i>Subtotal</i>			24	20.51
5	Am+Co+Dx+Te+Bt	18	1	0.85
	Am+Co+Lv+Te+Bt		2	1.71
	Am+Ge+Co+Dx+Bt		1	0.85
	Am+Ge+Co+Dx+Te		1	0.85
	Am+Ge+Co+Lv+Bt		1	0.85
	Am+Ge+Cz+Lv+Bt		1	0.85
	Am+Ge+Dx+Te+Bt		1	0.85
	Am+Sm+Co+Dx+Bt		1	0.85
	Am+Sm+Co+Lv+Bt		3	2.56
	Am+Sm+Co+Te+Bt		3	2.56
	Am+Sm+Dx+Te+Bt		1	0.85
	Am+Sm+Ge+Ak+Lv		1	0.85
	Am+Sm+Ge+Co+Bt		4	3.42
	Am+Sm+Ge+Dx+Bt		1	0.85
	Am+Sm+Ge+Dx+Lv		2	1.71
	Am+Sm+Ge+Lv+Bt		1	0.85
Am+Sm+Ge+Te+Bt	1	0.85		
Am+Sm+Lv+Te+Bt	2	1.71		
<i>Subtotal</i>			28	23.93
6	Am+Co+Dx+Lv+Te+Bt	11	1	0.85
	Am+Sm+Co+Dx+Te+Bt		1	0.85
	Am+Sm+Co+Lv+Te+Bt		3	2.56
	Am+Sm+Dx+Lv+Te+Bt		2	1.71
	Am+Sm+Ge+Co+Dx+Lv		1	0.85
	Am+Sm+Ge+Co+Te+Bt		1	0.85
	Am+Sm+Ge+Cz+Cu+Bt		1	0.85
	Am+Sm+Ge+Dx+Lv+Bt		1	0.85
	Am+Sm+Ge+Dx+Te+Bt		1	0.85
	Am+Sm+Ge+Lv+Te+Bt		3	2.56
	Sm+Ge+Co+Lv+Te+Bt		1	0.85
<i>Subtotal</i>			16	13.68
7	Am+Ge+Co+Dx+Lv+Te+Bt	6	2	1.71
	Am+Sm+Ge+Co+Dx+Lv+Bt		1	0.85
	Am+Sm+Ge+Co+Lv+Te+Bt		3	2.56
	Am+Sm+Ge+Cu+Lv+Te+Bt		1	0.85
	Am+Sm+Ge+Cz+Cu+Lv+Bt		1	0.85
	Am+Sm+Ge+Dx+Lv+Te+Bt		4	3.42
<i>Subtotal</i>			12	10.26
<i>Total</i>		<b>66</b>	<b>117</b>	<b>100.00</b>

*E. coli* causing edema in post-weaning pigs in Vinh Long province was multidrug resistant to 2-7 antibiotics with 66 phenotypes which were diversified and complicated. In which, 18 multi-resistant phenotypes to five antibiotics had the highest percentage (23.93%), followed by 13 phenotypes (20.51%) to four antibiotics; 11 phenotypes (18.8%) to three antibiotics; 11 phenotypes (13.68%) to six antibiotics. The result of this study showed that *E. coli* causing edema disease in post-weaning pigs in Vinh Long province was multidrug resistant to many antibiotics.

#### 4 CONCLUSIONS

The morbidity proportion of edema disease in post-weaning piglets in Vinh Long province was 19.51%, and the mortality rate was 57.33%. Edema disease in post-weaning pigs was age-dependent. F4 and F18 strains of *E. coli* caused edema disease in post-weaning pigs in Vinh Long; and F18 was the most common strain (59.32%). Stx2e toxin caused high edema mortality rate in post-weaning pigs (42.37%).

*E. coli* strains of F4 and F18 isolated from edema piglets were highly resistant to ampicillin, bactrim, streptomycin, moderately resistant to gentamycin. *E. coli* strains were multidrug resistant to 2-7 antibiotics with very diversified and complicated phenotypes.

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