Veterinary Integrative Sciences 2025; 23(1): e2025005-1-10. DOI: 10.12982/VIS.2025.005.

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Prevalence of antibiotic resistance genes of *Escherichia coli* at the pig slaughterhouses in the Mekong Delta

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Abstract

The study was conducted to determine the antimicrobial susceptibility and antibiotic-resistance genes of *Escherichia coli* at the pig slaughterhouses in the Mekong Delta, Vietnam. A total of 60 samples were collected at the slaughterhouse in the Mekong Delta, including 24 feces, 24 carcasses, 8 floors, and 4 wastewater samples. The prevalence of *E. coli* in feces, wastewater, carcasses, and floor samples was 91.67%, 50.00%, 29.17%, and 25.00%, respectively. A total of 79 *E. coli* isolates were examined for antimicrobial susceptibility to 15 antibiotics using the disc diffusion method according to CLSI 2021 guidelines. *E. coli* strains were highly resistant to amoxicillin (83.54%), ampicillin (78.48%), streptomycin (63.29%), and florfenicol (63.29%). However, those *E. coli* strains were sensitive to amoxicillin + acid clavulanic and amikacin (97.47%), cefaclor (95.45%), cefuroxime (93.67%), and enrofloxacin (92.41%). The results showed that 96.2% of examined *E. coli* strains were resistant from one antibiotic to eight antibiotics, and the most frequent multidrug-resistance phenotype was amoxicillin + ampicillin + florfenicol + streptomycin (13.92%). The prevalence of antibiotic resistance genes (*strA, sulII, qnrA, tetA, blaampC, blaTEM, and blaCTX-M*) in *E. coli* was determined by PCR. The prevalence of *strA, sulII, qnrA, tetA, blaampC,* and *blaTEM* genes in *E. coli* strains was 75.67%, 72.97%, 75.67%, 83.78%, 91.89%, and 83.78% respectively; however, *blaCTX-M* gene was not detected. Therefore, the contamination of *E. coli* exhibiting antibiotic resistance in pig slaughterhouses should be controlled to prevent public health.

Keywords: Antimicrobial resistance, *Escherichia coli*, Mekong Delta, Pig, Slaughterhouse.

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Funding: This study is supported by Can Tho University, Kien Giang University, and the Sub-department of Animal Health in Kien Giang, Hau Giang provinces

Article history; *received manuscript: 24 June 2023,*
revised manuscript: 06 February 2024,
accepted manuscript: 10 February 2024,
published online: 28 March 2024,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Antimicrobials are essential for treating animal infections. The emergence and spread of antimicrobial resistance have been recognized globally as a serious threat to public health (WHO, 2014). Antibiotics can lead to alterations in the microbiome of animals and the selection of antimicrobial-resistant bacteria. Furthermore, the use of many antibiotic groups to prevent and treat livestock diseases has created multi-resistant strains of antibiotics, affecting not only the health of consumers but also the health of animals and the course of treatment later. In addition, antibiotic residues in animal products have been recognized as a hazard to human health due to toxicity or allergenicity.

Among the most critical antibiotic-resistant bacteria, *E. coli* is listed on the "WHO: Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics" (WHO, 2017). These partially metabolized antimicrobials are excreted in wastewater along with urine and feces and may end up in surface waters and cropland via wastewater systems (Verburg et al., 2019). Moreover, antibiotic-resistant intestinal bacteria have a high potential for animal-to-human transmission (Tacconelli et al., 2018).

In recent, pig production in the Mekong Delta has developed rapidly. Diarrhea is a common disease in piglets, and *E. coli* has been identified as one of the causes of this disease in post-weaning and post-weaning piglets (Ly et al., 2001). Most of the published studies in the Mekong Delta have just focused on detecting hematological reactions, serotypes of O antigens and F antigens, or certain toxins (Nguyen and Le, 1996; Ly et al., 2003; Bui et al., 2004). Moreover, various reports have indicated that the distribution and characteristics of *E. coli* remarkably varied by geographical region, sampling time, and even on different herds. These changes significantly influence the effectiveness of disease control measures for pigs in geographical economic areas. However, the reports about antimicrobial susceptibility and prevalence of antibiotic-resistance genes in *E. coli* isolated from slaughterhouses have also been limited in the Mekong Delta, Vietnam.

Identifying pathogenic and antibiotic-resistance genes is crucial to guide treatment, which will reduce the economic losses caused by *E. coli* infection in animal husbandry and public health (Choi et al., 2001). Therefore, this study helps to detect the antibiotic resistance and circulation of genes encoding resistance factors of *E. coli* in pigs at slaughter facilities to improve food safety and protect human health.

MATERIALS AND METHODS

Sampling method

Experimental samples (feces, carcasses, slaughter floor, wastewater) were collected according to the Vietnamese Government Standards (QCVN 01-04-2009-BNNPTNT).

Fecal sampling: Pre-slaughter storage pigs are rectally sampled by inserting a sterile cotton swab through the anal sphincter, gently rotating it towards the rectal mucosal epithelium, and then inserting the swab with a stool sample into a sterile vial containing Cary-Blair transport.

Carcasses sampling: After the pig was slaughtered, samples were taken using sterile tools to cut at different meat sections, about 20 g each cut; each sample was cut 4 places, including the buttocks, back, chest, and cheeks. Combine the tissue pieces and just cut them into a pattern.

Slaughter floor sampling: Use a sterile cotton swab to lightly smear the slaughter bed in five positions on the platform, then place the swab in the Cary-Blair transport.

Wastewater sampling: At the slaughterhouse, it was taken at the outlet of the clarifier. Using a sterile 1,000 ml glass bottle, bring the mouth of the bottle to the surface of the water 20 cm, then turn the mouth upside down to fill the bottle with water.

Isolation and identification of *E. coli*

The *E. coli* was isolated according to the procedure of Barrow and Feltham (2003) and TCVN 8400-16:2011. The biochemical characterization was done according to the guidelines of Gyles and Fairbrother (2010).

Samples for analysis are inoculated on MacConkey agar. The suspicious *E. coli* colonies were pink-red in color, whole cover, round, slightly convex, and slightly smooth, with a diameter of about 1-2 mm colonies (after 24 hours of culture in 37°C). Biochemical reaction tests of *E. coli* were examined by Indole, Methyl Red, Voges-Proskauer, Citrate, H_2S generation, gas, and mobility.

Antibiotic sensitivity tests

E. coli was cultured on Nutrient Agar and incubated at 37°C for 24 hours. The disc diffusion tests were carried out according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2021). Then, those *E. coli* colonies were diluted in saline broth 9‰. The suspension's turbidity was adjusted to be equivalent to 10^8 CFU/ml with standard McFarland 0.5.

Then, 100 µL of the culture was spread on Mueller Hinton agar (Oxoid, UK), and the antibiotic discs were applied later. The fifteen antibiotics used included amoxicillin (Ax, 10 µg), amoxicillin/acid clavulanic (Ac, 20/10 µg), cefuroxime (Cr, 30 µg), cefaclor (Cf, 30 µg), gentamicin (Gm, 10 µg), streptomycin (Sm, 10 µg), kanamycin (Km, 30 µg), amikacin (Ak, 30 µg), enrofloxacin (Ef, 5 µg), florfenicol (Fl, 30µg), doxycycline (Dx, 10 µg), ampicillin (Am, 10 µg), trimethoprim/sulfamethoxazole (Bt, 1.25/23.75µg), colistin (Co, 10 µg), ofloxacin (Of, 5 µg). Those antibiotic discs were produced by Nam Khoa Services and Trading CO., LTD, Vietnam. The plates were then incubated at 37°C for 18–24 h, and the results were recorded by measuring the inhibition zone according to the CLSI (2021).

The detection of genes encoding antibiotic resistance

Determination of genes encoding antibiotic resistance as *blaTEM, blaCTX-M, strA, tetA, sulII, qnrA, blaampC, cat1* by PCR method. The nucleotide sequences of the antibiotic resistance gene primers designed according to the authors are presented in Table 1.

Bacterial DNA was extracted by dispersing *E. coli* colonies from an overnight culture on LB agar into 100 µL of sterile 1 × TE buffer (10 mM Tris-Cl, 1 mM EDTA buffer, pH 7.6). The suspension was heated to 100°C for 30 min to rupture bacterial cells and then centrifuged at 13,000 rpm for 15 min. The crude DNA in the supernatants was transferred into sterile microcentrifuge tubes and stored at –20°C until use (total DNA).

The mixture of one PCR reaction contained Master Mix 2X (Promega, USA) (12.5 µL); forward and reverse primers (IDT, USA) at 10 µM (0.5 µL/primer); distilled water (9.5 µL), and DNA template (2.0 µL). The thermocycling PCR reactions followed the references used in Table 1. The PCR products were electrophoresis in agarose gels 1.5% at 100V for 30 minutes. Then, gels were dyed in ethidium bromide, and figures were captured under UV to detect the results.

Table 1 Sequence of nucleotides of primer pairs used in this study

Primer	Nucleotide sequence of primer (5'-3')	Size (bp)	References
strA-F	CCTGGTGATAACGGCAATTC	546	Merkeviciene et al. (2022)
strA-R	CCAATCGCAGATAGAAGGC		
sulII-F	CGGCATCGTCAACATAACC	722	Gow et al. (2008)
sulII-R	GTGTGCGGATGAAGTCAG		
qnrA-F	AGAGGATTTCTCACGCCAGG	580	Cattoir et al. (2008)
qnrA-R	TGCCAGGCACAGATCTTGAC		
tetA-F	GGTTCACTCGAACGACGTCA	577	Kurnia et al. (2018)
tetA-R	CTGTGACAAGTTGCATGA		
blaampC-F	AATGGGTTTTCTACGGTCTG	191	Caroff et al. (1999)
blaampC-R	GGGCAGCAAATGTGGAGCAA		
blaTEM-F	ATTCTTGAAGACGAAAGGGC	1.150	Jouini et al. (2007)
blaTEM-F	ACGCTCAGTGGAACGAAAAC		
cat1-F	AGTTGCTCAATGTATATAACC	547	Santos et al. (2014)
cat1-R	TTGTAATTCATTAAGCATTCTGCC		
blaCTX-M-F	TTAGGAARTGTGGCTGAA	688	Dallenne et al. (2010)
blaCTX-M-R	CGATATCGTTGGTGGTTAT		

Statistical analysis

Data were collected and statistically processed by the Chi-square method with 95% confidence on Minitab 17.0

RESULTS

Prevalence of *E. coli* isolates in pigs at the slaughterhouse

Of 60 samples collected from slaughterhouses, 32/60 (53.30%) samples were in the presence of *E. coli*. The present rate of *E. coli* in fecal samples was the highest (22/24, 91.66%), followed by meat samples (7/24, 29.17%) and 25.00% in wastewater and slaughter floor samples.

Table 2 The prevalence of *E. coli* in the slaughterhouses

Samples	No. of examined samples	No. of positive samples	Percentage (%)
Pig feces	24	22	91.66
Carcass	24	7	29.17
Wastewater	8	2	25.00
Floor	4	1	25.00
Total	60	32	53.30

Antibiotic resistance of *E. coli* isolated at the slaughterhouse

Of 32 positive samples with *E. coli*, 79 *E. coli* isolates were selected to examine the antimicrobial susceptibility. The results (Figure 1) showed that *E. coli* isolates were resistant to Ax (83.54%), Am (78.48%), Sm and Fl (63.29%), while those isolates were sensitive to Ef (92.41%), Cr (93.67%), Co (94.94%), Cf (95.45%), Ak (97.47%), and Ac (97.47%). Moreover, 76 of the 79 *E. coli* isolates (96.20%) were multidrug-resistant (MDR) strains.

Those *E. coli* isolates were resistant to one to eight antibiotics. Out of 79 isolates, three strains were phenotypically 100% sensitive to antibiotics. Results are presented in Table 3.

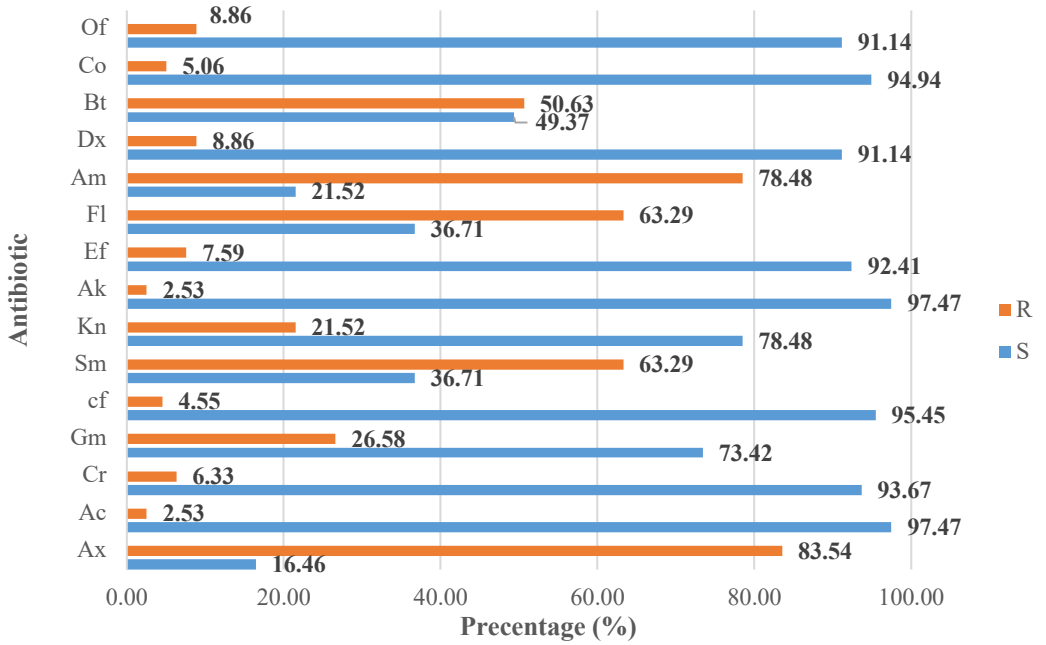

Figure 1 The antibiotic resistance of 79 *E. coli* strains to 15 antibiotics
Blue bars: sensitive (S), Orange bars: resistant (R)
Ax: amoxicillin; Ac: amox/acid clavulanic; Cr: cefuroxime; Cf: cefaclor; Gm: gentamicin; Sm: streptomycin; Kn: kanamycin; Ak: amikacin; Ef: enrofloxacin; Fl: florfenicol; Dx: doxycyclin; Am: ampicillin; Bt: trimethoprim/sulfamethoxazole; Co: colistin; Of: ofloxacin

Prevalence of genes encoding antibiotic resistance

Of the 79 *E. coli* isolates used in the antimicrobial susceptibility test, 37 represented antibiotic-resistance phenotypes. Antibiotic resistance genes of *E. coli* strains were identified by PCR with specific primers for different antibiotic groups. The resistance genes were *strA, sulII*, *qnrA*, *tetA, blaampC, blaTEM, cat1*, and *blaCTX-M*. The results are shown in Table 4.

The results of Table 4 show that the highest prevalence of antibiotic resistance genes was *blaampC*, at 91.89%, followed by *blaTEM* and *tetA*, which accounted for 81.78%, *strA*, and *qnrA*, at 75.67%, and *sulII*, at 72.97%. The *cat1* and *blaCTX-M* genes were not detected.

DISCUSSION

The results of this study indicated that *E. coli* could be isolated frequently at slaughterhouses. *E. coli* was not only detected in pig feces but also in carcasses, floors, and wastewater. It exhibited that *E. coli* could be contaminated from pig feces to others (Bouvet et al., 2002). Therefore, hygiene in slaughterhouses should be considered to prevent the contamination of bacteria in feces to carcasses and the environment. It is essential to improve food safety for public health.

Table 3 Antibiotic resistance combinations found in *E. coli* isolates

Number of antibiotic resistance	Number of isolates	Number of phenotypes	Phenotypes of resistance
0	3	3	Sensitive to all
1	6	1	Kn
		2	Sm
		1	Ak
		2	Fl
2	9	1	Ax, Kn
		6	Ax, Am
		1	Ax, Sm
		1	Sm, Bt
3	11	1	Gm, Kn, Of
		2	Ax Am, Bt
		4	Ax, Am, Sm
		1	Ax, Am, Fl
		1	Ax Am, Dx
		1	Ax, Sm, Kn
		1	Sm, Dx Bt
4	20	11	Ax, Am, Fl, Sm
		5	Ax, Am, Fl, Bt
		2	Ax, Am Kn, Bt
		1	Sm, Kn, Dx, Bt
		1	Ax, Sm, Fl, Bt
5	11	1	Ax, Am Fl, Sm, Ge
		5	Ax, Am, Fl, Sm, Bt
		1	Ax, Am, Fl, Ac, Bt
		1	Ax, Am, Fl, Sm, Kn
		1	Ax, Sm, Ef, Am, Of
		1	Ax, Ge, Sm, Fl, Bt
		1	Ax, Am,Gm, Kn, Fl,
6	12	1	Ax, Am, Fl, Dx, Bt, Of
		8	Ax, Am, Fl, Sm, Ge, Bt
		1	Ax, Am, Ge, Sm, Kn, Co
		1	Ax, Am, Fl, Sm, Kn, Bt
		1	Ax, Ac, Ge, Sm, Fl, Bt
7	3	1	Ax, Am, Fl, Sm, Ge, Gm, Bt
		1	Ax, Am, Fl, Sm, Kn, Co, Bt
		1	Ax, Am, Fl, Sm,Ge, Kn, Bt
8	4	1	Ax, Sm, Am, Fl, Gm, Ge, Kn, Bt
		1	Ax, Sm, Am, Fl, Ef, Dx, Of, Bt
		1	Ax, Sm, Am, Fl, Ef, Kn, Of, Bt
		1	Ax, Sm, Am, Fl, Ge, Kn, Dx, Bt
Total	79	79	

Ax: amoxicillin; Ac: amox/acid clavulanic; Cr: cefuroxime; Cf: cefaclor; Gm: gentamicin; Sm: streptomycin; Kn: kanamycin; Ak: amikacin; Ef: enrofloxacin; Fl: florfenicol; Dx: doxycyclin; Am: ampicillin; Bt: trimethoprim/sulfamethoxazole; Co: colistin; Of: ofloxacin

Table 4. Prevalence of antibiotic-resistance genes of *E. coli* isolated at the slaughterhouses

Gene	Number of examined isolates	Number of positive isolates	Percentage (%)
strA	37	28	75.67
sulII	37	27	72.97
qnrA	37	28	75.67
tetA	37	31	81.78
blaampC	37	34	91.89
blaTEM	37	31	81.78
cat1	37	0	0.00
blaCTX-M	37	0	0.00

In this study, the results also showed a high rate of antibiotic resistance of *E. coli* isolated at slaughterhouses. Those antibiotics, which were resistant, have

been used in animal husbandry for a long time. Therefore, *E. coli* could establish a natural resistance or obtain resistant factors from other bacteria in the intestinal fauna. Moreover, the slaughterhouses' environment could be a reservoir of *E. coli*, which are resistant to antibiotics (Barco et al., 2015). Those bacteria could circulate and contaminate pigs and carcasses during the slaughter process. The origin of pigs gathered at slaughterhouses seemed to be an essential factor affecting the prevalence of antibiotic-resistant *E. coli* here. Boerlin et al. (2005) examined antibiotic resistance of *E. coli* isolated from pigs and found that those *E. coli* isolates were highly resistant to ampicillin 57%, cephalothin 63%, kanamycin 64%, sulfamethoxazole 93%, tetracycline 96%, chloramphenicol 70%. In China, Wang et al. (2010) isolated 167 strains of *E. coli* from sick piglets and tested them for susceptibility to 12 antibiotics, and the results also showed antibiotic resistance to sulfamethoxazole 95%, tetracycline 94%, chloramphenicol 89%, streptomycin 84%, ciprofloxacin 72%, doxycycline 68%, kanamycin 56%. On the other hand, the results of this study showed various multi-resistant phenotypes were obtained. The history of antibiotics used in each farm was different; thus, the antibiotic sensibility level of *E. coli* isolates in other places was diverse. From that, multiple antibiotic-resistance phenotypes were obtained in this study. Yang et al. (2020) also reported that *E. coli* isolates at slaughterhouses in the USA could be multi-resistance to six antibiotics; the most common phenotype was resistant to two antibiotics. Further research should be conducted to determine the exact origin and antimicrobial susceptibility of *E. coli* at slaughterhouses in the Mekong Delta, Vietnam.

Those E. coli isolates examined in this study were resistant to antibiotics and harbored many antibiotic-resistance genes. The high prevalence of antibiotic-resistance genes (*blaampC*, *blaTEM*) in Table 4 proved the antimicrobial susceptibility of *E. coli* in Table 3 and Fig. 1. It indicated that *E. coli* isolates detected at slaughterhouses seemed to be naturally resistant to beta-lactam antibiotics. Although *strA*, *sulII*, and *qnrA* genes were present at a high rate, the resistance of *E. coli* was still low to those antibiotic groups (aminoglycoside, quinolone, etc.). Thus, the antibiotic-resistance performance of bacteria was still affected by other factors, such as the pressure of using antibiotics, the genes encoded in genomes, and the interaction among genes, etc. (Mkuhlu et al., 2020). At slaughterhouses, *E. coli* could be contaminated and have various origins, including pigs, the environment, workers, etc. It distributed for the prevalence of diverse *E. coli* harbored multiple antibiotic-resistance genes and multi-drug resistance patterns. Therefore, controlling *E. coli* contamination at slaughterhouses is necessary to prevent the spread of antibiotic-resistant *E. coli* to humans by consuming pig carcasses or contacting the environment. In previous reports, Boerlin et al. (2005) isolated 318 strains of *E. coli* and indicated the prevalence of antibiotic resistance genes was *tetA* (89.00%), *aadA* (89.00%), *sul1*(72.00%), *strA* (31.00%), and *sulII* (31.00%). Nguyen et al. (2015) examined the antibiotic resistance of 215 strains of ETEC isolated from diarrhea piglet feces by PCR method and found that a high prevalence of antibiotic-resistance genes included *blaTEM* (87.44%), *floR* (92.56%), *aadA* (95.35%), *qnrS* (88.37%), *blaCMY* (73.49%), *tetA* (86.98%), *qnrA* (69.30%). Wang et al. (2021) isolated 20 strains of *E. coli* from slaughterhouse wastewater, and the results showed that 95% of those strains carried the *blaCTX-M* resistance gene. Savin et al. (2021) isolated 97 wastewater samples, of which 22/97 were present with *E. coli*, accounting for 22.7%. In those 22 isolates, *blaTEM* accounted for 27.3%, followed by blaCTX-M-1 (22.7%) and *blaSHV-12* (13.6%).

CONCLUSIONS

There was a high prevalence of *E. coli* (53.30%) at slaughterhouses in the Mekong Delta, Vietnam. Most strains of *E. coli* isolated at slaughterhouses were

antibiotic-resistant and produced multi-resistant patterns. However, those *E. coli* isolates were sensitive to cefaclor, cefuroxime, amikacin, and enrofloxacin. Those *E. coli* isolates harbored various examined antibiotic-resistance genes. Gene *tetA*, *blaampC,* and *blaTEM* were detected frequently, and many different genotypes were obtained. Those *E. coli* isolates could circulate and contaminate slaughterhouses in the Mekong Delta. Thus, controlling the prevalence of antibiotic-resistant *E. coli* isolates is essential to improve food safety and prevent transmission between animals and humans.

AUTHOR CONTRIBUTIONS

Ngo Van Thong, Nguyen Khanh Thuan, Bui Thi Le Minh: Conceptualize and design the experiment, investigate, supervise, edit, perform formal analysis, and prepare the manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

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How to cite this article;

Ngo Van Thong, Nguyen Khanh Thuan, Bui Thi Le Minh. Prevalence of antibiotic resistance genes of *Escherichia coli* at the pig slaughterhouses in the Mekong Delta. Veterinary Integrative Sciences. 2025; 23(1): e2025005-1-10.