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Research Article

Study on antibiotic resistance profiles of *Escherichia coli* isolated from the surface of pork-selling counters in wet markets in Gia Lam, Hanoi

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Abstract

The presence of *Escherichia coli* (*E. coli*) on food contact surfaces is considered a significant risk to food safety and can have a major impact on public health. This study aimed to examine the contamination rate, antimicrobial resistance profiles, and several genes encoding virulence factors of *E. coli* recovered from the surfaces of pork-selling counters in wet markets in Gia Lam, Hanoi City. The findings in our study show that 50% (20/50) of swab samples carried *E. coli*. Resistance was most prevalent against tetracycline (75%), while ampicillin, streptomycin, and sulfamethoxazole/trimethoprim all demonstrated a slightly lower but identical resistance rate of 70%. The PCR test indicates that only one isolate (5%) harbored the *stx2* gene and one (5%) carried the *eae* gene, while none of the isolated strains possessed *stx1* and *ehxA*.

Keywords: *Food contact surface, antibiotic resistance, Escherichia coli, virulence genes, pork*

1. INTRODUCTION

Food safety is a global challenge that seriously affects human health and social life [1]. In Vietnam, wet markets play a major role in providing food sources. However, unsanitary conditions provide opportunities for the growth and infection of foodborne pathogens [2].

Fresh meat is often chosen for most daily meals in Vietnam. In particular, pork is the most common meat, with an average consumption of 24.7 kg/person/year [3]. According to a study in Hanoi, pork purchased at wet markets is the preferred choice of the majority of consumers instead of supermarkets [4].

Misuse and overuse of antibiotics lead to the emergence of antibiotic-resistant bacteria, especially antibiotic-resistant *E. coli* strains. WHO emphasizes that antibiotic resistance is one of the 10 biggest global threats [5]. A study in Gia Lam district, Hanoi, showed that 100% of *E. coli* isolates originating from chicken and pork in wet markets were found to be resistant to more than one antibiotic, with the highest levels of resistance to tetracycline (96.3%) and erythromycin (92.6%) [6]. The presence of antibiotic-resistant pathogens on the surfaces of pork-selling counters can be a source of infection and a threat to consumer health. In addition, some *E. coli* strains also carry virulence genes, increasing the risk of food safety [7].

The contamination level and antimicrobial resistance of *E. coli* on food contact surfaces, especially in processing facilities, have been published in several studies [8, 9]. However, information on the contamination rate and antimicrobial resistance profile of *E. coli* on the surface of pork-selling counters at wet markets in Vietnam is still limited. Therefore, the objective of this study was to examine the contamination rate and

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antimicrobial resistance of *E. coli* recovered from the surface of pork-selling counters in wet markets in Gia Lam, Hanoi.

2. MATERIALS AND METHODS

2.1. Materials

A total of 40 swab samples of pork-selling counters were collected at wet markets in Gia Lam, Hanoi.

The media and reagents include MacConkey Agar, Eosin Methylene Blue (EMB), Triple Sugar Iron (TSI), Sodium Chloride (NaCl), Nutrient Broth (NB), MR-VP Broth, Glycerol, Kovac's reagent, Methyl Red, α-naphthol, Potassium Hydroxide, and Simmon Citrate Agar.

Antibiotics used in the study include ampicillin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefoxitin (30 µg), meropenem (10 µg), tetracycline (30 µg), streptomycin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), and sulfamethoxazole/trimethoprim (23.75 /1.25 µg).

2.2. Methods

2.2.1. Sample collection

Samples were collected according to TCVN 8129:2019, National Standard on "*Microbiology of the food chain - Horizontal method for surface sampling*". Swab samples (40 samples) were obtained by wiping the surfaces of raw pork-selling counters at 8 wet markets in Gia Lam, Hanoi, with an area of 100 cm² and placed into a sterile zip bag containing 10 mL of Buffered Phosphate Saline (PBS). The samples were then kept in an icebox and transferred to the laboratory for *E. coli* isolation.

Table 1. *Primer sequences for the detection of virulence genes and STEC serogroup*

Target gene	*Primer*	*Primer sequence (5'- 3')*	*Amplicon Size (bp)*	*Reference*
stx1	stx1-F	TGTCGCATAGTGGAACCTCA	655	[13]
	stx1-R	TGCGCACTGAGAAGAAGAGA		
stx2	stx2-F	CCATGACAACGGACAGCAGTT	477	
	stx2-R	TGTCGCCAGTTATCTGACATTC		
eae	eae-F	CATTATGGAACGGCAGAGGT	375	
	eae-R	ACGGATATCGAAGCCATTTG		
ehxA	ehxA-F	GCGAGCTAAGCAGCTTGAAT	199	
	ehxA-R	CTGGAGGCTGCACTAACTCC		
rfb_O157	O157-F	CAGGTGAAGGTGGAATGGTTGTC	523	[14]
	O157-R	TTAGAATTGAGACCATCCAATAAG		
wzx_O26	O26-F	AGGGTGCGAATGCCATATT	417	
	O26-R	GACATAATGACATACCACGAGCA		
wzx_O45	O45-F	GGGGCTGTCCAGACAGTTCAT	890	
	O45-R	TGTACTGCACCCAATGCACCT		
wzx_O103	O103-F	GCAGAAAATCAAGGTGATTACG	740	
	O103-R	GGTTAAAGCCATGCTCAACG		
wzx_O111	O111-F	TGCATCTTCATTATCACACCAC	230	
	O111-R	ACCGCAAATGCGATAATAACA		
wzqE_O121/ wzqF_O121	O121-F	TCAGCAGAGTGGAATTTTGT	587	
	O121-R	TGAGCACTAGATGAAAAGTATGGCT		
wzx_O145	O145-F	TCAAGTGTTGGATTAAGAGGGATT	523	
	O145-R	CACTCGCGGACACAGTACC		

2.2.2. E. coli isolation and identification

E. coli was isolated from swab samples using the protocol previously described by Zhao *et al.* [11] with some modifications: The swab sample in a zip bag was thoroughly homogenized before being spread on MacConkey agar. The plates were then incubated at 37°C for 24 h. On MacConkey agar, *E. coli* formed typical pink colonies. After incubation, 3-5 typical colonies of *E. coli* on MacConkey agar were subcultured onto an Eosin Methylene Blue (EMB) agar plate and incubated at 37°C for 24 h. Typical *E. coli* colonies on EMB have a metallic blue sheen and a black center. These colonies were transferred to 10 mL of Nutrient Broth (NB) medium and incubated at 37°C for 24 h. The culture was used for biochemical tests. The identified *E. coli* strains were preserved with 20% glycerol at -86°C.

2.2.3. Antimicrobial susceptibility testing

The antibiotic resistance of *E. coli* isolates was assessed by the agar diffusion method according to the protocols of the Clinical and Laboratory Standards Institute [12]. The reference *E. coli* strain ATCC 25922 was used to control quality control in this experiment.

2. 2.4. Molecular characterization of E. coli isolates

DNA extraction of *E. coli* isolates was conducted using the Dneasy Tissue kit (Qiagen, Germany).

Virulence genes (stx*1, stx2, eae, ehxA*) and STEC serogroups (rfbO*157, wzxO26, wzxO45, wzxO103, wzxO111, wzqE_O121/wzqF_O121, wzxO145)* of *E. coli* isolates were detected by Multiplex-PCR. The sequences of primer pairs used for PCR are presented in **Table 1**.

Thermal cycles of PCR amplification include one cycle of denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 67°C for 80 s, elongation at 72°C for 90 s, and a final elongation at 72°C for 10 min.

3. RESULTS AND DISCUSSION

3.1. *E. coli* isolation and identification

A total of 40 swab samples were obtained at wet markets in Gia Lam, Hanoi, for isolation of *E. coli*. The results showed that *E. coli* was present in 20/40 (50%). **Figure 1** shows the Colony morphology of *E. coli* on MAC and EMB.

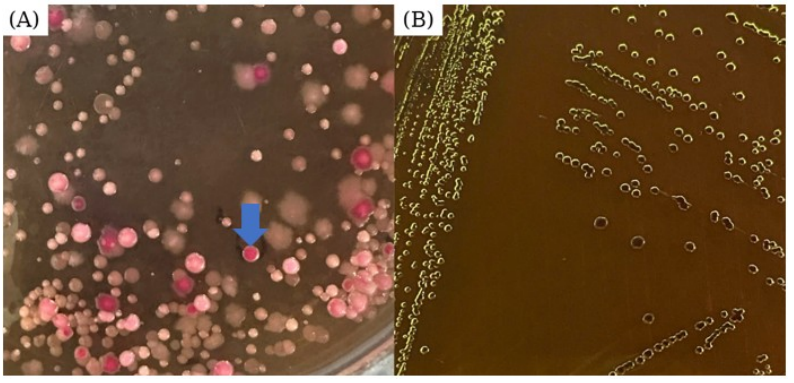


***Figure 1**. Colony morphology of E. coli on MAC (A) and EMB (B)*

In this study, *E. coli* was found in 50% (20/40) samples, indicating the high level of contamination on food contact surfaces at some markets in Gia Lam, Hanoi. *E. coli* contamination may result from unsanitary slaughtering and transportation processes. In addition, poor hygiene practices of sellers, such as not washing hands, failing to clean pork-selling counters and utensils before and after selling, or using unsafe washing water sources, also contribute to increasing the risk of *E. coli* contamination. Some studies in Vietnam also recorded high contamination rates of *E. coli* on pork and contact surfaces. According to Le Hong Phong *et al.* in 2019, the rate of *E. coli* contamination at slaughterhouses in the Southwest region of Vietnam was 38.89%, while at retail stores it was 11.11% [15]. A study by Hoang Minh Duc *et al.* in 2022 in Gia Lam, Hanoi, showed that 83.3% of pork samples were contaminated with *E. coli* [6]. In particular, the study by Le Minh Duc & cs (2023) in Hue revealed that 100% of pork samples were contaminated with *Coliforms*, and 88.33% were

contaminated with *E. coli.* [16]. A study in the UK analyzed 1,330 samples of food contact surfaces and found that 5.5% of them carried *E. coli* [17]. Another study in Makkah, Saudi Arabia, 17.7% of 294 food contact surface swab samples from 43 restaurants were positive for *E. coli* [18].

3.2. Antimicrobial resistance of *E. coli* isolates

The results of the antimicrobial resistance susceptibility of 20 *E. coli* isolates are presented in **Table 2**. *E. coli* strains recovered from pork butchery counters had high resistance rates to tetracycline (75%), ampicillin, streptomycin, and sulfamethoxazole/trimethoprim (70%), and chloramphenicol (65%). Lower resistance levels were recorded for nalidixic acid (30%), gentamicin (25%), ciprofloxacin (15%), and cefotaxime and cefoxitin (10%). Notably, all *E. coli* isolates were not resistant to ceftazidime, cefepime, and meropenem.

***Table 2**. Antimicrobial resistance profile of E. coli isolates*

Antimicrobial class	Antimicrobial agent	No. isolates (n = 20)	Resistance rate (%)
Penicillin	ampicillin	14	70
Cephalosporins	ceftazidime	0	0
	cefotaxime	2	10
	cefepime	0	0
Cephamycin	cefoxitin	2	10
Carbapenems	meropenem	0	0
Tetracyclines	tetracycline	15	75
Aminoglycosides	streptomycin	14	70
	gentamicin	5	25
Quinolones	nalidixic acid	6	30
Fluoroquinolones	ciprofloxacin	3	15
Phenicols	chloramphenicol	13	65
Sulfonamides	sulfamethoxazole/ trimethoprim	14	70

To our knowledge, the antibiotic resistance of E. coli recovered from pork-selling counters remains under-investigated, with very few studies published to date. However, *E. coli* contamination on the surface of pork-selling counters is a matter of concern due to the potential risk of transmitting antibiotic-resistant bacteria from the surface of counters to meat. Our study showed that *E. coli* isolates have high resistance rates to tetracycline (75%), ampicillin, streptomycin, and sulfamethoxazole/trimethoprim (70%). According to Bukhari's study và cs. (2021), *E. coli* isolated from contact surfaces at a meat shop had a high resistance rate to ampicillin (91.2%) and low resistance to ceftazidime, cefotaxime, and ciprofloxacin, with a rate of 2.9% [19].

Data regarding the antibiotic sensitivity of *E. coli* sourced from slaughterhouses and pork meat has also been made available in recent studies. In Brazil, a study of Coelho và cs. (2024) revealed that *E. coli* isolated from pig carcasses at slaughterhouses were highly resistant to ampicillin (88.4%), chloramphenicol (82.6%), streptomycin (80.7%), and nalidixic acid (73%) but low resistant to ciprofloxacin (25%) and ceftazidime (7.6%). Another study in São Paulo State, Brazil, showed that *E. coli* isolated from environmental samples at slaughterhouses (including water and tool surfaces) were resistant to tetracycline (44.2%), chloramphenicol (30.2%), sulfamethoxazole (14%), and ciprofloxacin (11.6%) [20]. In contrast, isolates had low resistance rates to the Cephalosporins class (cefotaxime and ceftazidime at 2.3%) [21]. In addition to the slaughterhouse environment, pork sold at wet markets is also a source of antibiotic-resistant *E. coli* and can contribute to the spread of the bacteria to food contact surfaces, leading to cross-contamination of other carcasses. Sampaio's study in Beijing, China, reported that a resistance rate of *E. coli* to sulfamethoxazole/trimethoprim was 92.3%, gentamicin was 18%, ceftazadime and cefotaxime were 5.1% and 7.7%, respectively [22].

The results in **Table 3** reveal that *E. coli* isolates showed resistance to at least one tested antibiotic, with 15 resistance patterns.

Of which, the resistance pattern "AMP-TET-STR-CHL-STX" was the most commonly detected. In addition, the results also showed that 84.2% (16/19) of antibiotic-resistant *E. coli* isolates were identified as multidrug-resistant strains.

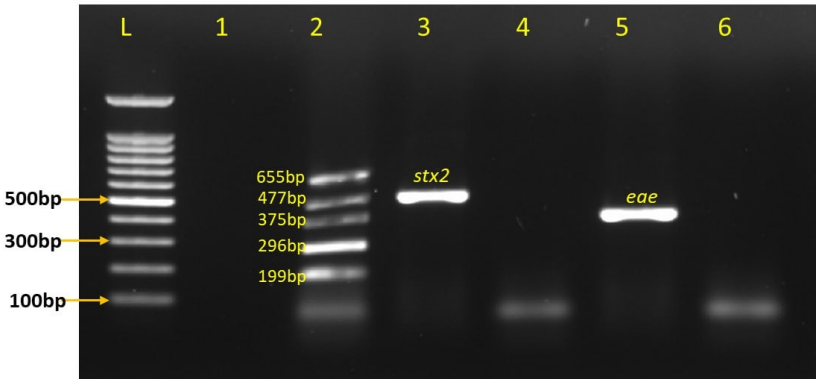
Table 3. *Antibiotic resistance patterns of E. coli isolates*

No. Antibiotics	*Resistance pattern*	*No. Isolates*
1	AMP	1
1	FOX	1
2	STX-FOX	1
3	AMP-TET-CHL	1
3	STR-CHL-STX	1
4	AMP-TET-CHL-STX	1
5	AMP-TET-STR-CHL-STX	4
5	AMP-TET-STR-NAL-STX	2
5	AMP-TET-STR-GEN-CHL	1
5	TET-STR-GEN-NAL-STX	1
5	TET-STR-GEN-CHL-STX	1
6	AMP-CTX-TET-STR-CHL-STX	1
6	AMP-TET-STR-NAL-CIP-CHL	1
8	AMP-TET-STR-GEN-NAL-CIP-CHL-STX	1
9	AMP-CTX-TET-STR-GEN-NAL-CIP-CHL-STX	1

Notes: AMP: ampicillin, CTX: cefotaxime, FOX: cefoxitin, TET: tetracycline, STR: streptomycin, GEN: gentamicin, NAL: nalidixic acid, CIP: ciprofloxacin, CHL: chloramphenicol, STX: sulfamethoxazole/trimethoprim.

3.3. Detection of virulence genes

The PCR results in **Figure 2** indicated that only one strain (5%) carried the *stx2 gene,* and one strain (5%) carried the *eae* gene. None of the isolated strains harbored *stx1* and *ehxA* genes. Also, STEC serogroup O157, *O26, O45, O103, O111, O121,* and *O145,* were not detected in this study.



Note: Well L: Ladder; Well 1: Negative control; Well 2: Positive control; Wells 3-6: Isolated E. coli strains

Figure 2. *Detection of virulence genes of E. coli isolates*

The O157:H7 strain of STEC is a notorious cause of major foodborne outbreaks, potentially leading to life-threatening conditions such as hemolytic-uremic syndrome (HUS) and hemorrhagic colitis [23]. Shiga toxin encoded by the *stx gene* plays an important role in the pathogenesis of STEC, but the presence of *stx* alone is not sufficient for the bacteria to cause disease [24]. Therefore, the detection of other virulence genes, including *eae* and *ehxA,* is necessary to evaluate the virulence potential of *E. coli* isolates. This is the first study in Vietnam on investigating the presence of STEC on the surface of the pork-selling counters in wet markets. Although one strain (5%) carried the *stx2* gene and one strain (5%) carried the *eae* gene was detected in 40 swab samples of the surface of pork-selling counters, none of the *E. coli* strains in this study is classified into

STEC serogroups (O157, O26, O45, O103, O111, O121, O145). However, a broader sampling scale is necessary to provide a more comprehensive verification regarding the prevalence - or lack thereof - of STEC serogroups in this environment.

4. CONCLUSION

In this study, 50% of swab samples collected from the surface of pork-selling counters at wet markets in Gia Lâm, Hanoi, were positive for *E. coli*. The isolated *E. coli* strains exhibited resistance to multiple antibiotics, with 15 distinct resistance patterns. One isolate was found to carry *stx2,* and another harbored the *eae* gene. The findings in this study emphasize the need for measures to reduce contamination of antibiotic-resistant *E. coli* on the surface of pork-selling counters.

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