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Research Article

Prevalence and antibiotic resistance characteristics of extended-spectrum beta-lactamase (ESBL)-Producing *Escherichia coli* Isolated from pork sold at retail markets in Hanoi

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Abstract

Antibiotic-resistant bacteria, particularly Extended-Spectrum β-Lactamase (ESBL)-producing *Escherichia coli*, have emerged as a serious threat to public health. The widespread use of antibiotics in agriculture, especially pig farming, contributes to the transmission of these resistant bacteria through the food chain. Pork, a staple food in Vietnam, represents a critical vector for this transmission. This study aimed to determine the prevalence, phenotypic antibiotic resistance profiles, and genetic characteristics (ESBL-encoding genes, phylogenetic groups, and clonal relatedness) of ESBL-producing *E. coli* isolated from pork sold at retail markets in Hanoi, Vietnam. A cross-sectional study was conducted to collect 70 fresh pork samples from retail markets across Hanoi. The prevalence of cefotaxime-resistant *E. coli* in pork samples was 88.6% (62/70). Among the 167 resistant isolates recovered, 87.4% (146/167) were confirmed as ESBL producers. These isolates exhibited high rates of multidrug resistance (MDR), with 71.2% (104/146) resistant to three or more antibiotic classes. Critically, resistance to last-resort antibiotics was detected, including colistin (10.3%) and carbapenems (2 isolates). Genotypically, *bla*TEM was the most prevalent gene, detected in 60.3% (88/146) of isolates, followed by *bla*CTX-M-9 (24.7%) and *bla*CTX-M-1 (22.6%). Phylogenetic analysis showed a dominance of group D (54.8%). MLVA revealed high genetic diversity, with 71.9% of strains showing no close clonal relationship. This study reveals an alarmingly high prevalence of MDR ESBL-producing *E. coli* circulating in retail pork in Hanoi.

Keywords: *Antibiotic resistance, microbial contamination, Escherichia coli, extended-spectrum beta-lactamase (ESBL), retail pork.*

1. INTRODUCTION

The alarming global rise of antimicrobial resistance (AMR) represents one of the foremost threats to public health, significantly increasing morbidity, mortality, treatment costs, and imposing substantial socioeconomic burdens [1, 2]. The proliferation of small-scale pig farming and slaughter operations, coupled with the uncontrolled application of antibiotics in livestock production, creates conducive conditions for the emergence and dissemination of resistant bacteria [3].

Among antimicrobial-resistant pathogens, *E. coli* producing extended-spectrum β-lactamases (ESBLs) are of particular concern due to their role as opportunistic pathogens and their capacity to harbor and disseminate

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resistance genes [3]. ESBLs hydrolyze a broad range of β-lactam antibiotics, including third-generation cephalosporins and monobactams, thereby severely complicating the clinical management of infections [4].

Pork, a staple protein source in the Vietnamese diet, has been identified as a critical link in the zoonotic transmission of antimicrobial-resistant bacteria from animals to humans [5, 6]. Previous studies have documented substantial *E. coli* contamination in retail meat across various regions of Vietnam [7, 8]. Hanoi, characterized by high population density and a complex food distribution network, is potentially a hotspot for ESBL-producing *E. coli* contamination from pork. Moreover, suboptimal hygiene practices during transportation, storage, and handling at retail points may further amplify the risk of contamination.

In Vietnam, several studies have investigated ESBL-producing *E. coli* contamination in food. A study by Le Quoc Phong *et al.* (2015) reported a prevalence of 40.6% ESBL-producing *E. coli* in retail food collected from markets in Ho Chi Minh City, with a multidrug resistance rate of 85.9% in these isolates [7]. Another study by Le Thi Kieu Hanh *et al.* (2017) evaluating ESBL-producing *E. coli* in foods sold at two markets in Vu Thu district, Thai Binh province, found 113/173 food samples contaminated with ESBL-producing *E. coli* [8].

To date, very few studies have assessed the prevalence and genotypic characteristics of ESBL-producing *E. coli* in pork sold in Hanoi. Therefore, this study was conducted to determine the current prevalence, antibiotic resistance phenotypes, distribution of major ESBL-encoding genes, and genetic diversity of ESBL-producing *E. coli* isolated from retail pork in Hanoi. The findings are intended to provide data on the circulation of these resistant agents in the food chain, thereby informing risk assessment and guiding future intervention strategies.

2. MATERIALS AND METHODS

2.1. Study design and sample collection

A cross-sectional study was conducted from August 2020 to June 2021. Fresh pork samples (*Sus scrofa domesticus*), comprising skin, lean meat, and fat, and slaughtered and sold on the same day, were collected. Speciality pork products (e.g., wild boar, mountain pig), imported pork, and frozen pork were excluded.

Pork samples were purchased from 8 inner-city districts of Hanoi: Ha Dong, Thanh Xuan, Hoang Mai, Dong Da, Hai Ba Trung, Ba Dinh, Cau Giay, and Hoan Kiem. Two retail markets were randomly selected from each district. Samples were collected according to the guidelines specified in TCVN 7925:2018 (Vietnamese Standard for sampling methods for microbial detection and enumeration on carcass surfaces).

The sample size (N) was calculated using the formula:

$$N = \frac{Z^2_{(1-\alpha/2)} * p(1-p)}{(d)^2} \qquad (1)$$

Where:

α: Statistical significance level, chosen as α = 0.05.

d: Margin of error d = 0.1.

p: The prevalence of pork contaminated with ESBL-producing *E. coli* from a previous survey. This study used p = 68.4% based on the research by Le Quoc Phong (2015) [7].

A total of 70 samples were collected (the calculated target was 82 based on an expected prevalence of 68.4% [7], a 95% confidence level, and a 10% margin of error). All samples were coded and immediately transported to the Department of Food Microbiology and Molecular Biology at the National Institute of Nutrition.

2.2. Media, reagents, and reference strains

The study used the following media and chemicals: Tryptone bile X-glucuronide (TBX) agar (Merck, Germany); Mueller-Hinton (MH) agar (Oxoid, UK); Tryptone broth (Merck, Germany); Buffered Peptone Water (BPW) (Merck, Germany); Kovac's reagent (Merck, Germany); and Cefotaxime (Wako, Japan).

Antibiotic disks (Oxoid, UK) included: Ampicillin (AMP), Fosfomycin (FOF), Cefoxitin (FOX), Ceftazidime (CAZ), Meropenem (MEM), Gentamicin (GEN), Kanamycin (KAN), Streptomycin (STR),

Tetracycline (TET), Ciprofloxacin (CIP), Chloramphenicol (CHL), Nalidixic acid (NAL), Sulfamethoxazole-trimethoprim (SXT), Cefotaxime (CTX), Ceftazidime/clavulanic acid (CAZ/C), and Cefotaxime/clavulanic acid (CTX/C).

Molecular biology reagents included: Multiplex PCR kit (Primerstar, Takara, Japan); agarose (Sigma, USA); GelRed Nucleic Acid Stain, 10,000X (Biotium); 100 bp DNA ladder (Invitrogen); and PCR primers (Phu Sa Co., Ltd, Vietnam).

Control strains included the antibiotic-susceptible control *Escherichia coli* ATCC 25922 and positive control *E. coli* strains containing *CTX-M* genes for PCR reactions, which were previously isolated from food and clinical samples and provided by the Osaka Public Health Institute, Japan.

2.3. Sample processing and analytical methods

ESBL-producing *E. coli* were isolated from the collected pork samples following the technical protocol of the World Health Organization (2021) [9]. Briefly, 25 g of each meat sample was homogenized with 225 mL Buffered Peptone Water (BPW) and incubated at 37°C for 18–24 h. Enrichment broth (10 µL) was streaked onto Tryptone Bile X-glucuronide (TBX) agar supplemented with 4 µg/mL cefotaxime (CTX) and incubated at 37°C for 18–24 h. Blue colonies on TBX-CTX agar that were Indole-positive were considered typical *E. coli*. From each plate, 2–3 typical colonies were selected for ESBL confirmation using the combination disk method with cefotaxime, ceftazidime, cefotaxime/clavulanic acid, and ceftazidime/clavulanic acid according to CLSI guidelines (2020) [10].

The antimicrobial susceptibility of the confirmed ESBL-producing *E. coli* isolates was determined using the Kirby-Bauer disk diffusion technique, as per CLSI guidelines [10].

The ESBL-producing *E. coli* isolates were classified into phylogenetic groups using a multiplex PCR method according to Clermont *et al.* (2000) [11].

The presence of *CTX-M* group antibiotic resistance genes in the ESBL-producing *E. coli* strains was determined by multiplex PCR, referencing the protocol described by Le Quoc Phong *et al.* (2015) [7].

The genetic relatedness of the ESBL-producing *E. coli* strains was analyzed using Multilocus Variable-Number Tandem Repeat Analysis (MLVA) as described by Caméléna *et al.* (2019) [12].

2.4. Data processing and analysis

Data were entered and managed using Microsoft Excel and analyzed using SPSS 22 software. Chi-square test (χ^2-test) was performed to compare multidrug resistance rates and gene carriage between groups, with statistical significance set at $p < 0.05$. BioNumeric 7.0 software was used to process MLVA results.

3. RESULTS AND DISCUSSION

3.1. Prevalence of ESBL-producing *E. coli* isolated from pork

Analysis of 70 pork samples collected from retail markets revealed a very high prevalence of cefotaxime-resistant *E. coli* at 88.6% (62/70 samples). From cefotaxime-positive samples, 2–3 typical colonies were randomly selected, confirmed as *E. coli* by the Indole test, and tested for ESBL production using the disk diffusion method (CTX, CTX-C, CAZ, CAZ-C).

Among 167 cefotaxime-resistant *E. coli* isolates, 87.4% (146/167) were ESBL-producing, while only 12.6% (21 isolates) lacked this capability (**Table 1**).

Table 1. *Prevalence of ESBL-producing E. coli in pork*

	Number of isolates (n)	Percentage (%)
ESBL-producing E. coli	146	87.4
Non-ESBL-producing E. coli	21	12.6
Total CTX-resistant *E. coli* isolates	167	100

This prevalence is higher than reported by Le Quoc Phong *et al.* (2015) in Ho Chi Minh City (40.6%) [7] and considerably higher than similar regional studies, such as in Chiang Mai, Thailand (69%) [18] or in pig offal at Hong Kong markets (0.5–52.4%) [19]. These disparities may stem from differences in antibiotic use

regulations and practices in pig farming among localities, as well as food safety conditions at traditional retail markets where cross-contamination risk is high.

3.2. Antimicrobial resistance profiles

Among the 146 ESBL-producing isolates, resistance to ampicillin (AMP) was universal (100%) (**Figure 1**). High resistance rates were also observed for chloramphenicol (CHL, 62.8%), trimethoprim/sulfamethoxazole (SXT, 55.8%), gentamicin (GEN, 40.4%), aztreonam (ATM, 29.5%), and cefepime (FEP, 24.4%). Resistance to amoxicillin/clavulanic acid (AMC), cefoxitin (FOX), and ciprofloxacin (CIP) was 9.0%, 11.5%, and 19.9%, respectively. Critically, 15 isolates (10.3%) were resistant to colistin-a last-resort drug for multidrug-resistant Gram-negative infections-and two isolates (1.4%) exhibited resistance to carbapenems (imipenem and meropenem). Although the latter prevalence is low, the presence of carbapenem-resistant *Enterobacterales* (CRE) in the food chain is a major red flag for public health. Carbapenems are the final therapeutic line for severe multidrug-resistant infections; their resistance genes, if disseminated via food, could accelerate the establishment of community reservoirs of resistance beyond healthcare settings [2, 5]. The detection of an isolate resistant to 10 antibiotic classes-including both colistin and carbapenems-suggests the potential co-accumulation of multiple resistance mechanisms on a single plasmid or strain, heralding the emergence of virtually untreatable "superbugs".

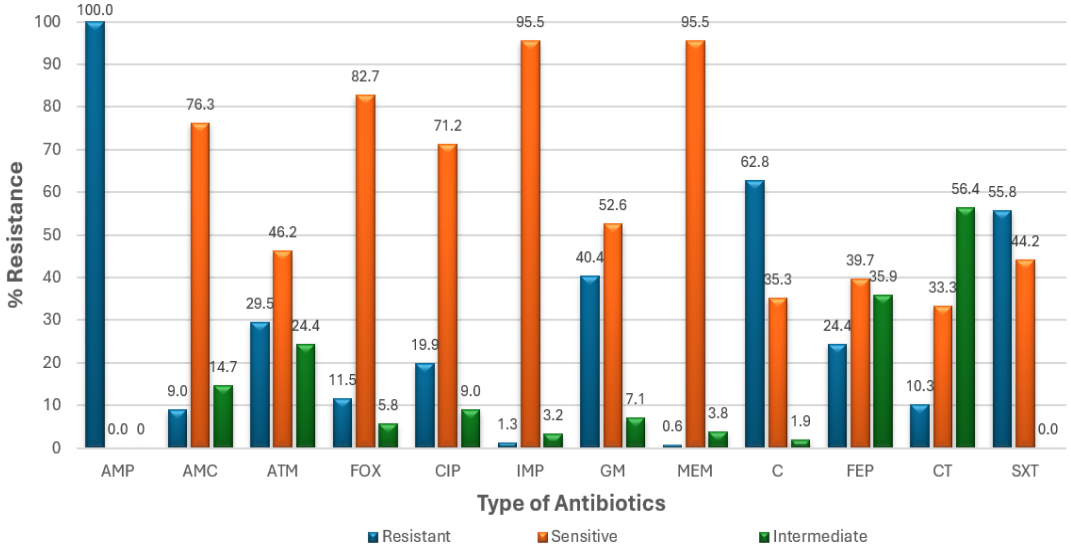

Figure 1. *Antibiotic resistance rates in ESBL-producing E. coli isolates (n = 146)*
Note: Resistance rates for each antibiotic are expressed as percentage of resistant isolates.

These findings align with recent reports of colistin-resistant Gram-negative bacteria on pork in Bac Ninh and Thai Binh provinces [13, 14].

The results showed that 71.2% of isolates were multidrug-resistant (resistant to ≥ 3 antibiotic classes), with 21.2% resistant to ≥ 6 classes. Notably, one isolate exhibited resistance to 10 antibiotics, including both colistin and carbapenems (**Table 2**).

Table 2. *Multidrug resistance patterns in ESBL-producing E. Coli*

Number of antibiotic classes with resistance		3	4	5	6	7	8	9	10	> 10
ESBL-producing E. coli (n = 146)	n	104	78	49	31	14	7	3	1	0
	%	71.2	53.4	33.5	21.2	9.5	4.7	2	0.6	0

3.3. ESBL-encoding gene profiles of *E. coli* isolates

CTX-M enzymes are currently the most prevalent ESBL group globally, particularly in drug-resistant *E. coli*. To investigate the genotype and resistance mechanisms, we determined CTX-M resistance genes using multiplex PCR.

Results showed that 71.9% (105 isolates) carried at least one CTX-M group ESBL-encoding gene. The most common was *bla*TEM (60.3%, 88 isolates), followed by *bla*CTX-M-1 (22.6%, 33 isolates) and *bla*CTX-M-9 (24.7%, 36 isolates). Less common genes included *bla*CTX-M-8/25 (8.2%, 12 isolates) and *bla*SHV (2.1%, 3 isolates). Notably, 41 isolates (28.1%) carried none of the surveyed CTX-M genes, suggesting the presence of other ESBL genes or resistance mechanisms. Many ESBL-producing *E. coli* carried multiple ESBL genes: 45.2% (66 isolates) carried 2 different genes, 12.3% (18 isolates) carried 3 different genes, while only 42.5% (62 isolates) carried a single ESBL gene.

A notable finding was the predominance of *bla*TEM (60.3%). This differs from Nguyen Do Phuc *et al.* (2016) in poultry, where CTX-M-9 (31.2%) and CTX-M-1 (29.8%) were most common [16] (**Table 3**). However, our results align with Le Quoc Phong *et al.* (2015), who reported beta-lactam resistance gene prevalence as: CTX-M-1 (50.7%), CTX-M-9 (41.5%), *bla*Tem (59.9%), and BlaSHV (2.8%) [7].

Table 3. *Prevalence of ESBL-encoding genotypes in ESBL-producing E. coli*

CTX-M genotype	Number of isolates	Rate (%)
*bla*Tem	88	60.3
*bla*SHV	3	2.1
*bla*CTXM-1	33	22.6
*bla*CTX-M-8/25	12	8.2
*bla*CTXM-9	36	24.7
*bla*CTXM-2	0	0
None Detected	41	28.1

While many global and regional reports indicate *bla*CTX-M dominance as the primary ESBL-encoding gene, studies since the early 2000s show that *bla*CTX-M, particularly *bla*CTX-M -15, has rapidly become a pandemic genotype, dominating clinical isolates across continents [20]. In the veterinary and food safety sectors, reports from China also show coexistence of *bla*CTX-M variants (97.33%), *bla*TEM (76.72%), and *bla*SHV (3.05%) in ESBL-producing *E. coli* from poultry during 2016–2019 [21]. Our results demonstrate that *bla*TEM remains dominant in pork in Hanoi, possibly reflecting a "legacy" from prolonged use of older-generation beta-lactams in Vietnamese livestock farming, creating selective pressure that maintains classical ESBL genes alongside newer variants.

3.4. Phylogenetic group distribution of ESBL-producing *E. coli* isolates

E. coli is classified into phylogenetic groups A, B1, B2, and D. Groups A and B1 typically comprise commensal, non-pathogenic strains residing in the intestinal tract, while groups B2 and D often include strains causing serious extraintestinal infections such as urinary tract infections, sepsis, and meningitis [15]. As shown in **Table 4**, analysis of phylogenetic groups among pork-derived ESBL-producing *E. coli* revealed diversity, with group D predominating (54.8%, 80 isolates), followed by group A (27.4%, 40 isolates), group B1 (12.3%, 18 isolates), and group B2 (5.5%, 8 isolates). The high prevalence of group D may reflect the transmission risk of pathogenic *E. coli* from animals to humans via the food chain.

Table 4. *Multidrug resistance distribution by phylogenetic group in ESBL-producing E. coli*

Phylogenetic group	Rate % (n_1)	Multidrug resistance rate % (n_2)	*p* (χ^2-test)
A	27.4 (n_1 = 40 isolates)	62.5 (n_2=25 isolates)	

B1	12.3 (n_1 = 18 isolates)	83.3* (n_2=15 isolates)	$p \leq 0.05$
B2	5.5 (n_1 = 8 isolates)	87.5* (n_2=7 isolates)	
D	54.8 (n_1 = 80 isolates)	71.3 (n_2=57 isolates)	

* χ^2-test ($p < 0.05$)

All isolates were resistant to ampicillin and cefotaxime. Significant differences in multidrug resistance rates (≥ 3 antibiotic classes) were observed among phylogenetic groups. Strains in groups B1 and B2 showed significantly higher multidrug resistance than groups A and D ($p < 0.05$). Notably, *E. coli* group B2, often associated with extraintestinal infections, demonstrated greater capacity to carry and accumulate resistance genes than commensal groups [15].

3.5. Genetic relatedness of ESBL-producing *E. coli* strains

MLVA was performed on 146 ESBL-producing *E. coli* isolates with determined CTX-M genotype. MLVA functions as a "genetic fingerprinting" method, analyzing variable-number tandem repeats (VNTRs) at multiple loci in the bacterial genome (**Figure 2**). As repeat numbers change rapidly across generations, genetically unrelated strains exhibit distinct repeat combinations, creating characteristic banding patterns in gel electrophoresis.

MLVA revealed considerable genetic diversity: 105 isolates (71.9%) showed no close genetic relationship (similarity < 80%), 41 isolates (28.1%) showed close relationship (similarity 80–95%), and no isolates showed very close relationship (similarity 95–100%).

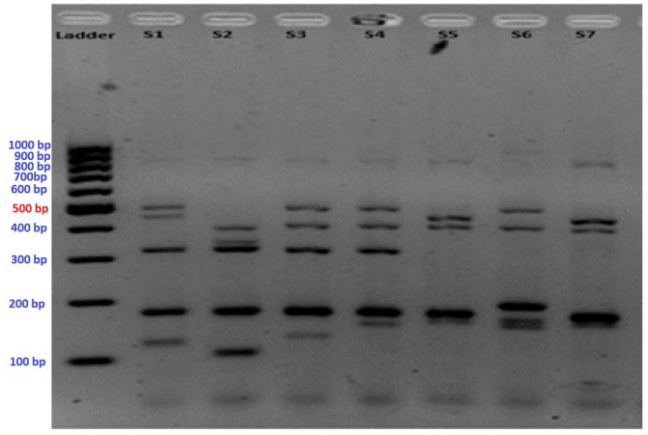

Figure 2. *Representative agarose gel electrophoresis of MLVA analysis of ESBL-producing E. coli strains. Lane 1: 100 bp DNA ladder for fragment size determination. Lanes are numbered (S1, S2, S3, etc.) corresponding to MLVA PCR products from individual ESBL-producing E. coli isolates*

These results demonstrate that ESBL-producing *E. coli* spread in the food system is a complex, diverse process involving multiple sources and mechanisms. This high genetic diversity reinforces global studies showing that ESBL-producing *E. coli* continuously evolves and adapts rapidly to antibiotic pressure, and that dissemination results not from a single clonal lineage but from multiple independent transmission events under antibiotic selective pressure [17].

This study has several limitations. First, the scope was limited to pork samples and did not evaluate environmental factors that could cause cross-contamination at sales points (knife surfaces, cutting boards, counters, vendors' hands). Therefore, the origin of isolated ESBL-producing *E. coli* (from the animal itself vs. contamination during slaughter, transport, or sales) cannot be definitively determined. Second, the sample size (n=70) may be insufficient for generalization to all of Hanoi. Future "Farm-to-Fork" studies are needed to accurately identify critical control points for drug-resistant bacterial transmission.

Nevertheless, the results emphasize the urgent need to strengthen antimicrobial resistance surveillance in the food chain, particularly in meat products, while implementing stricter controls on antibiotic use in livestock and stringent food safety hygiene measures.

4. CONCLUSION

This study reveals an alarming prevalence of multidrug-resistant ESBL-producing *E. coli* in pork at retail markets in Hanoi, with a remarkably high detection rate (87.4% of cefotaxime-resistant isolates) compared with many regional and global reports. A critical warning is the first-time detection of carbapenem-resistant *E. coli* strains-antibiotics of the "last-resort" class-in the retail food supply chain in Hanoi. This finding, together with a significant colistin resistance rate (10.3%), indicates that pork represents not only a source of common drug-resistant strains but also a potential reservoir for the most dangerous resistance genes, posing a serious and direct threat to consumer health and medical security.

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