



Genetic Characterization of the *blaTEM* Gene in *Escherichia coli* Isolated from Pork and Slaughterhouse Environments in the Mekong Delta

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ARTICLE INFO	ABSTRACT
Published Online: 04 August 2026	This study aimed to characterize the genetic features of the antibiotic resistance gene <i>blaTEM</i> in <i>Escherichia coli</i> isolates recovered from pork and slaughterhouse environments in the Mekong Delta, Vietnam. A total of 435 <i>E. coli</i> isolates recovered from feces, pork carcasses, wastewater, and slaughterhouse floor surfaces were screened for the <i>blaTEM</i> gene using polymerase chain reaction (PCR). Ten representative <i>blaTEM</i> -positive isolates were selected for nucleotide sequencing. The obtained sequences were analyzed using BioEdit and MEGA 11 to determine nucleotide sequence identity and reconstruct a phylogenetic tree using the Neighbor-Joining method with 1,000 bootstrap replicates. The <i>blaTEM</i> gene was detected in 117 of 435 (26.90%) <i>E. coli</i> isolates. The prevalence of <i>blaTEM</i> among sample types ranged from 16.67% to 29.33%, with no statistically significant differences ($P > 0.05$). Nucleotide sequence analysis revealed a high degree of sequence identity (99.992–100.000%) among the 10 representative isolates. Phylogenetic analysis grouped the sequences into two major genetic clusters with short genetic distances. No clustering according to sample origin was observed, indicating that the <i>blaTEM</i> gene is highly conserved among <i>E. coli</i> isolates circulating within the slaughterhouse environment.
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KEYWORDS: <i>blaTEM</i> , antimicrobial resistance, <i>Escherichia coli</i> , phylogenetic analysis, pig slaughterhouse, One Health.	

1. INTRODUCTION

Escherichia coli is an important indicator of fecal contamination in food production systems and an opportunistic pathogen responsible for a wide range of diseases in humans and animals. Although many *E. coli* strains are commensal, pathogenic strains frequently harbor virulence factors and antimicrobial resistance genes that can disseminate throughout the food chain, posing a significant threat to public health within the One Health framework (Bajaj et al., 2016). In recent decades, the increasing use of antimicrobial agents in livestock production, particularly β -lactam antibiotics, has accelerated the emergence and dissemination of antimicrobial-resistant *E. coli*, especially extended-spectrum β -lactamase (ESBL)-producing strains (Paterson and Bonomo, 2005; Bajaj et al., 2016).

Among the mechanisms of antimicrobial resistance in *E. coli*, the production of β -lactamase enzymes is the predominant mechanism responsible for inactivating penicillins and cephalosporins. The *blaTEM* gene was one of the first β -lactamase genes identified and remains one of the most

prevalent β -lactamase-encoding genes in *E. coli* isolated from humans, animals, and food. This gene is commonly located on plasmids and other mobile genetic elements, facilitating horizontal gene transfer among bacterial populations. Under continuous antimicrobial selective pressure, point mutations in *blaTEM* have generated numerous variants, some of which encode extended-spectrum β -lactamases capable of hydrolyzing expanded-spectrum cephalosporins, thereby compromising the effectiveness of antimicrobial therapy against Gram-negative bacterial infections (Bradford, 2001; Caniça et al., 1997; Bajaj et al., 2016).

Pig slaughterhouses represent a critical control point in the food production chain because they provide opportunities for the dissemination of antimicrobial-resistant bacteria among live animals, carcasses, processing equipment, wastewater, and the surrounding environment. During slaughter and carcass processing, inadequate hygienic practices may facilitate cross-contamination, allowing antimicrobial-resistant *E. coli* to spread throughout the processing environment. Previous studies have demonstrated that

slaughterhouses can serve as important reservoirs for *E. coli* carrying ESBL genes, including *blaTEM*, *blaCTX-M*, and *blaSHV*, thereby increasing the risk of antimicrobial resistance transmission through food products and environmental contamination (Silva *et al.*, 2023). Vietnam is one of the major pig producing countries in Southeast Asia, and the Mekong Delta is an important region for commercial pig production. The widespread use of antimicrobial agents for disease prevention and treatment in pig farming has contributed to the emergence of multidrug resistant bacteria. Recent studies have reported a high prevalence of antimicrobial resistant *E. coli* isolated from pork and slaughterhouse environments in the Mekong Delta. In addition, the *blaTEM* gene has been frequently detected among these isolates, suggesting that pig slaughterhouses may play an important role in the dissemination of β -lactam resistance genes along the food production chain (Thuan *et al.*, 2024; Thong *et al.*, 2025). Although the prevalence of the *blaTEM* gene has been extensively investigated, information regarding its genetic characteristics, nucleotide sequence diversity, and phylogenetic relationships among *E. coli* isolates from pork and slaughterhouse environments in the Mekong Delta remains limited. Comparative nucleotide sequence analysis coupled with phylogenetic reconstruction provides valuable insights into gene conservation, evolutionary relationships, and the dissemination pathways of antimicrobial resistance genes. Such information is essential for molecular epidemiological surveillance and supports the development of effective antimicrobial resistance control strategies within the One Health framework (Bajaj *et al.*, 2016). Therefore, the present study aimed to characterize the genetic characteristics of the *blaTEM* antimicrobial resistance gene in *Escherichia coli* isolates recovered from pork and slaughterhouse environments in the Mekong Delta, Vietnam. Nucleotide sequencing was performed to determine sequence variation, followed by comparison with reference sequences deposited in GenBank and phylogenetic analysis to evaluate the evolutionary relationships among the isolates. The findings provide valuable molecular epidemiological data on the *blaTEM* gene in Vietnam and contribute to antimicrobial

resistance surveillance and risk assessment programs for the food production chain under the One Health approach.

2. MATERIALS AND METHODS

2.1. Study Design, Location, and Duration

A cross-sectional study was conducted at selected pig slaughterhouses in Hau Giang, Kien Giang, Tra Vinh, and Ben Tre provinces in the Mekong Delta, Vietnam. Sampling was performed from December 2024 to June 2025. The selected facilities represent typical small- and medium-scale commercial pig slaughterhouses operating under routine hygienic conditions.

2.2. Sample Size and Sampling Strategy

A total of 240 samples were collected from 16 slaughterhouses located in Hau Giang, Kien Giang, Tra Vinh, and Ben Tre provinces. The samples comprised 96 fecal samples, 96 pork carcass samples, 16 wastewater samples, and 32 slaughterhouse floor samples.

2.3. Sample Collection and Transport

Fecal samples were collected directly from the rectum of pigs using sterile swabs. The swabs were placed in Cary-Blair transport medium, individually labeled, refrigerated at 4–8°C, and transported to the laboratory within 24 h for *Escherichia coli* isolation. Paired fecal and carcass samples were collected from the same pig. Carcass samples were collected at the slaughterhouse according to QCVN 01-04:2009/BNNT and processed following the procedures described by Barrow and Feltham (2003) and TCVN 8400-16:2011.

Slaughterhouse floor samples were collected in accordance with QCVN 150:2017/BNNT. At each slaughterhouse, two floor samples were obtained: one before slaughtering and one during slaughtering. Each sample consisted of pooled material collected from five different floor locations. Wastewater samples were collected from the final discharge point before release into the environment using the same sampling procedure previously applied for livestock wastewater.

Detection of the *blaTEM* gene was performed by polymerase chain reaction (PCR) using the primer pairs listed in Table 1.

Table 1. The primer nucleotide sequence identifies the gene encoding the antibiotic resistance factor *blaTEM*

Antibiotics	gene	Nucleotide sequence of the primer (5'-3')	size (bp)	References
Tetracyclin	<i>blaTEM-F</i>	ATTCTTGAAGACGAAAGGGC	1150	Jouini et al. (2007)
	<i>blaTEM-R</i>	ACGCTCAGTGAACGAAAAC		

The *blaTEM* gene was sequenced using Sanger sequencing (Sanger *et al.*, 1977). The obtained nucleotide sequences were compared with reference *blaTEM* sequences of *Escherichia*

coli available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). Sequence alignment, nucleotide sequence similarity analysis, and

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phylogenetic analysis of *bla*TEM-positive *E. coli* isolates from the Mekong Delta were performed using MEGA 11 software. Phylogenetic trees were reconstructed using the Maximum Likelihood (ML) method with 1,000 bootstrap replicates.

2.4 Parameters for analysis

Prevalence of *E. coli* in the analyzed samples; proportion of strains carrying the *bla*TEM gene; genetic similarity among the studied strains.

2.5 Data analysis

Raw data were processed using Microsoft Excel 2010 software. The Chi-square test in Minitab version 16 software was used to compare the prevalence of *E. coli* and the prevalence of genes encoding antibiotic resistance factors.

3. RESULTS

3.1. Prevalence of *Escherichia coli* in different sample types

The prevalence of *Escherichia coli* in 240 samples collected from pig slaughterhouses in four provinces of the Mekong Delta, Vietnam, is presented in Table 2.

Table 2. Prevalence of *Escherichia coli* in different sample types

Locatio n	Stool sample			Carcass sample			Wastewater sample			Slaughterhouse floor swab sample		
	N	+	%	N	+	%	N	+	%	N	+	%
HG	24	16	66,66 ^b	24	16	66,66 ^a	4	1	25,00 ^a	8	2	25,00
KG	24	22	91,66 ^a	24	7	29,17 ^b	4	1	25,00 ^a	8	3	37,70
TV	24	24	100,00 ^a	24	3	12,50 ^b	4	3	75,00 ^b	8	5	62,50
BT	24	22	91,66 ^a	24	3	12,50 ^b	4	3	75,00 ^b	8	4	50,00
P			<0,05			<0,01			<0,05			>0,05
Total	96	84	87,50	96	29	30,21	16	8	50,00	32	14	43,75

HG: Hau Giang, KG: Kien Giang, TV: TTra Vinh, BT: Ben Tre, N: Number of samples surveyed, +: Number of positive samples, %: Percentage

Values within the same column bearing different superscript letters (a, b, c, d) indicate a statistically significant difference.

3.2 Prevalence of the *bla*TEM gene among *Escherichia coli* isolates

The prevalence of the *bla*TEM gene among 435 *Escherichia coli* isolates recovered from different sample types is

presented in Table 3. Overall, the gene was detected in 117 isolates (26.90%).

Table 3. Prevalence of the *bla*TEM gene in the surveyed samples

Gene	Stool sample (n=283)		Carcass sample (n=70)		Slaughterhouse floor swab sample (n=46)		Wastewater sample (n=36)		P
	+	%	+	%	+	%	+	%	
<i>bla</i> TEM	83	29,33	15	21,43	13	28,26	6	16,67	>0,05

3.3 Phylogenetic analysis of *bla*TEM-positive *Escherichia coli* isolates

The genetic characteristics of the *bla*TEM gene were evaluated by nucleotide sequence analysis and phylogenetic

reconstruction. The phylogenetic relationships among the *bla*TEM-positive *Escherichia coli* isolates are presented in Figure 1.

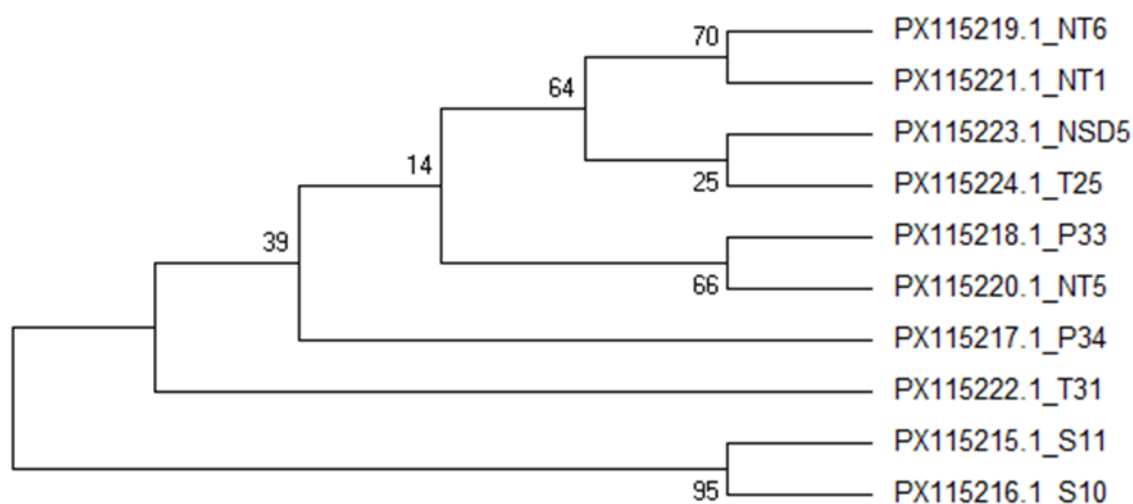


Figure 1: Phylogenetic tree of the *bla*TEM gene.

The pairwise nucleotide sequence identity matrix of the *bla*TEM gene among the *Escherichia coli* isolates was generated using MEGA 11 software

PX115215.1_S11										
PX115216.1_S10	99,997									
PX115217.1_P34	99,995	99,996								
PX115218.1_P33	99,994	99,995	99,997							
PX115219.1_NT6	99,994	99,995	99,997	99,996						
PX115220.1_NT5	99,995	99,996	99,998	99,999	99,997					
PX115221.1_NT1	99,994	99,995	99,997	99,996	100,000	99,997				
PX115222.1_T31	99,996	99,996	99,999	99,998	99,998	99,999	99,998			
PX115223.1_NSD5	99,992	99,993	99,996	99,995	99,996	99,996	99,996	99,996		
PX115224.1_T25	99,995	99,996	99,998	99,997	99,999	99,998	99,999	99,999	99,997	

4. DISCUSSION

The prevalence of *Escherichia coli* isolated from different sample types collected at pig slaughterhouses in four provinces of the Mekong Delta is presented in Table 2. Overall, *E. coli* was detected in 84 of 96 stool samples (87.50%), 29 of 96 carcass samples (30.21%), 8 of 16 wastewater samples (50.00%), and 14 of 32 slaughterhouse floor swab samples (43.75%).

The prevalence of *E. coli* in stool samples differed significantly among provinces ($P < 0.05$), ranging from 66.67% in Hau Giang to 100.00% in Tra Vinh. Likewise, the prevalence in carcass samples varied significantly ($P < 0.01$), with the highest detection rate observed in Hau Giang (66.67%) and the lowest in Tra Vinh and Ben Tre (both 12.50%). Significant differences were also observed in wastewater samples ($P < 0.05$), where the prevalence ranged from 25.00% in Hau Giang and Kien Giang to 75.00% in Tra Vinh and Ben Tre. In contrast, the prevalence of *E. coli* in slaughterhouse floor swab samples ranged from 25.00% to 62.50%, with no statistically significant differences among provinces ($P > 0.05$).

The high prevalence of *E. coli* in stool samples confirms that pigs entering the slaughterhouses were important reservoirs

of this bacterium. The detection of *E. coli* in carcasses, wastewater, and floor swab samples indicates the occurrence of contamination during slaughter and processing, suggesting that the slaughterhouse environment may facilitate the dissemination of *E. coli* throughout the production chain.

In the present study, the *bla*TEM gene was detected in 26.90% (117/435) of *Escherichia coli* isolates recovered from feces, carcasses, wastewater, and slaughterhouse floor swab samples. The prevalence of the *bla*TEM gene among the different sample types ranged from 16.67% to 29.33%, with no statistically significant differences ($P > 0.05$), indicating a relatively uniform distribution of this resistance determinant throughout the slaughterhouse ecosystem. These findings suggest the widespread circulation of β -lactam-resistant *E. coli* among animals, carcasses, and the slaughterhouse environment. Paterson and Bonomo (2005) reported that β -lactamase-encoding genes, particularly *bla*TEM, represent one of the most prevalent mechanisms of β -lactam resistance in the family *Enterobacteriaceae*. Likewise, Bajaj et al. (2016) emphasized that the dissemination of β -lactamase genes through the food chain constitutes an important public health concern within the One Health framework.

The prevalence of *bla*TEM observed in the present study was comparable to that reported by Thong et al. (2025) in pig slaughterhouses in the Mekong Delta but was lower than that reported in several studies conducted in Europe and North America, where ESBL-producing *E. coli* frequently harbor multiple β -lactamase genes, including *bla*CTX-M, *bla*TEM, and *bla*SHV (Madec et al., 2017). These differences may be attributed to variations in antimicrobial use in pig production, biosecurity practices, slaughter hygiene, and antimicrobial stewardship policies among countries. Nevertheless, the detection of *bla*TEM-positive *E. coli* in carcasses, wastewater, and slaughterhouse floor swab samples indicates that slaughterhouses remain critical control points for the dissemination of β -lactam-resistant bacteria into the food production chain.

Nucleotide sequence analysis demonstrated that the ten *bla*TEM sequences shared an exceptionally high level of nucleotide sequence identity, ranging from 99.992% to 100.000%. Isolates PX115219.1_NT6 and PX115221.1_NT1 exhibited identical nucleotide sequences (100%), whereas all remaining isolate pairs showed sequence identities greater than 99.99%, indicating that the *bla*TEM gene is highly conserved among *E. coli* isolates circulating in the Mekong Delta. Bradford (2001) reported that the TEM β -lactamase family possesses a highly conserved functional structure because of prolonged selective pressure imposed by β -lactam antimicrobial use, with relatively few point mutations giving rise to novel TEM variants. Likewise, Caniça et al. (1997) demonstrated that most *bla*TEM variants retained a remarkably high level of nucleotide sequence identity despite being recovered from diverse hosts and environmental sources.

Phylogenetic analysis revealed that the *bla*TEM sequences clustered into two major phylogenetic clades. The first clade comprised isolates NT6, NT1, NSD5, T25, P33, and NT5, whereas the second included isolates P34, T31, S11, and S10. Isolates S10 and S11 clustered together with 95% bootstrap support, while NT6 and NT1 formed a separate clade with 70% bootstrap support and shared 100% nucleotide sequence identity. These findings indicate a close genetic relationship among isolates within each clade, suggesting that they may have originated from a common bacterial lineage or carry closely related *bla*TEM-harboring plasmids. Carattoli (2013) reported that plasmids are the principal vehicles responsible for the horizontal transfer of antimicrobial resistance genes among *Escherichia coli* and other Gram-negative bacteria, thereby contributing to the maintenance of highly conserved resistance gene sequences within geographically related bacterial populations.

Although the phylogenetic tree was resolved into two major clades, several internal nodes exhibited relatively low bootstrap support values (14–39%). This observation is consistent with the nucleotide sequence identity matrix, in

which sequence identities exceeded 99%. A limited number of polymorphic sites provides insufficient phylogenetic information, thereby reducing statistical support for internal branches. Consequently, the low bootstrap values observed in this study most likely reflect the highly conserved nature of the *bla*TEM gene rather than substantial evolutionary divergence among the isolates. This interpretation agrees with the findings of Hall et al. (2013), who demonstrated that the evolution of the TEM β -lactamase family is characterized by limited nucleotide variation despite the emergence of numerous functional variants.

Notably, isolates recovered from different sample types did not cluster according to their sampling origin. Isolates obtained from feces, carcasses, wastewater, and slaughterhouse floor swab samples were distributed across both phylogenetic clades, indicating no apparent association between sample origin and the genetic characteristics of the *bla*TEM gene. This finding suggests that the same bacterial lineages or closely related plasmids circulate among animals, carcasses, and the slaughterhouse environment during processing. Similar observations have been reported by Madec et al. (2017) and Bajaj et al. (2016), who proposed that plasmid-mediated horizontal gene transfer plays a more important role than clonal evolution in the dissemination of ESBL genes among *E. coli* populations.

From the One Health perspective, the detection of *bla*TEM-positive *E. coli* on carcasses, in wastewater, and on slaughterhouse floor surfaces highlights the potential for antimicrobial resistance gene transmission from animals to humans through food products and environmental contamination. If slaughterhouse wastewater and solid waste are inadequately treated, *bla*TEM-positive bacteria may serve as environmental reservoirs of antimicrobial resistance genes. Therefore, strengthening antimicrobial resistance surveillance at slaughterhouses, improving slaughter hygiene, and promoting prudent antimicrobial use in livestock production are essential measures to limit the dissemination of plasmid-mediated β -lactamase genes (Paterson and Bonomo, 2005; Carattoli, 2013).

Although the present study provides important insights into the genetic characteristics of *bla*TEM, sequencing only a partial *bla*TEM gene fragment does not allow precise discrimination among TEM variants (e.g., TEM-1, TEM-2, TEM-52, and other ESBL-associated variants). Future studies should therefore integrate whole-genome sequencing (WGS), plasmid incompatibility (Inc) typing, and multilocus sequence typing (MLST) to elucidate the evolutionary origins and transmission dynamics of *bla*TEM throughout the food production chain. Such integrated genomic approaches are now regarded as the gold standard for molecular epidemiological investigations of antimicrobial resistance in food-producing animals and foodborne bacteria (Madec et al., 2017).

5. CONCLUSION

This study demonstrated that the *bla*TEM gene is widely distributed among *Escherichia coli* isolates recovered from pork and slaughterhouse environments in the Mekong Delta. The high nucleotide sequence identity (99.992–100.000%) and limited genetic divergence observed among representative isolates indicate that the *bla*TEM gene is highly conserved and may disseminate through mobile genetic elements. These findings provide important molecular epidemiological evidence supporting antimicrobial resistance surveillance and control in the food production chain under the One Health framework. Further investigations using whole-genome sequencing and plasmid characterization are required to better understand the evolution and dissemination of *bla*TEM among *E. coli* populations.

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