TROPICAL GENETICS

Volume 5, No. 2, November 2025
https://ojs.genetikawan-muda.com/index.php/tg

Original Research

Phylogenetic Analysis of *Escherichia coli* Bacteria Based on BlaTEM Genomic DNA from NCBI Sequence Data

Indria Palupi[1*], Afifatul Achyar [2]
[1,2] Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University, West Sumatra 25132, Indonesia
[*]**Corresponding author:** e-mail address: afifatul.achyar@fmipa.unp.ac.id

Article Info

Article history:
Received, 6 November 2025
Accepted, 29 November 2025
Available online 30 November 2025

Keywords:
Escherichia coli, blaTEM, antibiotic resistance, phylogenetic analysis, NCBI

How to cite: Palupi, I. and Achyar, A. (2025). Phylogenetic Analysis of *Escherichia coli* Bacteria Based on BlaTEM Genomic DNA from NCBI Sequence Data. Tropical Genetics, 5(2), 52-

Abstract

Antibiotic resistance caused by beta-lactamase–producing bacteria such as Escherichia coli remains a major global issue. The blaTEM gene is especially important because it is widely distributed and strongly associated with resistance to penicillins and third-generation cephalosporins. This study analyzed the phylogenetic relationships of ten E. coli isolates based on blaTEM nucleotide sequences from the NCBI database (2016–2025). Sequences were aligned using ClustalW in MEGA X, and phylogeny was reconstructed using the Neighbor-Joining method with the Kimura 2-parameter model and 1000 bootstrap replications. The results revealed four main clades, with isolates from 2021–2024 showing close genetic relatedness, while those from 2019 and 2025 displayed the greatest divergence. These patterns indicate ongoing evolutionary variation in the blaTEM gene, likely influenced by antibiotic selection pressure. Such findings reinforce the need for molecular surveillance of resistance genes to support early detection and improve antibiotic stewardship strategies.

Introduction

Antibiotic resistance is one of the major challenges in global health today. Pathogenic bacteria that were once susceptible to antibiotics are now becoming increasingly resistant, reducing the effectiveness of treatment and increasing the risk of therapeutic failure. *Escherichia coli* is a Gram-negative bacterium commonly found in the digestive tracts of humans and animals, and is one of the main examples of bacteria that are becoming increasingly resistant to antibiotics (Kurniawan *et al.,* 2023).

Several strains of *Escherichia coli* can cause various infections such as urinary tract infections, bacteremia, diarrhea, bloody diarrhea, and neonatal meningitis in humans and animals. In addition, other infections including pneumonia and sepsis in humans, as well as mastitis in dairy cows, are also caused by *Escherichia coli* bacteria. The main habitat of *Escherichia coli* is the digestive tract of humans and animals (Anggaeni *et al.,* 2023). There are strains of *Escherichia coli* that are commensal, harmless, and others that are pathogenic to humans and animals. E. coli can be found in soil and water due to fecal contamination and is used as an indicator of poor water and/or food quality. This bacterium can also be found in the environment (such as water, soil, air, and dust), production equipment, and workers. *Escherichia coli* is often used as an indicator of antimicrobial resistance (Pratiwi *et al.,* 2023).

Extended Spectrum β-lactamase (ESBL) is an enzyme capable of hydrolyzing and causing resistance to various types of β-lactam antibiotics, including third-generation cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime, as well as monobactams such as aztreonam. However, ESBL

cannot affect cephamycins such as cefoxitin and cefotetan, as well as carbapenems such as imipenem, meropenem, and ertapenem. Their activity can be inhibited by clavulanic acid (Normaliska *et al.,* 2019). ESBLs cause resistance to penicillin, cephalosporin, and aztreonam antibiotics, as well as to other classes of antibiotics such as aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones (Elise *et al.,* 2023). There are many types of ESBL, such as TEM, SHV, CTX, OXA, AmpC, etc., but the majority of ESBL are derivatives of the TEM or SHV enzymes, and these enzymes are most commonly found in *Escherichia coli*. (Prasetya *et al.,* 2018) Furthermore, ESBL gene transmission in E. coli typically occurs via plasmids, facilitating the transfer of antimicrobial resistance between bacteria. This has led to an increase in cases of difficult-to-treat infections, particularly urinary tract infections and nosocomial infections (Zhong *et al.,* 2025).

The continuous use of antibiotics over long periods can trigger the emergence of resistance (Gina *et al.,* 2020). Resistance refers to the ability of bacteria to survive the effects of antibiotics, allowing them to persist even after treatment (Hamida *et al.,* 2019). The incidence of resistance continues to increase in both humans and animals, particularly in Escherichia coli, which can acquire and spread resistance genes among bacterial populations (Agustin *et al.,* 2022). Therefore, this study aims to analyze the phylogenetic relationships and genetic distances of E. coli isolates based on blaTEM genomic sequences from the NCBI database.

Method

2.1. Materials

The data utilized in this study were secondary data obtained from the National Center for Biotechnology Information (NCBI) database. The dataset consisted of nucleotide sequences of the blaTEM gene derived from the genomic DNA of *Escherichia coli* isolates collected between 2016 and 2025.

2.2. Methods

This study uses secondary data obtained from the NCBI (National Center for Biotechnology Information) website. Secondary data is data created by people who did not participate in the event or condition being studied (Hidayat *et al.,* 2018). The data collected was the nucleotide sequence of the BlaTEM gene in the genomic DNA of *Escherichia coli* bacteria over the last ten years, from 2016 to 2025. A total of 10 *Escherichia coli* blaTEM nucleotide sequences retrieved from the NCBI GenBank database. The accession numbers of the sequences were: OQ674126.1 (2023), PQ409411.1 (2025), PP723879.1 (2024), OP870148.1 (2022), MZ768814.1 (2021), MW345820.1 (2020), MN158355.1 (2019), MG653169.1 (2018), KX171188.1 (2017), and KT792683.1 (2016). All sequences were obtained directly from the GenBank submission records corresponding to each accession number. The BlaTEM gene nucleotide sequence data obtained was aligned with Clustal-W using MEGA X software (Zhong *et al.,* 2011). Next, phylogenetic and genetic distance analysis was performed using the Neighbor joining method and the Kimura 2-parameter evolutionary model with 1000 bootstraps.

Results and Discussion

The phylogenetic tree generated using the Neighbor-Joining (NJ) method with bootstrap analysis shows the evolutionary relationships among *Escherichia coli* isolates based on blaTEM gene sequences collected from the years 2016 to 2025. The resulting tree forms four major clades, each reflecting distinct patterns of genetic proximity among isolates from specific years.

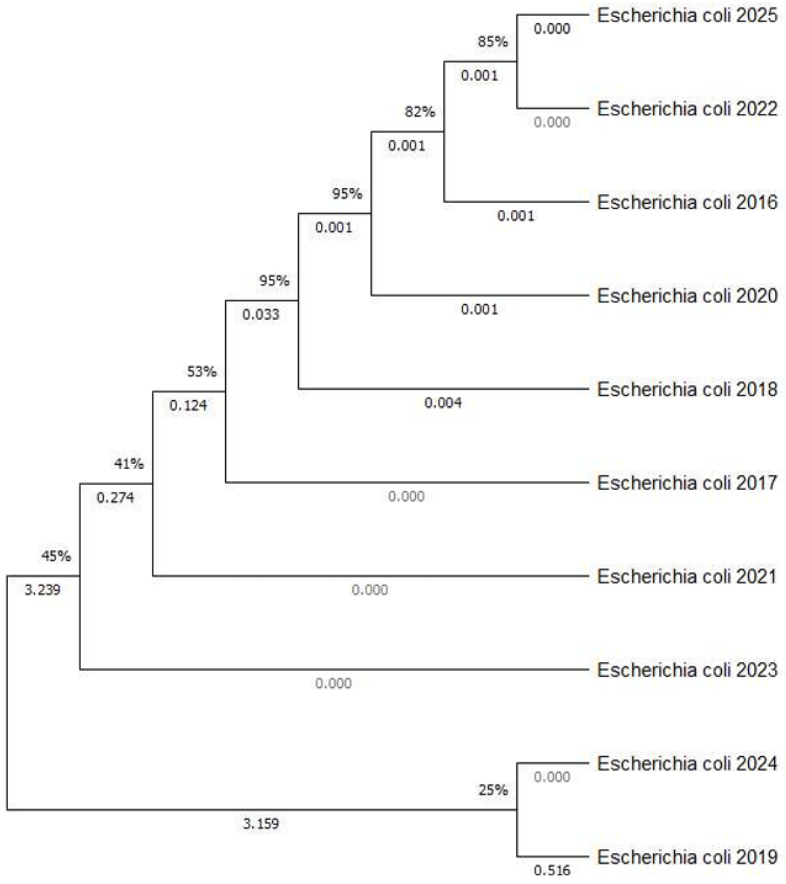

Figure 1. Phylogenetic tree of *Escherichia coli* with 10 species based on blaTEM using the Neighbor Joining method

The first clade, consisting of isolates with accession numbers OQ674126.1 (2025) and OP870148.1 (2022), shows a very small evolutionary distance (0.000). Although the bootstrap value supporting this grouping is 85%, which falls within the range of moderate support rather than strong support (Alfiana *et al.*, 2022; Hall, 2013), the extremely close genetic distance still indicates a high degree of sequence similarity between the two isolates. Similarly, the isolates with accession numbers KT792683.1 (2016) and MW345820.1 (2020) isolates also form a strong separate clade, supported by a bootstrap value of 95% and a low genetic distance (0.001), indicating that the blaTEM gene in these years underwent few mutations and is in the same evolutionary lineage. Meanwhile, the isolate MG653169.1 (2018) forms a separate branch but is still close to the KT792683.1 (2016) – MW345820.1 (2020) cluster, indicating slight genetic differences but still within the same large clade. The isolate of KX171188.1 (2017) isolate also forms a separate clade but is quite close to MG653169.1 (2018), which may indicate local variation or differences in gene acquisition time.

Isolates from accession number MZ768814.1 (2021), OQ674126.1 (2023), and PP723879.1 (2024) show greater differences. Specifically, the 2024 and 2019 isolates form a separate clade that is significantly distinct from the other isolates, with a relatively large evolutionary distance (0.516) and low bootstrap support (25%). This may indicate that these isolates have considerable variation or may have undergone higher recombination or mutation events in the blaTEM gene. The longest evolutionary distance was found in the branch between the MN158355.1 (2019) isolate and the other main groups (distance 3.159), indicating significant genetic changes. Relatively low bootstrap values in several other branches (e.g., 41% and 45%) suggest that the relationships in these clades are less stable and may change depending on the method or additional data used.

Table 1. Genetic Distance Matrix of *E. coli* Isolates (2016–2025)

Sampel	1	2	3	4	5	6	7	8	9
OQ674126.1 (2023)									
PQ409411.1 (2025)	0,005								
PP723879.1 (2024)	5,636	6,293							
OP870148.1 (2022)	0,005	0,000	6,293						
MZ768814.1 (2021)	0,000	0,008	5,959	0,008					
MW345820.1 (2020)	0,008	0,001	6,293	0,002	0,011				
MN158355.1 (2019)	6,408	7,591	0,000	7,521	6,619	7,499			
MG653169.1 (2018)	0,000	0,005	6,294	0,006	0,003	0,006	7,521		
KX171188.1 (2017)	0,007	0,000	6,293	0,000	0,008	0,000	6,959	0,009	
KT792683.1 (2016)	0,008	0,001	6,293	0,001	0,011	0,003	7,486	0,007	0,002

The analysis results show that the isolate with accession number OQ674126.1 (2023) has a very high genetic similarity to the isolates OP870148.1 (2022) and PQ409411.1 (2025), each with a genetic distance value of 0.005. This indicates that the three isolates likely originate from a similar source or have undergone very few genetic changes in the blaTEM gene. The isolate PP723879.1 (2024) also shows high genetic similarity to OQ674126.1 (2023) and OP870148.1 (2022), particularly to OP870148.1 (2022) (distance 6.293), as well as to PQ409411.1 (2025) (distances 5.636 and 6.293), although these values are slightly higher, indicating the possibility of minor mutations or local variations that distinguish these isolates. The isolate MN158355.1 (2019) shows the highest genetic distance from most other isolates, including MZ768814.1 (2021) (distance 7.521), MW345820.1 (2020) (6.619), and OP870148.1 (2022) (7.591). This indicates that MN158355.1 (2019) is one of the most genetically divergent isolates, possibly due to the influence of different selection pressures, major mutation events, or originating from a geographically distinct environment. The isolates MW345820.1 (2020) and MN158355.1 (2019) also show significant differences from PQ409411.1 (2025), with genetic distance values of 7.521 and 7.499, confirming the existence of separate evolutionary pathways.

In contrast, the isolates KT792683.1 (2016), KX171188.1 (2017), and MG653169.1 (2018) showed a more stable pattern. These three isolates displayed very low genetic distances between each other, such as between KT792683.1 and KX171188.1 (0.007), KT792683.1 and MG653169.1 (0.005), and KX171188.1 and MG653169.1 (0.000). This indicates that during the earlier period (2016–2018), the genetic variation of the blaTEM gene was relatively low, as reflected by the very small genetic distances among isolates. Low genetic distances generally correspond to limited sequence variation, a pattern typically observed when selection pressure remains stable (Thompson *et al.*, 1994), suggesting a homogeneous selection environment or minimal evolutionary pressure acting on this gene.

This close relationship is also observed in the isolate MZ768814.1 (2021), which shows small genetic distances to earlier isolates, such as MG653169.1 (2018) (0.008) and MW345820.1 (2020) (0.008). Overall, the phylogenetic tree demonstrates that although many *Escherichia coli* isolates carrying the blaTEM gene over the past decade exhibit high genetic proximity, several isolates particularly MN158355.1 (2019) and PP723879.1 (2024) show considerable divergence. High divergence such as that observed for MN158355.1 may reflect recombination events or gene acquisition from different lineages (Paterson & Bonomo, 2005). This pattern supports the possibility of independent evolutionary trajectories among these isolates. Independent evolution of the blaTEM gene has also been reported in various environments as a consequence of differing antibiotic selection pressures (Bush & Jacoby, 2010; Cantón & Coque, 2006). Understanding this pattern is important for elucidating the dynamics of antibiotic resistance gene dissemination and identifying evolutionary clusters that may become potential sources of outbreaks or targets for future surveillance efforts.

Conclusion

Phylogenetic analysis based on blaTEM gene sequences from *Escherichia coli* isolates over the past ten years shows significant genetic variation between isolates. The 2021–2024 isolate group forms a cluster with low genetic distance, indicating genetic stability and the possibility of a similar evolutionary origin. In contrast, isolates from 2020 and 2025 show striking genetic differences from other isolates, indicating the possibility of independent evolutionary events or different selection pressures. These findings emphasize the importance of molecular monitoring of antibiotic resistance genes, such as blaTEM, to provide insight into the evolutionary patterns and genetic variation of the blaTEM gene across different *E. coli* isolates. This information can serve as a basis for developing resistance surveillance strategies and more targeted antibiotic use policies in the future.

References

Agustin, A, L, D., Dan Ningtyas, N, S, I. (2022). Resistensi Eschericia Coli Terhadap Berbagai Macam Antibiotik Pada Pasien Kucing Di Rumah Sakit Hewan Pendidikan Universitas Pendidikan Mandalika. *Jurnal Resistensi Antibiotik* ,29(1):63-71

Alfiana, A, L, D., Ningtyas, N, S, I., Tirtasari, K, Mega. (2022). Resistensi Antibiotik Terhadap *Escherichia coli* Yang Diisolasi Dari Ayam Layer Di Desa Sesaot Kabupaten Lombok Barat. *Jurnal Resistensi Antibiotik* ,8(5):87-95

Anggaeni, T, T, K., Pratama, A. (2023). Isolasi Bakteri *Escherichia coli* Resistan Antibiotik Pada Ceker Ayam Di Pasar Tradisional Di Kecamatan Jatinangor, Sumedang Jawa Barat. *Jurnal Veteriner* ,24(1): 129-137

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy*, 54 (3), 969–976. https://doi.org/10.1128/AAC.01009-09

Cantón, R., & Coque, T. M. (2006). The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology*, 9(5), 466–475. https://doi.org/10.1016/j.mib.2006.08.011

Elise M., Ballo, E, M., Novalino, N, G., Nemay, A. (2023). Kajian Review Resistansi *Escherichia coli* Terhadap Antibiotik B-Laktam Dan Aminoglikosida Pada Ternak Ayam Dan Produk Olahannya Di Indonesia. *Jurnal Veteriner Nusantara* ,6(15):1-21

Gina, S., Nurjanah, G, S., Cahyadi, G, S., Windria, S. (2020). Resistensi *Escherichia coli* Terhadap Berbagai Macam Antibotik Pada Hewan Dan Manusia. *Jurnal Indonesia Medicus Veterinus* ,9(6): 970-983

Hall, B. G. (2013). *Building phylogenetic trees from molecular data with MEGA*. Molecular Biology and Evolution, 30(5), 1229–1235. https://doi.org/10.1093/molbev/mst012

Hamida, F., Aliya, L, S., Syafriana, V., Dan Pratiwi, D. (2019). *Escherichia coli* Resistansi Antibiotik Asal Air Keran Di Kampus Istn . *Jurnal Kesehatan* ,12(1):63-73

Hidayat, I. W., Ariana, A. D., Hendriani, W., Zein, R. A., Cahyono, R., & Wicaksono, D. A. (2018). *Keterampilan Belajar (Study Skills) Untuk Mahasiswa (Pertama)*. Kencana.

Kurniawan, H, M., Zuhdi, N., Nasution, A, N. (2023). Uji Sensitivitas Antibiotik Terhadap Bakteri *Escherichia coli* Dan Staphylococcus Aureus Secara In Vitro. *Jurnal Prosiding Seminar Nasional Teknologi Komputer Dan Sains* ,1(1):712-718

Normaliska, R., Sudarwanto, M, B., Latif, H. (2019). Pola Resistensi Antibiotik Pada *Escherichia coli* Penghasil Esbl Dari Sampel Lingkungan Di Rph-R Kota Bogor. *Jurnal Acta Veterinaria Indonesiana* ,7(2):42-4.

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Reviews*, 18(4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Prasetya, Y. A. (2018). Deteksi Gen SHV pada Isolat Klinik Escherichia Coli Penghasil Extended Spectrum Beta− Lactamases (ESBLs) dengan Metode Polymerase Chain Reaction (PCR) dari urin pasien. *Al Kauniyah Journal*, *11*(2), 91-98.

Pratiwi, E., Linosefa., Amelin, P. (2023). Perbandingan Pola Kepekaan Antibiotik Bakteri Penghasil Extended Spectrum Beta-Lactamase Penyebab Infeksi Saluran Kemih Di Rumah Sakit Umum Pusat Dr. M. Djamil Padang. *Jurnal Sari Pediatri* ,25(3):163 169

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment. *Nucleic Acids Research*, 22(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Zhong, J. Y., Zhu, B., Hua, L., Wang, C. B., Kuang, L., Xie, J. H., & Chen, Y. (2011). Complete genomic sequence analysis on human enterovirus 71 strain in Guangzhou, in 2008 and 2010. *Zhonghua liu Xing Bing xue za zhi= Zhonghua Liuxingbingxue Zazhi*, *32*(7), 700-704.

Zhong, W., Zhou, Y., Che, M., Wang, L., Tian, X., Wang, C., ... & Zhong, Z. (2025). Extended-spectrum β-lactamase-producing Escherichia coli isolated from captive primates: characteristics and horizontal gene transfer ability analysis. *PloS one*, *20*(4), e0321514.