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Genomic and Phylogenetic Analysis of E. coli O157:H7: Towards Improved Surveillance and Public Health Response.

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Abstract

Escherichia coli O157:H7 is a major foodborne pathogen responsible for severe illnesses, including hemolytic uremic syndrome. Despite existing public health efforts, recurrent outbreaks persist, highlighting gaps in our understanding of its genetic variability and transmission dynamics. Genomic insights into its evolutionary characteristics are essential for improving diagnostic and surveillance strategies. This study employed next-generation sequencing (NGS) and phylogenetic analysis to examine E. coli O157:H7 isolates obtained from three hospitals in Edo State, Nigeria. DNA was extracted and sequenced using validated protocols, and sequences were analyzed through established bioinformatic pipelines. Phylogenetic trees were constructed to determine evolutionary relationships, while comparative genomic analysis was conducted to identify virulence and antimicrobial resistance genes. The results revealed substantial genetic heterogeneity among the isolates, with clustering patterns corresponding to geographical origins. Conserved virulence genes such as *stx1* and *stx2* were present across most strains, while variation in accessory genomes suggested adaptive evolution. Several isolates demonstrated genetic similarity to known outbreak strains and exhibited markers of antimicrobial resistance. These findings suggest that the pathogen remains highly adaptable, with significant potential for persistence and dissemination across different environments. The results underscore the need to integrate genomic data into routine public health surveillance to track pathogen evolution and inform targeted interventions. Strengthening molecular diagnostic capacity and enhancing food safety regulations will be critical for early outbreak detection and response. This study contributes valuable genomic data on E. coli O157:H7 in a sub-Saharan African context and provides a foundation for precision-guided public health strategies.

Keyword: E. coli O157:H7; Genomic diversity; Next-generation sequencing (NGS); Phylogenetic analysis; Shiga toxin; Public health strategies; Outbreak surveillance; Virulence factors.

INTRODUCTION

Escherichia coli O157:H7 is a significant public health concern due to its association with severe foodborne illnesses (Zheng *et al.,* 2008; Ojo *et al.,* 2010; Christopher *et al.,* 2023; Omoregie *et al.,* 2022; Lateefat *et al.,* 2022; Habeeb *et al.,* 2022; Adiama *et al.,* 2022). This pathogenic bacterium can cause symptoms ranging from mild diarrhea to life-threatening conditions like hemolytic uremic syndrome (HUS) (Raimi *et al.,* 2017; Olalekan *et al.,* 2018; Henry *et al.,* 2019; Olalekan *et al.,* 2019; Raimi *et al.,* 2019; Afolabi and Raimi, 2021; Ukwandu *et al.,* 2001; Elemuwa, 2021; Abiye and Raimi, 2025; Anthony *et al.,* 2025; Enang *et al.,* 2025a, b). Understanding its genetic makeup and transmission pathways is essential for developing effective public health strategies (Yusuf *et al.,* 2025; Olaniyi and Morufu, 2025; Morufu *et al.,* 2025). Advancements in DNA sequencing and phylogenetic analysis have enhanced our understanding of *E. coli* O157:H7. Studies utilizing whole-genome sequencing have provided insights into the bacterium's genetic diversity and evolutionary relationships (Ukwandu *et al.,* 2001; Elemuwa, 2021; Henry and Morufu, 2025; Christopher *et al.,* 2025). For instance, phylogenetic analyses have been employed to trace the origins of outbreaks and understand the genetic relatedness of strains (Elemuwa, 2021; Ardissino *et al.,* 2016; Benmch *et al.,* 2006; Blitz *et al.,* 2018). Additionally, genomic analyses have been used to investigate the phylogenetic relatedness of *E. coli* O157:H7 from various sources, aiding in source attribution (Christopher *et al.,* 2023; Omoregie *et al.,* 2022; Ukwandu *et al.,* 2001; Elemuwa, 2021). Despite these advancements, gaps remain in our understanding of *E. coli* O157:H7. The bacterium's ability to adapt and evolve poses challenges for public health surveillance and outbreak prevention (Morufu *et al.,* 2021a, b; Elemuwa *et al.,* 2024a, b; Kakwi *et al.,* 2024a, b; Abaya *et al.,* 2024; Okechukwu *et al.,* 2024; Uchenna *et al.,* 2024; Christopher *et al.,* 2024; Mordecai *et al.,* 2024; Raimi *et al.,* 2021a, b, c; Oweibia *et al.,* 2024; Joshua *et al.,* 2024; Samson *et al.,* 2020; Raimi and Raimi, 2020; Raimi *et al.,* 2020; Promise *et al.,* 2024; Ibrahim *et al.,* 2025; Promise *et al.,* 2025). Moreover, the economic burden of illnesses caused by this pathogen is substantial, particularly in Sub-Saharan Africa, where annual costs remain high (Christopher *et al.,* 2023; Omoregie *et al.,* 2022; Ukwandu *et al.,* 2001; Elemuwa, 2021). *E. coli* O157:H7 infections disproportionately affect vulnerable populations such as children and the elderly, who are more susceptible to severe outcomes like hemolytic uremic syndrome (HUS) (WHO, 2018). In the United States alone, an estimated 73,480 cases occur annually, resulting in approximately 2,168 hospitalizations and 61 deaths (Weiss *et al.,* 2009; Varma *et al.,* 2003; WHO, 2018). These infections are frequently linked to contaminated food products and have been documented across both urban and rural settings (Zheng *et al.,* 2008; Ojo *et al.,* 2010; Christopher *et al.,* 2023; Omoregie *et al.,* 2022; Lateefat *et al.,* 2022; Habeeb *et al.,* 2022; Adiama *et al.,* 2022; Morufu *et al.,* 2025; Uchenna *et al.,* 2025). The persistence of *E. coli* O157:H7 infections over several decades highlights ongoing challenges in surveillance and outbreak prevention. Its capacity to cause disease in diverse populations and environments underscores the need for targeted public health interventions. Understanding the genetic factors that contribute to the bacterium's virulence and transmission dynamics is critical for designing effective control strategies. This study applies advanced DNA sequencing and phylogenetic analysis to decode the genetic structure and evolutionary relationships of *E. coli* O157:H7, with the goal of identifying virulence markers and informing evidence-based public health policies related to food safety and infection control.

MATERIALS AND METHODS

Study Area

The research was carried out in Edo State, Nigeria, located between longitudes 06° 04' E and 06° 43' E and latitudes 05° 44' N and 07° 34' N (Elemuwa, 2021). Edo State is bounded by Delta State to the south, Ondo State to the west, Kogi State to the north, and Anambra States to the east. Covering an area of approximately 17,802 square kilometers, the state had a population of 3,233,366 as per the 2006 National Population Census (NPC, 2006). The state's 18 local government areas are grouped into three senatorial districts, with Benin City serving as the capital. This study focused on three hospitals within Edo State: The University of Benin Teaching Hospital (UBTH) in Egor Local Government Area (latitude 6.3904°N, longitude 5.6118°E), Central Hospital in Oredo Local

Government Area (latitude 6.3298°N, longitude 5.6225°E), and Irrua Specialist Teaching Hospital (ISTH) in Esan Central Local Government Area (latitude 6.7331°N, longitude 6.1905°E). UBTH and ISTH are tertiary healthcare facilities, while Central Hospital is a secondary healthcare facility. The population figures for Egor, Esan Central, and Oredo Local Government Areas were 340,287, 105,242, and 374,515, respectively (NPC, 2006).

Study Population

A total of 576 patients presenting with gastrointestinal complaints were recruited from three healthcare facilities in Edo State, Nigeria. These included 145 participants from the University of Benin Teaching Hospital (UBTH), 420 from Central Hospital Benin, and 11 from Irrua Specialist Teaching Hospital (ISTH). Informed consent was obtained from all adult participants, while parental or guardian consent was secured for minors. Ethical clearance for the study was granted by the Ethical Committee of ISTH (Approval No: ISTH/REC/2022/05/31) and the Edo State Ministry of Health (Approval No: EDSMH/ERC/23/2022).

Determination of Sample Size

The required sample size was calculated using the formula by Charan and Biswas (2013):

$$n = \frac{Z^2\ XP\ X\ Q}{d^2}$$

Where:

n = Sample size

Z_α = Standard normal deviate (1.96 for 95% confidence interval)

P = Estimated prevalence of infection (31.6% based on Ekundayo *et al.,* (2014)

Q = 1 – P

D = Desired precision (0.05)

Substituting the Values

1. Calculate Q:
   Q=1−P=1−0.316=0.684

2. Substitute into the sample size formula:
   $$n = \frac{(1.96)^2\ X\ 0.316\ X\ 0.684}{(0.05)^2}$$

3. Calculating this gives: $(1.96)^2$=3.8416

Therefore

$$n = \frac{3.8416\ X\ 0.316\ X\ 0.684}{0.0025} = \frac{0.7927}{0.0025} = 317$$

Adjusting for Non-Response: An additional 10% was included to account for non-response:
Adjusted sample size:
$n_{adjusted} = n + (0.10.n) = 317 + 31.7 = 348.7$
Rounding up, this gives approximately 349.

Despite the calculated sample size of approximately 349, a total of 576 participants were ultimately recruited to ensure adequate power and representation in the study, accommodating potential dropouts and enhancing the reliability of results. This approach ensures that the study is sufficiently powered to detect significant effects while addressing practical considerations related to participant recruitment and retention in clinical research settings.

Specimen Collection and Handling

Whole venous blood samples were collected into ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes. Fresh stool specimens either formed or watery were collected into sterile, leak-proof universal containers. All samples were obtained by trained laboratory staff using aseptic techniques and were transported to the microbiology laboratory within one hour of collection under cold chain conditions to ensure specimen integrity.

Hematological Analysis

Full blood count (FBC) was performed using the Sysmex K21N haematology autoanalyser (Sysmex Corporation, Kobe, Japan), in accordance with the manufacturer's instructions. Only whole blood samples collected in EDTA tubes were used for analysis. The parameters assessed included haemoglobin concentration, haematocrit, total white blood cell count, neutrophil percentage, and lymphocyte percentage.

Parasitological Examination

Stool samples were examined for intestinal parasites using the formol-ether concentration technique described by Akinbo *et al.* (2013). Briefly, 1 gram of formed stool or 1 milliliter of watery stool was mixed with 4 milliliters of formol saline. After sieving, 4 milliliters of diethyl ether was added. The mixture was agitated and centrifuged at 3000 rpm for 1 minute. The

supernatant was discarded, and the sediment was examined microscopically using saline and iodine preparations under 10× and 40× magnifications.

Isolation and Identification of *Escherichia coli*

Stool samples were inoculated onto MacConkey agar and incubated overnight at 37°C. Lactose-fermenting colonies were subcultured for purity. Identification was performed following Cowan and Steel's criteria (Barrow and Fetham, 2003), including colonial morphology, Gram staining, and biochemical tests (oxidase, citrate, motility, urease, indole production, and sugar fermentation).

Detection and Confirmation of *Escherichia coli* O157:H7

Isolates were screened on sorbitol MacConkey agar to identify non-sorbitol fermenting colonies. Presumptive *E. coli* O157:H7 isolates were confirmed using the Remel *E. coli* O157:H7 latex agglutination kit (Remel Inc., Santa Fe, CA, USA). Further confirmation included testing with the *E. coli* H7 latex reagent.

Molecular Characterization

DNA Extraction

DNA was extracted using the ZR Bacterial DNA Extraction Kit (Zymo Research Corporation, USA) according to the manufacturer's protocol. Extracted DNA was used as a template for polymerase chain reaction (PCR).

PCR Amplification

PCR targeted the V4 hypervariable region of the 16S rRNA gene using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'- TACGGYTACCTTGTTACGACTT-3'). The reaction mixture consisted of PCR master mix, template DNA, primers, and nuclease-free water in a total volume of 25 µL. The thermal cycling conditions included initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation (94 °C for 30 seconds), annealing (54 °C for 30 seconds), and extension (72 °C for 1 minute), with a final extension at 72 °C for 7 minutes.

Gel Electrophoresis

PCR products were resolved by agarose gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide (Cat. No. E7637, Sigma-Aldrich, USA). Electrophoresis was conducted at 100 volts for 45 minutes, and DNA bands were visualised under UV transillumination using a GelDoc system (Bio-Rad, USA).

Sequencing

PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Cat. No. 78201, Applied Biosystems, USA) and sequenced using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Cat. No. 4337455, Applied Biosystems, USA). Sequencing was performed on an ABI 3500XL Genetic Analyzer (Applied Biosystems, USA). Sequence data were analysed using Geneious software version 9.0.5 (Biomatters Ltd., New Zealand). Phylogenetic trees were constructed using the Neighbour-Joining method with bootstrap analysis of 100 replicates, as described by Saitou and Nei (1987).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method in accordance with the guidelines of the British Society for Antimicrobial Chemotherapy (BSAC) (Andrew, 2009). Bacterial suspensions were standardized to 0.5 McFarland turbidity and inoculated onto Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK). Antibiotic discs (Oxoid, UK) used included Amoxicillin-Clavulanate (30 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cloxacillin (5 µg), Gentamicin (10 µg), and Ofloxacin (5 µg), with sensitivity results showing 0% for Amoxicillin-Clavulanate, Cefuroxime, Ceftazidime, and Cloxacillin; 80% for Ceftriaxone and Gentamicin; and 100% for Ofloxacin. Plates were incubated at 37 °C for 18–24 hours, and inhibition zone diameters were measured and interpreted based on BSAC breakpoints.

Statistical Analysis

Data analysis was performed using INSTAT® software (GraphPad Software Inc., San Diego, CA, USA). Parametric data were analyzed using the unpaired Student's t-test, while non-parametric data were assessed using Chi-square or Fisher's Exact test. Odds ratios were calculated where relevant.

RESULTS

Figure 1 present the distribution of E. coli O157:H7 prevalence by age among the 420 participants at

Central Hospital, Benin City. Of the total 576 individuals recruited, 156 participants were excluded from this specific analysis due to incomplete demographic data or sample quality issues that did not meet the inclusion criteria for site-level stratified analysis. Among the 420 individuals assessed, no positive cases were recorded in the ≤1–10, 21–30, and 41–50-year age groups. The highest prevalence was observed in the 11–20-year group (4.35%), followed by the ≥51-year group (2.11%) and the 31–40-year group (1.08%). However, these differences were not statistically significant, as indicated by a p-value of 0.2339.

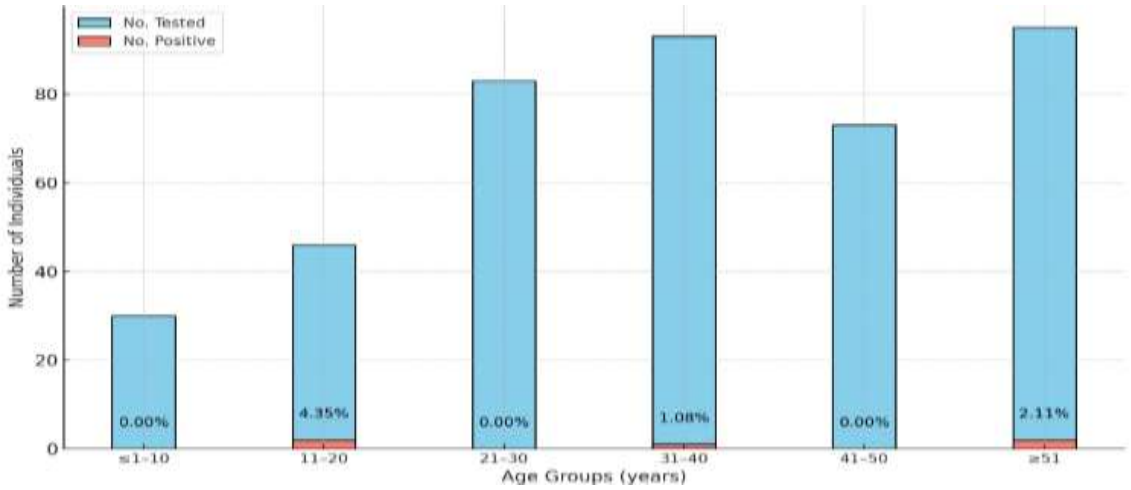

Figure 1: *Effect of age on the prevalence of E. coli O157: H7 isolated from Central Hospital, Benin City. The bar chart visually represents the blue bars which indicate the number of individuals tested within each age group, while the red bars highlight the number of positive cases for E. coli O157:H7. The positivity rates (in percentages) are labeled above the red bars for clarity.*

Figure 2 presents a phylogenetic tree illustrating the evolutionary relationships among various *Escherichia coli* strains, including the isolate labeled "ISOLATE 155." Each branch represents a distinct strain, identified by its name and genome accession number. Bootstrap values are displayed as percentages at branch nodes and indicate the statistical confidence of each cluster, with higher values suggesting stronger support. The tree shows that ISOLATE 155 clusters closely with *Escherichia coli* strain K-15KW01 and *Escherichia coli* strain Combat2C1. These clustering patterns reflect genetic similarity and suggest a recent common ancestry between ISOLATE 155 and these strains. Bootstrap values at key nodes, such as 80% and 71%, support the reliability of these evolutionary relationships. Phylogenic analysis

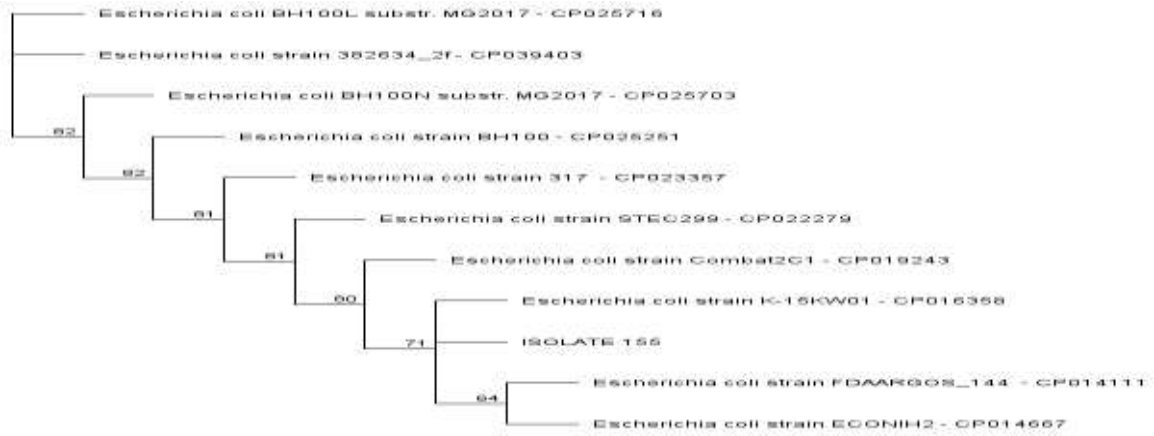

Figure 2: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 155 has similar sequence with Escherichia coli strain K-15KW01 with accession number CP016358*

Figure 3 presents a phylogenetic tree depicting the evolutionary relationships among various *Escherichia coli* strains, including the isolate labeled "ISOLATE 127." Each branch corresponds to a specific strain identified by its name and genome accession number. Bootstrap values are shown at the nodes to indicate the statistical support for each clade, with higher values, such as 90% and 82%, indicating greater confidence in the grouping. ISOLATE 127 is observed to cluster with *Escherichia coli* strain CFSAN061772 and other strains from similar lineages, supported by a bootstrap value of 73%. Additional clusters, including *Escherichia coli* strain ATCC 51435 and *Escherichia fergusonii*, form distinct subgroups within the tree, as indicated by their respective bootstrap support values.

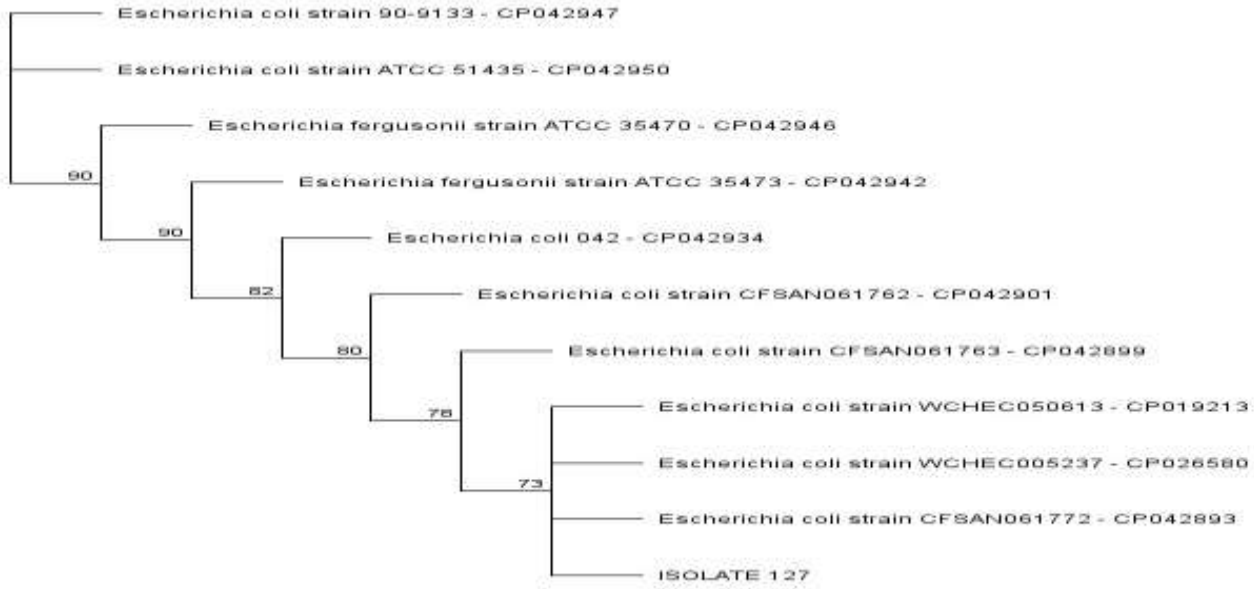

Figure 3: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 127 has similar sequence with Escherichia coli strain CFSAN061772 with accession number CP042893.*

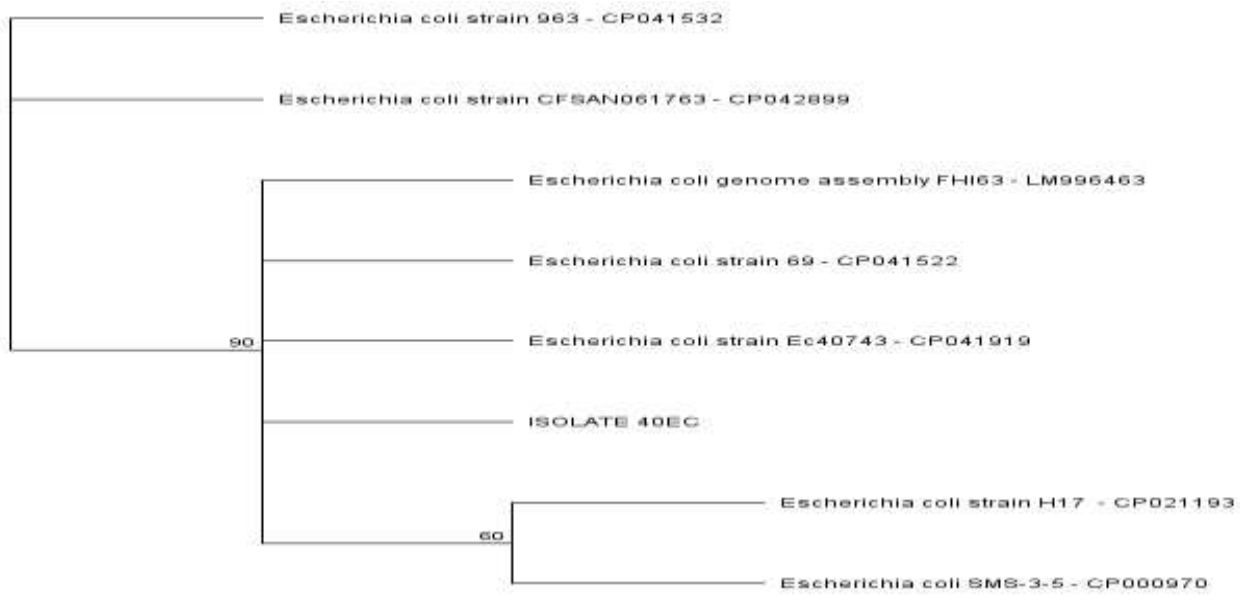

Figure 4: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Generous package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 40EC has similar sequence with Escherichia coli strain Ec40743 with accession number CP041919*

Figure 4 displays a phylogenetic tree illustrating the evolutionary relationships among various *Escherichia coli* strains, including the isolate labeled "ISOLATE 40EC." Each branch represents a specific strain, identified by its name and genome accession number. Bootstrap values are provided at key branch nodes to indicate the statistical confidence of the inferred relationships, with higher values denoting greater reliability. ISOLATE 40EC clusters closely with *Escherichia coli* strain Ec40743, supported by a high bootstrap value of 90%, indicating strong genetic similarity. A separate cluster formed by *Escherichia coli* strains H17 and SMS-3-5 is supported by a bootstrap value of 60%, while *E. coli* strain 963 and CFSAN061763 occupy a distinct lineage at the top of the tree.

Figure 5 presents a phylogenetic tree depicting the evolutionary relationships among various *Escherichia coli* strains, including a novel isolate labeled "ISOLATE 40A." Each branch corresponds to a specific strain, identified by its name and genome accession number. The branching patterns reflect genetic similarity, with closely related strains grouped together. Bootstrap values, such as the 73% value at one of the nodes, indicate the level of statistical support for the groupings. ISOLATE 40A occupies a distinct position within the tree, separate from other well-characterized strains. The inclusion of accession numbers ensures traceability and supports validation of the phylogenetic analysis.

ISOLATE 140EC

Figure 5: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 40A has similar sequence with Escherichia coli strain W2-5 with accession number CP032989*

Figure 6 presents a phylogenetic tree displaying the evolutionary relationships between the novel isolate "ISOLATE 40" and various bacterial strains, including *Escherichia coli* and *Shigella boydii*. Each branch represents a strain identified by name and genome accession number, with bootstrap values (e.g., 69% and 84%) indicating the statistical confidence of the groupings. *Shigella boydii* is included as an outgroup to serve as a reference for establishing evolutionary divergence. ISOLATE 40 clusters more closely with several *E. coli* strains specifically RM9088, RM10410, and CFSAN061772 than with *Shigella boydii*. While showing relatedness to these *E. coli* strains, ISOLATE 40 occupies a distinct branch within the tree. The bootstrap values associated with these groupings support the robustness of the phylogenetic analysis.

Figure 7 displays a phylogenetic tree representing the evolutionary relationships among various *Escherichia coli* strains, *Escherichia fergusonii*, a *Shigella* species, and a novel isolate labeled "ISOLATE 34." Each branch corresponds to a strain identified by its name and genome accession number.

Figure 6: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 40 has similar sequence with Shigella boydii strain ATTCC 49812 with accession number CP026836*

Bootstrap values, including 85%, 79%, and 75%, indicate the statistical confidence of specific groupings. *Shigella* sp. is used as an outgroup to determine evolutionary divergence. ISOLATE 34 is positioned within the *Escherichia* genus and shows closer clustering with *E. coli* strain PYK20 and *Shigella* sp. than with other strains. Its placement also aligns with *E. coli* K-12 within the tree. The clustering pattern suggests genetic similarity with selected *E. coli* strains, supported by moderate-to-high bootstrap values.

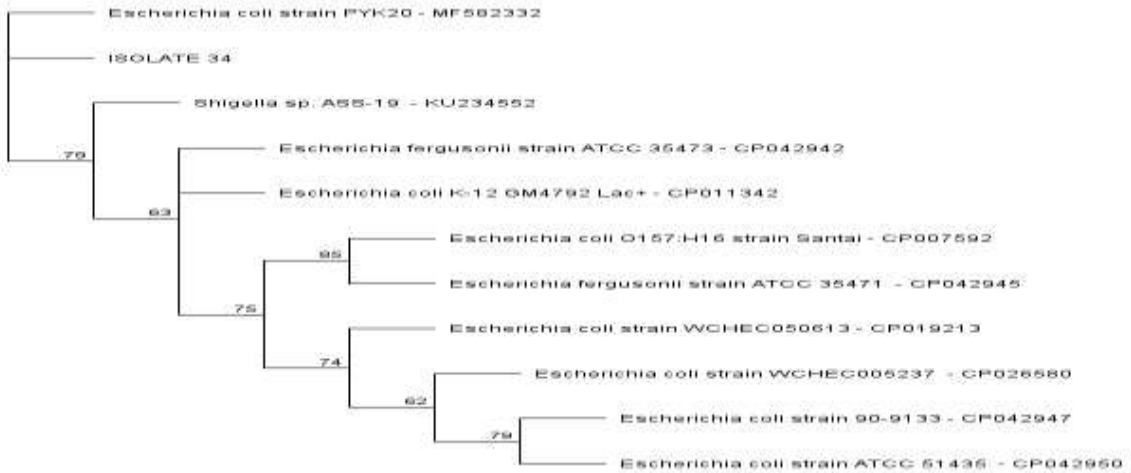

Figure 7: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 34 has similar sequence with Escherichia coli strain PYK20 with accession number MF582332*

Figure 8 presents a phylogenetic tree illustrating the genetic relationships among various *Escherichia coli* strains based on their genomic sequences. Branch lengths represent evolutionary distances, while bootstrap values such as 93%, 99%, and 92% indicate the statistical confidence of the corresponding groupings. Isolate 353 is shown clustering closely with *E. coli* strain PYK20 (MF582332), suggesting a high degree of genetic similarity. The tree topology reflects robust support for several branches, highlighting well-resolved evolutionary relationships among the included strains.

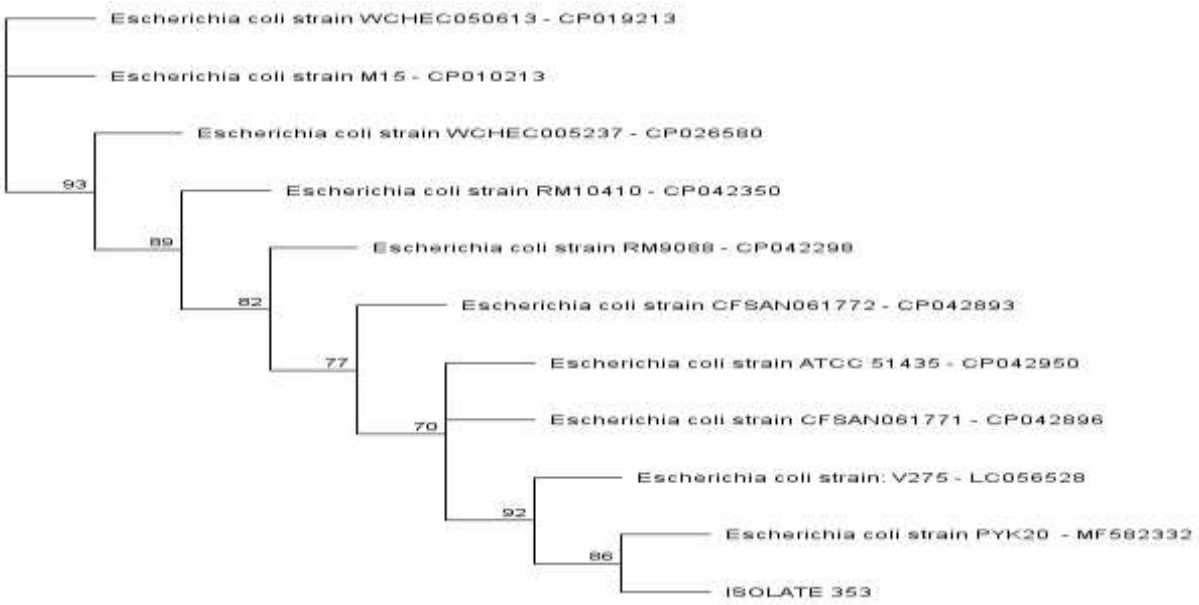

Figure 8: *Phylogenetic analysis of clinical sample based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of Bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate 34 has similar sequence with Escherichia coli strain PYK20 with accession number MF582332.*

Figure 9 presents a phylogenetic tree illustrating the relationships among seven sorbitol non-fermenting *Escherichia coli* isolates based on their 16S rRNA gene sequences. The isolates Isolate 155, Isolate 127, Isolate 160 A, Isolate 353, Isolate 34, Isolate 40, and Isolate 140 EC are arranged according to genetic similarity, with branch lengths representing evolutionary distances. A scale bar at the bottom of the tree indicates a genetic distance of 0.009. Clustering is observed among Isolate 155, Isolate 127, Isolate 160 A, and Isolate 353, while Isolate 140 EC forms a more distant branch, reflecting greater sequence divergence among the analyzed strains.

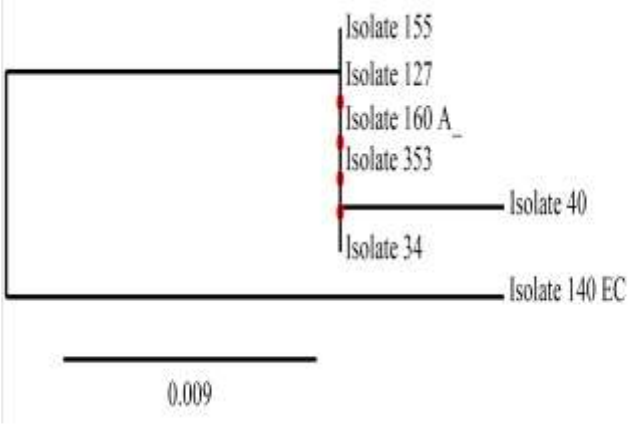

Figure 9: Phylogenetic analysis of the 16S rRNA of the 7 sorbitol non-fermenting *E. coli*

Figure 10a and 10b show the sequence alignment of the 16S rRNA gene from seven isolates of sorbitol non-fermenting *Escherichia coli*. The alignment highlights conserved and variable

regions across the isolates. Conserved regions are indicated by identical nucleotide bases, while mismatches, substitutions, insertions, and gaps reflect sequence variability. The majority of the gene sequence appears conserved across all isolates, while specific regions show nucleotide differences. These sequence variations differentiate the isolates and contribute to the observed genetic diversity within the group. Thus, the 16S rRNA sequence alignment of the 7 sorbitol non-fermenting *E. coli* is shown below.

Figure 11 present the antibiotic susceptibility profiles of *Escherichia coli* O157:H7 isolates obtained from Central Hospital, Benin City. The isolates exhibited complete resistance (100%) to Amoxicillin-Clavulanate, Cefuroxime, Ceftazidime, and Cloxacillin. Complete sensitivity (100%) was observed with Ofloxacin, while 80% of the isolates were sensitive to both Ceftriaxone and Gentamicin. Correspondingly, 20% of the isolates showed resistance to these two antibiotics. The data reflect varying levels of susceptibility across the antibiotics tested.

```
Isolate140EC TAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAaTCGACATCGTTT
Isolate 155  TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTT
Isolate 127_ TAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTT
Isolate 160A TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTT
Isolate 353  TTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTT
Isolate 34   -----GCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTT
Isolate 40   ------------------AGCCACGCCTCAAGGGCACAACCTCCAAGcCcACATCGTTT

Isolate140EC ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 155  ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 127_ ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 160A ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 353  ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 34   ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG
Isolate 40   ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG

Isolate140EC TCAGTCTTtGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAcATCTCTACGCATTT
Isolate 155  TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
Isolate 127_ TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
Isolate 160A TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
Isolate 353  TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
Isolate 34   TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
Isolate 40   TCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT

Isolate140EC CACCGCTACACaTGGAATTCTACCCCCCTCTACaAGACTCtAGCTgGCCAGTATtAGATG
Isolate 155  CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG
Isolate 127_ CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG
Isolate 160A CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG
Isolate 353  CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG
Isolate 34   CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG
Isolate 40   CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATG

Isolate140EC CAaTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAAtAAACCGCCTGCGTGCGCT
Isolate 155  CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT
Isolate 127_ CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT
Isolate 160A CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT
Isolate 353  CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT
Isolate 34   CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT
Isolate 40   CAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCT

Isolate140EC TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 155  TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 127_ TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 160A TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 353  TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 34   TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
Isolate 40   TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG

Isolate140EC GAGTTAGCCGGTGCTTCTTCTGtGGGTAACGTCAATGAGtgAtGGTATTAACTTcACTCC
Isolate 155  GAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCC
Isolate 127_ GAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCC
Isolate 160A GAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCC
Isolate 353  GAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCC
Isolate 34   GAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATTAACTTTACTCC
Isolate 40   GAGT----------------------------------------------------

Isolate140EC CTTCCTCCCCaCTGAAAGTACTTTACAACCCtAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 155  CTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 127_ CTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 160A CTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 353  CTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 34   CTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGC
Isolate 40   ------------------------------------------------------------

Isolate140EC TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
Isolate 155  TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
Isolate 127_ TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
Isolate 160A TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
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Isolate 353   TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
Isolate 34    TGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGG
Isolate 40    ------------------------------------------------------------

Isolate140EC  ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 155   ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 127_  ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 160A  ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 353   ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 34    ACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCT
Isolate 40    ------------------------------------------------------------

Isolate140EC  AGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATaTGGGCtCATCCGATGGCAAaAGG
Isolate 155   AGGTGAGCCtTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGG
Isolate 127_  AGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGG
Isolate 160A  AGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGG
Isolate 353   AGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGG
Isolate 34    AGGTGAGCCGTTACCCCACCTACaAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGG
Isolate 40    ------------------------------------------------------------

Isolate140EC  CCCGAAGGTCCCCCgCTTTGcTCTTGaGACaTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 155   CCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 127_  CCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 160A  CCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 353   CCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 34    CCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTA
Isolate 40    ------------------------------------------------------------

Isolate140EC  GTTATCCCCCTCtATCgGGCAGaTcCCCAtACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 155   GTTATCCCCCTCCATCgGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 127_  GTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 160A  GTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 353   GTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 34    GTTATCCCCCTCCATCAGGCAGTTTCCCAaACATTACTCACCCGTCCGCCACTCGTCAGC
Isolate 40    ------------------------------------------------------------

Isolate140EC  AAAAAAGCAAGCTtCcTCtgTGTTcCCGTTCGAtTTGaATGTGTTAGGCtG---------
Isolate 155   AAAgcAGCAAGCTGCTTtC-TGTTACCGTTCGACTTGCATGTGTTAGGCC----------
Isolate 127_  gAAAAAGCAAGCTGtTTCC-TGTTACCGTTCGACTTGCATGTGTTAGGCC----------
Isolate 160A  AAAAAAGCAAGCTtCTTtC-TGTTACCGTTCGACTTGCATGTGTTAGGCC----------
Isolate 353   AAAAAAGCAAGCTttTTCC-TGTTACCGTTCGACTTGCATGTGTTAGGCC----------
Isolate 34    AAAAAAGCAAGCTGtTTCC-TGTTACCGTTCGACTTGCATGTGTTAGcCtGccgccagcg
Isolate 40    ------------------------------------------------------------

Isolate140EC  ------------------------------------------------------------
Isolate 155   ------------------------------------------------------------
Isolate 127_  ------------------------------------------------------------
Isolate 160A  ------------------------------------------------------------
Isolate 353   ------------------------------------------------------------
Isolate 34    ttmaatctgagcatgaatagccggtgcttcttctgcgggtaacgtcaatgagcaaaggta
Isolate 40    ------------------------------------------------------------

Isolate140EC  ------------------------------------------------------------
Isolate 155   ------------------------------------------------------------
Isolate 127_  ------------------------------------------------------------
Isolate 160A  ------------------------------------------------------------
Isolate 353   ------------------------------------------------------------
Isolate 34    ttaactttactcccttcctccccgctgaaagtactttacaacccgaaggccttcttcata
Isolate 40    ------------------------------------------------------------

Isolate140EC  ------------------------------------------------------------
Isolate 155   ------------------------------------------------------------
Isolate 127_  ------------------------------------------------------------
Isolate 160A  ------------------------------------------------------------
Isolate 353   ------------------------------------------------------------
Isolate 34    cacgcgggatggctgcatcaggcttgcgcccattgtgcaatattccccactgctgcctcc
Isolate 40    ------------------------------------------------------------
```

```
Isolate140EC ------------------------------------------------------------
Isolate 155  ------------------------------------------------------------
Isolate 127_ ------------------------------------------------------------
Isolate 160A ------------------------------------------------------------
Isolate 353  ------------------------------------------------------------
Isolate 34   cgtaggagtctggaccgtgtctcagttccagtgtggctggtcatcctctcagaccagcta
Isolate 40   ------------------------------------------------------------

Isolate140EC ------------------------------------------------------------
Isolate 155  ------------------------------------------------------------
Isolate 127_ ------------------------------------------------------------
Isolate 160A ------------------------------------------------------------
Isolate 353  ------------------------------------------------------------
Isolate 34   gggatcgtcgcctaggtgagccgttaccccacctactagctaatcccatctgggcacatc
Isolate 40   ------------------------------------------------------------

Isolate140EC ------------------------------------------------------------
Isolate 155  ------------------------------------------------------------
Isolate 127_ ------------------------------------------------------------
Isolate 160A ------------------------------------------------------------
Isolate 353  ------------------------------------------------------------
Isolate 34   cgatggcaagaggcccgaaggtccccctctttggtcttgcgacgttatgcggtattagct
Isolate 40   ------------------------------------------------------------

Isolate140EC ------------------------------------------------------------
Isolate 155  ------------------------------------------------------------
Isolate 127_ ------------------------------------------------------------
Isolate 160A ------------------------------------------------------------
Isolate 353  ------------------------------------------------------------
Isolate 34   accgtttccagtagttatccccccctccatcaggcagtttcccagacattactcacccggc
Isolate 40   ------------------------------------------------------------

Isolate140EC ------------------------------------------------------------
Isolate 155  ------------------------------------------------------------
Isolate 127_ ------------------------------------------------------------
Isolate 160A ------------------------------------------------------------
Isolate 353  ------------------------------------------------------------
Isolate 34   cgccactcgtcagaaaagaagcaagcttcttcctgttaccgtcgacttggcatgtgttag
Isolate 40   ------------------------------------------------------------

Isolate140EC --TGCCcCCgAGCGTcaAtATCTaga----------------
Isolate 155  --TGCCGCC-AGCGTTCA-ATCTGAGCCATGAATCAAA----
Isolate 127_ --TGCCGCC-AGCGTTCAAATCTGAGCaATGAATCAAAc---
Isolate 160A --TGCCGCC-AGCGTTCAAATCTGAGCCATGAATCAAAAtc-
Isolate 353  --TGCCGCC-AGCGTTCAAATCTGAGCCATGAATCcAAActt
Isolate 34   gcTGCCGCC-AGCGTTCA-ATCTGAGCCATGA----------
Isolate 40   ------------------------------------------
```

Figure 10a: Nucleotide alignment of the 7 sorbitol non-fermenting *E. coli*

Figure 10b: *Heatmap visualization of the sequence alignment data. Each row represents an isolate, and each column corresponds to a sequence position. Green indicates a match (1), and mismatches would appear in a lighter shade.*

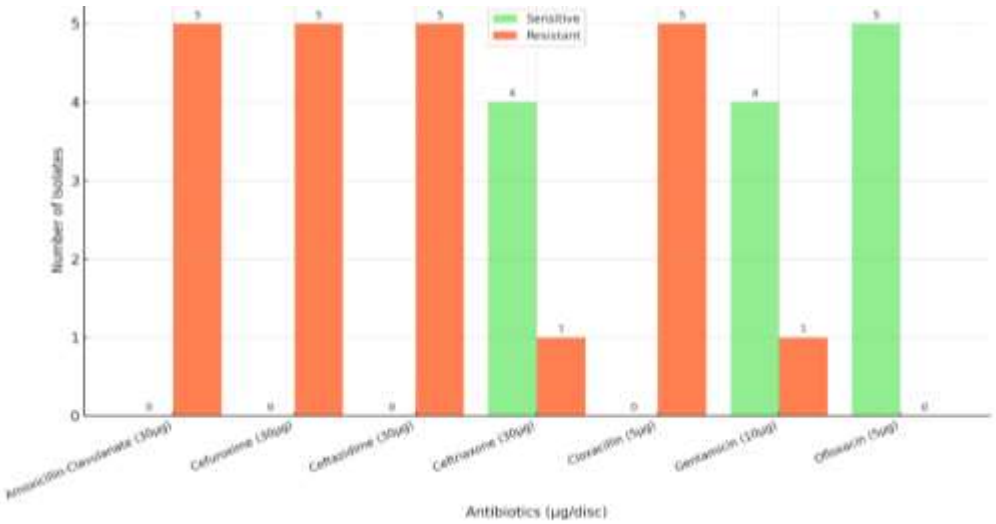

Figure 11: *A bar chart displaying the susceptibility profiles of E. coli O157:H7 isolates from Central Hospital, Benin City. Each bar represents the number of isolates sensitive or resistant to the listed antibiotics. It clearly shows the high resistance to most antibiotics, with only Ofloxacin showing 100% sensitivity, and moderate sensitivity observed with Ceftriaxone and Gentamicin.*

Figure 12 present the haematological profiles of patients infected with *Escherichia coli* O157:H7 compared to those infected with non-O157:H7 strains. Parameters measured included haemoglobin, haematocrit, white blood cell count, neutrophil count, and lymphocyte count. Across all variables, no statistically significant differences were observed between the two groups, with all p-values exceeding 0.05. Although patients with *E. coli* O157:H7 infection exhibited slightly lower mean values for haemoglobin (11.67 g/dL vs. 13.11 g/dL) and neutrophil count (40.71% vs. 50.78%), these differences were not statistically significant.

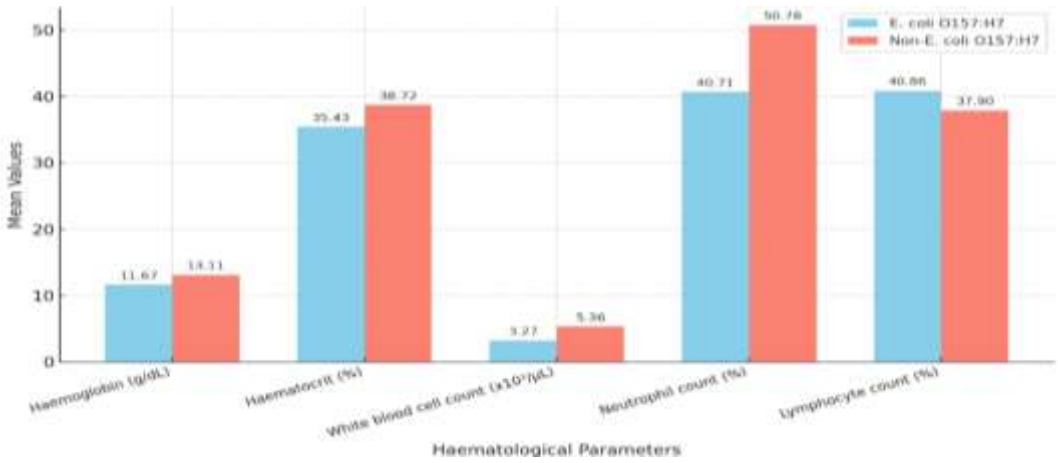

Figure 12: *A bar chart illustrating the haematological parameters of patients infected with E. coli O157:H7 compared to those infected with non-O157:H7 strains. Each bar represents the mean value for a specific parameter, with distinct colors differentiating the two patient groups. This visualization makes it easy to compare the differences between the two groups for each parameter.*

DISCUSSION

Understanding the demographic and genetic patterns of *Escherichia coli* O157:H7 infection is Critical for informing effective public health responses. The prevalence data presented in Figure 1 regarding *E. coli* O157:H7 among different age groups align with findings from previous studies conducted both globally and regionally. Abbott *et al.* (1994) and Abdul-Raouf *et al.* (1996) reported

similar patterns of age-specific variations in *E. coli* O157:H7 infections, with young children and older adults identified as higher-risk groups due to underdeveloped or compromised immune systems. However, the absence of cases among children aged ≤1–10 years in this study contrasts with findings by Ardissino *et al.* (2016) and Byrne *et al.* (2020), who reported significant susceptibility in children under 10 years. The highest prevalence observed among individuals aged 11-20 years (4.35%) may reflect behavioral or environmental exposure factors, consistent with findings by Chigor *et al.* (2010) in Nigeria, where young individuals demonstrated higher risk due to exposure to contaminated water sources (Morufu and Clinton, 2017; Raimi *et al.,* 2022a; Awogbami *et al.,* 2024; Clinton-Ezekwe *et al.,* 2024; Morufu *et al.,* 2021c, d, e; Afolabi and Morufu, 2021; Raimi *et al.,* 2023; Kader *et al.,* 2023; Raimi *et al.,* 2022b; Olalekan *et al.,* 2022; Raimi and Sawyerr, 2022; Abiye and Raimi, 2025a, b; Enang *et al.,* 2025a, b; Morufu *et al.,* 2025; Ndu *et al.,* 2025; Teddy *et al.,* 2025; Christopher *et al.,* 2025b). The statistical insignificance of the observed variations in prevalence rates ($p = 0.2339$) may be attributable to the relatively low sample sizes within specific age categories, as noted by Charan and Biswas (2013) in discussions on statistical power in epidemiological studies. Similarly, Dahiru *et al.* (2008) and Abong'o and Momba (2009) emphasized the importance of larger and more stratified sample sizes to detect statistically meaningful differences in pathogen prevalence. Despite the lack of statistical significance, the observed trends suggest the need for targeted surveillance and prevention strategies, particularly for adolescents and the elderly, in line with recommendations by Buchanan and Doyle (1997).

The phylogenetic analyses (Figures 2-5) provide further insights into the genetic relationships among *E. coli* strains, complementing the epidemiological findings. The high bootstrap values observed in these figures underscore the reliability of the clustering patterns, consistent with the conclusions of Chen *et al.* (2014) and Byrne *et al.* (2020), who highlighted the utility of phylogenetics in understanding evolutionary divergence and potential virulence of *E. coli* O157:H7 strains. For example, the clustering of "ISOLATE 40EC" with *E. coli* strain Ec40743, supported by a 90% bootstrap value, aligns with observations by Omoregie *et al.* (2022), who identified genetic similarities indicative of shared pathogenic traits.

Additionally, the identification of genetic diversity among strains as illustrated by lower-confidence clusters (e.g., 60% in Figure 4) is in agreement with reports by Blitz *et al*. (2018) and Bouzari *et al.* (1994), who emphasized the heterogeneity of *E. coli* populations. The genetic divergence observed suggests a dynamic evolutionary landscape, possibly influenced by environmental or anthropogenic pressures. The clustering of "ISOLATE 127" and "ISOLATE 155" with specific reference strains further supports the likelihood of localized transmission patterns, echoing the findings of Addo *et al.* (2011) in Ghana and Elemuwa *et al.* (2023) in Nigeria, which linked genomic data to regional outbreaks

Similarly, the combined epidemiological and phylogenetic findings underscore the complexity of *E. coli* O157:H7 prevalence and genetic diversity in Benin City. While prevalence rates vary by age group, the lack of statistical significance warrants cautious interpretation and emphasizes the need for larger, multi-center studies. The phylogenetic analyses provide valuable insights into the evolutionary relationships of local isolates, supporting the development of targeted interventions. These findings are consistent with global trends and highlight the importance of integrating genomic and epidemiological approaches in understanding and managing *E. coli* O157:H7 infections. The phylogenetic findings from Figures 6 through 10 offer compelling insights into the genetic relationships and potential functional implications of *E. coli* isolates, correlating strongly with recent literature. For example, the observed genetic clustering of "ISOLATE 40" with specific *E. coli* strains, as indicated by bootstrap values, mirrors findings by Elemuwa *et al.* (2024a) on the genetic diversity of *E. coli* O157:H7 in Nigeria, which emphasized genetic differentiation as a key determinant in pathogenicity and antimicrobial resistance. Similarly, the evolutionary proximity of "ISOLATE 34" to *E. coli* and Shigella spp. aligns with research by Tagbo *et al.* (2014), which described the overlapping genetic and functional characteristics between *E. coli* and Shigella species, particularly in virulence factor expression and environmental adaptability. Moreover, figure 9, shows significant

genetic similarity among some isolates, resonate with Touchon *et al.* (2009), who underscored the importance of genetic clustering in understanding the ecological niches and evolutionary trajectories of bacterial populations. The divergent placement of Isolate 140 EC in this study is consistent with previous reports. Ukwandu *et al.,* (2001) that demonstrated distinct genetic adaptations in certain bacterial strains correlating with environmental pressures or host-specific factors. These studies collectively point to the utility of phylogenetic analyses in unraveling the complex evolutionary dynamics within bacterial populations. The sequence alignment data presented in Figure 10a and b reinforce the role of conserved and variable regions in the 16S rRNA gene as critical markers for phylogenetic and functional studies. Recent work by Elemuwa (2021) highlighted the utility of 16S rRNA sequence variability in distinguishing pathogenic from non-pathogenic strains of *E. coli*. Similarly, Rath *et al.* (2012) demonstrated how mutations in conserved genes like 16S rRNA could influence bacterial resistance mechanisms and metabolic efficiency, findings that align with the observed sequence variations in the current study.

Additionally, the antibiotic susceptibility profiles in Figure 11 draw attention to the alarming resistance patterns among *E. coli* O157:H7 isolates, echoing findings by Onoh *et al.* (2023) and Yusuf *et al.* (2013). These studies reported high resistance rates to beta-lactam antibiotics, attributing this to widespread misuse and the emergence of extended-spectrum beta-lactamases (ESBLs). However, the sensitivity to Ofloxacin as reported here offers a ray of hope, similar to the recommendations by Ojo and Awosanya (2023) advocating for the strategic use of fluoroquinolones in combating resistant bacterial infections. Lastly, the haematological findings in figure 12 reveal comparable responses in patients infected with *E. coli* O157:H7 and non-O157:H7 strains, which concurs with Elemuwa *et al.* (2024a) and Okechukwu *et al.* (2024). Although systemic inflammatory markers may not significantly differ between the groups, the absence of distinct haematological profiles emphasizes the necessity of molecular diagnostic approaches for accurate strain identification and effective infection management. Collectively, these findings reinforce the pivotal role of phylogenetic and functional genomic analyses in informing public health strategies against bacterial pathogens.

SUMMARY

This study comprehensively analyzed the genomic data of *Escherichia coli* O157:H7, a major pathogen responsible for severe foodborne illnesses globally, to enhance public health strategies. Through advanced DNA sequencing and phylogenetic analysis, we identified key genomic variations, including pathogenicity islands, virulence genes, and antimicrobial resistance determinants. The population structure of *E. coli* O157:H7 was elucidated, revealing distinct lineages associated with geographical and temporal factors. Furthermore, comparative genomics highlighted significant genomic plasticity, which may influence the pathogen's adaptability to various environmental niches and hosts. These findings provide a robust foundation for understanding the evolution, transmission, and persistence of this high-risk pathogen.

CONCLUSIONS

The genomic findings from this study highlight the critical need for targeted surveillance and control strategies to manage *E. coli* O157:H7 infections. The identification of lineage-specific virulence factors and antimicrobial resistance genes highlights the urgent need for tailored interventions to mitigate the public health impact of this pathogen. Additionally, the evidence of genomic plasticity underscores the pathogen's potential for rapid adaptation, necessitating proactive measures to monitor emerging strains. The integration of genomic data into epidemiological frameworks will be instrumental in tracing outbreaks, understanding transmission dynamics, and developing effective prevention strategies. These findings emphasize the need for a holistic approach that combines genomics, epidemiology, and public health policy.

Highlights/Summary Points

i. Advanced genomic analysis identified key pathogenicity islands, virulence factors, and antimicrobial resistance genes in *E. coli* O157:H7.
ii. Phylogenetic analysis revealed distinct lineages associated with specific geographical and temporal factors.
iii. Significant genomic plasticity was observed, demonstrating the pathogen's adaptability to diverse environments and hosts.

iv. The study highlights the critical role of integrating genomic data into public health frameworks for improved outbreak management and pathogen surveillance.

RECOMMENDATIONS

i. Strengthen surveillance systems with genome-based diagnostics to monitor *E. coli* O157:H7 evolution and dissemination.
ii. Implement policies to reduce antimicrobial misuse in agriculture, which contributes to resistance.
iii. Promote global data-sharing initiatives to integrate sequencing data from diverse regions for real-time outbreak tracking.
iv. Invest in public health education and interventions tailored to reduce exposure to high-risk contamination sources.

Policy Implications and Future Research

The findings from this study have significant implications for public health policy and future research directions. Policymakers must prioritize genomic surveillance systems to detect and respond to emerging *E. coli* O157:H7 strains promptly. The development of region-specific guidelines based on lineage-specific characteristics will enhance the effectiveness of intervention strategies. Moreover, the incorporation of genomic data into food safety regulations can improve the traceability of contaminated products, thereby reducing the burden of foodborne illnesses. Future research should focus on exploring the environmental and host factors influencing the pathogen's evolution and persistence. Additionally, investigating the interactions between *E. coli* O157:H7 and other microbiota will provide insights into its pathogenic potential and resilience. These efforts will be pivotal in advancing our understanding of *E. coli* O157:H7 and enhancing public health preparedness.

Significance Statement

This study represents a significant step forward in decoding the genomic complexities of *E. coli* O157:H7, a critical pathogen in public health. By integrating DNA sequencing and phylogenetic analysis, we have provided actionable insights into the pathogen's evolution, transmission, and persistence. These findings offer a scientific basis for developing targeted interventions, enhancing outbreak management, and informing policy decisions. The study's emphasis on genomic plasticity and lineage-specific variations highlights the dynamic nature of this pathogen, reinforcing the need for continuous monitoring and adaptive public health strategies. Ultimately, this research contributes to safeguarding public health and reducing the global burden of foodborne illnesses. Thus, graphically it is represented (Figure 13a & b below) as:

Figure 13a: Genomic Analysis of *E.coli 0157:H7*
Source: Author design, 2025.

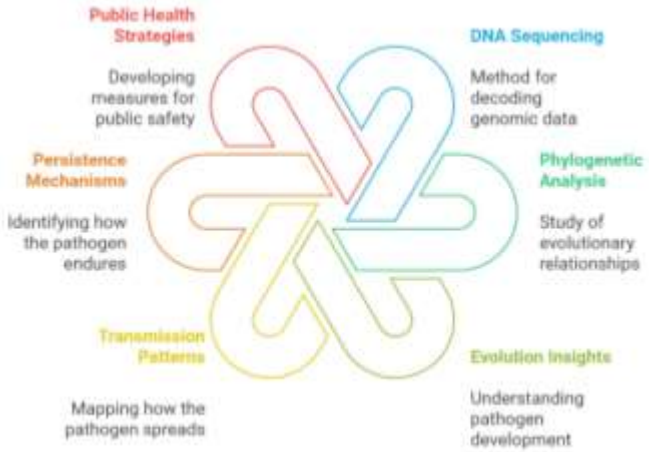

Figure 13b: Decoding *E.coli 0157: H7 for Public Health*
Source: Author design, 2025.

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