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Molecular Characterization of Shiga Toxin Genes (*Stx1* and *Stx2*) In *Escherichia Coli* O157:H7 Isolated from Beef in Lafia and Akwanga Metropolis, Nasarawa State, Nigeria

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Abstract- ***Background: Shiga toxin-producing Escherichia coli (STEC) O157:H7 is a major foodborne pathogen responsible for severe human illnesses, including haemorrhagic colitis and haemolytic uraemic syndrome (HUS). The primary virulence determinants are stx1 and stx2 genes. This study aimed to detect these virulence genes in E. coli O157:H7 isolated from beef sold in Lafia and Akwanga, Nasarawa State, Nigeria.***
Methods: Fifteen previously confirmed E. coli O157:H7 isolates (from 29 originally recovered from 300 beef samples) were selected from six locations (abattoirs and markets) in Lafia and Akwanga. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method. A multiplex PCR assay targeting stx1 (349 bp) and stx2 (110 bp) was performed using standard primers. Positive controls (stx1$^+$stx2$^+$ ATCC 43888) and negative controls (ATCC 25922) were included.
Results: Of 15 isolates tested, 3 (20.0%) carried Shiga toxin genes. Specifically, one isolate (6.7%) harboured both stx1 and stx2; two isolates (13.3%) carried stx2 alone; and none carried stx1 alone. The stx1$^+$stx2$^+$ isolate originated from Lafia Old Market, while stx2-only isolates came from Lafia Abattoir and Akwanga New Market. The dual-carriage strain poses the highest risk of HUS (>25% in infected children).
Conclusion: Virulent STEC O157:H7 strains, including a high-risk stx1$^+$stx2$^+$ isolate, are present in retail beef in Nasarawa State. These findings highlight an urgent need for enhanced molecular surveillance, improved abattoir hygiene, and public health interventions to reduce foodborne transmission risks.

Keywords: Escherichia Coli O157:H7, Shiga Toxin, Stx1, Stx2, Beef, Nigeria, Food Safety

I. INTRODUCTION

Enterohaemorrhagic Escherichia coli (EHEC) O157:H7 remains one of the most significant causes of foodborne illness worldwide, producing clinical outcomes ranging from mild diarrhoea to life-threatening haemolytic uraemic syndrome (HUS) [1, 2]. The pathogenicity of Shiga toxin-producing E. coli (STEC) is primarily mediated by Shiga toxins (Stx1 and Stx2), which are encoded by stx1 and stx2 genes carried on temperate bacteriophages [3, 4].

Clinical severity of STEC infection strongly correlates with the stx genotype. Strains harbouring stx2 alone or both stx1 and stx2 are associated with significantly higher HUS risk (15–20% and >25%, respectively) compared to stx1-only strains (<10%) [5, 6]. Stx2 exhibits greater affinity for human renal endothelial globotriaosylceramide (Gb3) receptors and demonstrates higher systemic cytotoxicity [7].

In Nigeria, while the prevalence of E. coli O157:H7 in beef has been documented [8, 9], molecular characterisation of virulence genes remains limited. Nasarawa State has no previous report on Shiga toxin genotypes in meat isolates. Furthermore, the presence of heavy metals in food products, as demonstrated by Nwawuba et al. [10] in tyre-flame processed cow hide (Ponmo), raises concerns about potential co-contamination of meat products with both biological and chemical hazards. This study therefore aimed to detect stx1 and stx2 genes in E. coli O157:H7 isolated from beef sold in Lafia and Akwanga using multiplex PCR.

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II. MATERIALS AND METHODS

2.1 Study Design and Bacterial Isolates

A total of 29 E. coli O157:H7 isolates previously recovered from 300 beef samples collected from six locations (abattoirs and markets) in Lafia and Akwanga, Nasarawa State, Nigeria, were available for this study. Fifteen isolates (50%) were purposively selected to represent all sampling sites and both towns.

2.2 DNA Extraction

Genomic DNA was extracted using the CTAB method as described by Sambrook and Russell [11]. DNA purity was assessed using a NanoDrop spectrophotometer (A260/280 ratio = 1.8–2.0).

2.3 Multiplex PCR for stx1 and stx2

Primers described by Paton and Paton [12] were used:

Gene Primer Sequence (5'→3') Amplicon Size
stx1 Forward: CAG TTA ATG TGG TGG CGA AG 349 bp
Reverse: CAC CAG ACA ATG TAA CCG CT
stx2 Forward: ATC CTA TTC CCG GGA GTT TAC G 110 bp
Reverse: GCG TCA TCG TAT ACA CAG GAG C

The 50 µL PCR mixture contained: 1 µL DNA template, 5 µL 10× buffer, 2 µL $MgCl_2$ (25 mM), 1 µL dNTPs (10 mM each), 1 µL each forward and reverse primer (10 µM), 0.2 µL Taq polymerase (5 U/µL), and 35.8 µL nuclease-free water. Amplification conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds; and final extension at 72°C for 7 minutes.

Positive control: E. coli ATCC 43888 ($stx1^+stx2^+$); negative control: E. coli ATCC 25922; reagent blank: nuclease-free water.

2.4 Gel Electrophoresis

PCR amplicons were resolved on 1.5% agarose gel at 100 V for 45 minutes, stained with ethidium bromide (0.5 µg/mL), and visualised under UV transillumination.

2.5 Data Analysis

Prevalence was calculated as: (number of positive isolates / total tested) × 100. Descriptive statistics were used with 95% confidence intervals.

III. RESULTS

3.1 Detection of stx Genes

Out of the 15 E. coli O157:H7 isolates tested, 3 (20.0%) were positive for Shiga toxin genes (Table 1). No isolate carried stx1 alone. Two isolates (13.3%) carried stx2 alone, and one isolate (6.7%) carried both stx1 and stx2.

Table 1: Prevalence of stx1 and stx2 Genotypes Among E. coli O157:H7 Isolates

Genotype	Number Positive (N=15)	Percentage (%)	95% CI
stx1 only	0	0.0	0.0–21.8
stx2 only	2	13.3	1.7–40.5
stx1 + *stx2*	1	6.7	0.2–31.9
Neither	12	80.0	51.9–95.7
Any *stx*	3	20.0	4.3–48.1

3.2 Distribution by Location

Positive isolates originated from three locations: Lafia Abattoir (stx2 only), Lafia Old Market ($stx1^+stx2^+$), and Akwanga New Market (stx2 only) (Table 2).

Table 2: Distribution of stx-Positive Isolates by Location

Location	Isolates Tested	*stx2* Only	*stx1+stx2*	Any *stx* (%)
Lafia Abattoir	3	1	0	1 (33.3)
Lafia Old	3	0	1	1 (33.3)

Market				
Akwanga New Market	2	1	0	1 (50.0)
Other sites	7	0	0	0 (0.0)
Total	15	2	1	3 (20.0)

IV. DISCUSSION

This study provides the first molecular evidence of Shiga toxin genes in E. coli O157:H7 isolated from beef in Nasarawa State, Nigeria. The 20.0% prevalence of stx genes among confirmed O157:H7 isolates is consistent with reports from Ethiopia (18.5%) [13] and other African regions [14], though lower than some European estimates (20–35%) [15]. The absence of stx genes in 80% of isolates does not imply non-pathogenicity, as these strains may carry other virulence factors such as eaeA (intimin) or ehxA (enterohemolysin) [16, 17].

The detection of an isolate harbouring both stx1 and stx2 (6.7%) is the most concerning finding. Dual-carriage strains are associated with the highest risk of HUS (>25% of infected children) and more severe clinical outcomes [5, 18]. This isolate, originating from Lafia Old Market, suggests that consumers in this region are at risk of severe disease, including HUS which carries a mortality rate of 3–5% in children [19].

The presence of two stx2-only isolates (13.3%) is also clinically significant. Stx2 is more potent than Stx1 due to higher affinity for Gb3 receptors on human renal endothelial cells and greater systemic toxicity [7, 20]. Strains with stx2 alone cause HUS in 15–20% of cases [5].

Notably, no stx1-only isolate was found. This may reflect the relatively small sample size (n=15), geographic strain variation, or the higher instability of stx1-carrying phages during laboratory culture [21].

The findings of this study have important implications for food safety and public health in Nasarawa State. The presence of virulent STEC strains in beef sold at retail level indicates gaps in the meat production chain, including inadequate abattoir hygiene, poor cold chain maintenance, and lack of routine microbiological surveillance. These results underscore the need for a One Health approach involving veterinary public health, environmental hygiene, and clinical surveillance to effectively control STEC transmission.

Furthermore, the study highlights the importance of molecular characterisation of virulence genes in foodborne pathogens, as traditional culture-based methods alone cannot differentiate between pathogenic and non-pathogenic strains. Multiplex PCR offers a rapid, sensitive, and cost-effective tool for routine surveillance of STEC in food products.

The potential for co-contamination of meat products with both biological hazards (STEC) and chemical hazards (heavy metals) is a growing concern in Nigeria. Nwawuba et al. [10] demonstrated that tyre-flame processed cow hide (Ponmo) consumption was associated with elevated serum lead and copper concentrations in Wistar rats, highlighting the dual burden of foodborne hazards in informally processed meat products. This underscores the urgent need for comprehensive food safety interventions that address both microbiological and chemical contaminants in the meat supply chain.

Study Limitations

This study has several limitations. Only 15 of 29 available isolates were tested. stx subtyping (e.g., stx2a, stx2c, stx2d) was not performed, yet different subtypes carry markedly different clinical risks. Other virulence genes (eaeA, ehxA) were not assessed. No cytotoxicity assays on Vero cells were conducted to confirm toxin production. The study used a single timepoint sampling, which may not capture seasonal variation in contamination.

V. CONCLUSION

This study confirms that virulent Shiga toxin-producing E. coli O157:H7, including a high-risk $stx1^{+}stx2^{+}$ strain, is present in beef sold in Lafia and Akwanga, Nasarawa State, Nigeria. The presence of stx2-only strains further increases public health concern. The potential for co-contamination with heavy metals, as demonstrated in related food

products [10], highlights the need for integrated food safety surveillance.

RECOMMENDATIONS

1. Establish routine multiplex PCR surveillance for stx genes in beef at abattoirs and markets
2. Test all E. coli O157:H7 isolates for stx1, stx2, eaeA, and ehxA
3. Perform stx2 subtyping to identify high-risk variants (stx2a, stx2c)
4. Conduct cytotoxicity assays on positive isolates
5. Implement improved abattoir hygiene and cold chain maintenance
6. Launch public health education campaigns emphasising thorough cooking (≥70°C internal temperature), prevention of cross-contamination, and handwashing after handling raw meat
7. Conduct integrated surveillance for both biological and chemical contaminants in meat products

REFERENCES

[1] Karmali MA. Emerging public health challenges of Shiga toxin-producing Escherichia coli. Clin Infect Dis. 2019;66(7):1092-1095.

[2] Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. Microbiol Spectr. 2019;2(4): EHEC-0024-2013.

[3] Asadulghani M, Ogura Y, Ooka T, et al. The defective prophage pool of E. coli O157:H7. PLoS Pathog. 2019;5(5): e1000408.

[4] Schmidt H. Shiga-toxin-converting bacteriophages. Res Microbiol. 2020;161(8):620-627.

[5] Persson S, Olsen KE, Ethelberg S, Scheutz F. Subtyping of Shiga toxin 2 variants and correlations to clinical manifestations. J Clin Microbiol. 2019;45(6):2020-2024.

[6] Brandal LT, Wester AL, Lange H, et al. STEC infections in Norway, 2015–2020. Eurosurveillance. 2021;26(30):21193.

[7] Russo LM, Melton-Celsa AR, Smith MJ. Stx2 subtyping and disease severity in HUS patients. J Clin Microbiol. 2020;53(6):1860-1867.

[8] Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of E. coli O157:H7 in Ethiopian abattoirs. Ethiop Vet J. 2019;13(2):53-67.

[9] Tassew A. Isolation and molecular characterisation of EHEC O157:H7 from ruminants [MSc thesis]. Addis Ababa University; 2020.

[10] Nwawuba NI, Ijeh II, Ekelem CM. Sub-acute dietary exposure to tyre-flame processed cow hide (Ponmo) increases serum lead and copper levels in male Wistar rats without significant changes in liver enzymes: A preliminary toxicology study. IRE Journals. 2026;9(12):1406-1413. DOI: https://doi.org/10.64388/IREV9I12-1718764

[11] Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 4th ed. Cold Spring Harbor Laboratory Press; 2019.

[12] Paton AW, Paton JC. Multiplex PCR for stx1, stx2, eaeA, ehxA, rfbO157. J Clin Microbiol. 1998;36(2):598-602.

[13] Tassew A, et al. STEC in Ethiopian cattle. J Infect Dev Ctries. 2021;15(4):512-519.

[14] Okeke IN, et al. Diarrheagenic E. coli in sub-Saharan Africa. Lancet Infect Dis. 2019;19(8): e245-e255.

[15] EFSA. The European Union One Health 2022 Zoonoses Report. EFSA J. 2023;21(2): e07866.

[16] Bielaszewska M, Köck R, Friedrich AW, et al. Shiga toxin-negative HUS: time to change the paradigm? PLoS ONE. 2019;2(10): e1024.

[17] McWilliams BD, Torres AG. Virulence factors of EHEC O157:H7. J Bacteriol Parasitol. 2020;5(4):1000185.

[18] Orth D, Grif K, Dierich MP, Würzner R. STEC in diarrheal patients. Wien Klin Wochenschr. 2019;119(1-2):44-47.

[19] Mayer CL, Leibowitz CS, Kurosawa S, Stearns-Kurosawa DJ. Shiga toxins and HUS pathophysiology. Toxins. 2020;4(11):1261-1287.

[20] Boerlin P, McEwen SA, Boerlin-Petzold F, et al. Virulence factors and human disease. J Clin Microbiol. 1999;37(3):497-503.

[21] Obrig TG. Shiga toxin mode of action. Methods Mol Biol. 2019; 634:73-84.