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Characterization and Antibiotic Susceptibility of *E. Coli* O157:h7 in Meat and Fish Sold in Major Ibadan Markets, Nigeria

Oluwafunmilayo Abosede Ayodele[1*], Anotu Mope Deji-Agboola[2], Adedayo Omotayo Faneye[3], Paul Akinniyi Akinduti[4]

[1] Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria
[2] Department of Medical Microbiology, Olabisi Onabanjo University, Sagamu campus, Nigeria
[3] Department of Virology, University of Ibadan, Ibadan, Nigeria
[4] Microbiology Unit, Department of Biological Sciences, Covenant University, Otta, Nigeria
[*]**Corresponding Author**
Dr. Ayodele Oluwafunmilayo Abosede
Department of Medical Microbiology
University College Hospital, Ibadan
Oyo State
Nigeria
Email: ayofunmi77@gmail.com
Phone: +23430706535

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Abstract

E. coli O157:H7 is one of the major causes of foodborne illness and it is of public health importance. Thus, the aim of this study was to isolate and characterize *Escherichia coli* O157:H7 from meat and fish sold in Ibadan markets. A total of 400 samples comprising of raw meat (beef, goat, pig, chicken, turkey) and fish (Sardine, Titus) were purchased from major markets in different parts of Ibadan. The samples were pre-enriched in tryptone soya broth at 37℃ for 24 hours, subcultured onto MacConkey, Blood and Sorbitol MacConkey agar plates. Thereafter, the organisms isolated were screened biochemically using Microbact GNB 12E. Antibiotic susceptibility and Double-disk synergy was performed using disc diffusion, Plate agglutination and PCR tests were used to identify *E. coli* O157:H7. 78 (19.5%) of the isolates obtained were identified as *E. coli*. The incidence of E. coli serotype O157:H7 was 6 (1.5%), 3 (50%) carries eaeA, hly, rfbE and flich7 genes, 3(50%) harboured flich7, rfbE and hly gene. The *E. coli* O157:H7 isolates were from beef 1 (0.8%), chevon - 1 (2.0%), pork 1 (4.0%), Sardine fish 2 (3.3%), Titus fish 1 (1.7%). 4 (66.7%) of the *E. coli* O157:H7 isolated were resistant to two or more antibiotics. The resistance rate was very high in Ampicillin - 66.7%, Cefuroxime - 66.7% and Gentamicin - 50.0%. The isolates were 100% sensitive to Ceftazidime and Ciprofloxacin. The isolation of *E. coli* O157:H7 in raw meat/fish and the existence of antimicrobial resistant isolates highlight the potential threat to public health.

Keywords:*Escherichia coli* O157:H7, Markets, Raw meat, Raw fish, Antibiotics

1. Introduction

Approximately 200 foodborne illnesses are recognized worldwide, but Enterohaemorrhagic *Escherichia coli* (EHEC) is considered among the most important bacterial pathogens to date [1]. *E. coli* O157:H7 is considered to be the archetypal and most infamous strain of the EHEC, because it is most commonly isolated from patients afflicted with haemorrhagic colitis and HUS [2, 3].The infectious dose of *E. coli* O157:H7 is reported to be as few as 1 to 100 CFU/mL [4,5] which is lower than most other enteric pathogens. The low infectious dose exemplifies the potent virulence of *E. coli* O157:H7, and the virulence of this microorganism stems primarily from the activities of three major virulence factors [1]. *E. coli* O157:H7 infections occur worldwide and this have been reported on every continent except Antarctica [6]. Cattle faeces are the most important source of *E. coli* O157:H7, however, the presence of *E. coli* O157:H7 in the faeces of other animal species has been well recognized. Thus, it is distributed globally in the soil, water, vegetation, decaying matter, and the large intestine of most animals and humans [7].

2. Materials and Methods

2.1 Sample collection

A total of 400 samples comprising of raw meat from beef (n = 125), chevon (goat) (n = 50), chicken (n = 40), turkey (n = 40) and pork (n = 25), fish; sardine (n = 60), titus (n = 60), were purchased from major markets in different parts of Ibadan during the rainy and dry seasons. The samples were placed in separate sterile plastic bags to prevent cross contamination. All samples collected were immediately transported to the Microbiology Laboratory of the University College Hospital, Ibadan and processed within 2 hours.

2.2 Isolation and Identification of *E. coli*

Twenty-five (25) grams of each sample were homogenized in 225 ml Tryptone Soya Broth and incubated at 37 ° C for 18 - 24 hours. All the incubated samples were inoculated onto MacConkey agar plates and were incubated at 37 ° C for 18 - 24 hours. Isolates were identified based on colonial appearance of pure colonies on the agar plates and Gram staining reaction. Gram negative, lactose fermenting isolates with colonial appearance resembling *E. coli* that were motile, positive for indole production and negative for oxidase test were subcultured on Sorbitol MacConkey agar and CHROMagarTM MH Orientation. The isolates were further identified using Microbat 12E identification kit.

2.3 Detection of *E. coli* O157:H7 serotype

Twenty-four hour old colonies of isolates confirmed to be *E. coli* were tested with latex dry spot agglutination kit for *E. coli* O157:H7 (*E. coli* O157:H7 latex Test Kit, Oxoid®, Missori, USA.) according to the manufacturer’s instruction. Saline (40µl) was placed on the small rings on both test and control reaction areas of the test card. A portion of the colony was picked with a sterile stick and was carefully emulsified in the saline in the test area and control. The suspension was then spread to cover the reaction area on the card. The O157 test latex was mixed and 1 drop was added to the emulsified colony and O157 control latex was mixed and 1 drop was added to the emulsified colony in the control area. The card was rocked for 30 seconds and observed for agglutination. Agglutination indicates the presence of *E. coli* serogroup O157.

The same procedure was repeated to check for H7 antigens. *Escherichia coli* O157: H7 ATCC 700728 was used as control.

2.4 DNA extraction from bacterial culture

Total DNA was extracted from bacteria isolates using commercially available bacteria DNA extraction kit (Jena Bioscience, Germany) according to the manufacturer’s instruction as described below.

E. coli were subcultured from Tryptone soy broth containing 25% glycerol to MacConkey agar plates and incubated for 24 hours at 37℃. One (1) pure colony was transferred into 1.5ml microtube and 300µl Cell lysis solution was added into the microtube, 1.5 µl of RNase A solution was added and the solution was mixed by inverting. This was incubated at 37℃ for 30 minutes and cooled on ice for 1minute. 100 µl of Protein precipitation solution was added and vortex vigorously for 30 seconds the solution was centrifuge at 15,000g for 5 minute.

The supernatant was transferred to a clean 1.5ml microtube containing 300 µl isopropanol > 99%. The sample was mixed by inverting gently for 1minute and centrifuge at 15,000g for 1minute. The supernatant was discarded and the tube was drained briefly on clean absorbent paper, 500 µl Washing buffer was added and the tube inverted several times to wash the DNA pellet. The solution was centrifuged at 15,000g for 1minute and the ethanol was discarded carefully. It was then air- dry at room temperature for 15 minutes and 100 µl of DNA Hydration Solution was added to the dried DNA pellet. The DNA was hydrated by incubating at 65℃ for 60 minutes and stored at 4℃.

2.5 Multiplex PCR

Five microliters (5µl) of the prepared cDNA of each sample was used in the multiplex reaction using PCR master mix from Jena Biosciences and primers for *E. coli* O157:H7 to make twenty-five microliters (25µl) reaction mix. The PCR master mix contains a premix of PCR buffer, Magnesium chloride, dNTPs, and Taq Polymerase enzyme in optimized concentrations. Nucleotide sequence of the primers is as shown in Table 1. Primers sequenced published by [8,9] obtained from a commercial company Jena Biosciences, Jena Germany were used for the PCR assay. The primers used were targeted at the conserved region of *E. coli* O157:H7 nucleoprotein and matrix gene.

Micro amps tubes' containing the PCR reaction mixes were placed in a thermal cycler (Master cycler gradient Eppendorff, Hamburg, Germany) programmed to run as follows:

There was an activation of the Taq polymerase enzyme at 94℃ for 2 minutes followed by 35 cycles of denaturation of the double stranded DNA at 94℃ for 20 seconds, primer annealing at 60℃ for 60 seconds, and an elongation of 60 sec at 72℃. There was a final extension time 72℃ for five minutes.

Table 1: Primers and primer sequences used in this study for the identification of *E. coli* O157: H7

Primers	Sequences (5′ - 3′)	Target gene	Amplicon size (bp)	Reference
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	$fliC_{h7}$	625	[8]
FLICH7-R	CAACGGTGACTTTATCGCCATTCC			
rfbE-F	CAGGTGAAGGTGGAATGGTTGTC	rfbE	296	[9]
rfbE-R	TTAGAATTGAGACCATCCAATAAG			
AE22	ATTACCATCCACACAGACGGT	eaeA	397	[8]
AE20-2	ACAGCGTGGTTGGATCAACCT			
MFS1-F	ACGATGTGGTTTATTCTGGA	hly	166	[8]
MFS1-R	CTTCACGTCACCATACATAT			

2.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (OXOID, UK), according to the Clinical and Laboratory Standards Institute [10]. Briefly, biochemically confirmed *E. coli* O157: H7 isolates were grown in Muller Hinton broth for 6 h at 37℃. Turbidity was adjusted to 0.5 McFarland standard and Muller Hinton agar plates were seeded with the cultures. Different antibiotic disks which include Ampicillin, Amoxicillin/Clavulanic acid, Gentamicin, Pefloxacin, Ciprofloxacin, Cefuroxime, Ceftazidime, and Meropenem (OXOID, UK) were placed on the inoculated medium. Antibiotic sensitivity plates were incubated at 37℃ for 24 h. *E. coli* ATCC 25922 was used as quality control strain. The zones of clearing around the disks were measured and compared with Clinical and Laboratory Standards Institute (CLSI) [10], standard and interpreted as sensitive, resistant or intermediate.

3. Results

Out of a total of 78 (19.5%) *E. coli* isolated from different meat and fish samples in this study, 4 (5.1%) were identified as *E. coli* O157:H7 serotype using plate agglutination method and were from meat samples (2), and fish samples (2). The *E. coli* isolated were further subjected to the multiplex PCR to detect four virulence genes in *E. coli* O157:H7. The multiplex PCR detected more *E. coli* O157:H7 6 (7.7%) serotype (Table 1). The *E. coli* O157:H7 isolates were from beef-1 (0.8%), chevon -1 (1.9%), pork-1 (3.9%), Sardine fish-2 (3.0%) and Titus fish-1 (1.6%). None was isolated from chicken and turkey.

Out of the six (6) *E. coli* O157:H7 identified by multiplex PCR assay 3 (50%) carries eaeA, hly, rfbE and flich7 genes, 3(50%) harboured flich7, rfbE and hly gene (Figure 1).

Antibiotic susceptibility profile of the *E. coli* O157:H7 from meat and fish characterized in this study displayed sensitivity to most of the antibiotics used (Figure 2), however, 4 (66.7%) of the isolates were resistant to one or more antibiotics as shown in Table 3. The resistance rate to Ampicillin and Cefuroxime was the highest 66.7% each, followed by Gentamicin 50.0%, Amoxicillin/Clavulanic acid, Pefloxacin and Meropenem 16.7% each. Ciprofloxacin and Ceftazidime had 100% sensitivity (Figure 3). Multidrug resistance was observed in 3 (50.0%) of the *E. coli* O157: H7 isolates (Table 3).

Table 2: Detection of *Escherichia coli* O157:H7 using plate agglutination method and PCR

Sample	No of Samples	Presence of *E. coli* O157: H7	
		Cultural	PCR
Meat Samples	280	3	3
Fish Samples	120	1	3
Total	400	4	6

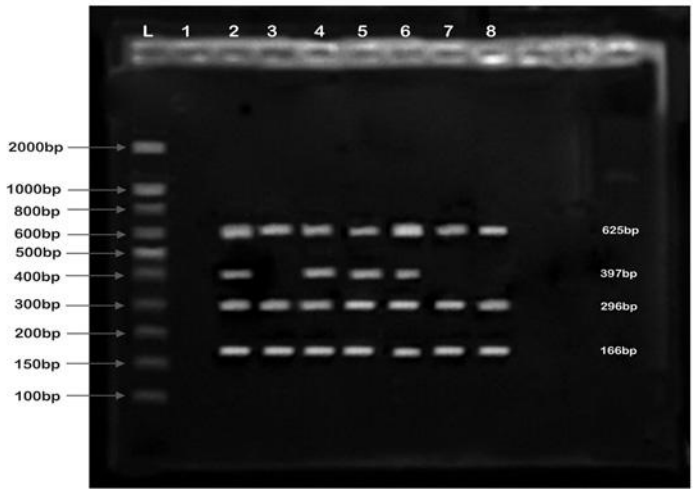

Figure 1: Gel electrophoresis of amplicons showing multiplex PCR to detect *flic, eae. Hly* and *rfbE* genes of *E. coli* O157:H7 in *E. coli* isolates. Lane L: DNA ladder (100 bp), lane 1: negative control, lane 2: positive control and lanes 3-8: amplicons from the isolates

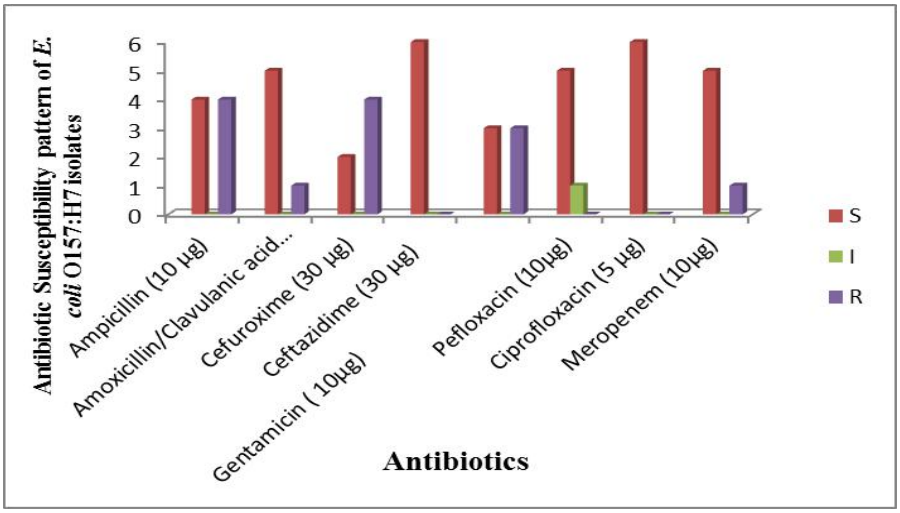

Figure 2: Antibiotic susceptibility pattern of *E. coli* O157: H7 isolates Antibiotics tested (µg/ml) *E. coli* O157: H7 isolates (n = 6)S, sensitive; R, resistant; I, intermediate

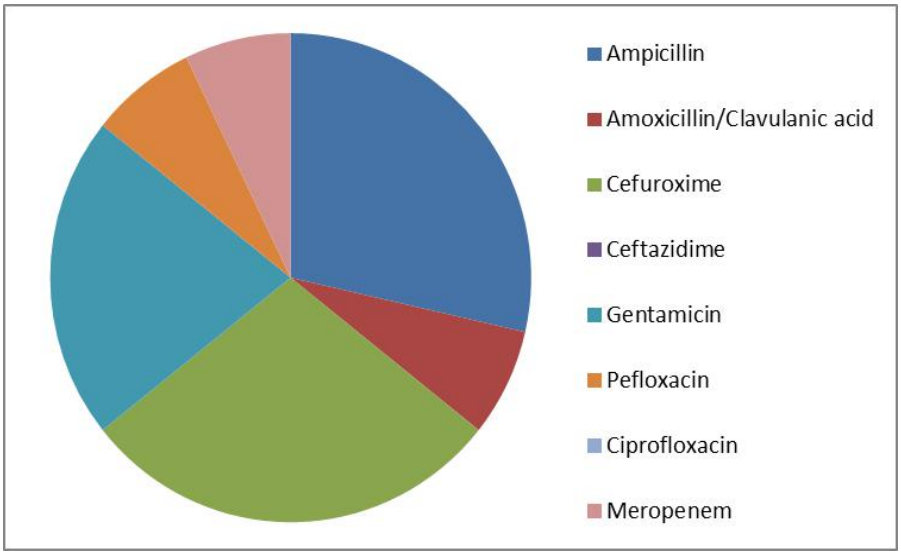

Figure 3: Resistance profile of *E. coli* O157:H7 isolates

Table3: Resistance pattern of *E. coli* O157: H7 showing the resistant phenotype

S/N	No. of Resistant Isolates	Resistant Phenotype
1	1	AMP, CXM
2	1	AMP, CXM, GEN
3	1	AMP, CXM, GEN, PEF
4	1	AMP, AMC, CXM, GEN, MEM

Note: AMP - Ampicillin, AMC - Amoxicillin/Clavulanic acid, CXM – Cefuroxime, GEN – Gentamicin, PEF – Pefloxacin, MEM-Meropenem

4. Discussion

This study isolated 78 (19.5%) *E. coli* isolates from different meat and fish samples. Plate agglutination method identified only 4 (5.1%) isolates as *E. coli* O157:H7 serotype while the multiplex PCR detected 6 (7.7%) *E. coli* O157:H7 serotype. In Nigeria, some studies have reported various prevalence of *E. coli* O157:H7 [11, 12, 13, 14]. Also *E. coli* O157:H7 have been isolated from the intestines of healthy cattle, deer, goats, and sheep [15].

Olatoye, Amosun and Ogundipe [16] reported a higher prevalence of *E. coli* O157: H7 in beef and in chicken from Ibadan. Enabulele, and Uraih, [17] isolated *E. coli* O157:H7 from meat sold at abattoirs and ready-to-eat grilled "Suya" meat sold in Benin City. Different authors reported higher prevalence *E. coli* O157:H7 in different meat and fish from various parts of Nigeria compared with that obtained in this study [18, 19, 20, 21, 22, 23].

Half (50%) of the *E. coli* O157:H7 isolates identified by multiplex PCR assay carries eaeA, hly, rfbE and flich7 genes, and the remaining, 3(50%) harboured flich7, rfbE and hly genes. Hessain, et al. [24] reported that 3 (27.27%) out of 11 *E. coli* O157:H7 isolated from raw beef, chicken and mutton carried eae. Oloyede, Afolabi, and Olalowo [23], did not detect any eaeA and hlyA virulence genes in *E. coli* O157:H7 isolated.

E. coli O157:H7 isolated in this study had a high resistance rate to Ampicillin, Cefuroxime, 66.7% each and Gentamicin 50.0%. Ciprofloxacin and Ceftazidime had 100% sensitivity. 3 (50.0%) of the *E. coli* O157: H7 isolates were resistance to three or more antibiotics. Previous studies such as Aibinu, et al. [18] recorded 100% sensitivity to Meropenem. Reuben, Owuna, (2013) reported that 15 (78.9%) and 17 (89.5%) of their isolates were sensitive to Ciprofloxacin and Gentamicin. Goncuoglu, et al. [26] had 100% sensitivity to Gentamicin and Ciprofloxacin for *E. coli* O157:H7 from cattle and sheep. Opere, et al. [27] also recorded 100% and 58.33% sensitivity to Gentamicin and Augmentin respectively. The studies of Abuelhassan, Fatima and Igwe, [28, 28, 22] reported 100% sensitivity of *E. coli* O157: H7 to Gentamicin, 20% resistant to Ciprofloxacin.

Limitations of the study: Often times, it was difficult obtaining samples from the retailers because their unhygienic attitude could be reported and poor method of sample preservation limit the number of samples to be collected.

5. Conclusion

E. coli O157:H7 is emerging foodborne pathogens whose outbreak can lead to death if not properly monitored. Isolation of *E. coli* O157:H7 in this study indicates the possible risk of food poisoning by *E. coli* O157:H7 among individuals in Ibadan.

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