Molecular Characterization of Salmonella Typhimurium and *E. coli* O157:H7 Isolated from Ready-to-eat Chicken Meat

Oluwatoiyin Hephzibah Ajulo a*, Olusola Victoria Adetunji b and Olugbenga Matthew Ajulo c

a Department of Animal Science, Faculty of Agriculture, University of Uyo, Uyo, AkwaIbom State, Nigeria.
b Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
c Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy University of Uyo, Uyo. AkwaIbom State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors OHA and OVA were involved in the design of the study. Authors OHA and OMA were involved in field works while author OVA supervised. Authors OHA wrote the manuscript and OMA edited and took responsibility for the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2022/v34i33B36132

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84204

Received 20 January 2022
Accepted 24 March 2022
Published 22 April 2022

Original Research Article

ABSTRACT

**Background:** Virulence genes are important in the pathogenesis of bacteria and in the mechanism of bacterial pathogenicity.

**Objective:** The study aimed at molecular detection of virulence genes such as sdiA, fimH, InvA and fliC and determination of similarity and links among the isolates.

**Method:** A total of 67 isolates including 4 controls were tested by Polymerase Chain Reaction (PCR) with 4 primer pairs including invA, fliC, sdiA and fimH to avoid bias. Full sequences of the 16S-Sequencing gene of both strains were carried out with 29 *Salmonella* and 30 *E. coli* isolates that were positive for amplification at 1500bp.ward hierarchical clustering model and agglomeration procedure was used. Clustered grouping and relational affinity test were conducted and depicted by the dendrogram. Molecular identification and interpretation were done using BLASTn Protocol.

*Corresponding author: E-mail: crownp4@yahoo.co.uk;
Result: None of the isolates were positive to the invA or fliC gene fragments. One isolate from each of *Salmonella* Typhimurium and *E. coli* O157:H7 was positive to sdiA and fimH respectively. Three *E. coli* isolates were positive with an amplification of 500bp which is specific for fimH genes. One of the isolates E459 showed amplification of fimH gene with multidrug resistance to 5 drugs namely Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin. Four *Salmonella* isolates had an amplification of 274bp specific for sdiA gene. Two variants of *E. coli* O157:H7 (unit g1 and unit g2) were identified. A mutant strain *Salmonella* Typhimurium LTS (STMD1) causing human gastroenteritis was identified also, *Salmonella* Typhimurium DT104 isolated exhibited multiple resistant genes (ACSSuT, SGI1) against several antibiotics. These are of public health significance. **Conclusion:** This study has indicated presence of sdiA and fimH genes in *Salmonella* typhimurium and *E. coli* O157:H7 respectively isolated from ready-to-eat chicken meats from public eateries. It also indicated association of sdiA and fimH genes with multi-drugs resistance.

**Keywords:** *E. coli* O157:H7; *Salmonella* typhimurium; PCR; multi-drug resistance; eateries; sequencing.

### 1. INTRODUCTION

*E. coli* O157:H7 belongs to a group of *E. coli* termed enterohemorrhagic *E. coli* strains (EHEC). These organisms are referred to as either VTEC or STEC and are of specific interest because it can be virulent, even in relatively healthy individuals [1]. They can cause severe diseases like Haemorrhagiccolitis, Haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura [2]. *Escherichia coli* possess many important virulence factors such as fimbriae, proteins, toxins, siderophores and capsular polysaccharides associated with adhesion, colonization and survival in different human tissues [3]. Virulence genes and pathogenic factors of bacteria are important in the pathogenesis of bacteria [4]. They are important factors in the mechanism of bacterial pathogenicity [5,6].

#### 1.1 Oligonucleotide Primer

FimH gene was important for detection of Type 1 fimbriae and was suggested to be involved in the adherence of some EHEC strains on the basis of inhibition by growth in mannose. Type 1 fimbriae was the first adhesion described in *E. coli* [7]. They are the most common adhesions produced by these bacteria and mediate adherence to mannose-containing glycoprotein found on the surfaces of many eukaryotic cells [8]. The gene 16S rRNA gene with set of primer 341F-907R targets *E. coli* O157:H7. The 16S rRNA gene is an accurate and reliable method for microbial identification. There is 16S rRNA which has both forward and reverse types. There is a lack of information about the role played by sdiA at controlling growth and survival of *E. coli* O157:H7 and *Salmonella* spp. in food sources, thus studies in this area are required. The study aimed at molecular detection of virulence genes such as SdiA, fimH, InvA and fliC and determination of similarity and links among the isolates.

### 2. METHODS

Ready-to-eat chicken meats were purchased from public eateries in Ibadan, Nigeria. Method of preparation and isolation of strains, antibiotic sensitivity Profiles of *Salmonella* Typhimurium and *E. coli* O157: H7 Isolates from ready-to-eat chicken meat and results of data of diarrhoea cases due to food poisoning have been earlier published [9]. PCR was done at International Institute of Tropical Agriculture (IITA). Four primers were used but only three of the primers could be amplified at the expected product size. A 16S rRNA gene specific PCR was conducted on all the *E. coli*0157:H7strains.

#### 2.1 Materials

Minor equipments used were petri dishes, flame, inoculating loops, electrophoresis, plates, casting comb, measuring spoons, foil papers, beakers, conical flasks, stoppers. Stir bars, magnetic stirrer, microwave oven, gel tank and tray (Sunrise 96R horizontal gel electrophoresis system), power pack (Sigma-tech ware PS250-2 power pack), UV trans -illuminator connected to computer, micropipettes (p10).

Major equipments used were IKA vortex genius, master-cycler nexus gradient (Thermal Cycler), Eppendorf flexid, My Gene TM Series, Pelter Thermal Cycler Model MG48+(Long Gene3), Electrophoresis cell (Model DYCP-31DN, Serial no 020-01, Power supply DYY-6C, Centrifuge
Reagents used included TBE (trisomic base, boric acid, EDTA.), loading dye, double distilled water, bacterial DNA isolation kit (Miniprep™), genomic DNA (template), master mix nuclease free water, GR green, 100bp ladder, 50bp ladder, agarose, positive control for E. coli O157:H7 and Salmonella Typhimurium (Spectra medics laboratories).

Bacterial DNA Extraction with some modification to the protocol used by the instruction manual of ZR Fungal/Bacterial DNA Miniprep™ (Catalog No. D6005) was used. The standard and bacteriologically positive strains were grown on nutrient agar at 37°C for about 48 hours. The pure culture plates were flooded with double distilled water with a wire loop. The bacterial colonies were scraped, mixed with the water and transferred to next plate to repeat the procedure. From a 1000µl tip Eppendorf, pipette was used to transfer the bacterial suspension into a labelled Eppendorf tube. The tube was secured in a bead beater and hand shaken vigorously for 5 mins. The ZR Bashing Bead™ Lyses Tube was centrifuged in a micro centrifuge at 10,000xg for 1 min. The flow through from the collection tube was discarded and the above step was repeated. A 200µl of DNA Pre-Wash Buffer was then added to the Zymo-Spin™ IIIC column and centrifuged at 10,000xg for 1 min. A 2.4µl of supernatant was transferred to Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000xg for 1 min. A 1,200µl of Fungal/Bacterial DNA Binding Buffer in which beta-mercuptoethanol had been incorporated was added to the filtrate in the collection tube from the previous step. A 800µl of the mixture from the above step was transferred to a Zymo-Spin™ IIIC column in a collection tube and centrifuged at 10,000xg for 1 min. The agarose was allowed to solidify and the comb was removed by flooding the surface of the gel with some of the 1xTBE and pulling out the comb, then the tray was inserted in the electrophoresis tank. Each well was loaded with a mixture of 2µl loading dye and 3µl genomic DNA. The tank was covered and two wires were fixed appropriately. The DYY-6C power supply was put on and the gel ran till the colour rays of the gDNA reached the edge of the gel then the power supply was stopped. The tray was removed and taken to the UV box connected to a computer, the gel was carefully transferred, appropriately placed in the UV box and the camera was adjusted to get a good shot.

2.2 Quantification of DNA

A spectrophotometer (Nanodrop 2000) connected to a laptop was used. The laptop was put on and connected to nanodrop. A 1µl of elution buffer was put in the pedestal, arm of the pedestal was covered and standardization was done on the laptop to measure the samples. The sample was shaken very well to mix the DNA, then a drop was put in the pedestal and the arm of the pedestal was covered. On the laptop, the concentration and quantity of DNA at 260/280 absorbance was noted. This was repeated for all the DNA samples, then the final work was exported to Microsoft excel, saved and correction was carried out.

2.3 Electropherogram of Genomic DNA (gDNA)

A 0.3g agarose powder was weighed and put in a 100ml conical flask, 30mls of 1x TBE was added and the gel was totally dissolved by microwaving. A 4µl of GR green was added to the agarose and the gel was poured into the appropriate tray with comb inserted into sufficient amount of 1x TBE buffer which was poured into the electrophoresis tank. The agarose was allowed to solidify and the comb was removed by flooding the surface of the gel with some of the 1xTBE and pulling out the comb, then the tray was inserted in the electrophoresis tank. Each well was loaded with a mixture of 2µl loading dye and 3µl genomic DNA. The tank was covered and two wires were fixed appropriately. The DYY-6C power supply was put on and the gel ran till the colour rays of the gDNA reached the edge of the gel then the power supply was stopped. The tray was removed and taken to the UV box connected to a computer, the gel was carefully transferred, appropriately placed in the UV box and the camera was adjusted to get a good shot.

2.4 Standardization of the PCR

The PCR protocol for each primer was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay. The annealing temperature and cycling condition were modified around the temperature given by the reference material. The concentration of the primer and the volume of each of the reaction mixture such as (DNA template, primer, Master Mix and nuclease free water) was adjusted. All the primers were subjected to this modification so as to get the
right condition that the amplicon produced at the sharpest band after being analysed by agarose gel electrophoresis. *Salmonella* isolates were 25 in number represented as A-Y plus 2 extra serving as control while *E. coli* isolates were 38 and represented as 1-38 plus 2 extra representing the control.

2.4.1 Cycling Conditions for Amplification with (Gene ampr PCR System 9700)

1. 94°C for 3mins
2. 94°C for 15secs
3. 65°C for 30secs
4. 72°C for 30secs
5. Go to step 2, 9 times
6. 94°C for 15secs
7. 55°C for 30secs
8. 72°C for 30secs
9. Go to step 6, 26 times
10. 72°C for 5mins
11. 10°C for forever

2.4.2 Electrophoresis Procedure

1) The gel tanks and tray were washed.
2) Stoppers were inserted to the tray, followed by the comb.
3) The tray was balanced on the gel casting system

2.4.3 Sample Preparation

1. 3ul of sample was added to 3ul of loading dye.
2. Then it was spun at 1200 rpm for 1min.

2.4.4 Running of Sample

1. The solidified gel was placed into tank.
2. 1L of 1x TBE was added and the combs were removed.
3. The samples were loaded into the well created by the comb.
4. The gel ran at 80volts for 45mins for the DNA or 2hrs for the PCR products.
5. Bands were viewed in UV trans-illuminator (ENDUROT M Gel Documentation System).

### Table 1. Cocktail Mixture for PCR

| Reagents                     | Volume(µl) |
|------------------------------|------------|
| 10× PCR buffer               | 1.0        |
| 25mM MgCl₂                   | 0.8        |
| 5pMol forward primer         | 0.5        |
| 5pMol reverse primer         | 0.5        |
| DMSO                         | 0.8        |
| 2.5Mm dNTPs                  | 0.5        |
| Taq (homemade)               | 0.2        |
| 10ng/µl                      | 3.0        |
| H₂O                          | 2.7        |
| Total                        | 10         |

### Table 2. Cocktail Mixture for 16S –Sequencing Primer

| Reagents                     | Volume(µl) |
|------------------------------|------------|
| 10× PCR buffer               | 1.0        |
| 25mM MgCl₂                   | 1.0        |
| 5pMol forward primer         | 0.5        |
| 5pMol reverse primer         | 0.5        |
| DMSO                         | 1.0        |
| 2.5Mm dNTPs                  | 0.8        |
| Taq 5u/ µl                   | 0.1        |
| 10ng/µl DNA                  | 2.0        |
| H₂O                          | 3.1        |
| Total                        | 10µl       |
| Initial denaturing | Denaturing temp. | Annealing temp. | Extension temp. | No. of circles | Final Extension Hold temp |
|-------------------|-----------------|-----------------|-----------------|----------------|--------------------------|
| 94°C 5min         | 94°C 30sec      | 56°C 30sec      | 72°C 45sec      | 36             | 72°C 7min 10°C∞        |

The amplicon from the reaction above was loaded on 1.5% agarose gel and the gel picture was attached as PCR. The ladder used is 1kbplus ladder from Invitrogen. The expected base pair of the amplicon was around 1500bp.

### 2.4.5 PCR Product Purification

1. 2vol (20ul) of absolute ethanol was added to the PCR product and Incubated at room temperature for 15minutes.
2. There was an initial spinning down at 10000rpm for 15minutes and the supernatant was decanted.
3. After spinning at10000rpm for 15minutes, 2vol (40ul) of 70% ethanol was added.
4. Supernatant was decanted and air dried.
5. 10ul of ultrapure water was added and amplicon were viewed on 1.5% agarose gel.

The PCR product was used for another PCR reaction that is now sequencing reaction.

### 2.5 Sequencing of 16s-Sequencing Primer

The BigDye® Terminator v1.1/3.1 Sequencing Buffer (5X)* was supplied at a 5X concentration. When in used for sequencing reactions, the final reaction volume was at a concentration of 1X. For a half reaction in 20 µL final volumes, 4 µL of ready reaction premix and 2 µL of BigDye sequencing buffer were used. The use of this buffer without optimization would result in deterioration of sequence quality. Applied Biosystems would not support diluted reactions or guarantee the performance of Big Dye® chemistry if diluted. *The Big Dye Terminator v1.1/3.1 Sequencing Buffer was intended for use only with Big Dye Terminator v1.1/3.1 Cycle Sequencing Kits. Full sequences of the 16S-Sequencing gene of both strains were carried out at 1500bp amplification.

### Table 4. Sequencing primer

| Reagent                        | Concentration | Volume |
|--------------------------------|---------------|--------|
| Ready Reaction Premix          | 2.5X          | 4 µL   |
| Big Dye Sequencing Buffer      | 5X            | 2 µL   |
| Primer                         | —             | 3.2 pmol |
| Template                       | —             | 10-40ng |
| Water                          | —             | 20 µL |
| Final vol                      | 1 X           | 20 µl  |

### Table 5. The amount of templates require in a cycle sequencing reaction

| Template                   | Quantity |
|----------------------------|----------|
| 100–200 bp                 | 1–3 ng   |
| 200–500 bp                 | 3–10 ng  |
| 500–1000 bp                | 5–20 ng  |
| 1000–2000 bp               | 10–40 ng |
| >2000 bp                   | 20–50 ng |
| Single-stranded            | 25–50 ng |
| Double-stranded            | 150–300 ng|
| Cosmid, BAC                | 0.5–1.0 µg|
| Bacterial genomic DNA      | 2–3 µg   |
Cycle sequencing on the system 9700, 9600, 2700, or 2400:

1. The tubes were placed in a thermal cycler and set to the correct volume.
2. An initial denaturation was performed;
   a. Rapid thermal ramp to 96 °C for 1 min
3. The following was repeated for 25 cycles:
   • Rapid thermal ramp* to 96 °C for 10 secs
   • Rapid thermal ramp to 50 °C for 5 secs
   • Rapid thermal ramp to 60 °C for 4 min
   *Rapid thermal ramp is 1 °C/second.
4. Rapid thermal ramp to 4 °C and was put on hold until it was ready to purify.
5. The contents were spun down in a microcentrifuge and purifying extension products.

To precipitate 20-µL sequencing reactions in 96-well reaction plates

1. The 96-well reaction plate was removed from the thermal cycler and briefly spun
2. 5 µL of 125mM EDTA was added to each well and ensured that it reaches the bottom of the wells.
3. 60 µL of 100% ethanol was added to each well.
4. The plate was sealed with aluminium tape and mixed by inverting 4 times.
5. It was then incubated at room temperature for 15 mins and moved to the next step immediately.
6. Beckman Allegra 6A centrifuge with a GH-3.8A rotor was set at 4°C and the plate was spun at 1650g for 45 mins.
7. The plate was inverted and spun to 185g, then removed from the centrifuge.
8. 60µL of 70% ethanol was added to each well with the centrifuge set at 4 °C and spun at 1650 g for 15 min.
9. The plate was inverted and spun to 185g for 1 min, then removed from the centrifuge.
10. The samples were suspended in injection buffer and covered with aluminium foil and stored at 4 °C.

2.6 Methods for Molecular Similarity and Links among the Isolated Strains

Step 1: The sequences were coded per fragment of each strain of microorganism. First the distance travelled by each gene fragment was measured and compared with the marker from the output molecular graph of the electrophoresis’s migration.

Step 2: The distances of each fragment were recorded and statistically standardized.

Step 3: Thereafter, using the Ward hierarchical clustering model. The similarities and its reverse (differences) of the genetic codons were carried out. This enabled a scaled comparison among the whole group of strains.

Step 4: An agglomeration procedure was then used. Agglomeration means a form of algorithm (process commands written as soft codes in order to align and realign and compare a set of data on a case by case per variable basis).

Step 5: These enabled a squared Euclidean distance to be operated from the Ward's cluster schedule.

Step 6: R language syntax was used to compute the pooled molecular scaling using the control as standard. Hence a clustered grouping and relational affinity test was conducted and depicted by the dendrogram of each microorganisms with respect to the various strains sampled [10-15].

The agglomeration distance that indicated similarity to the control was horizontally represented while the vertical branching represented the clustering of similarity only among the entire sequence launched in the molecular algorithm. The 4 nucleotide bases were coded into numerical variables; reiteration was modeled to cater for all possible combination of sequences in each fragment. Length of fragment was also coded in terms of base pairs per fragment. The measured distance from the primer was also recorded and ranked. The outcome input variables were pooled together and an Agglomeration procedure was launched via Claude’s hierarchical clustering model. Software used for the analysis included R language and SAS.

2.7 Molecular Identification of The Sequenced 16S rRNA of Selected Strains via Nucleotide BLASTn Protocol

BLAST is an abbreviation for Basic Local Alignment Search Tools. The comparison was based on position dependent Algorithm which
involved the pooling of several data bases (based on relevance of query) to compare the isolated strain [16]. This was conducted after the genes were mapped at the conserved regions and were retrieved from the entire genome data base of the probable strains [17]. In this research, the queried bacteria are food related strains of *Salmonella* sp. and *E. coli*. The Nucleotide sequences of conserved regions were then compared using the Programmed Homologous protocol and codes launched in the NCBI Data Bases [18]. The Total Score, Percentage Identity, e-value, and query cover was then obtained as the outcome and interpreted to identify the given strains based on the prokaryotic criteria specified.

### 2.8 Procedure for Molecular Identification

The first stage involved the Data Mining and Gene mapping while the second stage involved the custom comparative Nucleotide (BLASTn) protocol [19]. The following describes the methods by which these were conducted. Data mining was conducted from relevant data bases and GenBank. The genes were mapped, and conserved regions were separated which were used as probable Nucleotide probes. Alignment of these nucleotides were then inputted and optimized parameters such as Query sub-range; sequence specifications, the linking of all the pooled standard data bases were specified. The simulation was executed using the customized procedure via R language which compared the input with the sequences of the pooled conserved regions. The strain identity with the highest total score and smallest E-value and highest percentage identity is the molecular identity of the isolate queried. The results as mentioned above was retrieved, Interpreted, and reported accordingly [20].

### 3. RESULTS

#### 3.1 Molecular typing using PCR

None of the isolates was positive to the invA or fliC gene fragments. Three (10%) *E. coli* 0157:H7 isolates were positive by PCR. One (3.45%) of the isolates, E459, showed an amplification of fimH gene and multidrug resistance to 5 drugs, namely, Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin. The other 2 samples that were previously identified as negative samples with SMT were positive with PCR using the above primer pairs. Four (13.79%) *Salmonella* isolates were positive by PCR with amplification of 274bp fragments specific for sdiA gene. The isolate S6 (3.45%) showed positive to sdiA gene and was also positive with Standard Microbiological Technique (SMT) with multidrug resistance to 4 drugs namely Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin while the remaining 3 (10.34%) *Salmonella* isolates that were positive on PCR were negative with SMT.

From the 29 sequences in samples with alphabet, about 560 hits were obtained while from the 34 sequences in those samples with numbers, 496 hits were obtained using the NCBI blast. The blast hits were aligned to get single alignment. The first samples with alphabetical numbering were put into 5 major groupings based on their similarities and origin. The second sample with numbers had 3 major groupings based on their similarities and origin.

Agarose gel electrophoresis showed positive amplification of 500bp fragments for the fimH gene of *E. coli* 057:H7 in lanes 19, 30 and 31. A total of 38 *E. coli* isolates were subjected to PCR, only 3(7.9%) showed positive amplification to the fimH gene.

Lanes 2, 5, 13 and 15 showed positive amplification of 274bp fragments for the sdiA gene of *Salmonella* species. A total of 25 *Salmonella* isolates were amplified and only 4(16%) isolates showed positive amplification to sdiA gene. Lane M showed PCR markers.

On a maximum rescaled Ward distance of 25, the agglomeration program revealed that E27, E30, E21, E20 and E6 in decreasing order were the closest in molecular similarities to the control sequence used in priming the *Escherichia coli* sequences. Apart from the first group, there were other strains that also showed similarity such as E25, E28, E2, E19, E24, E16 and E7. It is important to note that the following representatives of the *E. coli* strains such as E30, E31 and E19 also had amplification for the fimH gene. The following Strains of *Escherichia coli* such as E8, E10, E11 and E12 had the highest dissimilarity among all the *Escherichia coli* strains sampled. These were based on the coefficient of agglomeration and the Ward linkage distance model. This model is particularly useful in molecular analysis because it inclusively factored in the diversity and proper multivariate characterization of the molecular base alignments.
Fig. 1. Agarose gel electrophoresis showed positive amplification of 500bp fragments for the fimH gene of *E. coli* 057:H7
Fig. 2. Agarose gel electrophoresis showing amplification at 1500bp to 16S-sequencing primer for *E. coli* O157:H7 genome
Fig. 3. Agarose gel electrophoresis showing amplification at 1500bp for 16S-sequencing primer for *Salmonella* specie
Fig. 4. The Case by case clusters of the four base pairs
Fig. 5. The Case by case clusters of the four base pairs
Fig. 6. Dendrogram showing the level of similarity among the isolated *Escherichia coli* strains as sampled in this study.
Fig. 7. Dendrogram showing the similarity of each sample’s strains of Salmonella species
Importantly, the clusters in *Escherichia coli*’s molecular dendrogram showed that the strains were very similar to one another. They were likely to be only four different strains from the 2 by 4 cluster pattern. This was unlike the dendrogram outcome of *Salmonella* spp. where the strains were very diverse and complexly related to the control and one another. The clusters that were represented under the dendrogram were indicators of % similarity in the sequence alignment and fragment analysis. Hence, the similarity of the strains to the probable characteristics of the control sequence.

On a maximum rescaled Ward distance of 25, the agglomeration program revealed that *Salmonella* spp. U and *Salmonella* spp. W were the closest in molecular similarities to the control used in priming the *Salmonella* sequences. These strains were followed by other strains such as A1, B2, and P. *Salmonella* spp. represented by U, W, A1, B2 and P. The number B2 which represented S6 was the only one among these strains that was positive to the sdiA gene.

However, the following strains of *Salmonella* spp., C, D and Y had the dissimilarity among all the *Salmonella* spp. strains sampled. This was based on the coefficient of agglomeration and the Ward linkage distance model. This model is particularly useful in molecular analysis because it caters for diversity and proper multivariate characterization of the molecular base alignments.

**E. coli** 0157:H7 Based on Primer Similarity and Functions of The Genes

(Example of role of genes in antimicrobial resistance and plasmid contents)

### Table 6. E27

| Scientific Name       | Max Score | Total Score | Query Cover | E value | % Identity | Accession Length | Accession     |
|-----------------------|-----------|-------------|-------------|---------|------------|------------------|--------------|
| *Escherichia coli*    | 62.6      | 62.6        | 100%        | 9e-11   | 95.35%     | 1238             | MT215717.1   |
| O157: H7 unitig1      |           |             |             |         |            |                  |              |

166 aa protein and 332 aa protein. Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. The 20 amino acids that are found within proteins convey a vast array of chemical versatility. The precise amino acid content, and the sequence of those amino acids, of a specific protein, is determined by the sequence of the bases in the gene that encodes that protein.

**Query 70**

```
TGGGAACGTGCATTCAAACTGGCGAGGCTTGAGTCTTGTA
```

**Sbjct 209**

```
TGGGAACGTGCATTCAAAACTGGCAGGCTAGAGTCTTGTA
```

### Table 7. E30

| Scientific Name       | Max Score | Total Score | Query Cover | E value | % Identity | Accession Length | Accession     |
|-----------------------|-----------|-------------|-------------|---------|------------|------------------|--------------|
| *Escherichia coli*    | 75.5      | 75.5        | 100%        | 5e-10   | 90.58      | 1460             | CP017446.1   |
| O157:H7 strain 9234   |           |             |             |         |            |                  |              |

Submitted by United State Department of Agriculture [21]. Produces a clinically threatening *E. coli* biofilm which reportedly increases its virulence, hence high level of toxicity [22].

**Query 70**

```
TGGGAACGTGCATTCAAACTGGCGAGGCTTGAGTCTTGTA
```

**Sbjct 152**

```
TGGGAACGTGCATTCAAAACTGGCAGGCTAGTGTTGTA
```
Table 8. 21

| Scientific Name | Max Score | Total Score | Query Cover | E value | Percentage Identity | Accession Length | Accession |
|-----------------|-----------|-------------|-------------|---------|---------------------|------------------|-----------|
| Escherichia coli O157:H7 strain 1175 unitig2 | 84.2      | 84.2        | 100%        | 4e-9    | 97.5%               | 1460             | MKIV01000003.1 |

This strain was among the isolates from which aminoglycoside resistance genes: aadA1, aadA2, aacC2, Kn, aph(3)-IIa, and aac(3)-Iva were purified [23].

Query 60 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTATAGA 100

Sbjct4395312 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTATAGA 4395352

Table 9. E20

| Scientific Name | Max Score | Total Score | Query Cover | E value | Percentage Identity | Accession Length | Accession |
|-----------------|-----------|-------------|-------------|---------|---------------------|------------------|-----------|
| Escherichia coli O157:H7 strain FLT | 77.4      | 77.4        | 100%        | 8e-5    | 99.1%               | 1790             | CP051835.1 |

Has these plasmids plasmid pFLT_1997A-2 which is currently being used by Biotech companies to genetically modify yeast (in production of probiotics among others as a purifiable and efficient molecular vector in gene cloning) [24].

Query 70 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTGTAGA 100

Sbjct4351767 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTGTAGA 4351807

Table 10. E6

| Scientific Name | Max Score | Total Score | Query Cover | E value | Percentage Identity | Accession Length | Accession |
|-----------------|-----------|-------------|-------------|---------|---------------------|------------------|-----------|
| Escherichia coli O157:H7 strain 9234 | 84.6      | 84.5        | 100%        | 9e-11   | 95.35%              | 1467             | CP017446.1 |

Cases of hemorrhagic diarrhea had been previously reported from many food poisoning cases from beef sources. E. coli O157:H7 adheres to the wall of the large intestine by secreting virulence factors directly into host cells which makes the progression of the diarrhea very fast and more threatening [25].

Query 70 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTGTAGA 100

Sbjct4100769 TGGGAACTGCTTCAGAAACTGGCAGGCTTGAGTCTTGTAGA 4100809

*Salmonella* Typhimurium: Based On In That Decreasing Order Were The Closest In Molecular Similarities To The Control Sequence Used As Primers

37
Table 11. U

| Scientific Name | Max Score | Total Score | Query Cover | E value | % Identity | Acc. Len | Accession |
|-----------------|-----------|-------------|-------------|---------|------------|----------|-----------|
|                 | 76.8      | 537         | 100%        | 8e-16   | 100.00%    | 4857450  | NC_003197.2 |

Major cause of human gastroenteritis. The incidence of non-typhoid salmonellosis is increasing worldwide, causing millions of infections and Morbidity annually [26]

Query 70  TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 100

Sbjct 289804 TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 289844

Table 12. W

| Description | Max Score | Total Score | Query Cover | E value | % Identity | Acc. Len | Accession |
|-------------|-----------|-------------|-------------|---------|------------|----------|-----------|
| AF233324. 1 | 76.8      | 76.8        | 100%        | 5e-15   | 100.00%    | 9608     | AF233324.1 |

A similar but variant strain mutant of Salmonella typhimurium LT2. The Salmonella virulence plasmid (pSTV) was purified as reported by Wiesner [27] in STMD1 variant of Salmonella typhimurium LT2

Query 70  TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 100

Sbjct 2801039 TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 2800999

Table 13. A1

| Scientific Name | Max Score | Total Score | Query Cover | E value | % Identity | Acc. Len | Accession |
|-----------------|-----------|-------------|-------------|---------|------------|----------|-----------|
| Salmonella Typhimurium STMF1 | 76.8 | 76.8 | 100% | 5e-15 | 100.00% | 43339 | AF170176.1 |

The plasmid-borne betalactamase cmy-2 was isolated in the genome of Salmonellatyphimurium STMF1. The most abundant integron, IP-1 (dfrA12, orfF and aadA2) which may be a clue to the model of its gene expression as well as mechanism of its infection

Query 70  TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 100

Sbjct 3571390 TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 3571350

Table 14. B2

| Scientific Name | Max Score | Total Score | Query Cover | E value | % Identity | Acc. Len | Accession |
|-----------------|-----------|-------------|-------------|---------|------------|----------|-----------|
| Salmonella Typhimurium 10/02 phagetype DT193 | 76.8 | 76.8 | 100% | 5e-15 | 97.50% | 4324 | EF204550.1 |

Majtánová [28] reported that bacteriophages present in the DT198 strain carry integrons which has embedded aadA1, BLA(PSE) genes which are partly implicated in multi-drug resistance characteristics of the strain

Query 70  TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 100

Sbjct 4100769 TGGGAACGTCAATTGAACACTGGCAGGCTTGAGTCTTTGAGA 4100809
Table 15. P

| Scientific Name | Max Score | Total Score | Query Cover | E value | % Identity | Acc. Len | Accession  |
|-----------------|-----------|-------------|-------------|---------|------------|----------|------------|
| Salmonella enterica subsp. enterica serovar Typhimurium DT104 | 71.3 | 71.3 | 100% | 1e-16 | 97.56% | 1544 | HF937208.1 |

Mather et al., [29] reported that resistance profiles are becoming more common in the Plasmid regulated antimicrobial resistance nature of Salmonella Typhimurium DT104 strain. Multidrug resistance spanning antibiotics like ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Examples of such plasmids are: ACSSuT, SGI1. ((which also aids speedy replication of Ribosomal RNA (rRNA))

Query 70  TGGGAACTGCGATCGAAGCTGGGGCTTGAGTCTTGTAGA 100

Sbjct 4196696 TGGGAACTGCGATCGAAGCTGGGGCTTGAGTCTTGTAGA 4196736

Table 16. The Isolates and Molecular ID

| Isolate code | Molecular ID                     |
|--------------|---------------------------------|
| E27          | Escherichia coli strain O157 H7 unitig 1 |
| E30          | Escherichia coli O157:H7 strain 9234 |
| E21          | Escherichia coli O157:H7 strain 1175 unitig2 |
| E20          | Escherichia coli O157:H7 strain FLT |
| E6           | Escherichia coli O157:H7 strain 9234 |

Salmonella spp. Isolates

| Code | Molecular ID                     |
|------|---------------------------------|
| U    | Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 |
| W    | Salmonella typhimurium STMD1     |
| A1   | Salmonella typhimurium STMF1     |
| B2   | Salmonella typhimurium 10/02 phagetype DT193 |
| P    | Salmonella enterica subsp. enterica serovar Typhimurium DT104 |

4. DISCUSSION

The isolated E. coli O157:H7 from ready-to-eat chicken meats were confirmed by PCR to contain fimH genes and possess multidrug resistance to 5 drugs namely Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoit and Ampicillin. This suggested that fimH gene represented high virulence of E. coli O157:H7 which also indicated their multidrug resistance. Previous study had indicated the isolation of E. coli O157:H7 strains with fimH gene from human body [30]. It also implied that occurrence of multidrug resistance would not only depend on the behaviour of patients towards inappropriate use of antibiotics but also through contaminated meals from ready to eat meals from public eateries. However, this study has a lesser prevalence of E. coli O157:H7 strains with fimH gene as compared to a previous study [31].

The observation of a sample of isolated E. coli O157:H7 and Salmonella typhimurium being confirmed for having sdiA gene with resistance to four antibiotics indicated the intensity of virulence and multi-drug resistance infection that could be contracted via purchased ready to eat chicken meats from public eateries. This poses a major public health concern considering the large number of persons that patronize public eateries. Earlier study indicated that E. coli and Salmonella spp do not synthesize AHLs but possess the AHL receptor, sdiA. A study indicated that sdiA bound to AHLs produced by other bacterial species; thereby allow E. coli and Salmonella spp to control gene transcription. The Salmonella spp sdiA gene controlled the rck gene that influenced Salmonella spp adhesion and invasion of epithelial cells and the resistance of the organism to antibacterial agents. In E. coli, study showed that sdiA control genes associated with acid resistance, virulence,
motility, biofilm formation, and autoinducer-2 transport and processing [31].

The ability of a bacterium to adhere to host tissues by specific fimbriae is important for the initiation of infection [32]. The gene encoding the adhesion of the type 1 fimbriae (fimH) was present in very few isolates of this study. This result is far lower than the result observed by another study [33] which reported an amplification of fimH gene of E. coli of APEC isolates and that of Roussan et al. [34] which identified fimH gene by using PCR. The fimH gene belongs to fimbrial adhesions virulence factors. Fimbriae are proteinaceous filaments or appendages expressed on the bacterial surface that is believed to mediate adherence to host cells [35] and helps the E. coli isolates to colonize the intestinal region [36]. Detection rate of fimH gene was 100% as reported by Tawakol and Younis [4].

The role of SdiA quorum-sensing-mediated signaling in Salmonella typhimurium has been controversial for many years. Although Salmonella harbors sdiA, it is not able to produce an acyl homoserine lactone (AHL) signal [37, 38]. The precise role of sdiA in quorum sensing was elusive for several years until Michael et al., [37] reported that sdiA was not sensing an auto-inducer produced by Salmonella itself but rather AHLs produced by other bacterial species. These authors also reported that sdiA-dependent phenotypes could be observed only in the presence of AHLs [39]. Two variants of E. coli O157:H7 (unit g1 and unit g2 were reported in this study. The Unit g2 showed resistance to aminoglycosides. E. coli O157:H7 strain 9234 isolate from this study has biofilm producing genes which is of clinical importance.

Another mutant strain Salmonella Typhimurium LTS (STMD1) causing human gastroenteritis was reported. Salmonella Typhimurium DT104 exhibits multiple resistant genes (AGSSuT, SG11) against the following Antibiotics: Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracycline.

5. CONCLUSION

This study has indicated presence of sdiA and fimH genes in Salmonella Typhimurium and E. coli O157:H7 respectively isolated from ready to eat chicken meats from public eateries in Ibadan. It also indicated association of sdiA and fimH genes with multi-drugs resistance.

6. RECOMMENDATION

Proper monitoring and enforcement of hygienic practices in public eateries is highly recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was granted by the Ethics Committee of the University College Hospital to access patients’ records for cases of gastroenteritis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davis CP. E. coli Infection, eMedicine Health; 2018.
2. Bindu K, Krishnaiah N, Subhashini N, Amaravathi P, Mani M and Ramya P. PCR analysis of mutton and chicken samples for the presence of Shiga Toxigenic E. coli. Archives of Clinical Microbiology; 2019.
3. Aljanaby AAJ, Affaham QMH. Phenotypic and Molecular Characterization of some Virulence Factors in Multidrug Resistance Escherichia coli Isolated from Different Clinical Infections in Iraq. American Journal of Biochemistry and Molecular Biology. 2017;7:65-78.
4. Tawakol MM, Younis AE. Role of Migratory Birds in Transmission of E. coli Infection to Commercial Poultry. Alexandria Journal of Veterinary Sciences AJVS. 2019;62(2):72-81.
5. Vandekerchove D, Vandemaele F, Adriaensen C, Zaleska M, Hernalsteens P, De Baets L, Butaye P, Van Immerseel F, Wattiau P, Laevens H, Mast J, Goddeiris B, Pasmans F. Virulence-Associated Traits in Avian Escherichia coli: Comparison between Isolates from Colibacillosis-Affected and Clinically Healthy Layer Flocks. Veterinary Microbiology Journal, 2005;108:75-87.
6. Cheng R, Sun C, Xu S and Gao S. Prevalence of LEE and HPI Pathogenicity Islands of Escherichia coli isolates from Weaned Piglets in China. Acta Microbiol. Sinica J. 2006;46:368-372.

7. Sokurenko EV, Courtney HS, Ohman DE, Klemm P, Hasty DL. FimH family of type 1 fimbrial adhesions: Functional heterogeneity due to minor sequence variations among fimH genes. Journal of Bacteriology. 1994;176 (3):748-755.

8. Johnson JR. Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev. 1991;4(1):80-108.

9. Ajulo HO, Adetunji VO, Ajulo MO. Antibiotic sensitivity of Salmonella typhimurium and Escherichia coli 0157:H7 isolates from ready-to-eat chicken meat in Ibadan-Nigeria. Asian Food Science Journal. 2019;10(1):1-17.

10. Kaufman L, Peter J. Rousseeeuw-finding groups in data: An introduction to cluster analysis. Wiley series in Probability; 1989.

11. Legendre P, Fortin MJ. Comparison of the Mantel test and alternative approaches for detecting complex relationships in the spatial analysis of genetic data. Molecular Ecology Resources. 2010;10:831-844.

12. Chipman H, Hastie T, and Tibshirani R. Clustering microarray data. In: Speed T, (ed.), Statistical Analysis of Gene Expression Microarray Data. Boca Raton, FL: Chapman and Hall. 2003:159-199.

13. Chipman H, Robert T. Hybrid hierarchical clustering with applications to microarray data. Biostatistics. 2006;7(3):286–301. DOI:10.1093/biostatistics/kxj007. 2005

14. Eisen M, Spellman P, Brown P and Botstein D. Cluster analysis and display of genomewide expression patterns. Proceedings of the National Academy of Sciences of the United States of America. 1998;95:14863–14868.

15. Frank. Hierarchical clustering: In Introduction to HPC with MPI for data science. In: Undergraduate topic in Computer Science. 195-211. Published by Springer, Berlin, Germany. DOI: 10.1007/978-3-319-21903-5_8. 2016

16. Federhen S. The NCBI taxonomy database Nucleic Acid Resources. 2012;40:D136-D143.

17. Rubinstein, Marcelo; Silva Junqueira de Souza, Flavio; Franchini, Lucia Florencia; Exaptation of transposable elements into novel cis-regulatory elements: is the evidence always strong? Oxford University Press; Molecular Biology and Evolution. 2013;30(6):1239-1251.

18. Wang Y, Zhang X, Zhang H, Lu Y, Huang H, Dong X, Chen J, Dong J, Yang X, Hang H, Jiang T. Coiled-coil networking shapes cell molecular machinery. Mol Biol Cell. 23(19):3911-22.

19. Maglott DR, Marchler-Bauer A, Miller V, Karsch-Mizrachi I, Ostell J, Panchenko A, Phan L, Pruitt KD, Schuler GD, Sequeira E, Sherry ST, Shumway M, Sirotkin K, Slotta D, Alexandre Souvorov A, Starchenko G, Tatusova TA, Wagner L, Wang Y, Wilbur WJ, Yaschenko E, Ye J. Database resources of the National Center for Biotechnology Information. Nucleic Acids Research. 2012;40(D1):D13–D25. Available:https://doi.org/10.1093/nar/gkr1184.

20. Frickmann H, Schwarz GS, Raphael Rakotozandrindrainy, Jürgen May J, Ralf M. Hagen. PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us? Infectious Diseases. 2015;47(7): 491-498.

21. United States Department of Agriculture; 2016.

22. Costa JC, Espesicht Ide F, Pieri FA, Benjamin Ldos A, Moreira MA. Increased production of biofilms by escherichia coli in the presence of enrofloxacin. Vet Microbiol. 2012;160(3-4):488–90. DOI: 10.1016/j.vetssmic.2012.05.036.

23. Carattoli A, Tosini F, Giles WP, Rupp ME, Hinrichs SH, Angulo FJ, Barrett TJ, Fey PD. Characterization of plasmids carrying CMY-2 from expanded-spectrum cephalosporin-resistant Salmonella strains isolated in the United States between 1996 and 1998. Antimicrob. Agents Chemother. 2002;46:1269-1272.

24. Tarr P. Escherichia coli O157:H7: Clinical, Diagnostic, and Epidemiological Aspects of Human Infection. Clinical Infectious Diseases. 1995;20(1):1-8. Available:http://www.jstor.org/stable/4458261

25. Centers for Disease Control and Prevention. Outbreaks of Salmonella Serotype Enteritidis Infection Associated with Consumption of Raw Shell Eggs-United States, 1994-1995. MMWR. 1996;45:737-747.

26. McClelland M, Sanderson KE, Spieth J, Clifton SW, Latrelle P, Courtney L, Porwollik S, Ali J, Dante M, Du F, Hou S, Layman D, Leonard S, Nguyen C, Scott K,
Holmes A, Grewal N, Mulvaney E, Ryan E, Sun H, Florea L, Miller W, Stoneking T, Nhan M, Waterston R, Wilson RK. Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature. 2001; 413(6858):852-6. DOI: 10.1038/35101614.

27. Wiesner M, Zaidi MB, Calva E. et al. Association of virulence plasmid and antibiotic resistance determinants with chromosomal multilocus genotypes in Mexican Salmonella enterica serovar Typhimurium strains. BMC Microbiol. 2009;9:131. Available: https://doi.org/10.1186/1471-2180-9-131

28. Majtanova L, Majtan T, Matan V. Molecular characterization of class 1 integrons in Clinical strains of Salmonella Typhimurium isolated in Slovakia. Polish Journal of Microbiology. 2007; 56(1): 19-23.

29. Mather AE, Reid SW, Maskell DJ, Parkhill J, Fookes MC, Harris SR, Brown DJ, Coia JE, Mulvey MR, Gilmour MW, Petrovska L, de Pinna E, Kuroda M, Akiba M, Izumiya H, Connor TR, Suchard MA, Lemey P, Mellor DJ, Haydon DT, Thomson NR. Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. Science. 2013;341(6153):1514-7. DOI: 10.1126/science.1240578.

30. Holati Z, Zamanzad B, Hashemzadeh M, Molaie R and Gholipour A. The FimH gene in uropathogenic Escherichia coli strains isolated from patients with urinary tract infection. Jundishapur J. Microbiol. 2015;8(2):e17520.

31. Smith JL, Fratamico PM, Yan X. Eavesdropping by bacteria: the role of sdiA in E. coli and Salmonella enteric serovar Typhimurium quorum sensing. Foodborne Pathog Dis. 2011;8(2):169-178.

32. Kaczmarek A, Budzynska A and Gospodarek E. Prevalence of genes encoding virulence factors among Escherichia coli with K1 antigen and non-K1 E. coli strains. Journal of Medical Microbiology. 2012;61:1360–1365.

33. Mbanga J, Nyararai YO. Virulence gene profiles of avian pathogenic Escherichia coli isolated from chickens with colibacillosis in Bulawayo, Zimbabwe: Onderstepoort. Journal of Veterinary Research. 2015;82(1):850-858.

34. Roussan DA, Hana Z, Khawaldeh G, Shaheen I. Differentiation of Avian Pathogenic Escherichia coli strains from Broiler Chickens by Multiplex Polymerase Chain Reaction (PCR) and Random Amplified Polymorphic (RAPD) DNA. Open Journal of Veterinary Medicine. 2014’4: 211-219.

35. Rodriguez-Siek E, Giddings W, Doetkott C, Johnson J, Nolan K. Characterizing the APEC pathotype. Veterinary Research. 2005;36:241-256.

36. Leimbach A, Hacker J, Dobrindt U. E. coli as an allrounder: The thin line between commensalism and pathogenicity. Current Topics in Microbiology and Immunology Journal. 2012;358: 32.

37. Michael B, Smith J, Swift S, Heffron F. SdiA of Salmonella enterica is a LuxR homolog that detects mixed microbial communities. Journal ofBacteriology. 2001;183:5733–5742.

38. Swift S, Lynch MJ, Fish L, Kirke DF, Tomas JM, Stewart GS and Williams P. Quorum sensing-dependent regulation and blockade of exoprotease production in Aeromonas hydrophila. Infection and Immunity. 1999:67:5192– 5199.

39. Sperandio V. SdiA Bridges Chemical Signaling between Salmonella enterica Serovar Typhimurium and Yersinia enterocolitica in Mice. Commentaries. Journal Of Bacteriology. 2010;192(1): 21–22.

© 2022 Ajulo et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.