Molecular characterization of *Salmonella typhimurium* and *E. coli 0157:H7*isolated from ready-to-eat chicken meat

ABSTRCT

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 Blatn Protocol.

Result: None of the isolates was positive to the invA or fliC gene fragments. One isolate from each of *Salmonella typhimurium* and *E. coli 0157: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 we 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* 0157: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.

Keyword: *E. coli* 0157:H7, *Salmonella typhimurium*, PCR, Multi-drug resistance, Eateries, Sequencing

Introduction

E. coli 0157: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 Haemmorhagiccolitis, Haemolytic uraemic syndrome and thrombotic thrombocytopaenic 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].

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* 0157: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* 0157: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.

METHOD

Ready-to-eat chicken meats were purchased from public eateries in Ibadan. Method of preparation and isolation of strains were earlier published [9]. PCR was done at 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.

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 gradiant (Thermal Cycler), Eppendorf flexid, MyGene TM Series, Pelter Thermal Cycler,Model MG48+(LongGene®), Electrophoresis cell (Model DYCP-31DN, Serial no 020-01, Power supply DYY-6C, Centrifuge (TG16-WS), Refrigerated Centrifuge (Heraeus fresco 17 Centrifuge), weighing balance (Mettler Toledo AL204), thermo scientific (NANODROP 2000) connected to laptop, Spectrophotometer, UV Documentation unit (LUIYI) connected to computer, Microwave (RUSSEL Hobbs), Freezer, Low temperature freezer (-36 ˚C). Reagents used included TBE (trisma 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 0157:H7(*Spectra medics laboratories).

Bacterial DNA Extraction witha 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 labeled 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. A 400µl 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-mercaptoethanol 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™ IIC column in a collection tube and centrifuged at 10,000xg for 1min.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 Zmo-Spin™ IIC column and centrifuged at 10,000xg for1 min. A 500µl of Fungal/Bacterial DNA Wash Buffer was added to the Zymo-Spin™ IIC column and centrifuged at 10,000xg for 1min.The Zymo-Spin™ IIC column was transferred to a clean 1.5ml micro centrifuge tube and 100µl of DNA Ellution Buffer was added to the column matrix and centrifuged at 10,000xg for 30secs to elute the DNA. A low temperature micro centrifuge was used to produce ultrapure DNA.

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.

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 1xTBE 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.

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 were 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 analyzed by agarose gel electrophoresis. *E. coli* 0157:H7 isolates were38 and represented as 1-38 along with 4 extra representing the control.

Table 1: Cocktail Mixture for PCR

REAGENTS	**VOLUME(UL)**
10× PCR buffer	1.0
25mM Mgcl2	0.8
5pMol forward primer	0.5
5pMol forward primer	0.5
DMSO	0.8
2.5Mm DNTPs	0.5
Taq (homemade)	0.2
10ng/µl	3.0
H_2O	2.7
Total	10

Cycling Conditions for Amplification with (Geneamp^r PCR System 9700)

1. 94^0C for 3mins
2. 94^0C for 15secs
3. 65^0C for 30secs
4. 72^0C for 30secs
5. Go to step 2, 9 times
6. 94^0C for 15secs
7. 55^0C for 30secs
8. 72^0C for 30secs
9. Go to step 6, 26 times
10. 72^0C for 5mins

11. 10^0C for forever

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

5) 2g of Agarose gel was weighed into a conical flask.

6) 100ml of 1x TBE was added to dissolve the Agarose gel and then melted in microwave oven until the solution became clear.

7) The solution was stirred cooled to room temperature, then 5ul of ethidium bromide was added and stirred.

8) It was then poured into the balanced tray and allowed to solidify.

Sample Preparation

1. 3ul of sample was added to 3ul of loading dye.

2. Then it was spun at 1200 rpm for 1min.

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 (ENDUROTM Gel Documentation System).

Table 2:Cocktail Mixture for 16S –Sequencing Primer

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/ul	0.1
10ng/µl DNA	2.0
H_2O	3.1
Total	10µL

Table 3:PCR Condition

Initial den.	**Den.**	**Ann. Tempt**	**extension**	**No. of circles**	**Final Extension**	**Hold tempt**
94˚c	94˚c	56˚c	72˚c	36	72˚c	10˚c
5min	30sec	30sec	45sec	-	7min	∞

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.

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.

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 BigDye® chemistry if diluted.*The BigDye Terminator v1.1/3.1 Sequencing Buffer was intended for use only with BigDye Terminator v1.1/3.l Cycle Sequencing Kits. Full sequences of the 16S-Sequencing gene of both strains were carried out with, 29 *Salmonella typhimurium* and 30 *E. coli 0157:H7* isolates positive for amplification at 1500bp.

Table 4: Sequencing primer

Reagent	**Concentration**	**Volume**
Ready Reaction Premix	2.5X	4 µL
BigDye Squencing 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	15*0–300 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 micro-centrifuge 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 15mins 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 1650*g* for 45mins.

7. The plate was inverted and spun to 185*g*, 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 185*g* 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.

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' 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 modelled 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.

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

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].

RESULTS

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 SMT with multidrug resistance to 4 drugs namely Cefuroxime, Augumentin, 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.

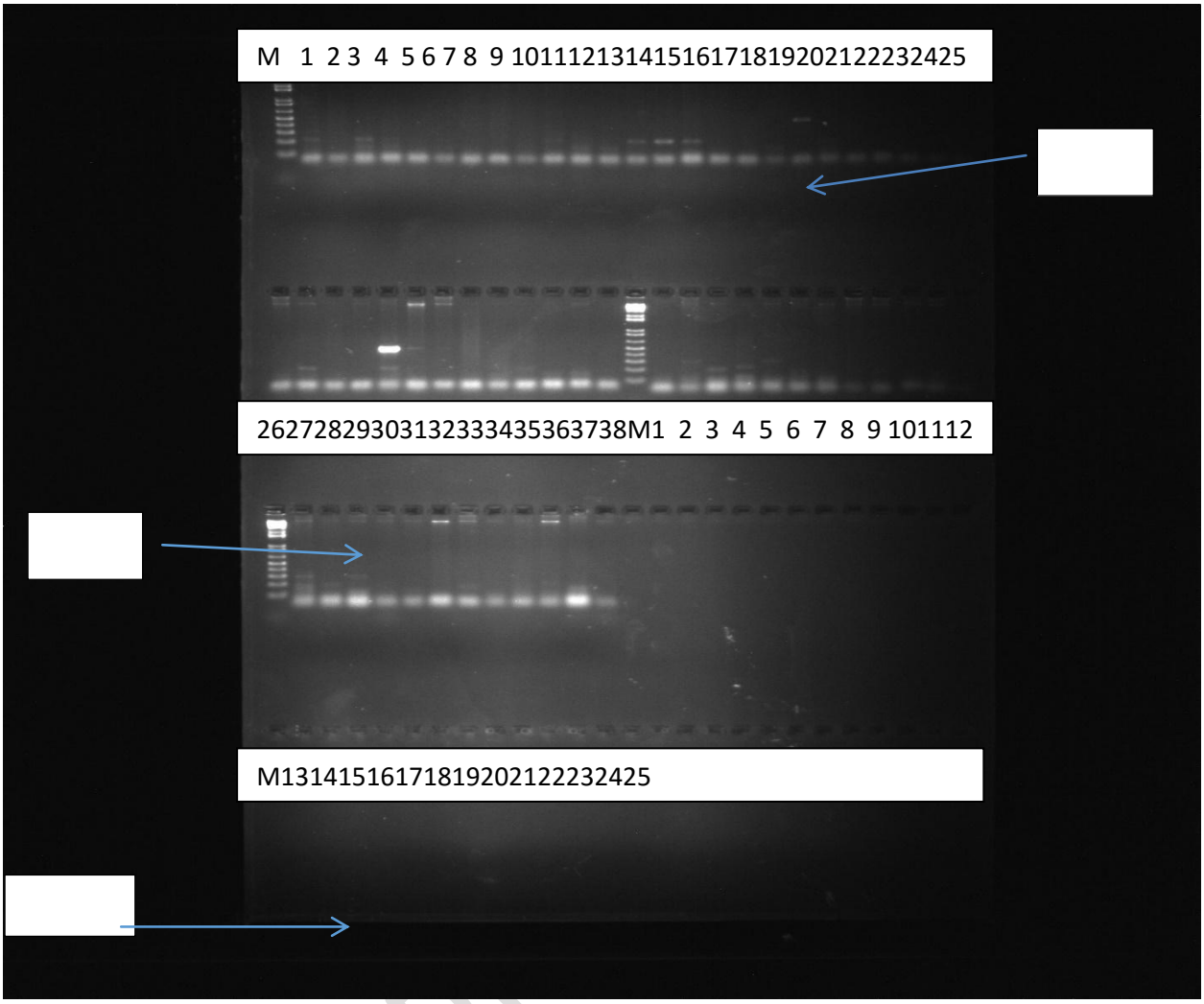

Figure 1: Agarose gel electrophoresisshowedpositive amplification of 500bp fragments for the fimH gene of *E. coli 057:H7*

Agarose gel electrophoresis showedpositive amplification of 500bp fragments for the fimH gene of *E. coli 057:H7* in lanes 19, 30 and 31.E181, E459, E484 were represented by the numbers 19, 30 and 31. Out of a total of 38 *E. coli* isolates, only 3(7.9%) showed positive amplification to the fimH gene.

Lanes 2, 5, 13 and15 showed positive amplification of 274bp fragments for the sdiA gene of *Salmonella* species represented by B= S6, E= S96, M= S148 and O= S196. A total of 25 *Salmonella* isolates were amplified and only 4(16%) isolates showed positive amplification to sdiA gene. Lane M showed PCR markers.

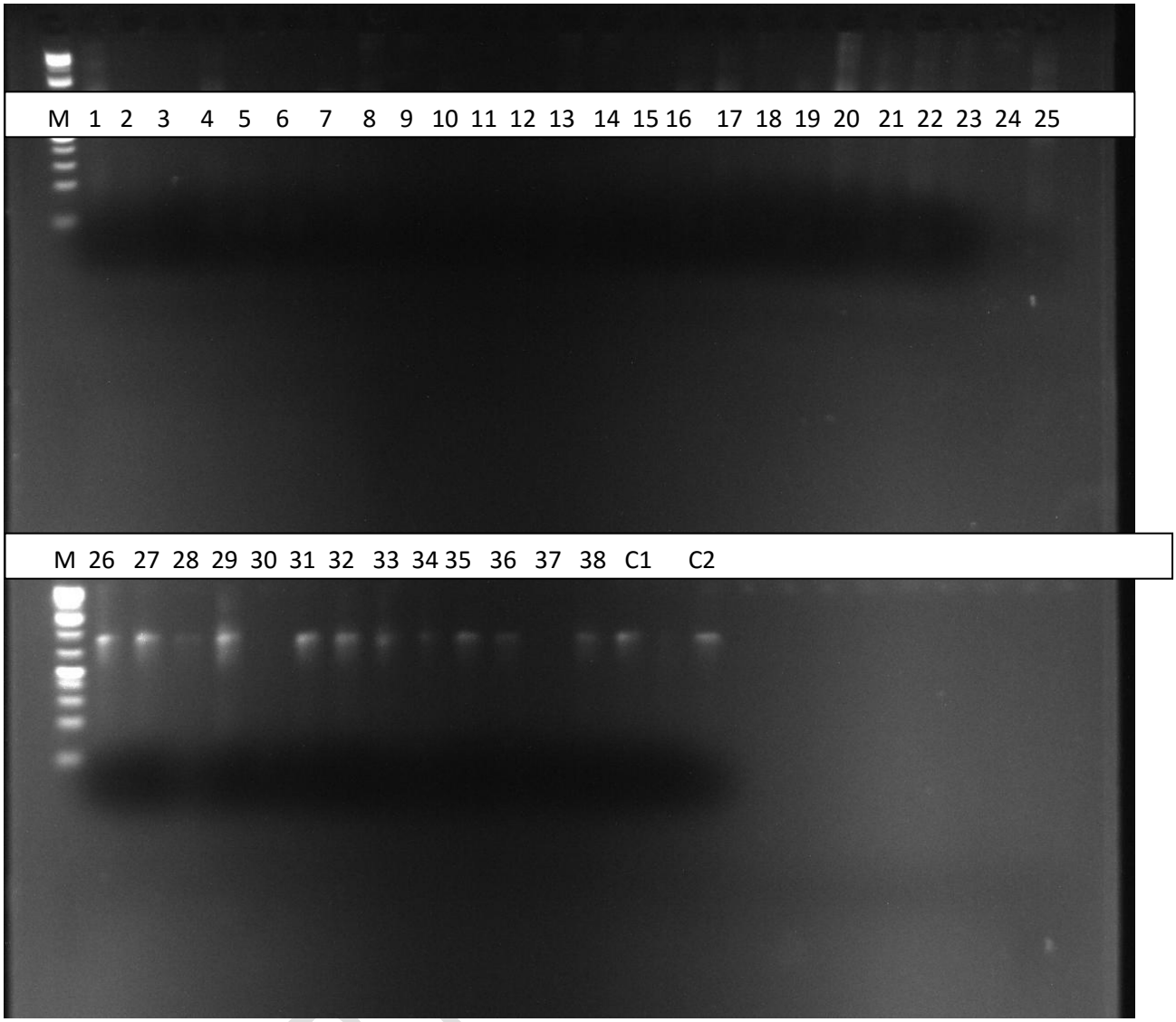

Figure 2: Agarose gel electrophoresis showing amplification of 1500bpfragments of 16S-sequencing primer for *E. coli* 0157:H7 genome

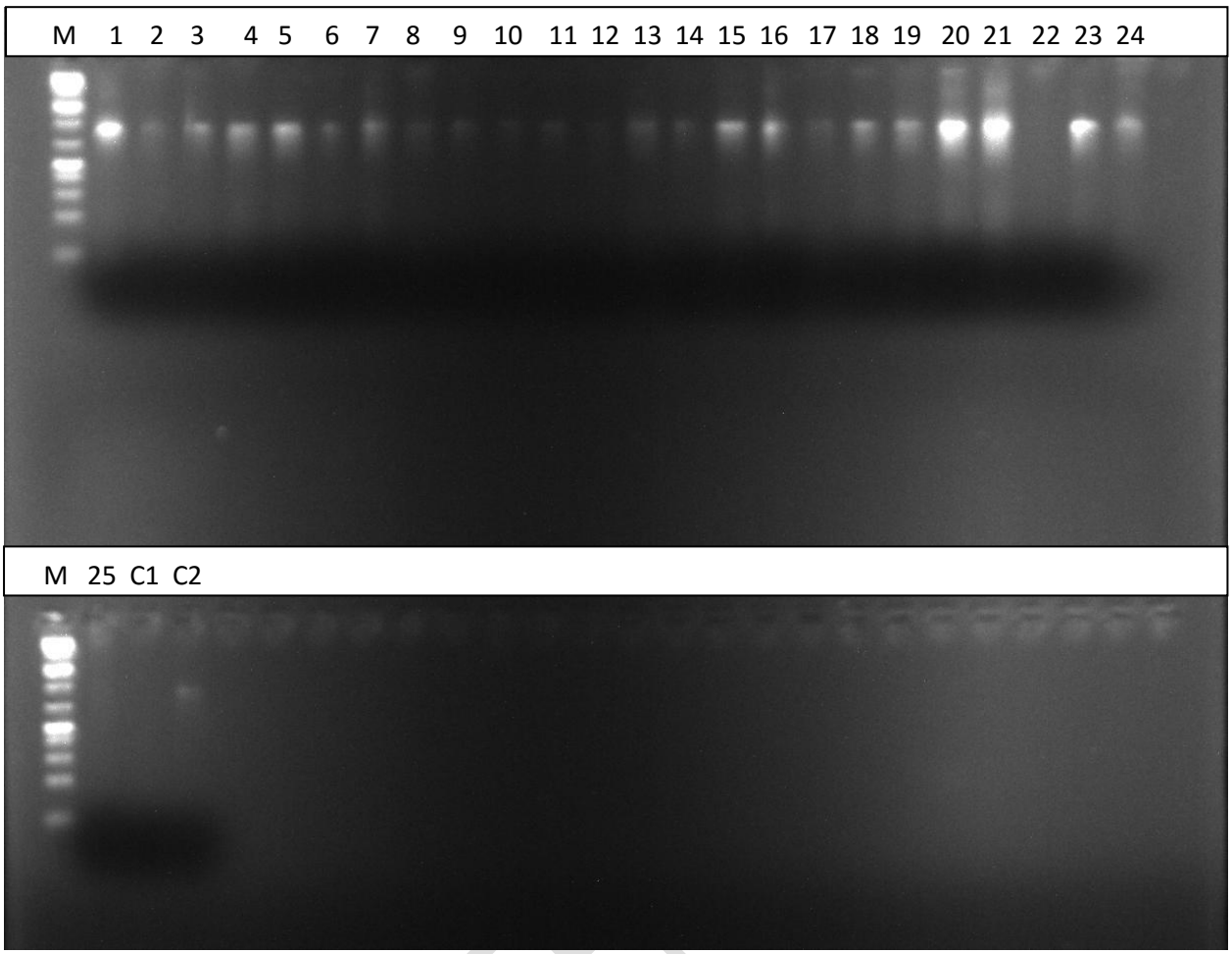

Figure 3: Agarose gel electrophoresis showing amplification of 1500bp fragments of 16S-sequencing primer for *Salmonella* specie

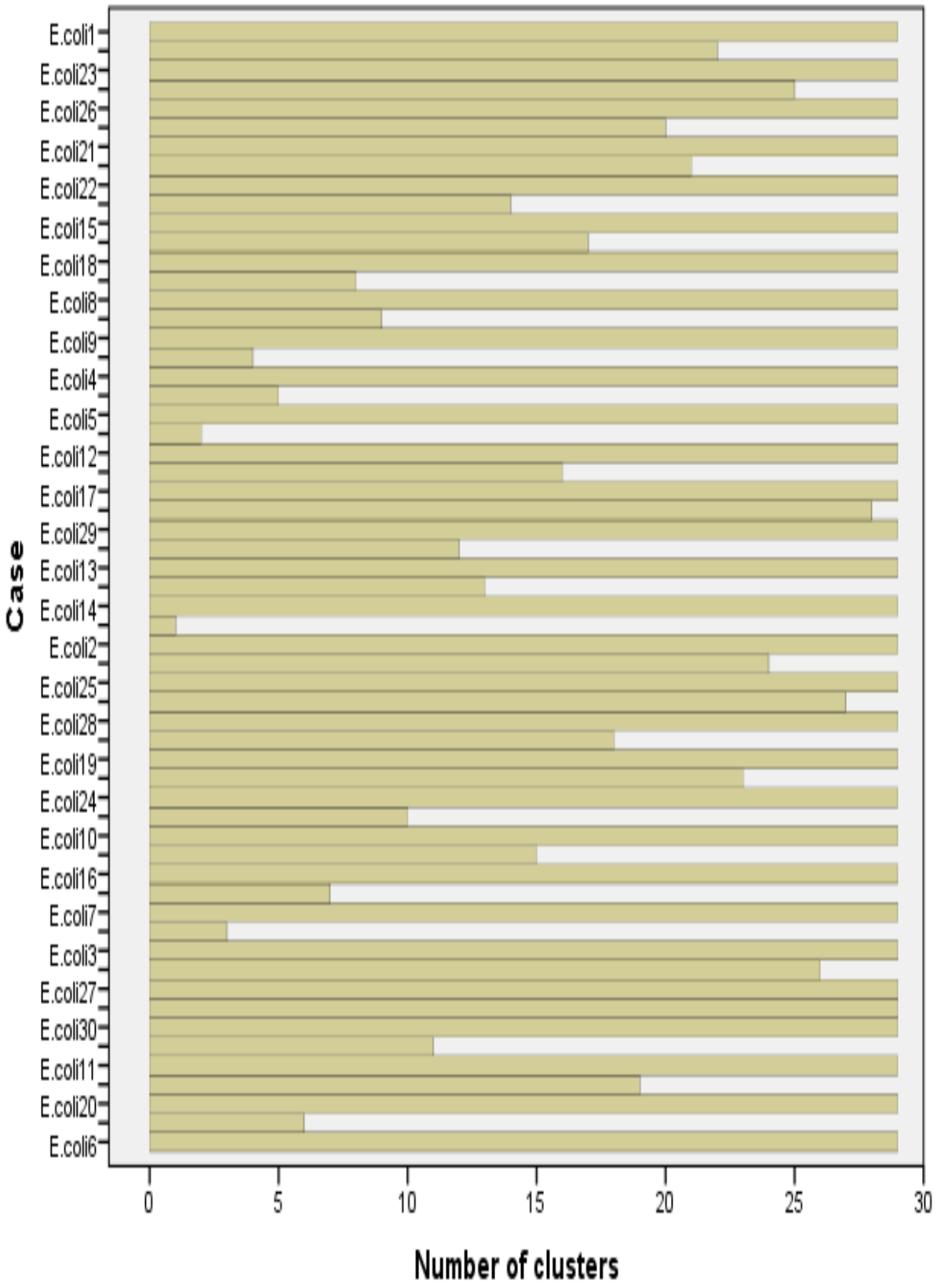

Figure 4: The Case by case clusters of the four base pairs.

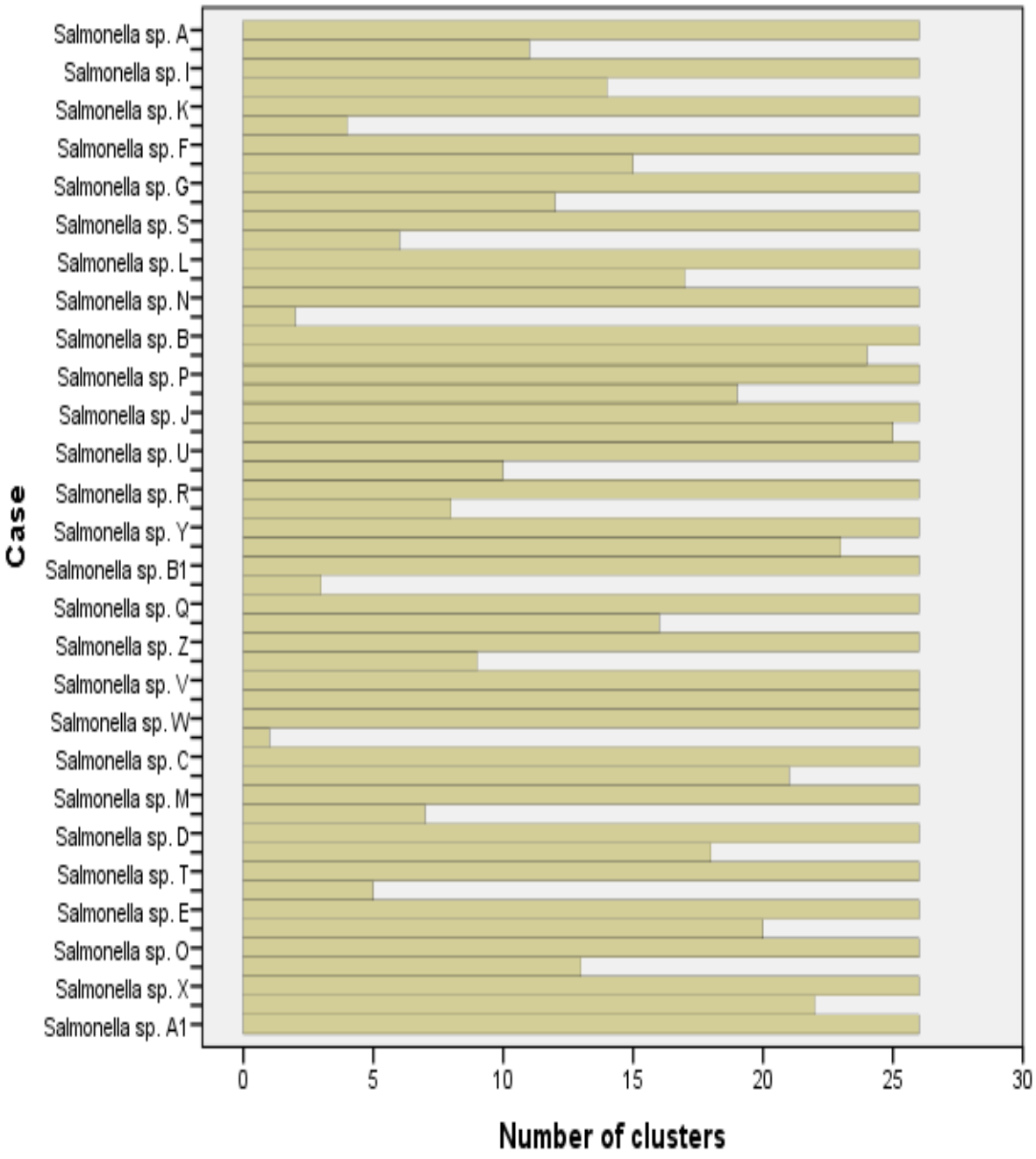

Figure 5: The Case by case clusters of the four base pairs.

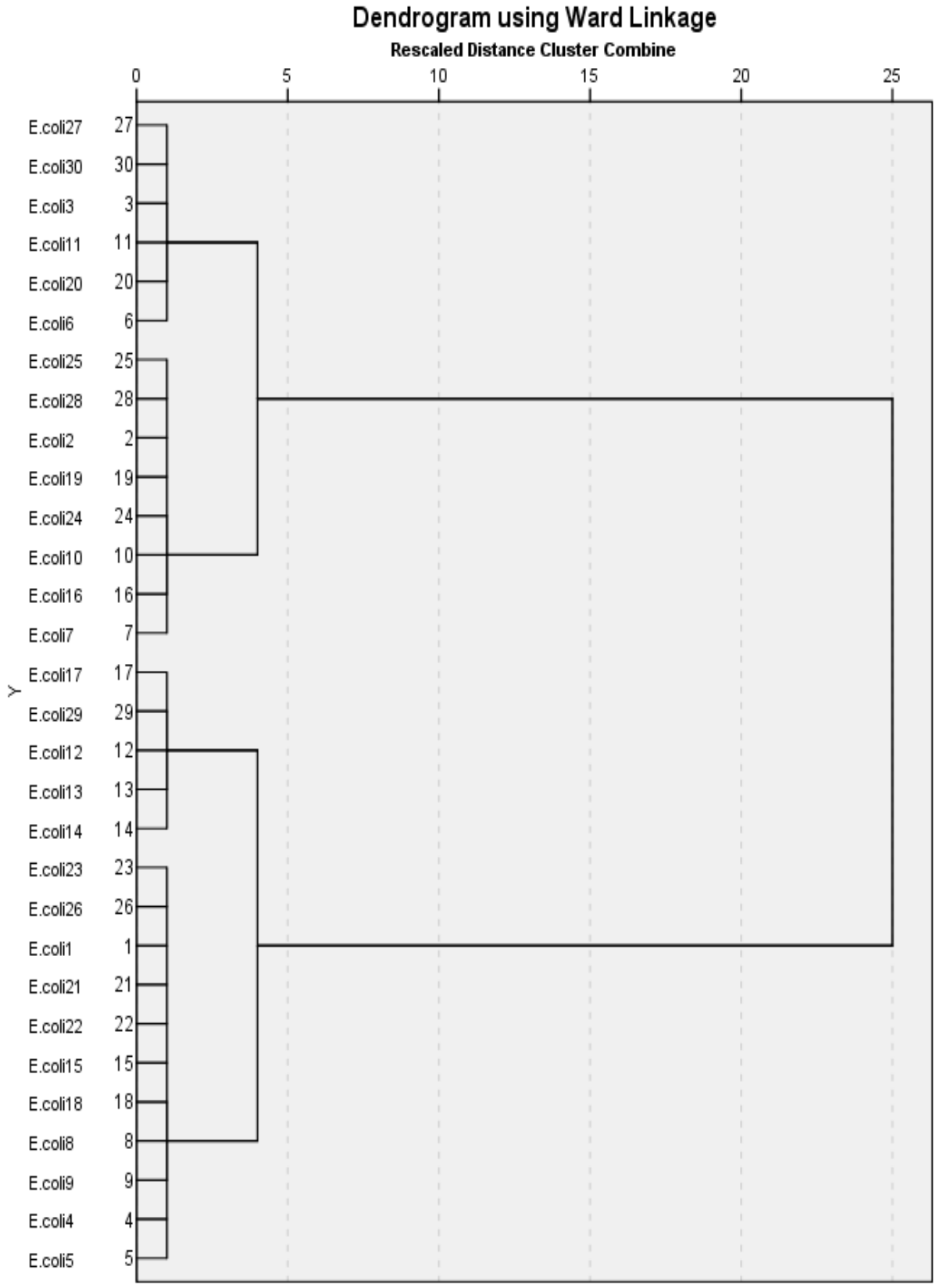

Figure 6: Dendrogram showing the level of similarity among the isolated *Escherichia coli strains as sampled in this study*

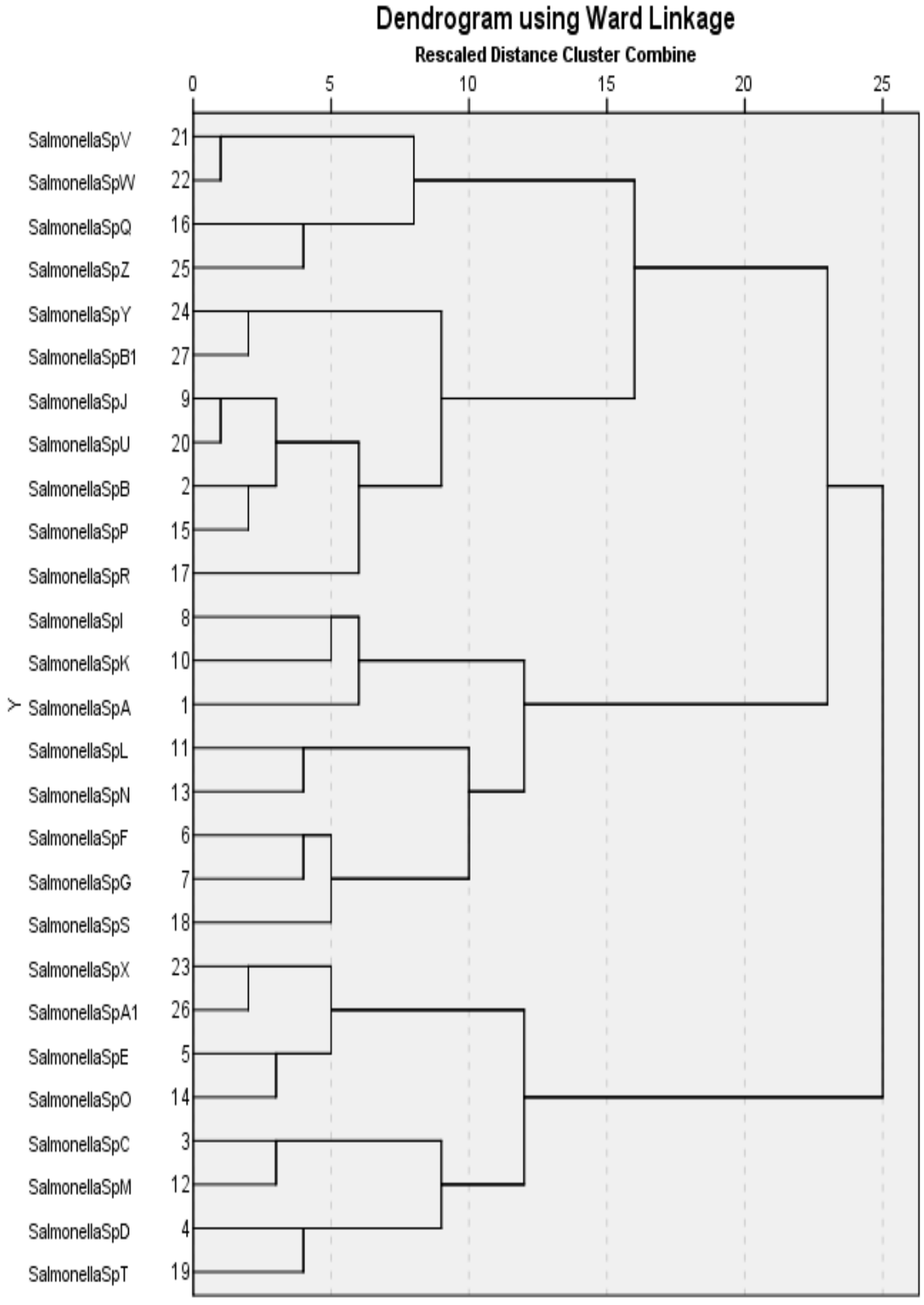

Figure 7: Dendrogram showing the similarity of each sample's strains of *Salmonella* species

INTERPRETATION

On a maximum rescaled Ward distance of 25, the agglomeration program revealed that E27, E30, E31, 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.

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. w*here 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.

INTERPRETATION

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 were also represented by the numbers 21, 23, 1, 2 and 16. The number 2 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 strain O157: H7 **unitig1**	62.6	62.6	100%	9e-11	95.35%	1238	MT215717.1

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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

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Sbjct 209 TGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA 249

Table 7: E30

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

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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

||||||||||||||||||||||||||| ||||||||||

Sbjct 152 TGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA 192

Table 8: E31

Scientific Name	Max Score	Total Score	Query Cover	E value	% 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	Acc. Len	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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

Sbjct 4351767 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 4351807

Table 10: E6

Scientific Name	Max Score	Total Score	Query Cover	E value	% Identity	Acc. Len	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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

Sbjct 4100769 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 4100809

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

Table 11: U

Scientific Name	Max Score	Total Score	Query Cover	E value	% Identity	Acc. Len	Accession
X	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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

||||||||||||||||||||||||||||||||||||||||

Sbjct 289804 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 289844

Table 12: W

	Description	Max Score	Total Score	Query Cover	E value	% Identity	Acc. Len	Accession
AF233324.1	*Salmonella*Typhimurium STMD1	76.8	76.8	100%	5e-15	100.00 %	96086	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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

||||||||||||||||||||||||||||||||||||||||

Sbjct 2801039 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

|||||||||||||||||||||||||||||||||||||||

Sbjct 3571390 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

|||||||||||||||||||||||||||||||||||||||

Sbjct 4100769 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 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 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 100

|||||||||||||||||||||||||||||||||||||||

Sbjct 4196696 TGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGA 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
E31	Escherichia coli O157:H7 strain 1175 unitig2
E20	Escherichia coli O157:H7 strain FLT
E6	Escherichia coli O157:H7 strain 9234
Salmonella spp. Isolates	
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

DISCUSSION

The isolated *E. coli* 0157: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, Nitrofurantoin and Ampicillin. This suggested that fimH gene represented high virulence of*E. coli* 0157:H7 which also indicated their multidrug resistance. Previous study had indicated the isolation of *E. coli*0157: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* 0157:H7 strains with fimH gene as compared to a previous study [31].

The observation of a sample of isolated *E. coli* 0157: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 invery 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 (ACSSuT, SGI1) against the following Antibiotics: Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracycline.

Conclusion

This study has indicated presence of sdiA and fimH genes in *Salmonella typhimurium* and *E. coli* 0157: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.

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

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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