Journal of Food and Nutrition Sciences
2019; 7(2): 25-33
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20190702.11
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Bacteriological Quality and Occurrence of *Escherichia coli* O157:H7 and *Salmonella* Species in Smoked Rat Meat Sold in Zaria, Nigeria

Terseer Iyene Addai*, Emmanuel Chukwudi Okolocha, Beatty Viv Maikai

Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria

Email address:
Terseeraddai@gmail.com (T. I. Addai), Eokolocha@yahoo.com (E. C. Okolocha), Beatt18@gmail.com (B. V. Maikai)

*Corresponding author

To cite this article:
Terseer Iyene Addai, Emmanuel Chukwudi Okolocha, Beatty Viv Maikai. Bacteriological Quality and Occurrence of *Escherichia coli* O157:H7 and *Salmonella* Species in Smoked Rat Meat Sold in Zaria, Nigeria. *Journal of Food and Nutrition Sciences*. Vol. 7, No. 2, 2019, pp. 25-33. doi: 10.11648/j.jfns.20190702.11

Received: March 31, 2019; **Accepted**: March 14, 2019; **Published**: June 10, 2019

Abstract: Rat meat is a good source of protein and it is traded and consumed in different parts of the world. The bacteriological quality with regards to the occurrence of *Salmonella* spp. and *Escherichia coli* (*E. coli*) O157:H7 in smoked rat meat sold in different parts of Zaria, Nigeria was determined in other to ascertain its safety for human consumption. A total of 384 smoked rat meat samples were examined from four purposively selected districts in the study area; Samaru, Basawa, Jushi and Sabon Gari, Zaria, Nigeria. The bacterial load was determined using the Total Aerobic Plate Counts (TAPC) by the spread plate technique. The samples were further screened for *E. coli* O157:H7 and *Salmonella* spp. using the conventional biochemical characterization methods, including standardized micro-substrate (Microgen GN-ID A+B) detection kit for Gram negative bacteria, Rapid latex agglutination test for *E. coli* O157:H7 and PCR for *salmonella* spp. The average TAPC ranged from 12 x 10^9 cfu.g^{-1} (lowest) in Basawa, Zaria, to 15 x 10^9 cfu.g^{-1} in Jushi, Zaria, sampling areas. *Salmonella* was isolated from 2(0.5%) and *E. coli* O157:H7 isolated from 5 (1.3%) of the total 384 samples. Although the prevalence of *E. coli* O157:H7 and *Salmonella* spp. in this study appeared to be low, the high bacteria count and occurrence of these two important foodborne pathogens is an indication of the poor bacteriological quality of smoked rat meat sold in Zaria, Nigeria and a potential source of food infection to consumers.

Keywords: *Salmonella*, *E. Coli* O157:H7, Smoked Rat Meat, Foodborne, Bacteriological Quality

1. Introduction

Meat refers to mostly skeletal muscles and associated fat, but it may also refer to organs, including lungs, livers, skin, brains, bone marrow, kidneys, and a variety of other internal organs as well as blood. Meat is animal tissue used as food and could be consumed fresh, smoked, salted, or sun-dried [1-2]. Rat meat is a regular staple, accepted as a popular source of protein and has been consumed by man in different parts of the world [3-6]. This important protein source is also a good income generator, and is widely traded and consumed in Nigeria [3, 6].

The nutrient composition of meat is diverse; this favors the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. Therefore, to maintain safety and quality of meat, the application of adequate preservation technologies is extremely important [7]. Among the methods used for meat preservation and processing, is smoking, which has been used for centuries and is still a widely practiced method [8-15]. Smoking hinders toxin formation in meat products [16], and lowers water activity thereby reducing bacteria growth [17-18]. Smoked meat is classified as "ready-to-eat" [19], which means it can be consumed after purchase without further processing. This preservation method is not totally effective since studies [20-21, 2] have shown bacterial contamination of smoked meat. This may be hazardous to the smoked rat meat consumers, due to the fact that the most serious safety issues resulting in immediate consumers' health problems is associated with bacterial pathogens [22-23], such as *Salmonella* spp. and *E.*

coli O157:H7 which have been isolated from processed meat in several studies [24-25].

Among shiga toxin producing *E. coli* (STEC) strains, *E. coli* O157:H7 is considered one of the most important food-borne pathogens [26]. *E. coli* O157:H7 strains are important zoonotic agents that cause diseases in animals and human. They cause diarrhoea that may result in life-threatening conditions ranging from haemorrhagic colitis (HC) to haemolytic-uremic syndrome (HUS) [27-29]. *Salmonella* causes self-limiting gastroenteritis and the more severe forms of systemic typhoid fever [30]. *Salmonella* infections in humans and animals have been identified as a major public health problem [31-33]. Though incidence vary between countries, the infections caused by *Salmonella* are believed to be one of the most widely spread food-borne zoonotic infection in developing countries [34].

Studies have indicated the presence of *E. coli* O157:H7 and *Salmonella* spp. in meat and meat products [35-37]. But despite the large populations that consume rat meat as a protein source, to the best of my knowledge, this is the first report on the bacterial contamination and occurrence of *E. coli* O157:H7 and *Salmonella* spp. in smoked rat meat sold in any part of the world. Therefore, the aim of this study was to determine the bacteriological quality and the occurrence of *E. coli* O157:H7 and *Salmonella* spp. to ascertain its safety for human consumption.

2. Materials and Methods

2.1. Study Area

The study was carried out in Zaria. Zaria is situated at the center of Northern Nigeria, on a plateau and at a height of 2200ft above sea level [38]. The area has a distinct humid, wet and dry season with fluctuation in temperature, with a monthly mean rising from January (21°C) and attaining a maximum in April (29°C) [39]. While the wet season occurs from May-October, which is the high-sun period; the dry season which is practically rainless occurs during November to April [38]. Zaria metropolis is divided administratively into Zaria and Sabon Gari Local Government Areas (L. G. A), each comprising of six (6) and five (5) district areas respectively. Zaria LGA comprises Zaria city, Dutse Abba, Tudun wada, Gyelesu, Tukur Tukur and Jushi; while Sabon Gari comprises Muciya, Bassawa, Samaru, Bomo and Hanwa [40].

2.2. Samples

Convenience sampling was carried out from January to May 2017, from four purposively selected district areas; Samaru, Sabon Gari, Basawa and Jushi within the study region. Sample size was calculated as described by [41] for a 50% prevalence. Three hundred and eighty-four samples were collected in total, 96 per district area. Samples were bought just the way they were sold to any customer, from sales point in each district area, but collected in separate sterile labeled polyethene bags and immediately transported to the bacteria zoonoses laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria for analyses.

2.3. Laboratory Procedures

The steps for the isolation of *E. coli* O157:H7 and *Salmonella* spp. were carried according to the isolation procedure of [42-43]. These steps included: Enrichment, Selective plating, biochemical characterization, serological confirmation by latex agglutination for *E. coli* O157:H7 and PCR for *Salmonella* spp.

2.3.1. Enrichment

Each sample was cut using a sterile scissors to make 10g which was weighed aseptically, and homogenized in 90ml 1.0% buffered peptone water, using a laboratory stomacher.

2.3.2. Total Aerobic Plate Counts

Standard aerobic plate count using spread plate methods as recommended [44] was used for the determination of total aerobic plate count. The homogenate, 10g of the sample homogenized in 90 ml 1.0% buffered peptone water, constituted the stock which was immediately used for serial dilutions. Serial dilutions of each sample were performed in hundred-fold to a dilution factor of 10^{-7} and plated out by spread plate technique on Nutrient agar (Oxiod, UK) plates using a sterile hockey stick. The plates were then incubated at 37°C for 24 h. The average bacterial loads of the samples collected were counted and expressed as colony forming unit per gram ($cfu.g^{-1}$) using the formula;

$$\text{Total aerobic plate counts per grams} = \frac{\text{number of counts per dilution}}{\text{dilution factor}} \quad (1)$$

2.3.3. Isolation and Identification of Salmonella and E. Coli O157:H7

The homogenate (10g of the sample homogenized in 90 ml 1.0% buffered peptone water) was incubated at 37°C for 24h and then used for the isolation and identification of *Salmonella* and *E. coli* O157:H7

i. Isolation of *Salmonella*

One (1) ml of the incubated homogenate was transferred using sterile pipette to 9ml of Rappaport Vassiliadis (RV) broth, which was incubated at 37°C for 24 h for selective enrichment. A loopful of the enriched Rappaport Vassiliadis (RV) broth was streaked onto Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37°C for 24 h. Two or three characteristic *Salmonella* colonies appearing pinkish-white, with or without black center on XLD agar as described by [45] were stored on nutrient agar (Oxiod, UK) slants, incubated at 37°C for 24 h and then refrigerated for biochemical characterization.

ii. Isolation of *E. coli*

One (1) ml of the incubated homogenate was transferred

using sterile pipette to 9ml of EC broth, which was incubated at 37°C for 24 h for selective enrichment. A loopful of the enriched EC broth was streaked onto Eosin Methylene Blue (EMB) agar plates using sterile wire loop and incubated at 37°C for 24 h. Colonies with greenish metallic sheen and dark centers suggestive of *E. coli* were picked, stored on nutrient agar slants (Oxiod, UK), incubated at 37°C for 24 h and then refrigerated for biochemical characterization.

2.3.4. Conventional Biochemical Characterization of Presumptive Isolates

The following conventional biochemical tests were carried out on the presumptive *Salmonella* and *E. coli* isolates as described by instructions [46, 42, 47]): Carbohydrate fermentation test in Triple Sugar Iron (TSI) agar (Oxiod, UK); Sulfur reduction, indole production and motility test in Sulfide Indole Motility (SIM) agar (Oxoid, UK); glucose fermentation pathway in Methyl Red-Voges Proskauer (MRVP) broth; Citrate utilization in Simmons Citrate agar and hydrolysis of urea in Urea agar (Oxoid, UK), according to Manufacturer's instructions. The isolates were further tested for the fermentation of sugars: maltose, manitol, lactose, glucose, sucrose and manose, according to the Manufacturer's.

2.3.5. Isolation of E. Coli O157:H7

Biochemically confirmed *E. coli* isolates were streaked on Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC agar) supplemented with Cefixime 50µg/L and Potassium tellurite 2.5mg/L at 37°C [24]. The plates were then incubated at 37°C for 24 h. Colonies of suspect *E. coli* O157 appeared as non-sorbitol fermenters, characterized by slightly transparent, almost colourless colonies with a weak pale brownish appearance. Three to four colonies from the CT-SMAC agar plates were selected and stored on nutrient agar (Oxiod, UK) slants, incubated at 37°C for 24 h and then refrigerated for further analysis.

2.3.6. Standardized Micro-Substrate Detection Kit for Gram Negative Bacteria (Microgen GN-ID A+B)

Presumptive *Salmonella* and *E. coli* cultures (24 h) on respective media were obtained and the test was carried out and interpreted as recommended by the manufacturer (Oxoid, UK). A 4-digit code was then obtained which was fed into the computer identification software; which gave the probable identity of the organism tested in percentage score. The Microgen software recommends a 75% cut-off point for a probable identification. All tests that gave less than 75% were not accepted as *Salmonella* and *E. coli*.

2.3.7. Rapid Latex Agglutination Test for Escherichia Coli O157:H7

Commercially available latex agglutination kit having *E. coli* O157:H7 antisera was used to further confirm for *E. coli* O157:H7 [43]. The kit contains two test reagents (O157 test reagent and H7 test reagent). The O157 test reagent consists of a red latex particle coated with antibodies specific for *E. coli* O157 and H7 test reagent consists of blue latex particles coated with antibodies specific for *E. coli* H7 antigen. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instruction, hence colonies that agglutinated were considered to be *E. coli* O157:H7.

*2.3.8. Detection of Inv*A *Gene by PCR (Polymerase Chain Reaction) Method*

An overnight culture was prepared by inoculating a loop full of the *Salmonella* isolates stored on Nutrient agar slants in 10 mls of peptone water, and then incubated at 37°C for 24 hrs. The overnight suspension was centrifuged at 4000 rpm for 5 minutes. The supernatants were discarded and the sediments were transferred into a 1.5 mls ependorf tubes. Two hundred microliters of molecular grade water was transferred into each tube and boiled in a water bath at 95 degrees for 30 minutes. The supernatants were aseptically transferred into fresh sterile 1.5 micro liters ependorf tubes. Extracted DNA were quantified using a Nanodrop spectrophotometer (Nanodrop ®; Pretoria, South Africa). *Salmonella* specific primers pair S139 5' - GTG AAA TTA TCG CCA CGT TCG GGC AA - 3' and S141 5' – TCA TCG CAC CGT CAA AGG AAC C – 3' were used to amplify a 284 bp fragment within the conserved *inv*A gene sequence of *Salmonella* spp. [48]. Reaction was carried out in a 50 µl amplification mixture consisting of 25 µl master mix (Genei, Bangalore), 2 µl of each primer, 19 µl of molecular grade water and 2 µl of extraction for each isolate. Amplification was carried out in an Eppendorf Mastercycler Gradient (Hamburg, Germany) using the following cycling condition; initial denaturation at 94°C for 1minute, 35 cycles of denaturation at 94°C for 1 minute, annealing at 64°C for 30 seconds and elongation at 72°C for 30 seconds, followed by 7 minutes final extension period at 72°C. PCR amplicons were separated by 1.5% agarose gel in cooperated with 5 microliters of ethidium bromide and electrophoresed at 100 volts for 45 minutes. 100bp DNA Ladder was used. The gel was visualized by a Biorad gel documentation device.

3. Results

3.1. Total Aerobic Plate Counts

Mean total aerobic plate counts were lowest (12 x 10^9 cfu.g^{-1}) at Basawa and highest (15 x 10^9 cfu.g^{-1}) at Jushi sampling areas. The mean TAPC observed at Samaru and Sabon Gari were 12 x 10^9 and 14 x 10^9. Mean log ± SD TAPC from Samaru, Sabon Gari, Jushi and Basawa sampling areas were 10.01±0.27, 10.03±0.26, 10.10±0.29 and 9.9±0.29 respectively (Table 1).

3.2. Frequency of Isolation of E. Coli O157:H7

Table 2, shows the percentage distribution of *E. coli* O157:H7 isolates in relation to sampling areas. Out of 22 suspected *E. coli* isolates identified by conventional biochemical methods (Table A1), 15 isolates showed typical *E. coli* O157:H7 growth on CT-SMAC appearing as colorless colonies indicative for non-sorbitol fermenters. Five of these

isolates were confirmed as *E. coli* using the Microgen system (Table A3). The Microgen confirmed isolates were screened using commercially available rapid latex agglutination kit having O157 and H7 antisera and all five isolates tested positive as *E. coli* O157:H7.

3.3. Frequency of Isolation of Salmonella

Table 3, shows the percentage distribution of *Salmonella* isolates in relation to sampling areas. Out of 18 suspected *Salmonella* isolates identified by the conventional biochemical methods (Table A2), the Microgen system confirmed 6 to be *Salmonella* and the remaining 12 isolates as members of *Enterobacteriaceae*, including *Citrobacter freundi, Pseudomonas agglomerans,* and *Klebsiella ozaenae.* (Table A3)

The *inv*A gene was detected in two of the 6 suspect *Salmonella* isolates, as seen in lanes 3 and 6, (Figure 1).

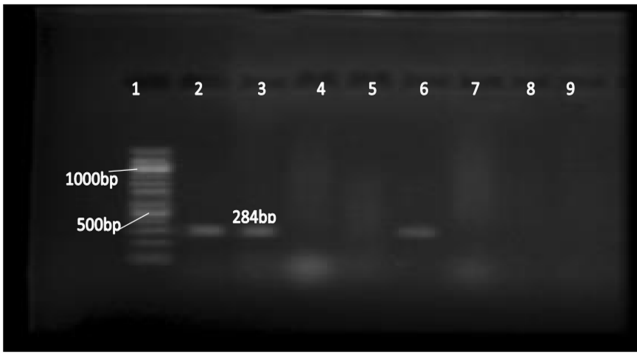

Figure 1. *PCR amplification of the invA gene in 6 Salmonella isolates. Lane 1, 100 bp ladder, Lane 2, positive control, Lane 9, Negative control and Lanes 3-8, Salmonella test isolates.*

Table 1. *Mean total plate counts of bacterial colonies from samples in the study area in relation to sampling areas.*

S/no	Sample areas	N	n	Mean count cfu.g^{-1} (10^9)	Log_{10}(mean±SD)	p-value 0.04
I	Samaru	90	70	12	10.01±0.27	
Ii	Sabon Gari	90	69	14	10.03±0.26	
Iii	Jushi	90	84	15	10.10±0.29	
Iv	Basawa	90	74	12	9.9±0.29	
	Total	384	297			

Key: N = Number of samples collected, n - number of samples with colonies within countable range (30-300), TAPC = Total aerobic plate count cfu.g^{-1}= colony forming unit per gram.

Table 2. *Percentage distribution of E. coli* O157:H7 *isolates in the study area in relation to sampling areas.*

S/N	Sampling area	Samples collected	Biochemically screened *E. coli*	Confirmed *E. coli* on CT-SMAC Agar	Confirmed *E. coli* O157:H7 by latex agglutination
		No (%)	No (%)	No (%)	№ (%)
I	Samaru	96 (25.00)	4 (4.17)	4 (4.17)	1 (0.26)
II	Sabon Gari	96 (25.00)	7 (7.29)	3 (3.13)	2 (0.52)
III	Jushi	96 (25.00)	8 (8.33)	5 (5.21)	2 (0.52)
IV	Basawa	96 (25.00)	3 (3.13)	3 (3.13)	0 (0)
V	Total	384(100)	22(5.73)	15(3.91)	5 (1.3)

Key: E.M.B - CT-SMAC – Cefixime Tellurite Sorbitol MacConkey agar.

Table 3. *Percentage distribution of Salmonella isolates in the study area in relation to sampling areas.*

S/N	Sample areas	Samples collected	Biochemically confirmed *Salmonella* isolates	*Salmonella* isolates confirmed by PCR
		No (%)	No (%)	No (%)
I	Samaru	96 (25.00)	5 (5.21)	0 (0)
II	Sabon Gari	96 (25.00)	2 (2.08)	0 (0)
III	Jushi	96 (25.00)	7 (7.29)	1 (0.26)
IV	Basawa	96 (25.00)	4 (4.17)	1 (0.26)
V	Total	384(100)	18(5.73)	2 (0.52)

Key: PCR – *Polymerase Chain Reaction.*

4. Discussion

Processed meat has been problematic, showing high levels of bacterial contamination. Results of this study showed that there was bacterial contamination and occurrence of *Salmonella* and *E. coli* O157:H7 in smoked rat meat sold in Zaria, Nigeria. Total aerobic plate count is a microbiological indicator for the quality of food conditions, that favors the multiplication of microorganisms as indicated by the presence of aerobic organisms [49]. The total aerobic plate counts observed in this study ranged from 12 x 10^9 cfu.g$^-$ in Basawa, Zaria to 15 x 10^9 cfu.g$^-$ in Jushi, Zaria sampling areas. The high values of TAPC observed; $>10^9$ is unsatisfactory [50], Microbial guidelines for food, which states that for smoked ready-to-eat meat, a total aerobic plate counts of $<10^6$ is satisfactory while $\geq 10^7$ is unsatisfactory. High values of bacterial contamination in processed meat have also been observed in other studies. A study [51]

reported a TAPC of between $6.70x10^8$ and $9.30x10^9$ $cfu.g^{-1}$ from traditionally prepared fried ground beef (Dambun nama) in Sokoto, Nigeria. A study [52] reported a TAPC of 9.0×10^9 $cfu.g^{-1}$ from locally processed meat sold in retail outlets in Trinadad and [2] reported a TAPC of 2.3×10^8 $cfu.g^{-1}$ from cane rats, in an assessment of microbial count loads of bush meats sold in different markets in Benin city, Nigeria. The high levels of bacterial contamination observed in this study could be attributed to the poor hygiene practices observed in the handling, processing, packaging and transportation practices as the result [53-54] explained that these practices may result in high levels of contamination. The implication of results of this study is that the product is not safe for consumption, since all the samples (100%) were positive for aerobic plate counts exceeding the permissible limit across all sampling areas.

Of the total 384 samples, *E. coli* O157:H7 was isolated from all but one sampling area with a prevalence of 5 (1.3%). This finding is similar to the findings of [36] who reported 2.2% prevalence from raw meat analyzed in same study region. Also, a study [35] reported a 4.3% prevalence of *E. coli* O157 from raw meat and smoked beef "suya" in Benin, Nigeria. The prevalence observed in this study however is at variance with the findings ~~of~~ [20] who recorded a 25% prevalence of *E. coli* O157:H7 from smoked beef "suya" in Kano, Nigeria. This result is an indication of poor hygienic practices involved in handling of smoked rat meat and may predispose the consumers to *E. coli* O157:H7 infections. Evidence of contamination as a potential source of *E. coli* O157:H7 has also been highlighted [55].

The results of *Salmonella* isolation in the present study demonstrates the presence of the pathogen in smoked rat meat sold in Zaria, Nigeria. The *inv*A gene which is essential for full virulence in *Salmonella* and thought to trigger internalization required for invasion of deeper tissue [56], was detected in two (2) of the (6) screened *Salmonella* isolates, giving an overall prevalence of 0.5%. Other reports have also shown the absence of the *inv*A gene in screened *Salmonella* isolates. A study [57] reported the *inv*A gene in 62 of 63 strains of *Salmonella* screened and [58] also reported 5 out of 8 *Salmonella* isolates harboring the *inv*A gene. The absence of this gene in the screened isolates could be due to lack of invasiveness of the isolates. Other studies have also shown the presence of *Salmonella* in the study region from different sources [59, 21]. The low prevalence (0.5%) of *Salmonella* observed in this study is similar to the findings ~~of~~ [21] who reported an overall prevalence of 2.3% of *Salmonella* spp. from 453 retailed beef and related meat products. The presence of *Salmonella* spp. in cooked foods is often attributed to inadequate refrigeration, sanitation and poor personal hygiene. Proliferation of this organism in foods may therefore, result from handling cooked foods by workers who are carriers of *Salmonella* which may be transferred from raw meat to cooked meat by hands, surfaces or utensils [60-61]. This may also be a probable reason for the presence of *Salmonella* spp. in smoked rat meat samples observed in this study.

Despite the high bacteria counts, the prevalence of *E. coli* O157:H7 and *Salmonella* spp. observed in this study appears to be relatively low. A possible explanation for this could be due to the fact that, during smoking of the rat meat, the high temperature is expected to have denatured microorganisms, as high temperatures reduce water content thereby reducing microbial activity [21]. However, A study [62] recovered 5 strains of *E. coli* O157:H7 at 56.8°C for 0, 15 and 30 minutes using a non-selective medium (Trypticase Soy Broth). This signifies that, *E. coli* O157:H7 could survive temperatures above 45°C. Also, *Salmonella* spp. may survive improper smoking of the rat meat product during processing. A study [63] stated that the presence of *Salmonella* spp. contaminant could be attributed to inadequate heating of meat product during its preparation. The unhygienic practices involved in the processing and preservation of the smoked rat meat could also be other potential sources of the organisms identified. This shows that the presence of these pathogens reported in this study could be from external sources like contamination from handlers, as well as internal sources like intestines and lymph nodes of the rats prior to processing. Notwithstanding the source, *Salmonella* spp. and *E. coli* O157:H7 are important foodborne pathogens and their presence in any food presents a serious health risk to the consumers.

5. Conclusion

The outcome of bacterial load from this study was higher than the permissible limit by (50) across all sampling areas within the study area. Two important food borne pathogens; *Salmonella* spp. and *E. coli* O157:H7 were detected in the study among others. In light of this, microbiological standards and guidelines should be enforced by relevant bodies on the sales of smoked rat meat, to ensure proper food hygiene standards are followed. The processing line should be carefully monitored by relevant bodies to ensure a safe and wholesome product for consumption. As a limitation, this study did not identify the exact source (s) of the foodborne pathogens isolated, therefore further studies should be carried out to identify potential sources of these pathogens with scientific based evidence to aid proper preventive and control measures.

Acknowledgements

Special gratitude goes to Mr. and Mrs. J.H. Addai, Prof. Helen Inabo and Dr. Chia Ahii Mathias for their financial and editorial contributions.

Appendix

Table A1. *Isolates of E. coli, from Different Sampled Areas and Their Reactions to Conventional Biochemical Tests.*

S/NO	isolate I.D	TSI	S	I	M	UR	MR	VP	MT	CT	MN	LC	SC	GL	ME
1	C17	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
2	R15	A/A, G	-	+	+	-	+	-	-	-	-	+	+	+	-
3	F10	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
4	K9	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
5	P.Z5	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
6	C24	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
7	P.Z1	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
8	F1	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
9	C6	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
10	P.Z7	A/A, G	-	+	+	-	+	-	+	-	+	+	+	+	-
11	T13	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
12	C3	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
13	K12	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
14	Z11	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
15	C16	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
16	P.Z9	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
17	R10	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
18	Z9	A/A, G	-	+	+	-	+	-	+	-	+	+	+	+	-
19	Z15	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
20	C30	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
21	R62	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
22	C21	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-

KEY; TSI- TRIPLE SUGAR IRON; S- SULPHIDE; I- IND-INDOLE; M- MOTILITY; UR-UREASE; MR- METHYL RED; VP- VOGES PROSKAUER; MT- MALTOSE; CT- CITRATE; MN- MANITOL; LC- LACTOSE; SC- SUCROSE; GL-GLUCOSE; ME- MANOSE; $\boldsymbol{A/A}$- ACID OVER ACID AND G-GAS (CO_2).

Table A2. *Isolates of Salmonella spp. from Different Sampled Areas and Their Reactions to Conventional Biochemical Tests.*

	S/NO isolate I.D	TSI	S	I	M	UR	MR	VP	OX	CT	MN	LC	SC	GL	ME
1	S175	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
2	B65	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
3	SR	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
4	Sa5	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
5	Sf11	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
6	B125	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
7	Sa1	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
8	Srt7	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	-
9	Sf11	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
10	K8ST	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
11	Sa2	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
12	Sa3	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
13	B126	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
14	5Tkm	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
15	R23	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
16	R53	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
17	Rsb	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
18	Rs5	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+

Table A3. *Identified Organisms Using the Microgen GN-ID A+B System for Gram Negative Bacteria.*

S/N	Identified organisms	Frequency
I	*Klebsiella ozanae*	6
II	*Escherichia coli*	5
III	*Citrobacter freundi*	5
IV	*Salmonella arizonae*	4
V	*Salmonella enterica* serovar Typhi	1
VI	*Salmonella* spp.	1
VII	*Pseudomonas agglomerance*	1

Reference

[1] Hammer, G. F. (1987). Meat processing ripened products. *Fleischwiritschaft* 67, 71-74.

[2] Gideon, E. and Joseph. I. (2018). Assessment of microbial count loads of bush meat sold at different markets in Benin city, Edo State, Nigeria. *International Journal of Agricultural Science* 3, 2367-9026.

[3] Addai, T. I. (2019). Sociodemographic features and practices of retailers and consumers of smoked rat meat sold (Study in Nigeria). *International Journal of Zambrut* 2(1), 37-43.

[4] Oyarekua, M. A. and Ketiku, A. O. (2010). The Nutrient Composition of the African Rat. *Advance Journal of Food Science and Technology* 2(6), 318-324.

[5] Doyle, K. (2014). Cambodian rat meat: A growing export market. http://www.bbc.com/news/world-asia-28863315

[6] Tee, T. N, Ikpa, T. F. and Tortange, V. (2012). Bush meat trade in Markudi; Implication for the conservation of wildlife in Nigeria. *Journal of Applied BioScience* 52, 3704–3715.

[7] Aymerich, T, Picouet, P. A, and Monfort, J. M. (2008). Decontamination technologies for meat products. *Meat Science* 78, 114−129.

[8] Maga, J. (1988). Smoke in Food Processing. Boca Raton, Florida: CRC Press, Inc.

[9] Huss, H. H. (1995). Quality and quality changes in fresh fish (Vol. 348). Rome: FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS.

[10] Rora, A. M, Kvale, A, Morkore, T, Rorvik, K. A, Steien, S. H, and Thomassen, M. (1998). Process yield, colour and sensory quality ofsmoked Atlantic salmon (Salmo salar) in relationto raw material characteristics. *Food Research International* 31, 601-609.

[11] Hattula, T, Elfving, K, Mroueh, U, and Luoma, T. (2001). Use of Liquid Smoke Flavouring as an Alternativeto Traditional Flue Gas Smoking of Rainbow Trout Fillets (Oncorhynchus mykiss). *Lebensmittel-Wissenschaft und-Techologies* 34, 521-525.

[12] Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B*, 770, 3-18.

[13] Martinez, O, Salmero´n, J, Guille´n, M, and Casa, C. (2007). Textural and physicochemical changes in salmon (Salmo salar) treated with commercial liquid smoke flavourings. *Food Chemistry* 100, 498-503.

[14] Alcicek, Z, and Atar, H. H. (2010). The effects of salting on chemical quality of vacuum-packed liquid smoked and traditional smoked rainbow trout (Oncorhyncus mykiss) fillets during chilled storage. *Journal of Animal and Veterinary Advances* 9, 2778–2783.

[15] Petridis, D, Zotos, A, Kampouris, T, and Roumelioti, Z. (2012). Optimization of a steaming with liquid smoke smoking process of Mediterranean mussel (Mytilus galloprovincialis). *Food Science and Technology International,* 19, 59–68.

[16] University of Florida. (2004). Guidance for processing smoked seafood in retail operations. Florida: IFAS Communication Services.

[17] Rørvik, L. M. (2000). Listeria monocytogenes in the smoked salmon industry. *International Journal of Food Microbiology* 62, 183–190.

[18] Swastawati, F, Suzuki, T, Dewi, E. N, and Winarni, T. (2000). The effect of liquid smoke on the quality and omega-3 fatty acids content of tuna fish (Euthynnus affinis). *Journal of Coastal Development* 3, 573-579.

[19] Monteiro, C. A, Levy, R. B, Claro, R. M, Castro, I. R, Cannon, G. A. (2010). A new classification of foods based on the extent and purpose of their processing. *Cad Saude Publica* 26 (11), 20, 39–49.

[20] Dahiru, M, Uraih, N, Enabulele, S. A. and Shamsuddeen, U. (2008). Prevalence of *E. coli* O157: H7 in fresh and roasted beef in Kano city, Nigeria. *Bayero Journal of Pure and Applied Sciences* 1(1), 39-42.

[21] Tafida, S, Y, Kabir, J, Kwaga, J. K. p, Bello, M, Umoh, V. J, Yakubu, S. E,… Nok, A. J. (2013). Occurrence of *Salmonella* in retail beef and related meat products in Zaria, Nigeria. *Food Control* 32, 119-124.

[22] Sousa, C. P. (2008). The impact of food manufacturing practices on food borne disease. *Brazilian Archives of Biology and Technology* 51(4), 815-823.

[23] Sofos, J. N. (2008). Challenges to meat safety in the 21st century. *Meat Science* 78, 3-13.

[24] March, S. B. and Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology* 23, 869-872.

[25] Eppinger, M, Mammel, M. K, LeClerc, J. E, Ravel, J. and Cebula, T. A. (2011). Genome signatures of *Escherichia coli* O157:H7 Isolates from Bovine Host Reservoir. *Applied and Enviromental Microbiology* 108(50), 20142-20147.

[26] Goncuoglu, M, Bilir Ormanci, F. S, Ayaz, N. D. and & Erol, I. (2010). Antibiotic resistance of *E. coli* O157:H7 isolated from cattle and sheep. *Annals of Microbiology* 60, 489–494.

[27] Nataro, J. P. and Kaper, J. B. (1998) Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews* 11, 142-201.

[28] Mead, P. S, Slutsker, L, Dietz, V, McCaig, L. F, Bresee, J. S, Shapiro, C, Griffin. P. M. and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases* 5(5), 607-625.

[29] Meng, J, Doyle, M. P, Zhao, T, Zhao, S. (2001). Enterohemorrhagic *Escherichia coli* O157:H7. In: Doyle, M. P, Beuchat, L. R, Montville, T. J (eds) Food microbiology: fundamentals and frontiers, 2nd edn. ASM Press, Washington, D. C, pp 193–213.

[30] Reda, A. A, Seyoum, B, Yimam, J, Andualem, G, Fiseha, S. and Vandeweerd, J. M. (2011). Antibiotic Susceptibility of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. *Journal of Infectious Diseases and Immunology* 3(8), 134-139.

[31] Crump, J. A, Luby, S. P, and Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization* 82, 346–353.

[32] Akinyemi, K. O, Phillip, W, Beyer, W. and Bohm, R (2007). In-vitro antimicrobial susceptibility Patterns of *Salmonella* enterica serovars and emergence of *Salmonella* phage type DT071 in a suspected community-associated outbreak in Lagos, Nigeria. *Journal of Infection in Developing Countries* (1), 48-54.

[33] Scallan, E, Hoekstra, R. M, Angulo, F. J, Tauxe, R. V, Widdowson, M-A, Roy, S. L, Jones, J. L, and Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases* 17(1), 7-15.

[34] Osterom, J. (1999). Epidemiological studies and proposed preventive measures in the fight against human Salmonellosis. *International Journal of Food Microbiology* 12, 41-52.

[35] Enabulele, S. and Uraih, N. (2009). Thrombotic Thrombocytopenic Purpura and Haemolytic Uremic Syndrome. *Mayo Clinical Proceedings* 76, 1154-1162.

[36] Tafida, S. Y, Kwaga, J. K. P, Bello, M, Kabir, J, Umoh, V. J, Yakubu, S. E. and Nok, A. J. (2014) Occurrence of *Escherichia coli* O157 in Retailed-Beef and Related Meat Products in Zaria, Nigeria. *Food and Nutrition Sciences*, 5, 481-487.

[37] Olukitibi, T. A., Adetuyi, F. C., Adeleke, B. S., and Abe, S. C (2017). Isolation and Antibiogram of Bacteria Isolated from Processed and Unprocessed Cow-Skin (Ponmo) in Ogbese Market. *Journal of Advances in Microbiology* 2(4): 1-8.

[38] Mortimore, M. J. (1970). Zaria and its Region, Occasional paper No. 4 Department of Geography, A. B. U. Zaria, published for the 14th *Annual conference of Nigerian Geographical Association, Zaria.* Pp 15.

[39] Yakubu, S. (2009). Effects of municipal soil waste application on soil properties and crop qualities in Zaria urban area northern Nigeria. Ph. D. Thesis, Department of Geology University of Abuja.

[40] Ministry of Economic Development (1996). Kaduna State Statistical year book, Kaduna Nigeria Ministry of Economic Development, Statistics Division.

[41] Thrusfield, M. (1997). Veterinary epidemiology. Blackwell Science Ltd, United Kingdom.

[42] International Organization of Standardization (ISO) (2002). Microbiology general guidelines on methods for the detection of *Salmonella.* International organization of standardization, Geneva, Switzerland.

[43] International Organization of Standardization (ISO) (2003). Isolation and Identification of Enterohaemorrhagic *Escherichia col*i O157. 1st Edition. International Organization for Standardization, Geneve, Switzerland.

[44] Sanders, E. R (2012). Aseptic Laboratory Techniques: Plating methods*: Journals of Visual Experiment.* 63: 3064.

[45] Doughlas WW, Gast RK, Mallinson ET. Salmonellosis In: Swayne, D. E, Glisson, J. R, Jackwood, M. W, Pearson, J. E. and Reed, W. M. (eds.) (1998). A Laboratory manual for the isolation and identification of avian pathogens, 4th ED. American Association of Avian Pathologists, Kennett Square, Pennsylvania. 4-13.

[46] Cowan, S. T and Steel, K. J (1993). Manual for Identification of medical bacteria. Cambridge University Press. 3rd Edition. 199–241.

[47] Harley, J. P and Prescott, L. M (2002). Laboratory exercises in microbiology. Biochemical activities of bacteria: Carbohydrates; fermentation and ß-galactosidase activity. 5th Edition. The McGraw−Hill Companies.

[48] Rahn, K, De Grandis, S. A, Clarke, R. C, McEwen, S. A, Galan, J. E, Ginocchio, C,…Gyles, C. L, (1992). Amplification of an *inv*A gene sequence of *Salmonella* Typhimurium by Polymerase Chain Reaction as a specific method of detection of *Salmonella. Molecular and Cellular Probes,* 6(4), 271-279.

[49] Nyenje, M. E, Odjadjare C. E, tanih N, F, Green, E, and Ndip, R. N. (2012). Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in alice, eastern cape province, south Africa: pblic health implications. *International Journal of Environment Research and Publc Health.* 9(8), 2608-2619.

[50] FAO/WHO (2002). Working paper on elaboration of a regional standard for microbiological levels in foods. *Codex Alimentarius commission.* (Accessed 20-23 january, 2003).

[51] Salihu, M. D, Junaidu, A. U, Magaji, A. A, Aliyu, R. M, Yakubu, Y, Shittu, A, and Ibrahim, M. A. (2010). Bacteriological quality of traditionally prepared fried ground beef (*dambun nama*) in Sokoto, Nigeria. *Advance Journal of Food Science and Technology.* 2(3), 145-147.

[52] Syne, S, Ramsubhag, A. and Adesiyun, A. A (2015). Microbial quality of locally processed meats sold in retail outlets in Trinidad, West, indies. *Journal of Food Protection.* 78, 333-339.

[53] Agbolagba, O. J. and Ibbinevbo, V. E. (2010). Microbial load of fresh and smoked fish marketed in Benin metropolis, Nigeria. *Research Journal of Fisheries and Hydrobiology,* 5(2), 99-10.

[54] Vliet, N. V, Moreno, J, Gómez, J, Zhou, W, Fa, J. E, Golden, C, Alves, R. R. N, and Nasi, R. (2017). Bushmeat and human health: Assessing the Evidence in tropical and sub-tropicalforests. *Ethnobiology and Conservation*. 3, 1-44.

[55] Elder, R. O, J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie. and W. W. Laegreid. (2000). Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci*. 97, 2999-3003.

[56] Khan, A. A, Nawaz, M. S, Khan, S. A and Cerniglia, C. E (2000). Detection of multidrug resistant *Salmonella Typhimurium* DT104 by multiplex polymerase chain reaction. *FEMS Microbiology. Letter*. 182, 355-360.

[57] Bacci, C, Boni, E, Alpigiani, I, Lanzoni, E, Bonardi, S. and Brindani, F. (2012). Phenotypic and genotypic features of antibiotic resistance in *Salmonella enteric* isolated from chicken meat and chicken and quail carcasses. *International Journal of Food Microbiology*, 160, 16-23.

[58] Oludairo, O. O, Kwaga, J. K. P, Dzikwi, A. A, and Kabir, J. (2013). Detection of *inv*A virulence gene by polymerase chain reaction (PCR) in *Salmonella* spp. Isolated from captive wildlife. *Bio-Genetic Journal,* 1, 12-14.

[59] Kwaga, J. K. P, Umoh, J. U, Addo, P. B. and Belino, E. D. (1985). Isolation of Salmonellae from lymph nodes of slaughtered cattle and market beef in Zaria, Nigeria. *Nigerian Veterinary Journal,* 14(1), 32-35.

[60] Jay, J. M. (2000). Modern Food Microbiology. 6th Edition. Aspen Publishers Inc. Gaithersburg. Maryland; 60-89.

[61] Lues, J. R, Rasephei, M. R, Venter, P. and Theron, M. M. (2006). Assessing food safety and associated food handling practices in street food vending. *International Journal of Environmental Health Research.* 16(5), 319-328.

[62] Clavero, M. R. S. and L. R. Beuchat. (1995). Suitability of Selective Plating Media for recovering Heat- or Freeze-Stressed *Escherichia coli* O157:H7 from Tryptic Soy Broth and Ground Beef; *Journal of Applied and Environmental Microbiology*. 61 (9), 3268–3273.

[63] Adams, R and Moss, M. O. (1999). Food microbiology. The Royal Society of Chemistry, Thomas Graham house, Service Park, Cambridge, UK. 192-202.