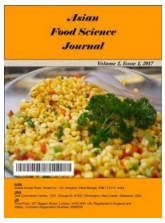

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Antibiotic Sensitivity Profiles of *Salmonella typhimurium* and *E. coli O157: H7* Isolates from Ready to Eat Chicken Meat in Ibadan-Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OHA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OVA supervised the field work. Author OMA managed the literature searches and the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The global increase in the use of antibiotics in poultry and livestock production has significantly resulted in the emergence of antibiotic resistant bacteria. There is a growing global concern of the effect of antibiotic resistance on both animals and humans. The aim of this study was to determine the antibiotic resistance profile of *Salmonella typhimurium* and *E. coli 0157:H7* in ready to eat chicken meat in Ibadan, Nigeria.
Method: A set of 500 pieces of chicken parts were purchased from sampled eateries in Ibadan, Nigeria. Standard microbiological techniques were used to isolate *Salmonella* and *E. coli* biochemically and serologically. All confirmed isolates were subjected to in vitro antibiotic

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susceptibility testing against 8 antibiotics of different classes of Gram negative antimicrobial-impregnated multidisks (ABTEK)
Result: The resistance pattern revealed *E. coli 0157:H7* and *Salmonella* isolates were resistant to 5 drugs namely; Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin. Highest resistance of *E. coli* isolates to Augmentin and Ampicillin were indicated both having a frequency of 11 (84.6%) and least resistance to Gentamicin, Ciprofloxacin and Ofloxacin 0 (0.00%) were indicated. Three antibiotics that showed 100% sensitivity by all the isolates were Gentamicin, Ciprofloxacin and Ofloxacin.
Conclusion: The study revealed that Isolated *E. coli* and *Salmonella* strains from ready to eat chicken parts were resistant to five antibiotics; Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin and very susceptible to three antibiotics namely; Gentamicin, Ciprofloxacin and Ofloxacin.

Keywords: E. coli; salmonella; antibiotics; antibiotic-resistance; ready-to-eat chicken; Ibadan.

1. INTRODUCTION

Chicken meat is the most popular type of poultry and it is the second most consumed meat in the world. The white meat is affordable, nutritious and it offers a wide range of nutrients for very few calories. The nutrition value of a whole chicken, meat and skin has been indicated to contain calories (223), protein (24 g), total fat (13.4 g), saturated fat (3.7 g), monounsaturated fat (5.4 g), polyunsaturated fat (2.9 g), cholesterol (76 mg), sodium (73 mg) and iron (1.3 mg) respectively [1].

The worldwide increase in the use of antibiotics in poultry and livestock production industry to treat and prevent infectious bacterial diseases and as growth promoters at sub-therapeutic levels in feeds has led to bacterial resistance to antibiotics during the past years [2]. This increase in the use of antibiotics has played a significant role in the emergence of antibiotic resistance bacteria [3]. Increasing episodes of multi-drug resistant pathogens can result in failure of antibiotic therapy in both animals and human and this also facilitates the transmission of antibiotic resistance between and among bacteria strains and species [2].

Food of animal origin represents the major route of human exposure to foodborne pathogens with antimicrobial resistance [4]. Wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings [5]. There is an increase in public and government interest in phasing out inappropriate antibiotic use in animal husbandry [12]. In recent years, the problem of resistant strains to multiple drugs (MDR) is increasing and most studies in Iran and other countries have shown high resistance of *Salmonella* strains to several antibiotics [6-8].

Similar findings on multiple drug resistance of *E. coli* strains have been reported from Nigeria and other parts of the world [9-14]. The prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades [9,15]. The frequent and unnecessary use of antimicrobial agents for farming and therapeutic purpose in animals and human are contributing to create resistant strains. Drug resistant bacteria are harder to treat with the common antibiotics [16].

The aim of this study was to identify and establish the antibiotic sensitivity profiles of the *Salmonella typhimurium* and *E. coli O157: H7* isolates from ready to eat chicken meat.

2. MATERIALS AND METHOD

A total of 500 ready-to-eat chicken meats were purchased from 25 eateries in Ibadan to prepare 1000 samples. The eateries were grouped into standard, semi standard and substandard based on the general outlook, perceived level of hygiene standard and the quality of food and service offered by the eateries. A combined method of the US Food And Drug Administration/ Association of Official Analytical Chemists - Bacteriological Analytical Manual.(FDA/AOAC BAM)Salmonella Isolation Procedure, [17] and some *modifications* were used to isolate *E. coli* and *Salmonella*.

2.1 Isolation of *Salmonella typhimurium* and *E. coli 0157:H7*

25 g of sample was added to 225 ml of 10% Buffer Peptone Water in a jar and incubated at 37ºC for 24 hr.100 µl of the aliquot was inoculated into bottles containing 10 mls of Rappaport Vassiliadis Soy Broth and incubated

at 37ºC for 24 hrs those bottles with milky residue were considered positive.

An inoculum loop was used to transferred from the broth to streak on Xylose lysine Deoxycholate (XLD) agar and incubated at 37ºC for 24 hrs (Pink to red± black centre or (yellow for lactose negative colonies) were chosen as presumptive positive colonies. A loop of the broth was also streaked on Hekteon Enteric agar and incubated at 37ºC for 24hr; positive strains appear as blue/grey to green with or without black centre).

Potentially positive colonies were streaked on nutrient agar and incubated at 37ºC for 24 hrs; this was use for biochemical test. The following biochemical test were conducted; Triple Sugar Iron (TSI), Urea hydrolysis, Indole Citrate and Motility.

Slide agglutination test was performed using commercial antisera which included: Salmonella poly O grp A-S, Salmonella grp 4-0 and Agglutinating serum H. Strong agglutination in polyvalent O as well as polyvalent H were strong presumptive evidence that it was a Salmonella though there was confirmation with biochemical testing.

Also, 25 g of chicken meat sample was shredded using sterile mortar and pestle and combined with 225 mL of EC-broth supplemented with novobiocin in a jar and incubated at 37ºC for 24 hrs. 1 µl of this enrichment media was transferred to empty petri dishes added and sorbitol MacConkey agar with BCIG supplemented with cefixime and tellurite (CT) (Oxoid) was added using pour plate method. The SMA BCIG-TC plates were incubated at 37ºC for 20 to 22 h.

-Sorbitol negative colonies were streaked on sorbitol MacConkey agar supplemented with 4-methyl umbelliferylD glucuronide (MUG) 0.2 g/L (Oxoid) and incubated at 37ºC for 24 hrs. The petri dishes were put under long wave ultra violet (365 nm) light, Strains of *E. coli* 0157:H7 are MUG negative (they don't fluorescence under UV light) [18].

Standard biochemical tests used for the identification of *E. coli*, included, Urease, Indole, Citrate and Motility. Typical colonies that were MUG negative and indole positive was sub cultured on Sorbitol Mac Conkey agar and screened by picking a portion of each isolated suspect colony from the agar and testing for O157 and H7 antigens using latex agglutination test kit (Remel, OXIOD).

A positive result was indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles. Isolates that were O157 and H7 positive is of the O157:H7 serotype. But those isolates that were O157 (+) but H7 (-), may be a non-motile variant (O157: NM). Those isolates that were sorbitol (-), indole (+), MUG (-) and serologically (+) for 0157 and H7 and was identified as *E. coli* is a confirmed positive for *E. coli 0157:H7.*

2.2 Data Analysis

The obtained data was analyzed using descriptive statistics such as frequency tables and charts. Statistical Package for Social Sciences (SSPS) version 21.0 (SPSS Inc. Chicago, III, USA) was used for Inferential analysis. Level of correlation between the eatery type and isolation of either of the organisms (*E. coli* and *Salmonella*) was determined and a $p \leq 0.05$ were considered significant.

2.3 Antibiotic Susceptibility Testing

Susceptibility tests was performed using the Kirby-Bauer method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) using method as described by Hudzicki which describes the Kirby-Bauer Disk Diffusion Test method on Mueller Hinton Agar, some modifications were however made [19]. All confirmed isolates were subjected to *in vitro* antibiotic susceptibility testing against 8 antibiotics of different classes Gram negative antimicrobial-impregnated multidisks (ABTEK) comprising Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin(10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg) and was carried out. A summary of the method is as follows; 38 g of Mueller-Hinton Agar was dispersed in 1L of distilled water allowed to soak for 10 minutes, heated till agar dissolved and sterilized by autoclaving at 121ºC for 15 minutes. The media was cooled to 47ºC and poured into plates; the plates were allowed to dry and appropriately labelled.

A sterile inoculating loop was used to touch a few isolated colonies from subcultures of the previous day. The organism was suspended in 2 ml of sterile saline and vortex or shake

vigorously to create a smooth suspension. The turbidity of this suspension was matched against 0.5 McFarland standard by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy, this suspension was used within 15 minutes of preparation

A sterile swab was dipped into the inoculum tube and rotated against the side of the tube using firm pressure, to remove excess fluid. The dried surface of a MH agar plate was inoculated by streaking the swab three times over the entire agar surface rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. The plate was rimmed with the swab to pick up any excess liquid and allowed to sit at room temperature no more than 15 minutes, The multi disk was placed on the surface of the agar, using a forceps to press it down to ensure complete contact with the agar surface. Once the multi disks are in place, then the lid were replaced and the plates inverted, and incubated at 37ºC for 16 to 18 hours.

The zone diameter was determined by measuring the radius of the zone, from the centre of the antibiotic disk to a point on the circumference of the zone where a distinct edge is present. This measurement was multiplied by 2 to determine the diameter of the zone of inhibition.

Interpretation and reporting of result was done using the CLSI susceptibility and interpretative breakpoints which indicated on each drug whether the zone sizes were susceptible (S), intermediate (I) or resistant (R) [20].

3. RESULTS

Out of 1000 samples tested, 27 biochemically confirmed isolates were subjected to antimicrobial testing against 8 different antimicrobial agents. Standard Values; The Antimicrobial Resistant Profile of *E. coli 0157:H7* with Abtek (Cm-12-8nr100) Rapid Lab shown (Table 1).

E. coli 0157:H7 isolates showed highest /resistance to both Augmentin and Ampicillin with a frequency of 11(84.6%) and least resistance to* Gentamicin, Ciprofloxacin and Ofloxacin 0 (0. 00%). Two antibiotics that showed 100% sensitivity by all the isolates were Gentamicin and Ciprofloxacin (Table 2).

The antimicrobial resistant pattern showed that 38.5% of the isolates had the highest resistant profile to 5 antibiotics namely Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin (Table 3). Antimicrobial Resistance Pattern (Phenotype) of *Salmonella typhimurium* Isolates (Table 4).

In the antibiogram results all *Salmonella* isolates (100%) were sensitive to Ciprofloxacin which is a member of the fluoroquinolones and was the most effective antibiotic followed by Gentamicin (92.3%). Almost all isolates (84.6%) showed considerable resistance to Cefuroxime, Augmentin and Ampicillin (Table 5). The

Table 1. Antimicrobial resistant profile of *E. coli 0157:H7* with abtek (Cm-12-8nr100) rapid lab

S/No	Presumptive isolate	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
	Sensitive values	**(≥21)**	**(≥18)**	**(≥15)**	**(≥21)**	**(≥16)**	**(≥18)**	**(≥17)**	**(≥17)**
1	E13	24	21	23	28	34	-	24	-
2	E20	23	22	32	36	36	-	18	-
3	E42	27	28	19	32	15	20	17	15
4	E48	30	22	22	36	34	22	26	21
5	E118	9	-	18	36	34	-	24	-
6	E121	12	-	18	30	34	-	26	-
7	E138	13	11	18	32	28	-	24	-
8	E276	-	-	20	38	34	-	25	-
9	E284	-	-	38	36	30	-	14	-
10	E409	-	-	20	26	22	-	-	-
11	E459	-	-	24	26	28	-	11	-
12	E487	-	-	24	34	26	-	12	-
13	E500	-	-	20	32	28	-	10	-

E (E. coli), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciprofloxacin (CPR), Oflaxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ampicillin (AMP)

Table 2. Antibiogram result of *E. coli 0157:H7* isolates

S/NO	Types of antibiotics	Disc potency	Number of isolate,T=13		
			Resistant {n(%)}	Sensitive {n(%)}	Intermediate :{n(%)}
1	Ceftazidime(CAZ)	30 µ	9(69.23)	4(30.77)	-(0.00)
2	Cefuroxime(CRX)	30 µ	9(69.23)	4(30.77)	-(0.00)
3	Gentamicin(GEN)	10 µ	-(0.00)	13(100.00)	-(0.00)
4	Ciprofloxacin(CPR)	5 µ	-(0.00)	13(100.00)	-(0.00)
5	Ofloxacin (OFL)	5 µ	-(0.00)	12(92.31)	1(7.69)
6	Augmentin (AUG)	30 µ	11(84.6)	2(15.38)	-(0.00)
7	Nitrofurantoin(NIT)	300 µ	5(38.46)	8(61.54)	-(0.00)
8	Ampicillin (AMP)	10 µ	11(84.62)	1(7.69)	1(7.69)

Table 3. Antimicrobial resistance pattern (Phenotype) *E. coli 0157:H7* isolates

Number	Antimicrobial resistant pattern(ARP)	ARP frequency (%)
2	AUG, AMP (2)	2 (15.4)
4	CAZ, CRX, AUG, AMP (4)	4 (30.8)
5	CAZ, CRX, AUG, NIT, AMP (5)	5 (38.5)

Number: number of antibiotics the isolate is resistant to, ARP Frequency (%) = the number of E. coli isolates that have the resistant pattern of the presented antibiotics

Table 4. Antimicrobial sensitivity profile for *Salmonella typhimurium* isolates

S/No	Presumptive isolate	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
	Sensitivity values	≥21	≥18	≥15	≥21	≥16	≥18	≥17	≥17
1	S3	23	-	22	30	30	20	16	16
2	S6	20	-	22	34	26	-	-	-
3	S20	24	24	24	36	36	-	25	-
4	S97	25	24	22	42	37	18	28	14
5	S157	-	-	18	30	28	-	10	-
6	S227	-	-	17	28	28	-	21	-
7	S229	-	-	22	24	20	-	25	-
8	S235	-	-	20	24	19	-	23	-
9	S313	-	-	18	34	24	-	-	-
10	S426	-	-	22	34	26	-	-	-
11	S436	-	-	20	40	28	-	-	-
12	S447	-	-	22	30	28	-	10	-
13	S492	-	-	22	40	26	-	-	-

S (Salmonella), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciprofloxacin (CPR), Oflaxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ampicillin (AMP)

Table 5. Antibiogram results of *S typhimurium* isolates

S/NO	Type of antibiotics	Disc potency	Number of isolates, T=13		
			Resistant {n (%)}	Sensitive {n (%)}	Intermediate {n (%)}
1	Ceftazidime (CAZ)	30 µg	9 (69.23)	3 (23.08)	1 (7.69)
2	Cefuroxime (CRX)	30 µg	11 (84.62)	2 (15.38)	0 (0.00)
3	Gentamicin (GEN)	10 µg	0 (0.00)	12 (92.31)	1 (7.69)
4	Ciprofloxacin (CPR)	5 µg	0 (0.00)	13 (100.00)	0 (0.00)
5	Ofloxacin (OFL)	5 µg	0 (0.00)	11 (84.62)	2 (15.38)
6	Augmentin (AUG)	30 µg	11 (84.62)	2 (15.38)	0 (0.00)
7	Nitrofurantoin (NIT)	300 µg	7 (53.85)	5 (38.46)	1 (7.69)
8	Ampicillin (AMP)	10 µg	11 (84.62)	0 (0.00)	2 (15.38)

Table 6. Antimicrobial resistance pattern (phenotype) for *S. typhimurium*

Number	Antimicrobial resistant pattern (ARP)	ARP frequency (%)
1	CRX (1)	1 (7.79)
2	AUG, AMP (1)	1 (7.79)
4	CRX, AUG, NIT, AMP (1) CAZ, CRX, AUG, AMP (3)	4 (30.8)
5	CAZ, CRX, AUG, NIT, AMP (5)	5 (38.5)

Number: number of antibiotics the isolate is resistant to; ARP Frequency (%) = the number of Salmonella isolates that are resistant to the presented antibiotics

*antimicrobial resistant pattern of Salmonella i*solates showed that 5(38.5%) of the isolates had the highest resistant pattern and were resistant to 5 antibiotics namely; Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin (Table 6).

This study showed that, most of the *Escherichia coli 0157:H7* and *Salmonella typhimurium* had multi-drug resistance and majority of the antibiotics were found inactive against them. Out of the thirteen isolated as *E. coli*, nine showed multidrug resistance to at least four antibiotics. Four isolates had multidrug resistance to four antibiotics while six isolates had multidrug resistance to five antibiotics namely; Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin. Thirteen *Salmonella* isolates had multidrug resistance to at least four antibiotics used in the study. Four of the isolates showed resistance to four antibiotics while another six isolates were resistant to five antibiotics.

In both *Salmonella* and *E. coli* isolates Ciprofloxacin and Gentamicin were found to be very active. For *Salmonella* isolates, 13 (100) were sensitive to Ciprofloxacin and twelve (92.31) isolates were sensitive to gentamicin. All the *E. coli* isolates were (100) sensitive to both Ciprofloxacin and Gentamicin irrespective of the eatery type.

4. DISCUSSION

Antimicrobial susceptibility of *Salmonella* strains is useful for epidemiological purposes [21]. In this study, based on the previous literature, resistance to 3 different classes of antibiotics was defined as a multi-drug resistance [22]. Some bacterial strains are now resistant to three or more classes of antimicrobial substances – the currently accepted definition of multi-drug resistance (MDR). CDC reported that while multi-drug resistance declined in *Salmonella* isolates in recent years, this was due to a reduction in numbers of *S. Typhimurium* isolated [23].

Previous reports had indicated that the amount of multidrug resistance ranges between 5-60% [21]. The reasons behind the increasing resistance of species that caused food borne diseases were due to the uncontrolled use of antibiotics on farm animals that resulted in destruction of sensitive bacteria and selection of resistant strains to multiple antibiotics. Through food, these strains could directly infect humans or transfer resistance genes to human endogen [24].

The use of antimicrobial agents in animal production has been identified as an important factor which select for antimicrobial resistant bacterial strains [25]. This might be due to an inevitable genetic response to the strong selective pressure imposed by antimicrobial agents which played a vital role in the evolution of antibiotic resistance among bacteria. These bacteria then pass the plasmid containing resistance gene among other bacterial cells and species [26]. Globally, antimicrobial resistant bacteria resident in the gut of carrier animals contribute significantly to environmental contamination and spread of antimicrobial resistant bacterial strains, hence the need to continuously monitor antimicrobial resistance in zoonotic and commensal bacteria of animal origin for the protection of public health [27-29]. The results of this study indicated highest resistance to Cefuroxime, Augmentin and Ampicillin by *Salmonella* isolates and resistance to Augmentin and Ampicillin by *E. coli* isolates. The most obvious reason that could be responsible for the high frequency of antibiotic resistance was due to the uncontrolled use of antibiotics for various purposes. Previous study observed a similar resistant profile among *E. coli* isolates from Chicken in Malaysia [30]. A local study indicated antimicrobial resistance in Nigeria [31]. This research indicated that two isolates were most sensitive to Ciprofloxacin, 100% sensitivity was observed. Similar study observed that some of the isolates were sensitive to Ofloxacin (100%), Gentamicin (100%), Nalidixic Acid (98%) and Nitrofurantoin (99%) [32]. It was reported previously that *E. coli*

isolates were sensitive to Levofloxacin (80%) and Ciprofloxacin (80%); a few number of isolates were sensitive to Azithromycin (30%) and Nalidixic acid (30%) and resistant to Tetracycline (80%), Ampicillin (90%), Erythromycin (90%), Amoxicillin (90%) and Metronidazole (100%) [32].

Fluoroquinolones were introduced in the 1980s and are fluorinated derivatives of quinolones. In Ciprofloxacin, the ethyl group of Norfloxacin was replaced by a Cyclopropyl group, increasing the spectrum of action to include Gram-positive bacteria and not compromising its activity against Gram-negative bacteria [33]. Fluoroquinolones, as all quinolones, are bactericidal antimicrobials that inhibit the catalytic activity of bacterial DNA gyrase (Topoisomerase II) and Topoisomerase IV, which are essential for the replication and transcription of bacterial DNA [33]. International organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE), as well as regulating authorities, had expressed their concern with the development of resistance in microorganisms that were pathogenic both for humans and animals, including zoonotic agents, such as *Campylobacter spp.* and *Salmonella spp.*, particularly to some antimicrobial classes, including fluoroquinolones [34]. Fluoroquinolones are intensively used in poultry production and have allowed better treatment of several diseases; however, their prudent, ethical, and professional use is essential [35]. The inherent risks of the inadequate use of antimicrobials in poultry production could include the introduction of bacterial resistance, environmental contamination, and the accumulation of residues in poultry products [35]. Consumer union in the US had concluded that overuse of antibiotics in food animals was increasing greatly. Human beings were at risk due to potential presence of the superbug in meat and poultry and its general migration into the environment. Numerous health organizations including World Health Organization, America Medical Association, American Public Health Association, just to mention but a few agreed and had called for a significant reduction in use of antibiotics for food animals, [36]. FDA has banned the use of Ciprofloxacin in 2005 after reported cases of Ciprofloxacin- resistant campylobacter in nearly 20% sampled chicken breast.

Antibiotics are often used for therapy of infected humans and animals as well as for prophylaxis and growth promotion of food producing animals. Many findings suggest that inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult [37]. Antimicrobial resistance in *E. coli* were reported worldwide. Treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents [38].

To deal with multi-drug resistant organisms, it is usually recommended that potentially synergistic antimicrobial combinations be used [39]. The issue of antibiotic resistance is very complex. In many parts of the world, poultry producers have proactively and voluntarily taken steps toward finding alternative ways to control disease while reducing antibiotic use. Two classes of antibiotics, that FDA deemed critically important to human medicine, especially for treating foodborne illness in human beings namely Fluoroquinolones and Cephalosporin, were phased-out of chicken production for a number of years. The FDA's proposed Veterinary Feed Directive (VFD) ensures that all antibiotics are administered to food producing animals under the supervision and prescription of licensed veterinarians [40].

Some commonly used antibiotics had become ineffective in treating infections from resistant bacteria resulting in choosing alternatives that could cause complications and more severe side-effects. It is for these reasons that fluoroquinolones have been banned in poultry production in the US [41]. These drugs were also prohibited in chicken farms in Australia, Finland and Denmark. The World Health Organisation (WHO) had issued warning about the risks for 18 years. There is a full ban of antibiotic use as a growth promoter in the European Union since 2006.

Care must be taken to reduce usage of antibiotics, with a particular focus on those antibiotics considered to be of critical importance for human medicine like the Fluoroquinolones and cephalosporin [41].

Other considerations include, timely clinical inspections to identify and treat sick animals before disease spreads to others; assessment of animal based welfare parameters to maintain a hygienic and healthy living environment; use of laboratory tests to detect animals at risk of developing disease [23].

5. CONCLUSION

This study confirmed that most of the isolated *Escherichia. coli* and *Salmonella* strains were multi-drug resistant. However, both isolated *Salmonella* and *E. coli* strains were susceptible to Ciprofloxacin and Gentamicin.

6. RECOMMENDATION

Based on the outcome of this study, there is need for a careful restrictions of antibiotics use in animals most especially those that are prescribed for human infections also there should be consideration for new food safety regulations and surveillance programs with a priority on molecular subtyping of zoonotic foodborne pathogens. Guidelines for antibiotic distribution in the country should be established by the Federal Ministry of Health (FMOH) and the National Agency for Food and Drug Administration and Control (NAFDAC) respectively. More research should be carried out on a possible and sustainable alternative to antibiotics from readily available sources. It is important that all poultry farms should be duly registered and representative of the regulatory body within the locality should examine samples from poultry farms to ensure the birds are antibiotic free before being sold or slaughtered. Finally, a one health approach should be deeply considered.

CONSENT

The study was explained to the Manager of the eateries who permitted the commencement of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table A1. Microbial identification of *E. coli isolates*

S/No	Presumptive isolates	SMAC+BCIG+CT BCG reaction pale (-)	SMAC+MUG MUG reaction No fluorescence (-)	Biochemical reactions			
				INDOLE Red/pink ring (+)	UREA Slant Pale (-)	S/Citrate Green (-)	Motility (+/-)
1	E13	-	-	+	-	-	+
2	E20	-	-	+	-	+	+
3	E24repeat	-	-	-	+	+	-
4	E42	-	-	+	+	-	-
5	E48	-	-	+	+	-	-
6	E113	-	-	+	+	+	+
7	E118	-	-	+	-	-	-
8	E121	-/+	-	+	-	-	-
9	E123	-/+	-	+	-	+	-
10	E128	-	-	+	-	+	+
11	E134	-	-	+	-	+	-
12	E135	-	-	+	-	+	-
13	E136	-	-	+	-	+	-
14	E137	-	-	+	-	+	+
15	E138	-	-	+	-	+	-
16	E147	-	-	+	-	+	-
17	E154	-	-	+	-	+	+
18	E165	-	-	+	-	+	-
19	E181	-	-	+	-	+	+
20	E183	-	-	-	-	+	+
21	E184	-	-	+	-	+	-
22	E185	-	-	-	-	+	-
23	E188	-	-	+		+	-
24	E215	-	-	+		+	+
25	E222	-	-	+	-	+	+
26	E229	-	-	+		+	+
27	E230	-	-	+	-	-	+
28	E264	-	-	+	-	-	+

S/No	Presumptive isolates	SMAC+BCIG+CT BCG reaction pale (-)	SMAC+MUG MUG reaction No fluorescence (-)	Biochemical reactions			
				INDOLE Red/pink ring (+)	UREA Slant Pale (-)	S/Citrate Green (-)	Motility (+/-)
29	E276(b)	-	-	+	-	-	+
30	E282	-	-	+	-	-	+
31	E284	-	-	+	-	-	+
32	E401	-	-	+	+	+	+
33	E409	-	-	+	--	-	+
34	E445	-	-	+	-	-	+
35	E459	-	-	+	+	+	+
36	E484(b)	-	-	+	-	-	+
37	E487	-	-	+	+	+	+
38	E500	-	-	+	-	-	+

MUG= 4-Methyl Umbelliferyl B D Glucuronide, SMAC= Sorbitol Macconkey Agar
CT= Cefixime Tellurite, BCIG =5-bromo-4-chloro-3-indolyl-β-D-glucuronide, E= E. coli samples, S/NO= Number of samples

Table A2. Latex agglutination test

S/No	Presumptive isolate latex agglutination test	Latex Agglutination	
		0157(+)	H7(+)
1	E13	+	-
2	E20	Auto agg	-
3	E24repeat	Auto agg	-
4	E42	+	+
5	E48	Auto agg	-
6	E113	Auto agg	-
7	E118	+	-
8	E121	Auto agg	-
9	E123	-	-
10	E128	-	-
11	E134	Auto agg	-
12	E135	Auto agg	-
13	E136	Auto agg	-
14	E137	Auto agg	-

S/No	Presumptive isolate latex agglutination test	Latex Agglutination	
		0157(+)	H7(+)
15	E138	+	-
16	E147	-	-
17	E154(r)	+	+
18	E165	-	-
19	E181	-	-
20	E184	Auto agg	-
21	E188	Auto agg	-
22	E230	-	-
23	E264	-	-
24	E276(b)	+	-
25	E282	-	-
26	E284	+	-
27	E401	-	-
28	E409	+	-
29	E445	-	-
30	E459	+	-
31	E484(b)	-	-
32	E487	+	+
33	E500	Auto agg	-

Autoagg= Autoagglutination, clumping of an individual's cells by his own serum

Table A3. Total positive *E. coli* 0157:H7 isolates

S/No	Presumptive positive strains	Biochemical reactions				Latex agglutination	
		INDOLE Red/pink ring(+)	UREA Slant Pale(-)	S/CITRATE Green (-)	Motility(+/-)	0157 (+)	H7(+)
1	E13	+	-	-	+	+	-
2	E20	+	-	-	+	Auto agg	-
3	E42	+	+	-	-	+	+
4	E48	+	+	-	-	Auto agg	-
5	E118	+	-	-	-	+	-
6	E121	+	-	-	-	Auto agg	-
7	E138	+	-	+	-	+	-
8	E154	+	-	+	+	+	+

S/No	Presumptive positive strains	Biochemical reactions				Latex agglutination	
		INDOLE Red/pink ring(+)	UREA Slant Pale(-)	S/CITRATE Green (-)	Motility(+/-)	0157 (+)	H7(+)
9	E276	+	-	-	+	+	-
10	E284	+	-	-	+	+	-
11	E409	+	-	-	+	+	-
12	E459	+	+	+	+	+	-
13	E487	+	+	+		+	+
14	E500	+	-		+	Auto agg	-

S/Citrate=Simmon Citrate agar slant; Latex agglutination = diagnostic test used to detect H7 and 0157 antibodies in E. coli isolates; (+) =positive, (-) =negative

Table A4. Microbial isolation of *Salmonella enterica* isolates

S/N	Presumptive isolate	Selective and differential media			Biochimical reactions				
		RVS (milky residue)(+ve)	HE (Green-Grey ± black center) (+ve)	XLD (PorY ±black center (+ve)	TSI R/Y±gas±H_2S	UREA pale (–ve)	INDOLE Red ring (+/-)	MOTILITY (+/-)	S/C Blue (+ve)
1	S3	+	+	+	Y	-	-	+	-
2	S6	+	+	+	+	-	-	+	+
3	S20	+	+	+	+	-	-	+	-
4	S92	+	+	+	-	+	-	+	+
5	S96	+	+	+	-	-	-	+	+
6	S97	+	+	+	+	+	+	+	+
7	S98	+	+	+	-	+	-	+	+
8	S111	+	+	+	+	+	-	+	+
9	S117	+	+	+	Y	-	-	+	+
10	S118	+	+	+	Y	-	-	+	+
11	S128	+	+	+	Y	-	-	+	+
12	S139	+	+	+	Y	-	-	-	+
13	S148	+	+	+	+	-	-	-	+
14	S157	+	+	+	Y	-	+	-	+
15	S196	+	+	+	Y	-	+	-	+
16	S227	+	+	+	+	+	-	-	+
17	S229	+	+	+	+	+	+	+	+
18	S235	+	+	+	+	-	+	-	+
19	S313	+	+	+	+	-	-	+	-

20	S426	+	+	+	Y	-	-	+	+
21	S436	+	+	+	+	-	-	+	+
22	S447	+	+	+	+	-	+	-	+
23	S492	+	+	+	+	+	+	+	+

S = Salmonella isolates, RVS = Rappaport-Vassiliadis Soya Peptone (Rvs) Broth, HE= Hektoen Enteric Agar, XLD = Xylose Lysine Decarboxylase (X.L.D.) Agar, TSI= Triple Sugar Iron Agar

Table A5. Serology for presumptive *Salmonella typhimurium* isolates

S/N	Presumptive isolates	*Salmonella* Poly O(Grp A-S)	*Salmonella* antiserum grp B factor4	*Salmonella* poly H(Phase1&2)
1	S3	-		+
2	S6	+	-	+
3	S20	+	-	+
4	S92	+	-	-
5	S96	+	-	-
6	S97	+	-	-
7	S98	-	-	-
8	S111	-	-	-
9	S117	-	-	-
10	S118	-	-	-
11	S148	-	-	-
12	S157	Auto agg	-	Auto agg
13	S227	+	-	-
14	S229	-	+	-
15	S235	+	+	-
16	S313	+	+	-
17	S426	+	Auto agg	Auto agg
18	S43 6	Auto agg	Auto agg	Auto agg
19	S447	+	+	-
20	S492	+	+	-

Poly O (A-S) = O-antisera, Poly H (Phase1&2) = H-antisera

Table A6. Total positive *Salmonella Typhimurium* isolates

S/N	Positive strains	TSI R/Y±gas±H2S	UREA pale (–ve)	INDOLE Redring(+/-)	MOTILITY (+/-)	S/CBlue (+ve)	Poly O Grp(A-S)	Grp B factor 4	Poly H(Phase1&2)
1	S3	Y	-	-	+	-	-	+	+
2	S6	+	-	-	+	+	+	-	-
3	S20	+	-	-	+	-	+	-	+
4	S97	+	+	+	+	+	+	-	-
5	S157	Y	-	+	-	+	Auto	-	Auto
6	S227	+	+	-	-	+	+	-	-
7	S229	+	+	+	+	+	NIL	+	-
8	S235	+	-	+	-	+	+	+	-
9	S313	+	-	-	+	-	+	+	-
10	S426	Y	-	-	+	+	+	Auto	Auto
11	S436	+	-	-	+	+	Auto	Auto	Auto
12	S447	+	-	+	-	+	+	+	-
13	S492	+	+	+	+	+	+	+	-

R/Y = Red/Yellow, H2S = Hydrogen sulphide, S/C = Simmon citrate

Table A7. Frequency of *Salmonella* and *E. coli 0157*:*H7* isolation from selected local governments

S/No	Local government	Eatery code	Total number sampled	Frequency*	
				Salmonella typhimurium	***E coli* 0157:H7**
1	Ibadan North	S1	20	3	2
		Semi 1	20	-	-
		S2	20	-	2
		Semi 2	20	-	-
2	Lagelu	S3	20	1	-
		S4	20	-	1
		Sub1	20	-	2
3	Egbeda	S5	20	1	1
		Semi3	20	-	1
		Sub 2	20	-	-
		Sub 3	20	1	1

S/No	Local government	Eatery code	Total number sampled	Frequency*	
				Salmonella typhimurium	***E coli* 0157:H7**
4	Ibadan South/West	S6	20	-	-
		S7	20	-	1
		S8	20	-	1
		Sub 4	20	1	-
5	Ibadan North/East	S9	20	3	-
		Semi 4	20	-	-
6	Ibadan North/West	S10	20	-	-
		S11	20	-	-
		Semi 5	20	-	-
		Sub 5	20	-	-
7	Oluyole	Semi 6	20	-	1
		S12	20	2	-
		Sub 6	20	1	-
		S13	20	-	1
Total	7	25	500	13	14

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