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Research Article

Prevalence of *Campylobacter* spp. and antibiotics resistant *E. coli* on poultry carcasses and handlers' hands at Ikpa slaughter, Nsukka, Nigeria

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Abstract

Handling and consumption of contaminated meat can lead to food poisoning and acquisition of antibiotics resistance genes. This study determined the prevalence of *Campylobacter* spp. and antibiotics resistant *E. coli* on poultry carcasses and handler's hands at Ikpa slaughter, Nsukka. Swabs collected from carcass surfaces and handler's hands were cultured and isolates were identified with phenotypic and molecular methods. Coliform enumeration, antibiotics susceptibility testing and data analysis were done. Out of 204 samples, 29 (14.2%) were *Campylobacter* positive, consisting of *C. jejuni* (27.6%) and *C. coli* (72.4%) while another 67 samples yielded 23 (34.4%) positive *E. coli.* Coliform counts were $3.2x10^3$cfu cm^{-2} and $1.06x10^3$cfu $cm^{-2}$for unwashed and washed carcasses, $2.5x10^5$ cfu cm^{-2} and $0.5x10^4$ cfu cm^{-2} for unwashed and washed handlers' hands respectively. *E. coli* was isolated from 45 and 22% of unwashed and washed carcasses respectively and *Campylobacter* from 16% of unwashed carcasses only. One and five sticks swabbing methods yielded *E. coli* in 20.5 and 60.9% and *Campylobacter* in 12 and 83.3% of carcass samples respectively. Only *E. coli* was detected in 10 (90.0%) of the unwashed hands. *E. coli* isolates were multi drug-resistant at 80% with index >0.2 and a range of 70 -100% resistant to enrofloxacin, tetracycline, erythromycin, streptomycin and ampicillin; 30 - 50% to gentamycin, chloramphenicol and ciprofloxacin while; 10% were to cephtriazone and amoxicillin/clavulanic acid. Prevalence of *Campylobacter* and antibiotics resistant *E. coli* spp. were significantly associated ($P<0.05$) with status of processor's hands and swabbing methods hence, the public health risk and the need for improved hygiene.

Keywords: antibiotics resistant; *Campylobacter* spp.; *E. coli*; humans; poultry; zoonosis

Introduction

Campylobacter and *E. coli* species are among the leading causes of food borne illnesses worldwide (Altalhi *et al.*, 2010; Colles *et al.*, 2016). Different species of thermotolerant *Campylobacter*, particularly *C. jejuni* and *C. coli* as well as antibiotics resistant *E. coli* and Shiga-toxin producing strains have been isolated in

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animals and food of animal origin (Lindqvist and Lindblad, 2008; Altalhi *et al.*, 2010; Made *et al.*, 2017). Chicken is one of the highly consumed meat products across the globe, in most of the countries, it is considered among the most affordable poultry species that are slaughtered at home or the slaughterhouses especially during festivity periods (Mpundu *et al.*, 2019). However, it serves as reservoir hosts for *Campylobacter* and *E. coli* species hence, contact and consumption of contaminated poultry have been the most frequently reported risk factor for the infections in humans (Altalhi *et al.*, 2010; Levallois *et al.*, 2014). This can occur mostly during poultry processing in the slaughterhouses as a result of carcasses having contact with fecal materials, contaminated equipment, tables and as well, cross contamination from workers (Zailani *et al.*, 2016). Different prevalence rates of *Campylobacter* species in poultry products at both the slaughter unit and retail outlets have been reported (Rodrigo *et al.*, 2005; Rosenguist *et al.*, 2006). The ingestion of *Campylobacter* cells as few as 500 cells in a contaminated meat can lead to campylobacteriosis hence, the inclusion of the agent in the European Directive 2003/99/EC on monitoring of zoonotic agents at all levels of the food chain (Anonymous, 2003). The species have caused about 2.5 million cases of food poisoning in USA (CDC, 2010). The UK, Food Standards Agency (FSA) has reported 72.9% *Campylobacter* species prevalence and more than 10,000 CFU g^{-1} in fresh whole retail chickens (FSA, 2014). On the other hand, *E. coli* species which are usually used as an indicator of feacal contamination and in the assessment of hygienic practices in abattoirs, have been found in poultry meat at different prevalence rates but more in developing countries including India with 98% (Sharma *et al.*, 2015).

E. coli contaminated poultry meats could serve as a foremost threat to human health through the transfer of antibiotics resistant gene to other bacteria of clinical importance. Moreover, the survival ability of *E. coli* as common flora in gut of both animals and humans has outfitted it as a vehicle for such transfer and this is of grave consequences especially if the pathogens are of high virulence (Schroeder *et al.*, 2004). This is added to the fact that antibiotics are wrongly used in the developing countries where resistance have been related with prophylactic usage, wrong dosage, substandard drugs and absence of regulation and control (Nwankwo *et al.*, 2018; Aworh *et al.*, 2019). In Nigeria, out of 66% antibiotics prescription rate, only 1.2% was done according to treatment guidelines (Olayinka *et al.*, 2020). Moreover, cephalosporins which has been classified among the critically important antibiotics in humans, also used in poultry, was the most prescribed class (WHO, 2017; Olayinka *et al.*, 2020). This could lead to high selection pressure on the entero-bacteria like *E. coli* which has a broad range of both pathogenic and commensal strains that can harbor antibiotic resistance traits of public health interest (Van den Bogaard *et al.*, 2001; Nsofor *et al.*, 2013). In Saudi Arabia, high antibiotics resistance rates of 78.4%, 70.3%, 48.6% against Ampicillin, Nalidixic acid and streptomycin respectively have been reported among *E. coli* species from poultry (Altalhi *et al.*, 2010) and among poultry farmers and processors (Van den Bogaard *et al.*, 2001). Nsofor *et al.* (2013) had also reported high prevalence of antibiotic resistant *E. coli* in both humans (85.7%) and animals (53.5%) which were related to over dependence on antibiotics in poultry production. Moreover, there are potentials for cross-contamination between raw meats and individuals or surfaces in the kitchen environment and the subsequent risk of contamination in ready-to-eat foods as well as transfer of resistant genes to humans through contacts and dietary exposure (Humphrey *et al.*, 2001; Luangtongkum *et al.*, 2009).

Food borne infections caused by *Campylobacter* and *E. coli* species are among the most common public health issues with grave consequences including acute gastroenteritis and other complications. However, the epidemiology of the infections as well as spread of antibiotics resistance species via handling of raw meat poultry have not been fully elucidated (Burgess *et al.*, 2005). The study therefore, was aimed at determining the prevalence of *Campylobacter* species and antibiotics resistant *E. coli* on poultry carcasses and handler's hands at Ikpa slaughter, Nsukka, Nigeria.

Materials and Methods

Study area

The study was conducted in Nsukka which is located at coordinates of 6°51′24″N and 7°23′45″E (Obiorah *et al.*, 2016). It has a tropical climate that support livestock farming. It has 3 slaughterhouses; Ikpa, Orba and Obollo. The Ikpa slaughter which was used in the study serves as the major converging point for the household communities who either sell or buy chicken for local consumption. Majority of the processed chicken are transported to the neighboring state of Kogi, where they are used for different food delicacies and in the preparations of commercial chicken 'suya' (Figure 1).

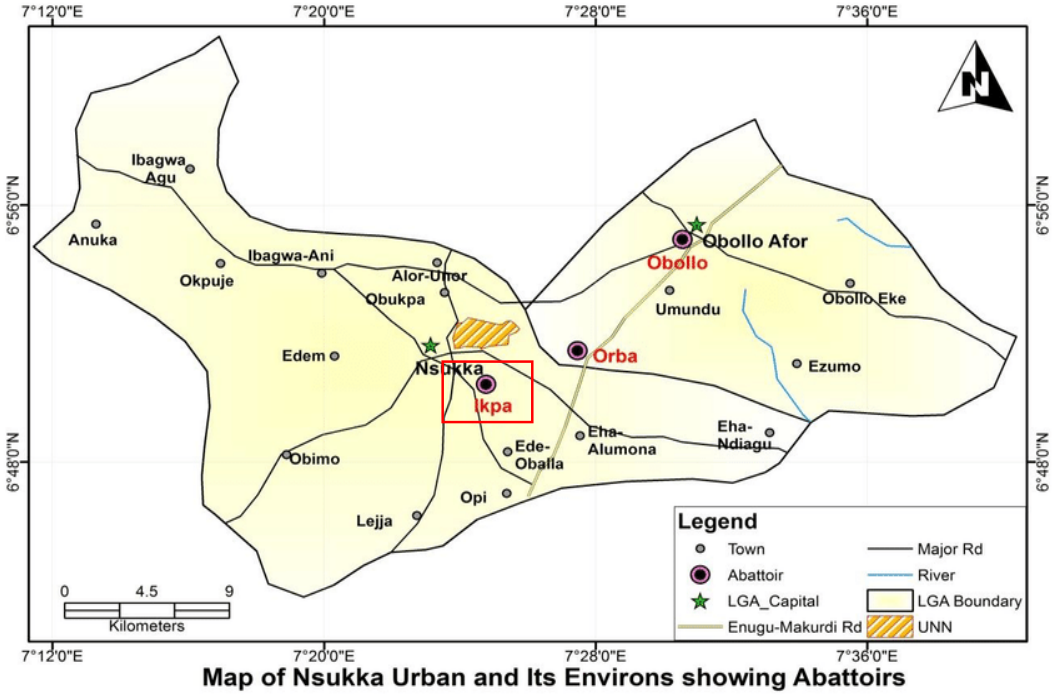

Figure 1. Map of Nsukka urban with red rectangle showing the study site (Ikpa slaughter)
Source: Modified from Obiorah *et al.* (2016)

Sample size determination

The minimum sample size was calculated for *E. coli* and *Campylobacter* spp. respectively using the formula $N=Z^2p\ (1-p)/d2$ (Thrusfield, 2005). Where: N = Sample size; Z = 1.96 (Standard error), d = Level of precision (5%), P = 12.75% and 4.1% (known prevalence for *Campylobacter* and *E. coli* species) respectively (Braide *et al.*, 2017; Chah *et al.*, 2018). A minimum sample size of 170 and 60 were for *Campylobacter* and *E. coli* species respectively as shown below; *Campylobacter* = 3.841x0.1275x0.8725/0.0025 = 170 samples, *E. coli* = 3.8416x0.041x0.959/0.0025 = 60 samples. However, in order to increase the level of precision, 204 and 67 carcass swabs were used for *Campylobacter* and *E. coli* species respectively while 22 hands surface swabs of processors that volunteered were equally assessed.

Sampling and sample collection

Visits were made to the Ikpa slaughter and 5 carcasses (unwashed with water and washed) were systematically (1 in 5) sampled every 2 weeks during poultry processing from May to November 2019. Five sterile swab sticks per carcass were rubbed firmly with slow rotation at 5 sites on each carcass covering a total area of 4 cm^2 including the skin of the neck and each suspended in 5 ml of peptone water. Swabs were equally

obtained using one sterile swab stick per carcass as in the processors' hands. The samples were transported in an ice packed container within 2h of collection to the Dept. of Veterinary Public Health and Preventive Medicine Lab, University of Nigeria, Nsukka where the samples were processed.

Phenotypic identification

For *Campylobacter*, samples were cultured on modified charcoal, cefoperazone deoxycholate agar (CCDA) with selective supplement, (Oxoid, Basingstoke, UK) at 42°C for 48 h under microaerobic condition generated by Campygen^{R} (Oxoid). Pure colonies appearing discrete, whitish and glistening were further subjected to biochemical analysis as previously described (Nwankwo *et al.*, 2017). For *E. coli*, suspected colony growths on MacConkey agar (Oxoid) were selected and purified by sub culturing aerobically on MacConkey agar (Oxoid) at 37 °C for 24 h. Pure typical colonies were then inoculated on eosin methylene blue agar (EMB) (Oxoid, England) for isolation of greenish metallic sheen colonies.

Total coliform count

Samples in 5ml peptone water were pulled together and tenfold dilution was made by dispensing 1ml into a tube containing 9ml of peptone water and subsequently till the tenth (10^{-1} to 10^{-10}). The diluted samples (0.1ml) were inoculated on MacConkey agar (Oxoid) and incubated aerobically at 37 °C for 24 h. The population of the lactose fermenters appearing as pinkish colonies was determined by counting the colony forming unit (cfu ml^{-1}) with colony counter and the mean recorded (cfu cm^{-2}).

Storage of isolates

Campylobacter isolates were kept in storage media (85% tryptone soya broth and 15% glycerol) at -20 °C prior to Polymerase Chain Reaction (PCR) analysis while pure colonies of *E. coli* were selected from individual samples and plated on nutrient agar slant prepared overnight and incubated at 37 °C for 24h, before storage at 4 °C (Nwankwo *et al.*, 2017).

Identification by Multiplex PCR

The genomic DNAs were extracted from the harvested cells of individual colony cultures using the Chelex extraction method. Briefly; harvested cells of each colony were re-suspended in 0.2 ml (200 µl) of Phosphate Buffered Saline (PBS), vortexed and centrifuged at 13000 rpm for 15min. The supernatant was discarded and 200 µl of 10% Chelex suspension was added and incubated at 57 °C for 15 min and vortexed for 10 sec, incubated at 100 °C for 8 min and vortexed again for 10 sec. It was centrifuged at 13000 rpm for 3 min and chilled immediately at -80 °C. The PCR primers (F 5'CCATAAGCACTAGCTAGCTGAT 3') and (R 5'CCA TAA GCA CTA GCT AGC TGAT3') for *C. jejuni*; (F 5' GTAAAACCAAAGCTTATCGTG 3) and (R 5' TCCAGCAATAGTGTGCAATG 3) for *C. coli*; (F 5' CCCCCTGGACGAAGACTGAC 3') and (R 5' ACCGCTGGCAACAAAGGATA 3') for *E. coli* (Inqaba Biotechnology Company, Pretoria, South Africa) were used to amplify the 16S rRNA (Moreno *et al.*, 2001; Yamazaki-Matsune *et al.*, 2007). The reaction of each sample was made up with the specific primer pair (1 µM each primer), 2X Polymerase Chain Reaction (PCR) Master Mix (QIAGEN), 100 ng of DNA template, and nuclease free water to 25 µl. The amplification was done in a DNA thermal cycler (Eppendorf) with an initial denaturation of 1.5 min at 95 °C, followed by 30 cycles of denaturation (0.5 min at 95 °C), annealing (0.5 min at 58 °C), and elongation (0.5 min at 72 °C) and final extension at 72 °C for 7 min (Wang *et al.*, 2002; Nwankwo *et al.*, 2017). Agarose gel (1.5%) electrophoresis was done in trisboric acid/EDTA (TAE) buffer which contained 5 µl of ethidium bromide (5 mg/ml) at 120 volts for 40 mins. Reference strains of *E. coli, C. jejuni* and *C. coli* from National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria were used as positive controls while sterile water was used as negative control. Band views of 323, 126 and 401 base pairs (bps) for C. *jejuni*, C. *coli* and *E. coli* respectively were visualized using the GelDoc-It™ 310 Imaging System (UVP, Cambridge, United Kingdom).

Antibiotics susceptibility testing

Ten antibiotics from eight different classes based on WHO (2017) recommendation list of Critically Important Antimicrobials for Human and animals were used (WHO, 2017). The ten antibiotics were: ampicillin, 10 µg; ciprofloxacin (CIP), 5 µg; erythromycin (E), 15 µg; tetracycline (TE), 30 µg; gentamicin (CN), 10 µg; streptomycin (S), 10 µg; enrofloxacin (ENR), 5 µg; ceftriazone (CEF), 30 µg; amoxicillin and clavulanic acid (AMC) 30 µg and chloramphenicol (CHL) 30 µg. Representative isolates were subjected to an antibiotic susceptibility test by employing the disc diffusion test method following Clinical and Laboratory Standards Institute (CLSI, 2010). The classifications of the isolates were done as resistant and sensitive. Resistance to ≥3 classes of antibiotics was termed Multidrug Drug Resistance (MDR) (Magiorakos *et al.*, 2012).

Data analysis and interpretation

An acceptable count of ≤10 CFU cm^{-2} (1 log) for *E. coli* count was adopted and used in the interpretation of the total coliform count (TCC) (NDVQPH, 2010).The results were presented in tables and Chi-Square was used to analyze the statistical significant relationship between poultry carcass and handlers' hands status, collection methods with contamination of both *E. coli* and *Campylobacter* spp. at $p \leq 0.05$. Data analysis was done using Open Epi Statistics (version 3.0) (Centers for Disease Control and Prevention, CDC).

Results

Out of the 204 and 67 cultured samples, 29 (14%) and 23 (34.3%) were positives for *Campylobacter* and *E. coli* respectively (Table 1). Of the 29 *Campylobacter* isolate, 8 (27.6%) and 21 (72.4%) were C. *jejuni* and *C. coli* respectively. For *Campylobacter*, 28/189 (14.8%) and 0/15 were positive in unwashed and washed carcasses respectively while 18/40 (45%) and 6/27 (22%) were positive for *E. coli* in unwashed and washed carcasses in that order (Table 2).

Table 1. Prevalence of *Campylobacter* and *E. coli* isolates on raw poultry carcasses at Ikpa slaughter, Nsukka, Nigeria

Pathogen (n)	No negative	No positive (%)	OR	95CI	p-value
Campylobacter (204)	175	29 (14.2)	1		
E. coli (67)	44	23 (34.3)	3.15	1.66 – 5.98	0.00

Table 2. Carcasses hygiene status and the detection of *Campylobacter* and *E. coli* isolates at Ikpa, slaughter, Nsukka, Nigeria

Pathogen	Sample source (n)	No +ve (%)	No -ve	OR	95%CI	p-value
E. coli	Washed carcass (27)	6 (22.22)	21	1		
	Unwashed carcass (40)	18 (45.00)	22	2.86	0.95 - 9.61	0.09
Campylobacter	Washed) carcass (15)	0	14	1		
	Unwashed carcass (189)	28 (14.81)	161	2.44	0.31 - 19.26	0.63

Out of the 67 carcass swab samples, 23 were used for coliform count and 17 (73.9%) had coliform growth at dilution 10^{-3} with mean counts of $1.06x10^2$ $cfucm^{-2}$ and $3.2x10^2$cfu cm^{-2} for washed and unwashed carcasses respectively. Three out of 10 (30%) of the hand samples used for coliform count had growth with mean counts of $2.5\ x10^5$ cfu cm^{-2} (unwashed) and $0.5x10^4$ cfu cm^{-2} (washed) (Tables 3 and 4).

Table 3. Total coliform counts of poultry carcass surfaces at Ikpa slaughter, Nsukka, Nigeria

Numbers for unwashed carcass	No of colonies at 10^{-3} dilution	Total coliform count per cm^2	Number for Washed carcass	No of colonies at 10^{-3} dilution	Total coliform count per cm^2
1	4	$2.0x10^3$	15	3	$1.5x10^3$
2	11	$5.5 x10^3$	16	0	0
3	25	$1.25 x10^4$	17	5	$2.5x10^3$
4	32	$1.6 x10^4$	18	4	$2.0x10^3$
5	12	$6.0 x10^3$	19	4	$2.0x10^3$
7	2	$1.0 x10^3$	21	1	$5.0x10^2$
8	0	0	22	0	0
9	0	0	23	0	0
10	0	0	Mean		$1.06x10^2$
11	2	$1.0 x10^3$			
12	1	$5.0 x10^2$			
13	3	$1.5 x10^3$			
14	1	$5.0 x10^2$			
24	1	$5.0 x10^2$			
25	1	$5.0x10^2$			
Mean		$3.2x10^2$			

Table 4. Total coliform counts of handlers' hands at Ikpa slaughter, Nsukka, Nigeria

Numbers for unwashed hands	No of colonies at 10^{-5} dilution	Total coliform count per cm^2	Numbers for Washed hands	No of colonies at 10^{-5} dilution	Total coliform count per cm^2
6	4	$2.0 x10^5$	26	0	0
20	6	$3.0 x10^5$	27	0	0
31	0	0	28	0	0
32	0	0	29	0	0
33	0	0	30	1	$0.5 x10^4$
Mean		$2.5 x10^5$			$0.5 x10^4$

Furthermore, 9/44 (20.5%) and 14/23 (60.9%) were positive for *E. coli* in samples collected with one and five sticks/sites swabbing methods respectively, while 24/198 (12%) and 5/6 (83.3%) were positive *for Campylobacter* with the same collection methods in that same order (Table 5). Out of 12 unwashed hands, 10 (83.3%) were positive for *E. coli* while no positive was recorded from the washed hands even for *Campylobacter* spp. in both washed and unwashed hands (Table 6).

Table 5. Sample collection techniques and detection of *Campylobacter* and *E. coli* isolates on carcasses at Ikpa, slaughter, Nsukka, Nigeria

Pathogen	Sampling collection methods (n)	No +ve (%)	No -ve	OR	95%CI	p-value
E. coli	One swab stick (44)	9 (20.45)	35	1		
	Five swab sticks (23)	14 (60.86)	9	6.0	1.99 - 18.40	0.00
Campylobacter	One swab stick (198)	24 (12.12)	174	1		
	Five swab sticks (6)	5 (83.33)	1	36.2	4.06 - 323.50	0.00

Table 6. Hand hygiene status and the detection of *Campylobacter* and *E. coli* isolates at Ikpa slaughter, Nsukka, Nigeria

Pathogen	Sample source (n)	No +ve (%)	No -ve	OR	95%CI	p-value
E. coli	Washed human palms (10)	0	10	1		
	Un washed human palms (12)	10 (83.33)	2	27	2.34 - 311.1	0.00
Campylobacter	Washed human palms (10)	0	10			
	Un washed human palms (12)	0	12			

Out of 20 *E. coli* isolates, 16 (80%) were resistant to 3 or more antibiotics. A range of 70 -100% resistance was recorded for enrofloxacin (70%), tetracycline (90%), erythromycin (100%), streptomycin (100%) and ampicillin (100%). Cephtriazone and amoxicillin/ clavulanic acid had 10% resistance each followed by ciprofloxacin (30%), chloramphenicol (40%) and gentamycin (50%). The antibiotics resistance profile and multi resistance index are shown in Tables 7 and 8 respectively.

Table 7. Antibiotics resistance profile of *E. coli* isolates from poultry carcass at Ikpa slaughter, Nsukka, Nigeria

Classes of antibiotics	Antibiotics Disk (µg)	*E. coli* isolate N=20	Zone diameter breakpoint (mm) R	Antibiotics Resistance in %
Fluoroquinolones	Ciprofloxacin (5)	6	≤15	30
Macrolides	Erythromycin (15)	20	≤13	100
Aminoglycosides	Streptomycin (10)	20	≤11	100
Fluoroquinolones	Enrofloxacin (5)	14	≤17	70
Penicillin	Ampicillin (10)	20	≤13	100
Aminoglycosides	Gentamycin (10)	10	≤12	50
Tetracyclines	Tetracycline (30)	18	≤14	90
Cephalosporin	Cephtriazone (30)	2	≤19	10
Penicillin/ beta-lactamase inhibitors	Amoxicillin/Clavulanic acid (30)	2	≤13	10
Chloramphenicol	Chloramphenicol (30)	8	≤12	40

Note: R= Resistance (CLSI, 2018)

Table 8. Multiple antibacterial resistance patterns and indices of *E. coli* isolates from carcass surface at Ikpa slaughter, Nsukka, Nigeria

No of antibacterial class	Resistance pattern	No of resistant isolates (%)	Total no of isolates (%)	MARI (a/b)
4	ERY, S, AMP, TET	4 (20)	20 (100)	0.4
6	CHL, ERY, S, ENR, CN, TET	2 (10)		0.6
9	CHL, CIP, ERY, S, ENR, AMP, CN, TE	2 (10)		0.8
5	ERY, S, ENR, AMP, TET	4 (20)		0.5
6	ERY, S, ENR, AMP, CN, TET	2 (10)		0.6
4	ERY, S, AMP, AMC	2 (10)		0.4
7	CHL, CIP, ERY, S, ENR, AMP, TET	2 (10)		0.7
8	CHL, CIP, ERY, S, ENR, AMP, CN, TET	2 (10)		0.8

Note: MARI=Multi Antibiotics resistance Index where a=No of antibiotics an isolate is resistant to. b=Total antibiotics the isolate was subjected to (Aworh *et al.*, 2019). Ampicillin (AMP), 10 µg; Ciprofloxacin (CIP), 5 µg; Erythromycin (E), 15 µg; Tetracycline (TE), 30 µg; Gentamicin (CN), 10 µg; Streptomycin (S), 10 µg; Enrofloxacin (ENR), 5 µg; Ceftriazone (CEF), 30 µg; Amoxicillin and Clavulanic acid (AMC) 30 µg and Chloramphenicol (CHL) 30 µg

Discussion

The detection of both *Campylobacter* and *E. coli* species in the study is of great concern as the zoonotic infections has been associated with both public health and economic consequences. The low prevalence rate (14.2%) for *Campylobacter* spp. in this study can be explained by the poor viability of the organism outside the poultry gut when compared with the 36% prevalence reported in poultry feaces in the same study area (Akwuoba *et al.*, 2010). However, the two species; *C. jejuni* and *C. coli* isolated in the study were the pathogenic strains responsible for severe gastroenteritis and other complications in humans both in the developed and developing countries (Nwankwo *et al.*, 2017; Jovanovic *et al.*, 2020). Higher prevalence rates of *Campylobacter* species in live chicken have been reported in many other studies including 38.1% and 39.1% in Malaysia and Nigeria respectively (Nwankwo *et al.*, 2017; Mohamed-Yousif *et al.*, 2019). Moreover, the lower rate on the surface of processed chicken carcasses could be due to the incompatible atmospheric condition and other surrounding pressures that would have caused them in viable but non culturable (VBNC) state (Magajna and Schraft, 2015).

On the other hand, the high prevalence rate (34.4%) for *E. coli* being the most common commensal enteric bacteria in humans and animals has revealed the level of contamination in the slaughterhouse during the poultry processing. Both *Campylobacter* and *E. coli* are zoonotic pathogens of significant public health concern and their prevalence on the surface of processed carcasses can cause further contamination of meat product and consequential infection in humans (Nwankwo *et al.*, 2017). Even though the 36.4% prevalence for *E. coli* was lower than 43.4% and 47.2% in retailed poultry and frozen meat in Oyo state, Nigeria and 38.3% in bison carcasses in USA, it was far higher than 4.7% and 7.5% in hatcheries and farms respectively in Zaria, Kaduna state, Nigeria. This implies that less contaminations may have occurred in the farms and hatcheries and has further shown the significance of hygiene in the abattoirs where high contamination rates have also been reported (Qiongzhen*et al.*, 2004; Raji *et al.*, 2007). The prevalence rate was also higher than 12.75% and 11.1% reported in processed poultry in neighboring states of Imo and Cross River respectively (Ukut *et al.*, 2010; Braide *et al.*, 2017). This may be a reflection of higher hygiene standard in the abattoirs where those studies were conducted.

Since the study has revealed the Total Coliform Count (TCC) of both poultry carcasses and handlers' hands to be above the 10 CFU cm^{-2} permissible limits as recommended in the EU Decision 2001/471/EC (MC Evoy *et al.*, 2004; NDVQPH, 2010), there is need for improved hygiene at Ikpa slaughter in order to reduce the high risk of infections among processors and consumers of affected meat. The lower TCC ($1.06x10^2$ cfu cm^{-2}) value in the washed carcass compared to ($3.2x10^2$cfu cm^{-2}) in the unwashed has further revealed the need for the use of uncontaminated water in the rinsing of poultry carcasses. It was observed that processors usually use the same water in a washing basin without regular changing throughout the day's operation and as such, could not achieve significant reduction. The poor reduction rate was in agreement with that of the TCC range; 5.0 - 6.3 mean log CFU cm^{-2} before washing and 4.6-6.3 CFU cm^{-2} after carcass washing in South Africa (Jaja *et al.*, 2018). Improved and significant reduction in the microbial contamination can further be achieved by washing carcasses over portable running water as contamination of drinking water has equally been reported (Cokal *et al.*, 2011). Furthermore, the high TCC value in the unwashed hands could possibly lead to the spread of *E. coli* infection as workers were seen shaking hands, eating and drinking during the abattoir operation (Adu-gyamfi *et al.*, 2012). The finding of 5 sticks swabbing method as being twice and four times more effective than the one stick swabbing in the isolation process of *E. coli* and *Campylobacter* species respectively, indicates that multiple swabbing is essential in the detection rate of these pathogens especially *Campylobacter* species that requires special conditions and capacities (Nwankwo *et al.*, 2017).

The prevalence of antibiotic resistant *E. coli* in this study may be associated with use-selection pressure and this is of great concern due to the public health and economic implications especially in a country where antibiotics are easily acquired and used with bias in poultry (Kabir *et al.*, 2004). The 100% resistance to AMP, STR and ERY in the study is worrisome since they are used as the first-line agents in the treatment of

colibacillosis in humans and may lead to narrowed option for therapy (Horner *et al.*, 2013). Indeed, there were increase in the pathogen resistance to AMP and STR compared to 48.6% and 78.4% for the antibiotics in that same order as reported in another study (Altalhi *et al.*, 2010). The 90% resistance for tetracycline was in agreement with other studies that have reported high resistant *E. coli* from animal origins including 89.63 % in China and 89% in Tunisia (Soufi *et al.*, 2009; Zhang *et al.*, 2012). Increased resistance from 19 to 30% for ciprofloxacin and 70% for enrofloxacin in the study may be due to the continuous use of flouroquinolone in poultry industry which has been banned in the developed world and this could jeopardize antibacterial therapy in humans (Li *et al.*, 2019).

Furthermore, even though the U.S. Food and Drug Administration has prohibited the use of chloramphenicol in food animals, the increased resistance for both chloramphenicol (40%) and gentamycin (50%), compared to 32.4% and 24.3% in that order as reported in another study can be associated with their uncontrolled uses in developing countries (DHHS, 2010). Amoxicillin/clavulanic acid and cephtriazone had low rates (10%) each and this could be ascribed to their low or more recent usage in poultry industry. The above finding was in agreement with the low resistant rate (8.9%) of *E. coli* to amoxicillin/clavulanic acid in Turkey (Gundogan and Avci, 2013). Many high prevalence of multidrug resistance among *E. coli* as revealed in the study have been reported in chicken and foods of animal origin (Schroeder *et al.*, 2004; El-Enbaawy and Yousif, 2006). Studies have equally established associations between urinary tract infections and gastroenteritis in humans with the consumption of *E. coli* and *Campylobacter* spp. contaminated poultry meat (Manges *et al.*, 2007; Karikari *et al.*, 2017). Hence, there is need for more active surveillance and improved hygiene practices in the slaughterhouses in order to prevent the infections spread and resistant gene transfers to other microbes in the food chain and to humans.

Conclusions

In conclusion, the prevalence of *Campylobacter* species and antibiotics resistant *E. coli* on poultry carcasses as well as handlers' hands as obtained in Ikpa slaughter, Nsukka has revealed the high risk of the infections spread in humans and antibiotics resistance gene transfer among other pathogens of clinical importance through handling and consumption of contaminated poultry meat.

Authors' Contributions

I.O.N conceptualized and designed the research outline with E.V.E. The investigation was conducted by I.O.N under the supervision of E.V.E and J.A.N. The molecular and data analysis was done by B.J.A. and A.J.O. respectively. The manuscript was drafted by I.O.N and reviewed by all the authors before the submission.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Ethical clearance was obtained from the Institutional animal care and use committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Poultry processors were informed and hand swabs were collected only from those that gave their consent after the purpose of the study was explained to them.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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