Prevalence and Antibiotic Resistance of *Escherichia coli* O157: H7 Serotype from Chicken Droppings Produced by Free - Ranged and Poultry Birds in Cross River, Nigeria

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Abstract: This study investigated the prevalence of *Escherichia coli* O157:H7 from chicken droppings produced by free ranged and poultry birds at different locations within Cross River State, Nigeria and their susceptibility to commonly used antibiotics. A total of 360 cloacal swab samples each were randomly collected from poultry (confined) and free ranged (unconfined) chickens. Standard cultural, biochemical, and serological (latex agglutination) methods were used to isolate *E. coli* O157:H7. The isolates were subjected to antimicrobial susceptibility testing using disc diffusion method. Out of a total number of 360 anal swab samples collected from poultry and free-ranged chicken, 24 (6.67%) strayed and 7 (1.94%) poultry samples were positive for *E. coli* O157:H7 and the prevalence values differed significantly ($p<0.05$) among the group of birds. Values for age category among the free-ranged birds also differed significantly ($p<0.05$) with strayed chicks (1-3weeks old) having highest value of 10.89%. Out of the 9 isolates screened, 8 (88.89%) were resistant to tetracycline, 7 (77.78%) to ampicillin and nitrofurantoin and 6 (66.67%) to chloramphenicol. All isolates were resistant to at least one antibiotic. This study reveals that the intestinal track of chicken harbors the bacterial pathogen hence interventions are needed to reduce transmission of *E. coli* O157:H7 via poultry products.

Keywords: *Escherichia coli*, Poultry, Antibiotic Resistance, Poultry Droppings

1. Introduction

Escherichia coli is documented as the most-studied bacterium [1]. The primary and secondary habitats of *Escherichia coli* are the intestinal tract of warm-blooded animals. In poultry, *E. coli* resides in the lower digestive tract, which it colonizes in the first 24h after hatching [2]. *Escherichia coli* O157:H7 is a serotype of the bacterial species *Escherichia coli* and it is an important food borne pathogen of public health importance [3-4].

Poultry is a major fast growing source of food in the world today [5]. Poultry is an essential component of the Nigerian economy, providing income for small-scale farmers and a good source of high quality protein for the ever growing population of Nigeria. In livestock production, poultry occupies a prominent position in the provision of animal protein and this account for about 25% of local meat production in Nigeria [6].

Food pathogens cause more than three-hundred diseases [7-8]. Pathogens can be transmitted to humans directly through contact with poultry litter or indirectly through contaminated poultry products [9] The microorganisms present in the poultry droppings could be due to the contamination of the feed and water used in feeding the poultry fowl, contamination may also be caused by the micro flora of poultry attendants [10].

Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine [11]. Antimicrobial resistance among *E. coli* in food animals such as chicken is of increasing concern due to the potential for transfer of these resistant pathogens to the human population [12]. The intestinal track of poultry contains resistant bacteria and accordingly their faecal flora contains a relatively high proportion of resistant bacteria.

Consequently, the objectives of this study was to determine the prevalence of *Escherichia coli* O157:H7 from chicken droppings produced by free ranged and poultry birds in different locations within Cross River State and their susceptibility to commonly used antibiotics.

2. Materials and Methods

2.1. Study Area

This research was carried out in selected highly populated communities in Cross River State. The state is located in the Niger Delta Region, Southern Nigeria and it occupies an area of $299,10km^2$ and an estimated population of 3,920,208 as at 2006 Nigeria population census [13]. It is bounded to the North by Benue State, to the West by Enugu and Abia States, to the East by the Republic of Cameroon and to the South by Akwa Ibom State and the Atlantic Ocean.

2.2. Sampling Design

The study area was mapped out according to political demarcations i.e. Northern, Central and Southern Senatorial Districts. Each senatorial district was further mapped out into two sampling areas each comprising of two local Government Areas found in the state. Each sampling area was designated SA.

2.3. Sampling Procedures

Sampling was restricted to communities within a particular sampling area during each sampling period. Bi-weekly sampling was performed during each sampling period. A total sampling duration of nine months was utilized with each area sampled three times for isolation of the pathogen giving a total of 18 sampling periods.

2.4. Collection of Feacal Samples from Domestic Birds

A total of 360 cloacal swab samples each were randomly collected from poultry (confined) and free ranged (unconfined) chicken. Five local poultry houses were randomly selected within each sampling area and visits made to obtain approval from the farm owners. Four birds were selected per poultry farm while the stray birds were sampled randomly from selected households during the night periods when they assembled to sleep. Information on the sex and age of each bird was provided by the farmer.

Each swab was immediately placed in Amies transport medium and kept in a flask with frozen ice packs (4°C). The samples were immediately transported to the Microbiology laboratory for analysis within 24 h.

2.5. Sample Processing

About 1.0 ml of each stool samples suspension from transport medium was introduced into 10ml of buffered peptone water containing cefixime (0.05mg/l) and vancomycin (8.0 mg/l) (BPW-CV) and vigorously vortexed for 30 seconds to homogenize the mixture. It was then incubated at 37°C for 24 hours for enrichment.

The Enzyme-linked immunosorbent assay (ELISA) technique was used for the qualitative detection of *E. coli* O157 antigens in all enriched samples [14]. Two drops of each sample was introduced into separate wells until all the required number of wells (excluding the control wells) were used depending on the number of enriched samples to be assayed. Two drops of the positive and negative control solutions were also dropped into their respective wells. The contents were incubated at 37°C for 30 min and 2 drops of the enzyme conjugate added to each well. After a period of 30 min incubation, the contents were washed three times using deionized water and 2 drops of chromogen added to each with gentle shaking. The results were compared to those of the positive and negative control wells. A significant colour change to yellow indicated the presence of the *E. coli* O157 antigen bound by the anti-*E. coli* O157 antibodies impregnated in the wells. An optical density (OD) reading greater than 0.15 also confirmed a positive result.

All enriched samples with positive ELISA results were analyzed using the standard *E. coli* O157:H7 culture technique as recommended by [14]. All enriched, ELISA positive stool samples were serially diluted to 10^{-3} using physiological saline (0.85% w/v NaCl). Approximately 0.1ml of 10^{-2} and 10^{-3} dilutions were spread plated on Sorbitol MacConkey agar supplemented with cefixime (0.5mg/l) and potassium tellurite (2.5mg/l) (SMAC-CT). All cultured samples were incubated overnight at 42°C for 24 h. Sorbitol-negative colonies that appeared colourless to grey on SMAC-CT were considered positive for *E. coli* O157:H7. Three randomly selected suspected colonies were isolated on each plate and separately subcultured on nutrient agar slants and stored at 4°C in a refrigerator.

All positive colonies isolated on nutrient agar slants were further inoculated into test tubes containing *E. coli* with MUG (*E. coli*-MUG) medium and incubated at 42°C for 18-24 h. The broth cultures were then observed under ultraviolet (UV) light of long wavelength (650nm) to detect the inability of *E. coli* O157:H7 to cleave MUG (about 92% of *E. coli* other than *E. coli* O157:H7 produce the enzyme

glucoronidase which cleaves MUG to produce a blue fluorescent product). Positive isolates were considered as those that fermented lactose (yellow broth), produced gas (collected at the tip of the immersed Durham tubes) and did not produce any fluorescence. Other confirmatory biochemical tests typical to *E. coli* such as indole, methyl red, Voges Proskauer, Citrate and Lysine decarboxylase were also performed on the isolates.

Antigen typing was performed using standard *E. coli* O157:H7 antisera (Difco Laboratories, Detroit, Mich.) produced from rabbits and preserved with glycerol using 1:2 dilution. Slide agglutination technique was used to test resuscitated colonies directly from Sorbitol MacConkey agar (SMAC) as recommended by [16]. Colonies that agglutinated rapidly with the *E. coli* O157:H7 antisera were considered as confirmed positive *E. coli* O157:H7 colonies.

2.6. Antimicrobial Susceptibility Testing

The *E. coli* O157:H7 isolates were tested for antimicrobial susceptibility against eleven antibiotics using the disc diffusion method according to the Clinical Laboratory Standard Institute guidelines [17]. *E. coli* ATCC 25922 was used as a reference strain for quality control. The antibiotics disc included Amoxycillin ($48\mu g/ml$), Ampicillin ($32\mu g/ml$), Ceftriaxone ($64\,\mu g/ml$), gentamicin ($16\,\mu g/ml$), cotrimozole ($25\mu g/ml$), tetracycline ($30\mu g/ml$), cefuroxime ($32\mu g/ml$), ofloxacin, ($16\mu g/ml$), ciprofloxacin ($8\mu g/ml$), chloramphenicol ($32\mu g/ml$) and Nitrofurantoin ($32\mu g/ml$).

2.7. Statistical Analysis

The data were analysed with SPSS version 11.0 software (SPSS Inc, Chicago, IL). Percentages were compared using a Pearson chi square (x^2) test for dependent samples or Fisher's extract "t"- test where appropriate. Means were compared using the student "t"-test for dependant samples.

The prevalence of *E. coli* O157:H7 strains from chicken droppings of free ranged and poultry birds were analyzed by statistical comparison of the survival values (log cfu/ml) of all pairs using one-way analysis of variance (ANOVA: Jump in version 4.0.3, SAS institute Inc, Cary, N. C., USA) to determine significant differences at a probability level of 0.05.

3. Results

The percentage prevalence of poultry birds had highest value of 3.33% from sampling areas 3 and 4 while no pathogen was isolated from samples obtained from sampling area 6. No significant difference (p>0.05) was observed in percentage prevalence among the poultry birds from various sampling areas as shown in Table 1.

Free-ranged birds had the highest percentage prevalence of 11.67% from sampling area 3 and least value of 3.33% from sampling area 6. Overall percentage prevalence of 1.94 and 6.67% were obtained for poultry and free-ranged chicken droppings, respectively. Though no significant difference (p>0.05), was observed in the prevalence values among the free-ranged birds, the values between the confined (poultry) and free-ranged birds differed significantly (p<0.05).

The percentage prevalence of *E. coli* O157:H7 in poultry droppings had highest value of 3.33% among the broilers (4-12weeks old) and lowest value of 1.11% among the chicks (1-3 weeks old) and layers (18-72 weeks old) though no significant difference (p>0.05) was observed in the prevalence among the different age categories of the poultry birds.

Faecal droppings from free-ranged chicken had highest percentage prevalence of 10.89% among chicks (1-3 weeks old) and lowest value of 2.22% among the layers (18-72 weeks old). The percentage prevalence among the different age categories of strayed chicken differed significantly at p<0.05. Prevalence values for both poultry and free-ranged chicken droppings according to age category are shown in Table 2.

Table 3 shows the susceptibility pattern of some commonly used veterinary antibiotics. Out of the 9 isolates screened, 8 (88.89%) were resistant to tetracycline, 7 (77.78%) to ampicillin and nitrofurantoin and 6 (66,67%) to chloramphenicol. All isolates were resistant to at least one antibiotic while the highest resistance (72.73%) was obtained with ICD1, ICD5 and ICD7. Total resistance of individual isolates to all antibiotic showed significant difference *(p<0.05)*.

Table 1. Prevalence of Escherichia coli O157:H7 in poultry and strayed chicken droppings from various sampling areas.

Sampling Area	Confined (Poultry)		Strayed	
	No of samples	No of positive samples (%)	No of samples	No of positive samples (%)
SA1	60	1 (1.67)	60	3 (5.00)
SA2	60	1 (6.67)	60	5 (8.33)
SA3	60	2 (3.33)	60	7 (11.67)
SA4	60	2 (3.33)	60	4 (6.67)
SA5	60	1 (1.67)	60	3 (5.00)
SA6	60	0 (0.00)	60	2 (3.33)
Overall prevalence	360	7 (1.94)	360	24 (6.67)

Test of significance at p<0.05

Table 2. Prevalence of Escherichia coli O157:H7 in strayed and confined chicken droppings according to age categories of birds.

Birds category (Age in weeks)	Strayed		Confined	
	Total number of samples	No of positive samples (%)	Total number of samples	No of positive samples (%)
Chicks (1-3)	101	11 (10.89)	90	1 (1.11)
Broilers (4-12)	112	6 (5.36)	90	3 (3.33)

Birds category (Age in weeks)	Strayed		Confined	
	Total number of samples	No of positive samples (%)	Total number of samples	No of positive samples (%)
Layers (18-72)	90	2 (2.22)	90	1 (1.11)
Old layer (>72)	57	5 (8.77)	90	2 (2.22)
Total	360	24 (6.67)	360	7 (1.94)

Test of significance at p<0.05

Table 3. *Antibiotics susceptibility profile of E. coli O157:H7 chicken isolates on some commonly used antibiotics.*

Antibiotics disc content in µg/ml	Diameter of inhibition (mm)	ICD1	ICD2	ICD3	ICD3	ICD4	ICD5	ICD6	ICD7	ICD8	ICD9	Total resistance (%)
AMP	32	17	12 (R)	0 (R)	0 (R)	19 (S)	0 (R)	11 (R)	6 (R)	19 (S)	9 (R)	7 (77.78)
AMX	48	18	0 (R)	20 (S)	21 (S)	23 (S)	6 (R)	25 (S)	19 (S)	27 (S)	11 (R)	3 (33.33)
CEF 1 (11.11)	32	17	22 (S)	18 (S)	23 (S)	28 (S)	10 (R)	24 (S)	20 (S)	19 (S)	21 (S)	1 (11.11)
CFT 5 (55.56)	64	35	38 (S)	28 (R)	37 (S)	39 (S)	21 (R)	29 (R)	32 (R)	36 (S)	30 (R)	5 (55.56)
CLM 6 (66.67)	32	17	0 (R)	14 (R)	8 (R)	10 (R)	19 (S)	23 (S)	9 (R)	21 (S)	11 (R)	6 (66.67)
COT 5 (55.56)	25	16	5 (R)	11 (r)	19 (S)	7 (R)	0 (R)	21 (S)	0 (R)	18 (S)	23 (S)	5 (55.56)
CPF 2 (22.22)	8	21	24 (S)	22 (S)	27 (S)	26 (S)	18 (R)	29 (S)	15 (R)	31 (S)	21 (S)	2 (22.22)
GEN 5 (55.56)	16	15	10 (R)	0 (R)	18 (R)	20 (S)	8 (R)	17 (S)	6 (R)	23 (S)	0 (R)	5 (55.56)
NTF 7 (77.78)	32	17	15 (R)	13 (R)	8 (R)	0 (R)	27 (S)	15 (R)	0 (R)	19 (S)	12 (R)	7 (77.78)
OFX 2 (22.22)	16	21	19 (R)	23 (S)	27 (S)	29 (S)	24 (S)	14 (R)	28 (S)	30 (S)	33 (S)	2 (22.22)
TET 8 (88.89)	30	19	0 (R)	11 (R)	17 (R)	8 (R)	10 (R)	13 (R)	15 (R)	15 (R)	22 (S)	8 (88.89)
% Resistance 51 (51.52)			72.73	63.64	36.36	36.36	72.73	45.45	72.73	9.09	54.55	51 (51.52)

Test of significance at p<0.05

KEYS: AMP = AmpicIllin; AMX = Amoxicillin; CEF = Cefurantoin; CFT = Ceftriazone; CLM = Chloramphenicol; COT = Cotrimoxazole; CPF = Ciprofloxacin; TET=Tetracycline; GEN = Gentamicin; NTF = Nitrofurantoin; OFX = Ofloxacin; S = Sensitive; R = Resistant

4. Discussion

This study confirmed that confined and free-ranged chicken droppings were found to harbor *E. coli* O157:H7 with overall prevalence of 1.94% and 6.67%, respectively. Though no significant difference (*p>0.05*) was observed among the strayed birds, values between the confined and free-ranged birds differed significantly (*P<0.05*). This may be due to regular administration of antibiotics by which most poultry farmers tend to protect the birds against infection. Ojo *et al*. confirmed the presence of *E. coli* O157:H7 strains in the faeces of poultry sampled from different farms in Nigeria [18]. In a similar study, [19] also reported 13 and 14% level of *E. coli* O157:H7 from Lagos and Ibadan poultry farms, respectively.

In another study conducted in Nigeria by [20], the authors reported that *E. coli* was the most prevalent bacteria (53.50%) isolated from Poultry droppings in Akure, Ondo State, Nigeria. A similar study conducted by [21] also reported that out of 194 cloacae samples examined for *E. coli* O157:H7 from Poultry farms in Ethiopia, 13.4% were found to be positive for *E. coli* O157:H7. These variations may probably be due to the sampling techniques, geographical location, hygiene of the poultry farms, or the sensitivity of the microbiological technique used for isolation of the bacteria.

Significant difference (*p<0.05*) was also observed in *E. coli* O157:H7 prevalence among the various age groups in free-ranged birds with the chicks (1-3weeks old) having the highest prevalence of 10.89%. This agrees with the findings of [21] who reported a higher prevalence of 18.8% among young birds (less than six months) than adult birds with a prevalence of 7.5%. This high prevalence among chicks between 1-3weeks old may be due to low immunity associated with young birds since all groups obtain food from the same source and were exposed to the same environmental conditions. Chadran and Mazumder observed that 93 *E. coli* isolates obtained from cloacal swabs and fresh droppings of 8 avian birds in British Columbia, Canada were carriers of *stx*2-gene [22]. Poultry meat has been shown to carry *E. coli* O157:H7 on its surface and the bacteria do persist in the caeca of experimentally infected chicks for as long as 11 months.

The antimicrobial susceptibility tests revealed that out of the 9 isolates screened against 11 commonly used veterinary antibiotics, there was resistance to tetracycline, ampicillin, nitrofurantoin and chloramphenicol. This agrees with the separate findings of [23-26] who reported resistance to these panel of antibiotics in poultry birds in China and Nigeria respectively.

5. Conclusion

The prevalence of *E. coli* O157:H7 was examined among poultry birds and free - range birds in Cross River State, Nigeria. This study confirms that confined and free-ranged chicken droppings harbor *E. coli* O157:H7 with the highest prevalence found among droppings of the free- ranged birds. All isolates of *E. coli* O157:H7 also exhibited antimicrobial resistance to a minimum of one antibiotics used in this study. Due to the relative increase in the consumption of chicken meat in the study area, extensive research is still required to establish the risk involved and to better understand the mechanisms of bacterial resistance to antimicrobial agents with emphasis on *E. coli* O157:H7 isolates. The cautious use

of antibiotic is recommended, and alternative means of therapy like the use of probiotics should be employed to reduce antimicrobial resistance.

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