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Research Article

Prevalence and Antibiotics Sensitivity of *Escherichia Coli* O157:H7 In Table Eggs from Poultry Farms in Ibadan, Oyo State, Nigeria

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ABSTRACT

The occurrence of antibiotics resistant food borne pathogens continues to be a growing concern to the food industry. However, as eggs continue to be a source of cheap protein, there are few information on the prevalence of these antimicrobial resistant *Escherichia coli* O157:H7 in table eggs. Therefore, this study was designed to determine the occurrence and antibiotic sensitivity profile of *Escherichia coli* O157:H7 in table eggs from poultry farms in Ibadan, Oyo State Nigeria. Eggshells and contents of 360 table eggs were sampled purposively from 12 farms (2 farms per local government) in Ibadan. Enumeration for *Escherichia coli O157:H7,* total aerobic bacteria and coliform counts was performed using standard procedures. The antibiotics sensitivity test was carried out using the Kirby Baeur disc diffusion method. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. The prevalence of *Escherichia coli* O157:H7 was 7(9.8%) which was entirely from egg shell. The Total aerobic bacteria count (Mean Log CFU) was 1.43 ±1.65 and 4.95 ± 0.24 for egg contents and shell, respectively, and the Total coliform count was 1.36±1.46 and 4.84 ± 0.33 for egg contents and shell, respectively. All isolates were resistant to Ampilillin, Ceftadizine, Cefuroxime and Amoxicillin. In all 100% was multidrug resistant. The isolates were mostly susceptible to Ciprofloxacin (87.5%). and Ofloxacin (87.5%). Improvement in the hygienic conditions of poultry farms and control of the misuse and overuse of antibiotics in poultry is therefore strongly recommended.

Keywords: *Escherichia coli O157:H7, Table eggs, Prevalence, Antibiotics Sensitivity*

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INTRODUCTION

The consumption of table eggs continues to increase worldwide as eggs are a source of cheap protein (Eid *et al.*, 2015). According to the United States Department of Agriculture, Nigeria is the leading country in Africa. Egg production and egg consumption in Nigeria is increasing daily as poultry production is considered the fastest growing economy in Nigeria due to its contributions to food security in the provision of protein (meat and egg) and other essential nutrients in man as well as its fast returns in investments (Mottet and Tempio, 2017; Anang *et al*., 2013; USDA, 2103). Eggs can be contaminated through shell indicating horizontal transmission or transovarially indicating vertical transmission (Elafify *et al.*,2016). Facal contamination of eggshell and leads to presence of Coliform and Enterobacteriacea which gives indication about the hygienic quality of egg (Jambalang, 2017). Also, a lot of intestinal pathogens have been isolated from eggs especially *Escherichia coli* and *Salmonella* (Al Momami *et al.*, 2018). These pathogens may cause food borne illnesses which is of serious public health importance since it may lead to asymptomatic or life-threatening infections (Al Momami *et al.*, 2018; Adeyanju, and Ishola, 2014).

Escherichia coli are Gram-negative, facultative anaerobic, motile, catalase positive, oxidase negative, non-spore forming rods. They also reduce nitrate to nitrite and produce acid from the fermentation of glucose (Sophie and Ruiting, 2014). *Strains of E. coli* that causes harmful effect on humans includes as enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enteroinvasive (EIEC), Shiga toxin-secreting (STEC), Enterotoxigenic (ETEC), diarrhea-associated hemolytic (DHEC), enteroaggregative (EAAggEC) and cytolethal distending toxin recreating (CDTEC) (Gonzales *et al*., 2013). Infections with EHEC causes hemorrhagic colitis and hemolytic uremic syndrome that leads to thrombocytopenia and renal injuries (CFSPH, 2016). *E. coli*

O157:H7 which is the most virulent member of the EHEC and STEC can cause severe diarrhea which are sometimes bloody and haemolytic ureamic syndrome leading to kidney damage (CDC, 2016; Okorie-Kanu *et al.*, 2016).

Antibiotics are medicines used to prevent and treat bacterial infections (WHO, 2016). Antibiotics are used in poultry farms to increase feed conversion ratio, to promote growth of birds, to prevent, treat and control diseases and to combats stress from environmental changes (Kalia *et al.*, 2017; Mubito *et al.*, 2014). The frequent exposure to antibiotics has provided has led to the emergence of antibiotics resistance strains as Antibiotics are administered to poultry in food and water almost throughout their life cycle (Zaman *et al.*, 2017). Antibiotics resistance is the ability of a microorganism to stop an antimicrobial agent from working against it, thereby leading to ineffectiveness of standard treatments, persistence and spread of infections (WHO, 2017b). The spread and emergence of resistance is aggravated in places without standard treatment guidelines where antibiotics are over-prescribed by health workers and veterinarians (WHO, 2016). The effect of antimicrobial resistant genes in poultry and the possible transmission to human's bacteria through animal food-borne pathogens cannot be emphasized, it causes treatment failure in both animals and man leading to increased cost of treatment and general loss (Bengtsson *et al.*, 2014, Wegener, 2012). This study was therefore design to assess the total bacterial and coliform contamination, prevalence and antibiotics susceptibility profile of *E. coli* O157: H7 in the shell and contents of eggs in Ibadan, Oyo,state, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Ibadan, located in the South-Western part of Nigeria and the capital city of Oyo State, with co - ordinates $7_0 23^{\cdot} 47''$N $3_0 55'0''$E. Ibadan is the most populous city in Oyo State with over 3 million inhabitants. There are 11 local governments in Ibadan.

Sampling Method

The sampling was purposive based on the numbers of registered farm in each local government and presence of farm in urban areas. Samples were taken from the following local government areas: Akinyele, Egbeda, Lagelu and Ido (Local government areas with high numbers of farms) as well as Ibadan south west and Ibadan North local government areas (Farms located in urban areas).

Sample Collection and Preparation

Thirty freshly laid unbroken eggs each were collected from 12 different poultry Farms (n=360) aseptically into sterile nylon bags on ice packs and was taken to the Laboratory within 6 hours for microbiological analysis. A total number of seven eggs were pooled to form one composite. Sterile swab sticks were used to clean the egg shells and was dissolved in 9ml of sterile distilled water. To culture from the egg contents, 70 % ethanol was used to disinfect the egg shell and was flamed using a Bunsen burner. The eggs were then broken using sterile forceps and the thoroughly mixed with a sterile glass rod. 1ml of the mixed egg was then transferred into 9ml of sterile distilled water.

Total Aerobic Bacterial and Total Coliform Count

The distilled water containing both the egg shell and egg content were serially diluted to five folds. Total Anaerobic and Total Coliform counts were done by placing 0.1ml of the serially diluted samples in Nutrient agar and MacConkey agar and it was incubated at 37_0 C for 24 hours and the plates were counted using a colony counter counts were expressed in Log_{10} colony forming units per ml.

Isolation of *E. coli* O157:H7

Isolation of *E. coli* O157:H7 was done according to the modified procedure of International Standard Organization. Pre-Enrichment of *E coli* O157:H7 was done by placing 5ml of the 10_0 dilution into 45ml of modified Tryptone soy broth supplemented with Novobiocin and incubated at 37_0C for 24 hours. SMAC (Sorbitol MacConkey agar) was used as the selective media for the detection of *E. coli* O157. SMAC was inoculated by placing 0.1ml of the broth cultures into the plates and it was incubated at 37_0C for 24 hours. The colourless distinct colonies were then sub cultured into nutrient agar for biochemical tests

Biochemical Characterization

Gram staining, catalase test, indole test, Voges Proskaeur-Methyl Red test and Sugar Fermentation test were carried out according to the standard procedure of Cheesebrough, (2006).

Serological confirmation of *E.coli* O157:H7

Suspected strains of *E..coli* O157 based on the Biochemical characterization were emulsified in loop full phenol saline on a slide and two drops of Oxoid DR0620M *E.coli* O157 latex were added to it and mix with a loop. It was then observed for agglutination which indicates a positive result.

Antibiotics Sensitivity Test

The Antibiotics Sensitivity was done according to the standard procedure on Mueller–Hilton agar using Kirby Bauer disk diffusion method (Shanthi *et al*., 2018) consisting of multidisc of Ampicillin (10µg), Ceftazidime (30µg), Cefuroxime (30µg), Gentamycin (10µg), Nitrofurantion (300 µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Amoxycillin/Clavulanate (30µg).

Preparation of Inoculum

Mueller Hinton broth was used to grow overnight culture of the isolates at 37°C and adjusted until the visible turbidity is equal to that of a 0.5 McFarland standard (Shecho *et al.*, 2017).

Inoculation on Plates

Sterile cotton swab was dipped into the adjusted culture and inoculated on Mueller Hinton agar plate by streaking the entire surface of the plate with the swab. The plates were allowed to dry before and antibiotics discs were applied.

Measurement of Zone of Inhibtion

Zones of inhibition were measured in mm using a transparent ruler and the result was interpreted according to the Clinical

and Laboratory Standards Institute (CLSI) Standards for Antimicrobial Susceptibility Testing (CLSI, 2016). The experiments were carried out in triplicates and *E.coli* ATCC 35218 was used as control.

Data Analysis: The total aerobic bacterial and total coliform counts were expressed in mean Log CFU/ml. The prevalence of *E. coli* O157:H7 was expressed in percentage. Data were analyzed using descriptive statistics and ANOVA (SPSS version 20.0 for windows).

RESULTS

Prevalence of *E. coli* O157: H7: The total prevalence of *E. coli* O157:H7 was 7 (9.8 %), with the highest prevalence found in Egbeda Local Government 3(4.2%), and followed by Akinyele and Ibadan South West Local Government areas 2 (2.8%). All the *E. coli* O157:H7 isolated were from the egg shell (Table 1).

Table 1:
Prevalence of *E. coli* O157: H7 in different Local government in Ibadan.

Source/LGA	No Of Composite Samples	Total No Of Isolates On Egg Shell (%)	Total No Of Isolates In Egg Contents (%)	Total (%)
Ibadan North	12	0 (0)	0 (0)	0 (0)
Ido	12	0 (0)	0 (0)	0 (0)
Lagelu	12	0 (0)	0 (0)	0 (0)
South West	12	2 (2.8)	0 (0)	2 (2.8)
Akinyele	12	2 (2.8)	0 (0)	2 (2.8)
Egbeda	12	3 (4.2)	0 (0)	3 (4.2)
Total	**72**	**7 (9.8)**	**0 (0)**	**7 (9.8)**

Table 2:
Total Aerobic Bacterial count(TABC) and Total Coliform Count (TCC)

Sources/Local Government Area	Egg Contents (Mean ± SD) CFU/ml		Egg Shell (Mean ±Sd) CFU/ml	
	LOG TAI	LOG CC	LOG TABC	LOG CC
Ibadan North	1.66 ± 2.2B	1.57±1.86B	5.31±0.18b	5.14± 0.23B
Ido	1.38 ± 1.59I	1.80 ±1.69B	4.40 ± 0.50B	4.35± 0.62B
Lagelu	2.23 ±1.89b	1.19 ±1.76B	5.07 ±0.11B	4.9 ±0.3B
South West	0 ±0B	0 ±0B	5.17 ±0.15B	5.0 ±0.17B
Akinyele	1.99 ±1.66B	1.98 ± 1.88B	4.68 ± 0.35B	4.8 ± 0.38B
Egbeda	1.26 ±0.97B	1.63 ±1.58B	5.05±0.15B	4.9 ± 0.3B
Total	1.43±1.65	1.36±1.46	4.95±0.24	4.84±0.33

Total Aerobic Bacterial count (TABC) Total and Coliform Count (TCC)

The Log mean CFU/ml ± Standard Deviation for Total Bacterial count and Coliform Count for egg shell was 4.95 ± 0.24 and 4.84 ± 0.33, respectively and for egg contents 1.43 ±1.65 and 1.36 ± 1.46, respectively (Table 2).

Antibiogram of the isolates

All the seven *E. coli* O157 isolates were resistant to Ampicillin, Augmentin, Ceftazidime, and Cefuroxime (100%). 86% of the isolates were resistant to Nitrofurantoin and 28.6% to Ciprofloxacin and Ofloxacin. Moderate susceptibility to gentamycin was also recorded (57%). All the isolates were multidrug resistant *E. coli* O157. Ciprofloxacin and ofloxacin appears to be the most effective antibiotics with 71.4% susceptibility each. One of the isolates has 100% resistance against all antibiotics used (Figure 1).

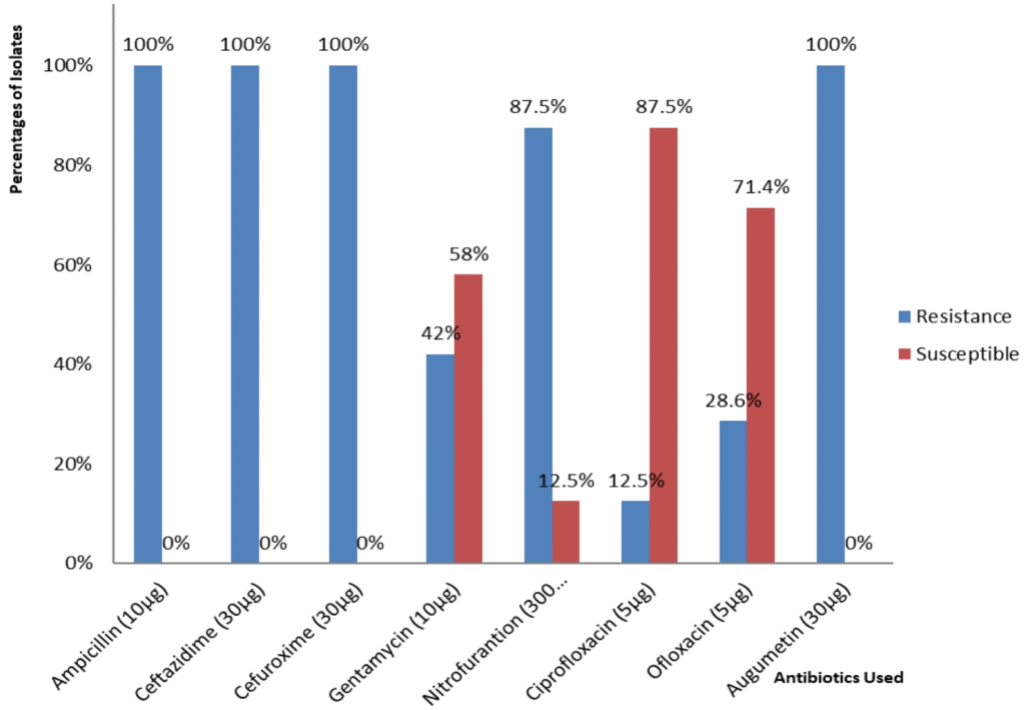

Figure 1:
Antibiogram of the *E. coli* O157:H7 isolates

DISCUSSION

Isolates of *E.coli* O157: H7 were obtained from all the egg shells sampled but were not isolated from the egg contents. Contamination of eggs by this pathogen may be by vertical process during the egg laying process in which egg comes in contact with the pathogen in the intestine and reproductive tract of the infected birds. Eggs may also be contaminated after deposition when it comes in contact with environmental bacteria (Spitzer, 2015). This is the major reason why hygienic practice is of major importance in poultry production.

The prevalence of *E.coli* O157: H7 observed in this study was 9.8% and all of the isolated *E.coli* O157: H7 was from egg shell. This report is different from the findings of Shecho *et al*. (2016) who reported higher prevalence of 13.6% of *E.coli* O157: H7 from chicken cloacae in Eastern Ethiopia. The variation may be accounted for by the difference in the sample used. Whereas, passage of egg through the chicken cloacae is expected to contributes to the contamination of egg shell

The egg contents contain other microbes but it is devoid of *E.coli* O157:H7 which is in line with the findings of Chaemsanit *et al*. (2015) who reported 0% prevalence of *E.coli* O157:H7 in egg content. The absence of the *E. coli* O157:H7 in egg content observed in this study may be as a result of the natural protective mechanism which makes contamination of egg content difficult. The egg shell and the outer shell membrane that separate the shell from the albumen serves as a major barrier to bacteria. In addition, the albumen gives a basic environment which discourages the proliferation of many bacteria (Spitzer, 2015).

Both egg shell and egg contents are contaminated with aerobic and coliform bacteria. The total mean of Log TABC and TCC CFU/ml were 4.95 ± 0.24 and 4.84±0.33 respectively. This was in line with the findings of Eman and Saad (2015) who reported 6.7±1.07 and 7.41±5.22 for Log TABC and TCC CFU/ml respectively from leaking chicken table eggs.

E.coli O157:H7 isolated from this study shows a high resistant pattern to most commonly used antibiotics. There was 100% resistance of the isolates to Ampicillin, Augumentin, Ceftazidime, and Cefuroxime. This is in agreement with the findings of Amézquita-López *et al*. (2016) who observed 100% resistance of *E.coli* O157:H7 isolated from domestic farm animals in rural communities in Northwestern Mexico to Ampicillin. It is also in line with the report of Mashak (2018) also reported 100% resistance of *E.coli* O157:H7 isolated from raw meat samples from ruminants and poultry to Ampicillin and Egbule *et al*. (2016) who reported 100% resistance of *E.coli* O157:H7 from stool samples collected from children with diarrhea to Ceftazidime and Cefuroxime.

It was also observed that only 87.5% of the *E.coli* O157:H7 isolates were resistant to Nitrofuratoin, however, 87.5% and 71.4% of the isolates were susceptible to Ciprofloxacin and Ofloxacin, respectively.. In this study Ciprofloxacin and Ofloxacin were found to be the most effective antibiotics against the *E.coli* O157:H7 isolates .These findings agrees with the study of Reuben and Owuna (2013) who observed 89.5% susceptibility of *E.coli* O157: H7 to Ciprofloxacin. These varying antimicrobial resistant pattern may be as a result of repeated exposure of the organisms to the antibiotics thereby causing the organism to build up resistance against them.

All the isolates (100%) were multidrug resistant, that is they were all resistant to three or more tested antibiotics, This report is in accordance with the earlier study by Abebe *et al*. (2014) who reported 93.2% multidrug resistant *E.coli* O157:H7, this slight variation may be due to change in resistant genes of *E.coli* O157:H7 which may be as a result of natural resistance in which the pathogen possess characteristics that inhibit the action of the antibiotics or acquired resistance in which there is a change in the genetic characteristics of the pathogen or horizontal gene transfer in which genetic characteristics are transferred by members of the same generation (Werner, 2014). In this study, one isolate was found to be resistant to all the antibiotics used which is of serious public health implication. Hygiene level should be increased in poultry (layers) farm to reduce the occurrence of food borne pathogens also the controlled use of antibiotics in food of animal origin should be encouraged.

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