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Prevalence of Multidrug Resistant *Escherichia coli* O157:H7 in Raw Chicken Sold in Ibadan, Oyo State, Nigeria

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Abstract: Indiscriminate use of antibiotics in livestock management could predispose meat consumers (humans) to risk of antibiotic resistance. This study investigated the prevalence of multidrug resistant *E. coli* O157:H7 in raw chicken sold in Ibadan. A total of sixty chicken samples were collected from three different markets in Ibadan. *E. coli* O157:H7 was isolated using Sorbitol MacConkey Agar and was serologically confirmed using latex agglutination test. Antibiotic susceptibility test was carried out using disc diffusion method. Out of all the 60 samples, the overall prevalence of 6.67% was obtained. The prevalence in Bodija market (10%) was higher than other markets. All the *E. coli* O157:H7 strains were resistant to all the antibiotics used. This study revealed that the isolated *E. coli* O157:H7 from chicken samples were multidrug resistant.

Key words: *Escherichia coli* · Chicken · Antibiotics · Antibiotic Resistance · Nigeria

INTRODUCTION

Escherichia coli is an important member of the genus *Enterobacteriaceae*. It is considered an important pathogen, comprising the normal flora of the gastrointestinal tract of both humans and animals [1]. *Escherichia coli* live a faecal-oral lifestyle and constitute about 1% of the gastrointestinal microbial population of mammals. *E. coli* is used as the preferred indicator of environmental faecal contamination in the safety assessment of food and water [2].

Escherichia coli O157:H7 is an enterohaemorrhagic strain of *Escherichia coli* and a cause of food borne illness [3]. *Escherichia coli* O157:H7 strains are responsible for disease in animals and man and have emerged to be important zoonotic agents. While most strains of *E. coli* are harmless and normally found in the intestines of mammals, these strains may produce Shiga-like toxins, which cause severe illness. They are also referred to as verocytotoxin producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) [3].

The most frequent mode of transmission for *E. coli* O157:H7 infection to human is through consumption of contaminated food and water. However, it may also spread directly from person to person and occasionally through occupational exposure [4]. Meat, milk, unpasteurized dairy products, ground beef, apple cider, egg, chicken meat and foods with animal origin have been associated with severe outbreaks of *E. coli* O157:H7 [5]. Domestic and wild animals as well as poultry are sources of the *E. coli* O157:H7 serotype. Contamination of meat with faecal materials in the slaughtering process is the main transmission route of *E. coli* O157:H7 [6].

Diseases caused by *E. coli* often require antimicrobial therapy. However, antibiotic-resistant strains of this bacterium cause longer and more severe illnesses than their antibiotic-susceptible ones. Several studies have shown that antibiotic resistance in *E. coli* strains has increased over time [7]. An epidemiological investigation revealed that the O157:H7 serotype of *E. coli* was the most commonly detected strains in foods with animal origins and that there was a high incidence of resistance

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(30-80%) to commonly used antibiotics [8]. Therefore, there is need to investigate the prevalence of this strain in poultry meat sold in Ibadan, Oyo State, Nigeria.

MATERIALS AND METHODS

Sample Collection: Sixty samples of frozen and unfrozen chicken were randomly collected from three different markets in Ibadan (Oja-oba, Bodija and Apata). The samples were collected into sterile polythene bag and transported on ice to the laboratory for analysis.

Isolation and Characterization of *E. coli* O157:H7: Isolation of *E. coli* O157:H7 from the samples was done using the method described by Tafida *et al*. [9]. Twenty five grams of each chicken sample were homogenised in 225 ml of sterile Peptone water and incubated overnight at 37°C. After incubation, a loop full of the broth culture was inoculated on Sorbitol MacConkey Agar (SMA) plates. The plates were incubated at 37 °C for 24 hours. Non-sorbitol fermenting *E. coli* colonies were selected and sub-cultured. The isolates were identified based on morphological and biochemical characterizations.

Serotyping of *E. coli* O157: H7: Serological test was carried out on all non- sorbitol fermenting *E. coli* using commercially prepared *Salmonella polyvalent* 'O' and 'H' antisera for *E. coli* O157:H7. Briefly, the non-sorbitol fermenting *E. coli* isolates were emulsified in a drop of normal saline on a clean slide to form a smooth suspension, then a drop of antiserum was added and mixed gently. It was rocked for two minutes and matched for agglutination. Agglutination indicated positive, while no agglutination indicated negative.

Antibiotics Sensitivity Test: Antibiotics sensitivity test was performed on *E. coli* O157:H7 using Kirby Bauers disc diffusion technique as described by Akinpelu and Kolawole [10]. Sterile Mueller-Hinton agar plates were inoculated with standardized cultures of *E. coli* O157:H7 ($1.5x10^6$ CFU/ml). Then antibiotic impregnated discs were placed on the surface of the agar using sterile forceps. The plates were incubated at 35°C for 18 hours after which zones of inhibition were measured. The antibiotics used were: Augmentin (30 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg), Cotrimoxazole (30 µg), Chloranphenicol (30, µg), Sparfloxacin (10 µg), Amoxacillin (30 µg), Pefloxacin (30 µg), Tarivid (10 µg) and Streptomycin (30 µg). These antibiotics were classified as 'Resistant' (R) or 'Susceptible' (S) using standard recommendations of Clinical and Laboratory Standard Institute (CLSI).

RESULTS

Out of the 60 raw chicken samples collected from three markets, a total of 60 non-sorbitol fermenting *E. coli* were isolated. Only 4 (6.67 %) were confirmed to be *E. coli* O157:H7. These *E. coli* O157:H7 were obtained from two samples of unfrozen chicken from Bodija market, one sample of unfrozen chicken from Oja-Oba market and one sample of frozen chicken from Bodija market.

The results of the prevalence of *E. coli* O157:H7 strains in the chicken samples in three markets in Ibadan are presented in Figure 1. The prevalence of *E. coli* O157:H7 strains in the unfrozen chicken samples from Bodija, Oja-Oba and Apata markets were 10 %, 5 % and 0% respectively while the prevalence of the pathogen in frozen chickens from Bodija, Oja-Oba and Apata markets

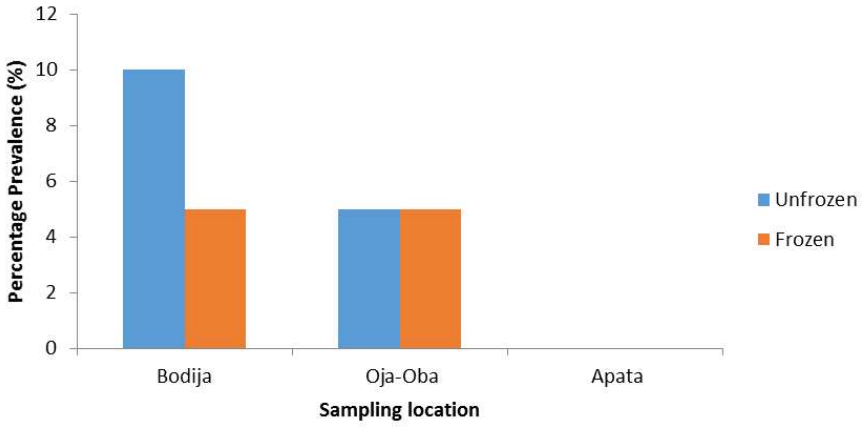

Fig. 1: Prevalence of *E. coli* O157:H7 in chicken samples

Table 1: Antibiotic sensitivity of *E. coli* O157:H7 on commercial antibiotics

S/N	Antibiotics	Zone of inhibition (mm)	Sensitivity
1	Augmentin	3	R
2	Ciprofloxacin	0	R
3	Gentamycin	2	R
4	Cotrimoxazole	0	R
5	Chloranphenicol	0	R
6	Sparfloxacin	4	R
7	Amoxacillin	2	R
8	Pefloxacin	3	R
9	Tarivid	4	R
10	Streptomycin	0	R

Key:
R –Resistant

were 5 %, 5 % and 0%. The prevalence of *E. coli* O157:H7 strain was significantly higher in unfrozen chicken compared to frozen chicken. Also, its occurrence was higher in Bodija market than other markets used in this study.

The *E. coli* O157:H7 strains obtained in this study were resistant to all the ten antibiotics used. This implies that the *E. coli* O157:H7 strains were multidrug resistant. The results of the antibiotic susceptibility patterns of the *E. coli* O157:H7 isolates are shown in Table 1.

DISCUSSION

In this study, *E. coli* O157:H7 strain was isolated from unfrozen and frozen raw chicken in Ibadan. This finding is in accordance with the report of Aibinu *et al.* [11] who isolated *E. coli* O157:H7 from cattle, pig and chicken samples in Lagos and Ogun states. Olatoye *et al.* [12] also isolated this pathogen from beef and chicken in municipal abattoirs in Lagos and Oyo states.

The overall prevalence of *E. coli* O157:H7 obtained in this study in the three sampled markets was 6.67%. The prevalence was different within the sampled markets. The prevalence obtained in this study was lower than that of Olatoye *et al.* [12] who reported a prevalence of 14.5 % in raw chicken samples. However, it was higher than prevalence of 4.2 % reported by Hiko *et al*. [13].

The study confirmed chicken as a reservoir of *E. coli* O157:H7 which have also been isolated from cattle, meat and milk from other parts of the country by different researchers including [11], Luga *et al.* [14] and Ojo *et al.* [15]. Cross contamination of ready-to-eat foods and food handlers with such organisms as well as other pathogenic bacteria could result into outbreak of food-borne illnesses [15].

All the *E. coli* O157:H7 strains isolated in this study were resistant to Augmentin, Ciprofloxacin, Gentamycin, Cotrimoxazole, Chloranphenicol, Amoxacillin, Pefloxacin and Streptomycin. This finding is also similar to the report of Islam *et al.* [6] who reported that *E. coli* O157:H7 strains were 100% resistant to Amoxicillin, Streptomycin, Gentamicin, Cotrimazole and Chloramphenicol. The results negated the report of Amosun *et al*. [16] who recorded 70% resistance to these antibiotics.

The high prevalence of antibiotic resistant bacteria has been associated with several factors including indiscriminate use of antibiotics due to unregulated access of non-professionals to different classes of antibiotics over-the-counter. Antibiotic use and misuse have been considered to be the most vital selecting force to antimicrobial resistance of bacteria development and spread in both veterinary and human medicine [17].

CONCLUSION

This study revealed that chickens sold for human consumption in the study area were contaminated with multidrug resistant *E. coli* O157:H7 with varying prevalence in the sampled markets. The public health and food safety implications of these results include the risk of meat borne food poisoning and spread of antibiotic resistance across the food chain.

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