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Research Article

Detection of Shiga toxins from *Escherichia coli* O157:H7 amongst Coliform Bacteria Isolated from Poultry Meats sold in some parts of Gombe Metropolis

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ABSTRACT

With the increase in global demand and consumption of poultry meat, as well as variety in poultry meat and products, microbial safety must be ensured. *Escherichia coli* has for decades been identified as an important zoonotic pathogen. This study aimed to investigate the presence of pathogenic *E. coli* from chicken meats obtained from various outdoor vendors in Gombe metropolis, Gombe State, Nigeria. A total of 60 chicken parts; 10 samples per chicken (including breast, drumstick, wings, thighs, backs and legs) were purchased. The samples were analysed via bacteriological culture-based techniques on MacConkey agar and Eosin Methylene Blue agar; Gram staining, further growth on Sorbitol MacConkey Agar and polymerase chain reaction (PCR) using Stx2 primer and enteropathogenic *E. coli* were characterised. The occurrence of *E. coli* was 50% (n=30). Other coliforms found were *Klebsiella pneumoniae* (25%), *Citrobacter* spp (10%) and *Enterobacter aerogenes* (6.7%). Meanwhile, the occurrence of *E. coli* was highest in the chicken legs with 33.3% (n= 10) and lowest in drumsticks with 6.7% (n=02). The highest contamination level of *E. coli* was in chicken legs with 3.00 x 10^3 CFU/g, while the lowest was observed in drumsticks at 1.32 x 10^3 CFU/g. Of the 30 *E. coli* isolates across all the chicken parts, only 16.6% (n=5) contained Stx2 genes. Educating the vendors on good hygiene practices is critical in eliminating contamination and thereby preventing outbreaks due to *E. coli O157:H7*.

Keywords: Chicken meats; Coliforms; Pathogenic *E. coli;* PCR; Shiga toxin

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INTRODUCTION

In both developed and developing nations of the world, chicken meat is among the most widely consumed meat products sourced from approximately 19 billion chickens globally (Mpundu *et al*., 2019). However, lack of strict hygiene practices in primary and secondary production lines, as well as the final product of the food chain, bacteria particularly those of public health importance contaminate the chicken and its products. These include *Salmonella* spp. and coliforms especially *Escherichia coli* which is among the normal microbial flora of gastrointestinal tracts of various animals including chickens and man. These bacteria are the global lead causes of foodborne ailments including gastroenteritis. It is a major global cause of chronic and acute foodborne diseases in humans and poultry (Mpundu *et al*., 2019). *Escherichia coli* is a bacterium that inhabits the intestines of both man and animals and its presence indicates faecal contamination that usually arises from contamination by human faeces via water or food source. Presence of *E. coli* in chicken meat is an indicator of poor hygiene practices in places where the chickens are slaughtered, processed or vended. In addition, *E. coli* was found to be prevalent in various parts of the world such as India with 98% prevalence (Sharma and Chattopadhyay, 2015), Pakistan with 43.5% prevalence (Zainab *et al*., 2022), Morocco with 48.4% (Cohen *et al*., 2020), Nigeria with prevalence of 99.4% (Agusi *et al*., 2024) and 82.3% in Malaysia (Suryadevara *et al*., 2020).

Prevalence reports of *E. coli* contamination in chickens from different countries around the globe are reflections of varying hygiene practices and quality controls. Countries with lower contamination levels are believed to have better hygiene practices in meat processing. High contamination of meat from chicken is an important public health issue with the potential for causing foodborne infections and intoxications in humans, and may consequently lead to outbreaks due to the organisms involved (Mpundu *et al*., 2019). Due to various food consumption patterns including consumption of chicken meat, globally people suffer from foodborne ailments throughout the year. Thus, reducing the levels of contamination at various stages of processing can significantly impact the reduction of incidences associated with consumption of contaminated poultry meats and their products (Keener *et al*., 2004; Adu-Gyamfi *et al*., 2012).

Studies have shown that the consumption of foods contaminated with shiga toxin-producing *E. coli* (STEC) is the major cause of human infections (Mead *et al*., 1999; Caprioli *et al*., 2005; WHO, 2018). The genes that encode virulence in STEC play a vital role in disease causation. Such genes encode for the two major virulence factors as Stx1 and Stx2 that is identical in many respect to *STX* of *Shigella dysenteriae*. These contribute to the pathogenicity of STEC. The Stx1 and Stx2 have 56% homogeneity (Momtaz andJamshidi, 2013; Scheutz and Strockbine, 2015; WHO, 2018). Diseases due to shiga toxin-producing *E. coli* are life-threatening, thus ensuring the safety of chicken meat parts vended in parts of Gombe metropolis is very essential. This research was therefore conducted with the objective of analyzing the incidence of *E. coli* in chicken parts with the purpose of determining the intensity of the contamination as well as the presence of shiga toxin among the isolated strains. This will help proffer possible solutions that will help to improve hygiene practices to avoid occurrence of an outbreak due to the organism and the toxin.

MATERIALS AND METHODS

Collection of samples

A total of 60 samples, 10 from each chicken (particularly the breast, drumstick, wings, thighs, backs and legs) were purchased from vendors at different locations in Gombe metropolis viz; Jekadafari, Pantami, Kumbiya-Kumbiya and Bolari. The samples were obtained in *STX* visits to each location for nine months; from July, 2016 to March, 2017. The samples were aseptically placed immediately after purchase into sterile plastic bags and transported to the Microbiology laboratory at the Department of Microbiology, Gombe State University.

Microbiological Analysis

Microbial analysis was carried out to isolate coliforms in the chicken meats. A 25 g of each of the samples was aseptically cut into smaller pieces using a sterile knife. They were then macerated and homogenized with 225 mL of sterile buffered peptone water in a bag using stomacher. Following homogenization, the samples were serially diluted into 10^{-1}, 10^{-2}, and 10^{-3} dilutions. To determine the bacteria growth, 0.1 mL from each dilutions series was spread-plated onto MacConkey agar (MA) and Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. The colony forming unit per gram (CFU/g) was counted after the incubation.

Phenotypic Identification of Coliforms and *E. coli*

Isolates presumably identified as coliforms on MA and *E. coli* on EMB agar were further subjected to gram staining and biochemical tests such as indole production, citrate utilization, urease, ornithine decarboxylase, motility, hydrogen sulphide, gas and acid production, Voges-Proskauer and methyl red. The isolates were biochemically identified as members of the coliforms, non-coliform members or *E. coli* in particular.

Presumptive Screening for Pathogenic *E. coli* (*O157:H7*)

Suspected *E. coli* strains from the EMB agar that showed colonies with greenish metallic sheen were further subcultured on Sorbitol MacConkey (SMAC) agar. The SMAC is selectively used to differentiate between *E. coli* O157:H7 and other strains of *E. coli* as, while the former cannot ferment sorbitol as such the colonies appear colourless, the latter can ferment sorbitol, eventually making the colonies appear pink or red (Al-Saadi *et al*., 2018).

Molecular Detection of Shiga Toxin (Stx2) in *E. coli*

Isolates negative for sorbitol fermentation were subjected to PCR for the detection of the gene that encodes for the presence of shiga toxin. These isolates were first subjected to DNA isolation, then PCR, gel electrophoresis and finally visualization of the electrophoretic PCR products.

DNA Genomic Extraction

The extraction of the genomic DNA was conducted using conventional boiling method. Isolates were first subcultured in Luria Bertani (LB) broth in test tubes for overnight. About 1 mL of an overnight culture of *E. coli* in the LB broth was aseptically transferred into an Eppendorf tube and centrifuged at 13,400 x g for 1 minute at room temperature. The supernatant was

discarded and pellets were re-suspended in 200 µLof Tris-EDTA buffer (TE buffer). The mixture was further boiled at 100°C, for 10 minutes and then allowed to cool at -20°C for 10 minutes. It was further centrifuged at 13,400 x g for 2 minutes. The supernatant containing the extracted DNA was kept at -20°C for further use.

Amplification of the Target Gene using PCR

The primers used in the amplification of the targeted gene *STX2* were the forward 5'-GCGGTTTTATTTGCATTAGC-3' and the reverse 5'-TCCCGTCAACCTTCACTGTA-3'. Their locations within the gene were 1228–1247 and 1342-1323 respectively. The amplicon size was 115 bp (Wang *et al.*, 2002). Products for the PCR were prepared in a 25 µL reaction volume that consisted of 1 µL of 50 ng genomic DNA, 1 µL each of 10 µM forward and reverse primers, 12.5 µL of 2X PCRBIO Taq Mix Red (PCR Biosystems Ltd, London, UK) that is composed of PCRBIO Taq DNA polymerase, 6mM $MgCl_2$ and 2mM dNTPs and 9.5 µL sterile distilled water.

Amplification of Stx2 PCR product was carried out in a thermal cycler (Model SC300, Kyratec Australia). The thermal cycler conditions of amplification included; 94 ^oC for 5 minutes of initial denaturation, 30 cycles of 94 ^oC for 1 minute, annealing at 55 ^oC for 1minute and extension at 72 ^oC for 1 minute. Finally, extension followed at 72 ^oC for 5 minutes.

Agarose gel Electrophoresis and Visualisation of the PCR Products

Following the PCR, the products were subjected to agarose gel electrophoresis in 1.5% agarose (Fisher Scientific, Hampton New Hampshire, USA) prepared by dissolving 0.45g of agarose in 1X TBE (Tris-Borate EDTA) buffer prepared from 54 g of Tris-base, 27.5 Boric acid and 20 mL of 0.5M EDTA at pH 8.3.The mixture was heated on a hot plate (Hanabishi, Japan) for the agarose to completely dissolve. The solution was cooled at 50 ^oC and 1.5 µL of ethidium bromide (EtBr) was dispensed. The solution was in turn dispensed into a casting tray containing a comb to make wells for loading the PCR products. The PCR products were loaded into the agarose wells inside a gel box containing the buffer. Following loading, the electrophoretic tank was connected to a voltage source and was run at 80 V for 40 minutes. The products were finally viewed under UV-illuminator using gel documentation system (SynGene, UK). Band for *STX2* gene were viewed at 115 bp.

RESULTS

As presented in Table 1, the most occurring coliform across the different chicken parts was *E. coli* (30.0%), followed by *K. pneumoniae* (16.0%) and *Citrobacter* spp (6.0%), while the least occurring was *Enterobacter aerogenes* (4.0%).

Based on the chicken parts, the wings and thighs were 50.0% each most contaminated with *E. coli.* However, the legs were most contaminated with *K. pneumonia* (50.0%). There were 20.0% contamination with *Citrobacter* spp on thighs and legs, but the backs were not contaminated with it. Also, *Enterobacter aerogenes* occurred mostly on the legs, but absent in the breasts, drumstick and backs.

As depicted in Table 2, Chicken part with the highest *E. coli* contamination was leg with a count of 3.0 CFU/g, followed by back and wing with 2.80 CFU/g each, breast and thigh with 2.61 CFU/g each while the least count of *E. coli* contamination was observed with chicken drumstick (1.50 CFU/g). Moreover, all the chicken parts showed evidence of shiga toxin except leg.

Figure 1 displays the amplified PCR products of the five shiga toxin producing *E. coli* obtained from the chicken breast, drumstick, wings, thighs and backs of the poultry meat obtained. The amplicon size was 115 bp.

Table 1: Occurrence of coliform bacteria in poultry meat samples

Meat type	No. of examined	*E. coli* No. (%)	*K. pneumoniae* No. (%)	*Citrobacter sp.* No. (%)	*E. aerogenes* No. (%)
Chicken breast	10	4 (40)	2 (20)	1 (10)	0 (0.0)
Drumstick	10	2 (20)	1 (10)	0 (0.0)	0 (0.0)
Chicken wings	10	5 (50)	2 (20)	1 (10)	1 (10)
Chicken thighs	10	5 (50)	3 (30)	2 (20)	1 (10)
Chicken backs	10	4 (40)	2 (20)	0 (0.0)	0 (0.0)
Chicken legs	10	10 (100)	5 (50)	2 (20)	2 (20)
Total	**60**	**30.0**	**16.0**	**6.0**	**4.0**

Table 2: Presence of *E. coli* count and the shiga toxin in poultry meat parts

S/N	Sample	*E. coli* Count (x10^3 CFU/g)	Meat part	Shiga Toxin (*STX*)
1	CBEC 1	2.52	Chicken breast	-
2	CBEC 2	2.12	Chicken breast	-
3	CBEC 3	2.11	Chicken breast	+
4	CBEC 4	2.61	Chicken breast	-
5	DEC 1	1.50	Drumstick	+
6	DEC 2	1.32	Drumstick	-
7	WEC 1	2.80	Wings	-
8	WEC 2	2.65	Wings	-
9	WEC 3	2.11	Wings	+
10	WEC 4	2.12	Wings	-
11	WEC 5	2.62	Wings	-
12	TEC 1	2.00	Thighs	-
13	TEC 2	2.00	Thighs	-
14	TEC 3	2.61	Thighs	-
15	TEC 4	2.23	Thighs	-
16	TEC 5	2.25	Thighs	+
17	BEC 1	2.22	Back	-
18	BEC 2	2.32	Back	-
19	BEC 3	2.63	Back	+
20	BEC 4	2.80	Back	-
21	LEC 1	2.52	Legs	-
22	LEC 2	2.24	Legs	-
23	LEC 3	2.52	Legs	-
24	LEC 4	2.70	Legs	-
25	LEC 5	2.21	Legs	-
26	LEC 6	2.20	Legs	-
27	LEC 7	2.50	Legs	-
28	LEC 8	2.80	Legs	-
29	LEC 9	3.00	Legs	-
30	LEC 10	2.20	Legs	-

Key: - = negative, + = positive, CB= Chicken breast, W= wing, T= Thigh, L= Leg, B= Back, D= Drumstick, EC= *E. coli*

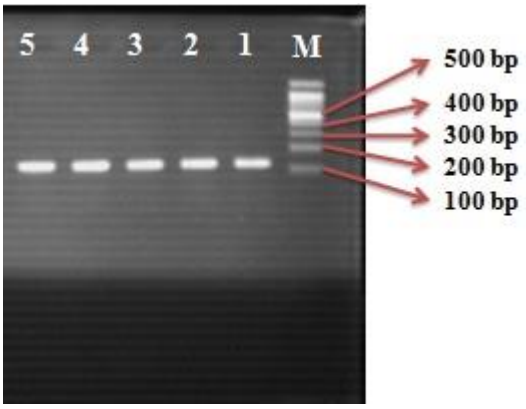

Fig 1: Amplified PCR products of Stx2 genes of *E. coli*. Lame M, 100 bp molecular weight marker; Lanes 1-5 Stx 2 genes of *E. coli* isolates of CBEC 3, DEC 1, WEC 3, TEC 5 and BEC 3

DISCUSSION

Poultry meats are vital components of diets in humans. This is owing to the presence of protein, mineral and vitamins. However, these meats are prone to contamination by bacterial pathogens that cause food poisoning (Zainab *et al*., 2022). Shiga-toxin producing *Escherichia coli* (STEC) is a highly recognized food pathogen that causes various outbreaks. Shiga-toxin producing *Escherichia coli* causes bloody diarrhea, haemorrhagic colitis (CS) and haemolytic uremic syndrome (HUS) (AmézquitaLópez

et al., 2012). The STEC can cause the life-threatening HUS due to the production of shiga toxin by *STX* 1 gene, *STX* 2 gene or both (Loo *et al*., 2013).

The results obtained from the incidence of coliforms in poultry meat from various parts showed highest occurrence (100%) of *E. coli*. This is because this organism is the most prevalent bacterial strain on earth as well as a model organism for biotechnological research. It is equally the simplest bacterium that serves as indicator for faecal contamination. This in turn indicates poor hygiene practice whenever they are found to be associated with poultry meats (Mpundu *et al*., 2019). The high numbers of coliforms particularly *E. coli* and *K. pneumoniae* in the poultry meats represent a potential source for human colonization and infection as well as spread within the community (Eibach *et al*., 2018). The presence of other coliforms (*Citrobacter* spp. and *E. aerogenes*) in the poultry meat samples analyzed is akin to the presence of *E. coli* and *K. pneumoniae* and is an indication of poor hygiene practice as well as improper handling of the meats (Mukesh *et al*., 2018). Higher occurrence of *E. coli* was found in chicken legs. This may be attributed to the fact that legs are the first contact of the poultry to the ground where faeces are found. Additionally, the scaly nature of the legs and the uneasy nature of the scale removal and cleaning may be another contributing factor. The lowest prevalence was observed in drumstick as that is the part of chicken meat that can be cleaned more easily than the other parts. This contradicts the findings of Enver *et al*. (2021) in which the drumstick had the highest incidence of *E. coli* (4.75×10^3 CFU/g) as against the chest, wing and offal.

Prior to consumption, meat from poultry should be absolutely free from *E. coli* due to the severity and nature of the infection or intoxication induced by the bacterium in humans (Ishola and Taiwo, 2014). For the screening of foodborne pathogens, *E. coli* is frequently employed as an indicator of safety for microbiological importance (Foddai and Grant, 2020). In this research, the rate of isolation of *E. coli* was 50.0 %; closely related to that obtained by Rafiq *et al*. (2024) and lower than 63.5 % rate of detection by Rahman *et al*. (2020).

In this study, the *E. coli* count indicates that the isolated strains were not within the acceptable limit (≤100 CFU/g) as per as the International Commission on Microbiological Specifications of food (ICMSF) specifications for the total coliform count is concerned. This indicates a threat of infections due to *E. coli* (Loo *et al*., 2013) and consequently outbreaks (Mpundu *et al*., 2019). The figures obtained in this study with regard to the *E. coli* and other coliform bacteria is much more than the acceptable limit (>2000 CFU/g). Similar results were obtained by Hassanien *et al*. (2015), Kim *et al*. (2018) and Enver *et al*. (2021). The coliform counts and in particular, *E. coli* counts increase during the course of processing to the final products from raw materials (Kim *et al*., 2018).

This study shows the low prevalence (16.7%; n= 5/30) of shiga toxin genes in *E. coli* obtained from chicken breast, drumstick, wings, thighs and backs samples. There was no *STX* detected from poultry leg sample although the highest prevalence of *E. coli* was 100% (n=10/10) from them. This shows that there is no association between contamination and *STX* gene detection. There is also low prevalence of Shiga toxin producing *E. coli* as compared to a study in Saudi Arabia by Bosilevac and Koohmaraie (2012) and Al-Zogibi *et al*. (2015), although their samples were not poultry meats, the *STX*2 was detected in *E. coli* as conducted in this study.

It has been reported that STEC strains have various capabilities of causing serious diseases in animals and man. This is linked to the amounts and types of the *STX* produced. Therefore, the type of *STX* toxin produced by STEC isolated from human infections has been extensively studied. However, only little is known about the frequency of the *stx2* subtype as well as the combination of virulence factors expressed by STEC from the intestinal tract of healthy animals (Momtaz and Jamshidi, 2013). In a nutshell, the prevalence of *E. coli* from poultry meat types does not influence the presence of *STX*2 in the samples.

CONCLUSION

Poultry meat types were screened for the presence of coliforms and *E. coli* in particular. Most of the samples obtained from the chicken parts were positive for *E. coli*. Moreover, all the *E. coli* strains detected were harbouring shiga toxins except those detected from the legs. Poor personal hygiene that may lead to transfer of coliform bacteria and *E. coli* as well as improper handling and processing of the poultry meats may be the main contributing factors toward the prevalence of the pathogenic bacteria. The presence of *STX*2 indicates a great threat to public health of the consumers. This can bring in addition to the rise in mortality rate and great economic loss. Thus, relevant authorities should develop a concerted mean to combat the occurrence of *E. coli* pathogenic strains especially those of serious global public health concern.

CONFLICT OF INTEREST

There was no conflict of interest among the authors of this research

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