Research Article

Risk of Shiga Toxigenic Escherichia coli O157:H7 Infection from Raw and Fermented Milk in Sokoto Metropolis, Nigeria

Yusuf Yakubu, 1 Abdulmalik Bello Shuaibu, 2 Aliyu Musawa Ibrahim, 1 Ummukulthum Lawal Hassan, 3 and Raymond Junior Nwachukwu 1

1Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria
2Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria
3College of Agriculture and Animal Science, Bakura, Zamfara State, Nigeria

Correspondence should be addressed to Yusuf Yakubu; yakubu.yusuf@udusok.edu.ng

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Escherichia coli O157:H7 is an enteric foodborne pathogen associated with life threatening disease conditions. The enterobacteria are frequently found in cattle gastrointestinal tract with high potential of contaminating animal products such as meat, milk, and cheese. A cross-sectional study was conducted to investigate the presence of Shiga toxin-producing Escherichia coli O157:H7 in milk products sold within Sokoto metropolis. Two hundred and sixty (260) samples (comprising 160 raw and 100 fermented milk samples) were collected from different sources within the study area. Bacteriological isolation and biochemical characterization yielded Escherichia coli with a detection rate of 9.23% (24/260). Molecular identification of the recovered isolates by PCR amplification of the Stx1 gene revealed Escherichia coli O157:H7 with a positive rate of 20.83% (5/24). The overall prevalence of Es. coli O157:H7 was 1.92% (5/260) and the positive proportions for raw and fermented milk samples were 1.86% (3/160) and 2.0% (2/100), respectively. Fisher’s Exact test showed a nonsignificant association between the isolates and the different milk types \( p = 0.943 \); \( OR = 0.94 \); [95% CI: 0.154–5.704]. The results revealed presence of Escherichia coli O157:H7 in raw and fermented milk sold within Sokoto metropolis, Nigeria. The findings indicate possible fecal contamination of the milk products, with serious public health consequences. This necessitates the need to screen other milk products produced in the area such as butter and cheese. Health authorities in the State need to enlighten dairy farmers on the zoonotic potential of Escherichia coli O157:H7 and the role of cattle in the spread of the pathogen.

1. Introduction

Escherichia coli is a Gram-negative, rod shaped, facultative anaerobic bacterium of the family Enterobacteriaceae. Based on its virulence, the bacterial organism is classified into five groups, namely, enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), attaching and effacing E. coli (AEEC), and Shiga toxin-producing E. coli (STEC) [1]. Escherichia coli O157:H7 is an emerging serotype of Escherichia coli that accounts for most human diseases caused by enterohaemorrhagic Escherichia coli (EHEC). It is associated with life threatening disease conditions in humans such as hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and hemorrhagic colitis (HC) [2]. The organism was first recognized as a human pathogen following outbreaks of hemorrhagic colitis associated with consumption of contaminated beef hamburger [3]. Cattle are the principal reservoir of Escherichia coli O157:H7 and are resistant to infection by the pathogen as they lack receptors responsible for the attachment of the bacteria to host cell [4]. However, humans are highly susceptible to infection following direct contact with contaminated animal faeces or consumption of contaminated animal products such as beef, milk, and cheese [5, 6]. Escherichia coli O157:H7 has worldwide distribution and determinants responsible for its
emergence in human population are yet to be confirmed [7, 8]. However, changes in livestock husbandry system, slaughter, and meat processing practices have been suggested to play important role in the emergence of the pathogen [9]. Enterohemorrhagic Escherichia coli expresses one or more potent cytotoxins known as Shiga toxins (Stx), which are the major virulence factors in the pathogenesis of diseases caused by the bacteria. Escherichia coli O157:H7 produces Shiga toxin 1 which is encoded by the Stxl gene and serologically indistinguishable with the toxin of Shigella dysenteriae [10].

Cow milk forms an important component of some traditional dairy dishes and beverages of the Fulani and Hausa tribes in northern Nigeria. It is sometimes served raw immediately after milking or allowed to ferment for Hausa tribes in northern Nigeria. It is sometimes served 

**2. Materials and Methods**

2.1. Study Area. The study area was Sokoto metropolis, the capital of Sokoto State Nigeria. It is made up of four local government areas, namely, Sokoto North, Sokoto South, Wamakko, and Dange-Shuni. The State is located on latitude 13°N and between longitudes 4°E and 6°34'E in Northwestern Nigeria. The State covers an area of approximately 36,000 square kilometers [11]. The State shares border with Niger republic to the north, Kebbi State to the south, and Zamfara State to the east. Based on the 2006 census, Sokoto State was estimated to have a population of about 4,344,399. The State is ranked second in livestock population with about 3 million cattle, 4 million goats, 3.85 million sheep, 0.8 million camels, and 1 million poultry [12].

2.2. Study Design and Sample Collection. A cross-sectional study was conducted where dairy cattle herds and milk retailing outlets within Sokoto metropolis were identified for the collection of raw and fermented milk, respectively. A total of 160 raw milk samples were collected from 16 dairy herds within 3 Local Government Areas (LGAs) comprising 62 samples from Sokoto North, 55 samples from Sokoto South, and 43 samples from Dange-Shuni. Sampling could not be done in Wamakko LGA as there are no established dairy cattle herds. To collects samples, lactating cows were randomly selected and 10 ml of milk was collected in sterile bottles from each cow by the livestock attendants. Ten milliliters (10 ml) of pooled fermented milk samples was also collected from 100 different sales outlets within the metropolis. Both milk samples (raw and fermented) were transported in an ice chest to the Public Health Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

2.3. Culture and Biochemical Characterization. The acidity of fermented milk samples was ascertained using a pH indicator paper (Whatman®). Both the raw and fermented milk samples were diluted tenfolds using sterile distilled water before inoculating 1 ml onto MacConkey agar (Oxoid, UK) and subcultured on Eosin Methylene Blue agar (Oxoid, UK) using spread plating technique. The plates were incubated at 37°C for 24 hours and bacterial colonies that are circular, moist, smooth, and pinkish on MacConkey agar but have green metallic sheen on Eosin Methylene Blue agar were presumed to be Escherichia coli. All presumptive colonies were subjected to a panel of conventional biochemical tests (IMViC) and isolates that were positive for indole and methyl red tests but negative for Voges Proskauer and Citrate utilization tests we identified as E. coli. The identified E. coli isolates were further subcultured onto sorbitol MacConkey agar (Oxoid, UK) and incubated at 37°C overnight. Non sorbitol fermenters that appear as smooth, circular, and colourless colonies were tentatively identified as Escherichia coli O157:H7 as earlier described [13].

2.4. DNA Extraction. The boiling method of nucleic acid extraction was employed as earlier described [14]. Briefly, a loopful of each isolate was suspended in two hundred microliters (200 μl) of TE buffer (Tris-HCl [10 mM]: EDTA [1 mM]) in a microfuge tube and heated at 96°C for 15 mins. The tubes were immediately transferred onto ice for 15 mins and then centrifuged at 15000 g for 2 mins at room temperature. The pellets were discarded while the supernatant containing the DNA templates was used for polymerase chain reaction.

2.5. Molecular Identification. A multiplex polymerase chain reaction (PCR) was done using Top Taq™ Master Mix PCR kit (Qiagen®). The 25 μl reaction mixture contained 12.5 μl Top-Taq Master Mix 2x, 7.5 μl RNase-free water, 2.5 μl of 200 ng DNA template, and 0.25 μM of two-primer cocktail (Table 1) amplifying the 180 bp of the Stxl gene as described earlier [15]. Samples were subjected to 35 PCR cycles, each consisting of 1 min of denaturation at 95°C, 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15, and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. Sterile RNase-free water and a confirmed E. coli O157:H7 isolate were used as negative and positive controls, respectively.

2.6. Agarose Gel Electrophoresis. A 1.5% agarose gel was prepared by suspending 1.5 grams of agarose powder in 100 ml of 1x Tris-Borate-EDTA (TBE) buffer and heated on a hot plate until completely dissolved. Ethidium bromide (2.5 μl) was added to the liquid agarose before pouring into a gel caster set and allowed to solidify at room temperature.

**Table 1: Sequences of oligonucleotides used for PCR.**

| Primer | Sequence (5’ to 3’) | Expected amplicon size (bp) |
|--------|---------------------|-----------------------------|
| StxlF  | ATAAATCGGCCATTCTGTTGACTAC | 180 |
| StxlR  | AGAACGCCACTGAGATCATC | |

*Note: The sequences were designed to amplify a 180 bp fragment of the Stxl gene.*
### Table 2: Frequency of *Escherichia coli* isolates from raw and fermented milk samples.

| Type of Milk | Number of samples positive for *Escherichia coli* | Number of samples negative for *Escherichia coli* | Total |
|--------------|-----------------------------------------------|-----------------------------------------------|-------|
| Raw milk     | 15                                            | 157                                          | 160   |
| Fermented milk | 9                                             | 98                                           | 100   |
| Total        | 24                                            | 236                                          | 260   |

$\chi^2 = 0.010$, $p = 0.919$, OR = 1.05, (95% CI: 0.44–2.49), OR: odds ratio, and CI: confidence interval.

![Agarose gel electrophoresis result for PCR detection](image)

The caster was rightly placed into an electrophoresis tank and flooded with 1x TBE buffer to the maximum level before carefully removing the comb. The PCR products (5 µl each) were then loaded into the wells using a 10 µl Eppendorf pipette. Four microliters (4 µl) of 100 bp ladder (BioLabs Inc. New England) mixed with 2 µl Gel Loading Dye Blue 6x (BioLabs Inc. New England) was also loaded into one of the wells before connecting the tank to a power pack and plugging it to the mains supply. The products were electrophoresed at 85 volts for 50 mins. Immediately after electrophoresis, the agarose gel was viewed using a Gel Doc™ XR+ (BioRad). The gel image was captured and labelled accordingly.

2.7. Data Analyses. The results obtained were presented in tables and narratives. Fisher’s Exact test was used to determine association between the *E. coli* O157:H7 isolates and type of milk (raw and fermented) using a significance level of 5% and 95% confidence interval. All analyses were performed using SPSS (version 23; SPSS Inc., Chicago, IL, USA).

### 3. Results and Discussion

The overall prevalence of *E. coli* was 9.23% (24/260) with an isolation rate of 9.38% (15/160) and 9.0% (9/100) for raw and fermented milk, respectively. Statistical analysis using Chi Square ($\chi^2$) test showed a nonsignificant association ($p = 0.919$, OR = 1.05, [95% CI: 0.44–2.49]) between the *E. coli* isolates and the different milk types (Table 2). Molecular identification by PCR amplification of the Shiga toxin 1 gene (*Stx1*) in *E. coli* O157:H7 showed 5 out of the 24 *E. coli* isolates to be positive with a prevalence of 20.83% (5/24) (Figure 1). The prevalence in raw and fermented milk was 1.86% (3/160) and 2.0% (2/100), respectively. Fisher’s Exact test revealed a nonsignificant association ($p = 0.943$; OR = 0.94; [95% CI: 0.154–5.704]) between the *E. coli* O157:H7 isolates and the different milk types (Table 3). The proportions of raw milk samples positive for *Escherichia coli* in three local government areas sampled were 5.45% (3/55), 12.70% (8/62), and 9.30% (4/43) for Sokoto South, Sokoto North, and Dange-Shuni, respectively. Univariable analysis by logistic regression showed a nonsignificant association between the *E. coli* isolates and the local government areas (Table 4). However, only 3 isolates of *E. coli* recovered from raw milk samples were *E. coli* O157:H7 with positive proportions of 25.0% (2/8) and 33.3% (1/3) for Sokoto North and Sokoto South LGAs, respectively. None of the *E. coli* isolates from Dange-Shuni LGA were STEC. Fisher’s Exact test revealed a nonsignificant association ($p = 0.998$; OR = 1.50; [95% CI: 0.084–26.86]) between the STEC isolates and the LGAs in the study area (Table 5). The two STEC identified among the nine *E. coli* isolated recovered from fermented milk were from different LGAs (Sokoto North and Sokoto South). However, no statistical analysis was undertaken to determine any association between the STEC isolates and the LGAs since both are areas had one positive sample each.

Milk is a good medium for the growth and proliferation of microorganisms and several disease-causing bacteria such as *Listeria monocytogenes*, *Salmonella* species, *Shigella* species, and *Campylobacter* species have been associated with consumption of contaminated milk [16]. Of all the pathogens responsible for milk-borne diseases, *E. coli* O157:H7 is the most pathogenic as it has been incriminated in various...
Table 5: Frequency of *Escherichia coli* O157:H7 isolates from raw milk in two Local Government Areas (LGAs).

| LGA        | Number of samples positive for *Escherichia coli* O157:H7 | Number of samples negative for *Escherichia coli* O157:H7 | Total |
|------------|----------------------------------------------------------|----------------------------------------------------------|-------|
| Sokoto North | 2                                                       | 6                                                        | 8     |
| Sokoto South | 1                                                       | 2                                                        | 3     |
| Total       | 3                                                       | 8                                                        | 11    |

*p = 0.998, OR = 1.50, (95% CI: 0.084–26.86), OR: odds ratio, CI: confidence interval, and LGA: Local Government Area. Wamakko LGA was not included in the analysis as none of the samples from the area were positive for *Escherichia coli* O157:H7.*

4. Conclusion

The findings of this study indicate presence of *E. coli* O157:H7 in raw and fermented milk sold within Sokoto metropolis. The milk products could be contaminated in the course of milk collection, processing, storage, and/or transportation, thus presenting serious public health problems, especially to children and the elderly. Emphasis should be made on the need for hazard analysis critical control point (HACCP) in the traditional methods of milk production in order to identify potential sources of microbial contaminants and introduce appropriate prevention and control measures. Veterinary extension services in the State should reiterate on farm hygiene practices with a view to educating the Hausa/Fulani nomads on the importance of farm hygiene and the risk associated with consumption of contaminated milk. Emphasis should be made on the zoonotic potential of *Escherichia coli* O157:H7 and the role of cattle in the spread of the pathogen. Health authorities in Sokoto State and the country at large need to regulate the production and sell of milk and milk products by introducing periodic screening and issuance of fitness certificate to farmers.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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