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Polymerase Chain Reaction, Characterization and Antibiogram of Conventional Antibiotics on *Escherichia coli* 0157:H7 Isolated from Sundried Tomatoes within Kaduna, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author JRW designed the study, wrote the protocol and performed the PCR along with the author BJM. Isolation, characterization and statistical analysis were carried out by authors AWD, HSD and LMY. The corresponding author and author HSD managed the literature searches. Drug sensitivity test was carried out by author BA. All authors read and approved the final manuscript

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ABSTRACT

Abstract: Enterohaemorrhagic *E. coli* 0157:H7 is a zoonotic pathogen associated with diarrhea, hemorrhagic colitis and hemolytic uremic syndrome.
Aim: The aim of this research was to carry out an assessment of the incidence of Enterohaemorrhagic *E. coli* 0157:H7 in sun dried tomatoes using PCR.
Study Design: Cross sectional study.

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Place and Duration of Study: The research was carried out during dry season, January - April in Kaduna state.
Methodology: A total of 250 samples of sundried tomatoes were collected from open field sun-drying sites and different markets within Kaduna state. *E. coli* was isolated using most probable number. Colonies showing green metallic sheen were further characterized on cefixime sorbitol macConkey agar at 37^{o}C for 48hours. PCR was used to characterize the shiga-toxin producing bacteria using sets of reported synthetic oligonucleotide probes derived from sequences of the shigatoxin genes: stx1, and *stx2.*
Results: PCR amplified products identifying the stx1 and stx2 gene sequences were observed in DNA extracted from pale coloured colonies isolated on the cefixime sorbitol macConkey agar. The moisture contents of the sundried tomatoes were variable. The different markets presented moisture content with means; 13.1, 12.9, 12.9, 12.8, 12.7% for Hunkuyi, Samaru-Zaria, Kafanchan, Birnin- Gwari and Kaduna Central market respectively. The total viable bacteria count ranged from 2.3 x 10^2 – 5.6 x 10^8 cfu/g. Samples from Hunkuyi drying site presented significantly (*P=.05)* higher mean total bacterial count of Log_{10}8.74±0.33 than Log_{10} 7.43±0.23 from Samaru-Zaria, Kafanchan (Log_{10}5.74±0.07), Birnin Gwari (Log_{10}5.26±0.05) and central market (Log_{10}2.36±0.04). Using disk diffusion method, the Shigatoxin producing *E. coli* isolates were resistant to chloramphenicol, sparflaxacin, amoxicillin and to some few other common conventional antibiotics but they were sensitive to ciproflacxacin. The prevalence of the isolated pathogens was 2.6% and 1.3% for stx1 and stx2 respectively.
Conclusion: This study has shown sun dried tomatoes could be a vehicle for Shiga-toxin-producing *Escherichia coli.*0157:H7. Therefore, intervention through the good manufacturing practices and establishment of improved sun drying processes is advocated to avoid life-threatening systemic manifestations often associated with human infections.

Keywords: Sun dried tomatoes; E. coli 0157:H7; stx1; Stx2; Nigeria.

1. INTRODUCTION

Enterohaemorrhagic *E. coli* 0157:H7 is a gastrointestinal pathogen [1]. They are zoonotic pathogens associated with diarrhea, hemorrhagic colitis and hemolytic uremic syndrome [2]. Diseases caused by entero-hemorrhagic *Escherichia coli* (EHEC) have become a major public health problem as reported by Fode-Vaughan [3] in human beings. The organism is commonly acquired through the consumption of contaminated food, water [4] and via contact with ruminant feces. This organism could cause attaching and effacing properties in diarrhea cases [5].

EHEC infections can result in bloody or non bloody diarrhea, which may be complicated by hemorrhagic colitis and severe renal and neurological sequelae, including hemolytic-uremic syndrome [6]. Ruminant gastrointestinal tract is the primary reservoir of *E. coli* 0157:H7 [7-8]. These organisms are shed along with faeces by the reservoir to open fields which could contaminate open field where agricultural products such as tomatoes are sun dried. Consequently, through rain wash off and dust, the organisms contaminate these products. For this reason the aim of this research was to carry out an assessment of the incidence of Enterohaemorrhagic *E. coli* 0157:H7 in sundried tomatoes within some parts of Kaduna state.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Total of two hundred and fifty (250) samples was collected during dry season of January – April, 2015. The samples were purchased from open sun drying sites; Hunkuyi, Samaru and some selected markets; Kafanchan, Birnin-Gwari and Kaduna Central market within Kaduna state.

2.2 Determination of Moisture Content

Percentage moisture content of the samples was determined according to the methods presented previously [9].

2.3 Total Viable Count

Serial dilution of the samples was prepared by arranging six test tubes containing sterile 9ml of peptone water. Ten (10g) of the sample was poured into 90mls of sterile peptone water. This was homogenized using magnetic stirrer to produce stock solution. A sterile pipette was

used to draw one (1 ml) aliquots of the stock solution and transferred aseptically into the first test tube containing sterile 9ml peptone water and mixed thoroughly to produce a dilution. This process was repeated serially to obtain dilutions 10^{-1} - 10^{-8}. These dilutions were inoculated on sterile plate count agar using spread plate method. Dilution with count 10 -300 colonies was adopted for subsequent analysis. The counts obtained were expressed as Log_{10}.

2.4 Screening for Coliforms and Faecal Coliforms Using Most Probable Number (MPN)

Coliforms and faecal coliforms were assayed according to methods presented earlier [10].

Gram staining was performed after completed test. All cultures appearing as Gram-negative, short rods were tested for the IMViC reactions.

2.5 Isolation of *E. coli* 0157:H7

Colonies showing green metallic sheen were further subcultured on cefixime sorbitol macConkey agar at 37℃ for 24hs. Colonies showing pale coloured appearance were recorded as *E. coli* 0157:H7.

2.6 Molecular Characterization of *E. coli* 0157:H7

2.6.1 DNA extraction

Pure submerged cultures of *E. coli* 0157:H7 isolated from sun dried tomatoes with characteristic green metallic sheen on Eosin Methylene Blue Agar, gram negative rod and pale coloured colonies on cefixime macConkey sorbitol agar was used as a source of DNA. The DNA was extracted by the Qiagen DNeasy Plant Mini-Kit. The concentration and purity of the extracted DNA were determined according to the reported method of [11].

2.6.2 PCR

PCR was carried out according to the methods of [12]. Amplification reaction for stx1 was carried out for 30 repeated cycles as follows; initial-ization - temperature of 94℃ for 2mins, denaturation reaction of 94℃ for 30sec, then annealing at 48℃ for 30sec then finally elongation at 72℃ for 60sec. The amplification reaction for *stx2* was carried as follows for 30 cycles in a thermal cycler (WD-9402C). The amplification reaction for stx2 was carried out for 30 cycles at 94℃/2 minutes, denaturation at 94℃ for 30sec, annealing at 56℃ for 30sec, and extension at 72℃ for 60sec by [13] in [12].

2.6.3 Pimers

A synthesized primer produced using Prime 3` soft ware programme was obtained from Sigma-Aldrich Co. LLC. U.S.A. and the sequences shown below.

stx-1 F ACACTGGATGATCTCAGTGG
R CTGAATCCCCCTCCATTATG
amplicon (614bp) [14] in [12]

stx-2 F CCATGACAACGGACAGCAGTT
R CCTGTCAACTGAGCAGCACTTTG
amplicon (779bp) [14] in[12]

2.6.4 Characterization of amplicon

The PCR products was analysed by electrophoresis according to the methods of [15] on a 1% agarose gel in 1 x TAE (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) stained with 1 µg of G-green per ml.

2.7 Antibiotic Sensitivity Test on *E. coli* 0157:H7 Isolates

Antibiotic sensitivity test was carried out according to the methods of authors [16].

3. RESULTS AND DISCUSSION

Table 1 presents mean ±S.E.M. of percentage moisture content and total viable bacteria count of sun dried tomatoes. Hunkuyi had the highest moisture content (13.10±1.10%) with lowest values on samples from central market (12.7±1.12%). The result showed no significant difference (*P=.05*) between the values obtained for moisture contents. Mean total bacteria viable count was highest for dried tomatoes obtained from Hunkuyi (Log_{10}8.74±0.33), with lowest count of Log_{10}2.36±0.04 on samples from central market. The high bacteria load on sundried tomatoes could be due to handling techniques. Traditionally, fresh tomatoes are cut into pieces and spread on open field where ubiquitous microorganisms could contaminate the exposed products. Comparatively, the mean bacteria counts ($2.3 \times 10^2 - 5.6 \times 10^8$ cfu/g) observed from this study is higher than 1.0×10^3 cfu/g recommended limits prescribed by Standard Organization of Nigeria for raw food material such as wheat flour [17].

Table 1. Percentage moisture content and total viable bacteria count of sun dried tomatoes (n=250)

Sample source	Parameter	
	Moisture (%)	Total viable count (Log_{10})
Hunkuyi drying site	13.10±1.10[a]	8.74±0.33[b]
Samaru drying site	12.9±1.32[a]	7.43±0.23[b]
Kafanchan market	12.9±1.08[a]	5.74±0.07[b]
Birnin-gwari market	12.8±1.31[a]	5.26±0.05[b]
Central market	12.7±1.12[a]	2.36±0.04[c]
P-value	0.99	0.51

Values are Mean ±S.E.M. and superscripts with different letters varied significantly

Table 2 showed the mean occurrence of coliforms, faecal coliforms and *E. coli* 0157:H7 isolated from sun dried tomatoes. Samples from Hunkuyi presented highest coliform counts (920.0±5.50 MPN/g), while Kafanchan and central markets presented lowest coliform counts (220±2.10 MPN/g). Dried tomatoes from Samaru had highest contamination of both faecal coliforms (34.0±2.22 MPN/g) and *E. coli* 0157:H7 (Log_{10}1.85±0.08). Analysis of results showed significant difference (*P>.05*) between the coliform counts of the different samples. However, no significant difference (*P=.05*) between loads of faecal coliforms and *E. coli* 0157:H7 on samples. The high occurrence of coliforms, faecal coliforms and detection of *E. coli* 0157:H7 observed in this research could be due exposure of the products to animal faeces as reported by author [18]. Herds of cattle ranches sited within the vicinity of the drying sites especially in Samaru locale that registered highest faecal coliforms (34.0±2.22MPN/g) and *E. coli* 0157:H7 (Log_{10}1.85) could be responsible for the contamination. These organisms could be transferred to the drying points by either wind or pre contaminated during Irrigation with sewage water or unhygienic sun drying on floors and other surfaces [19]. Similarly, the use of animal dung as a fertilizer is a common practice in the sample areas and thus could also be a medium for high bacteria loads on exposed foods. Cattle manure function as a reservoir for the pathogens, with most cattle isolates had been reported clustered with *E. coli* 0157:H7 [6].

In Table 3, prevalence of *E. coli* 0157:H7 and faecal coliforms on sun dried tomatoes is presented. The result shows that samples from Samaru recorded the highest occurrence of *E. coli* 0157:H7 (11%) and faecal coliforms (31%). The prevalence of *E. coli* 0157:H7 and faecal coliform obtained from this study could be due to exposure of the drying sites to faeces of infected humans. Most of the drying sites are within the locale of the villagers with lacking sewage disposal facility. Therefore, indiscriminate passing of faeces in nearby bush around drying sites could be a result of microbial contaminants observed on the sundried tomatoes.

The detection of stx-1 and stx-2 at 2.6% and 1.3% respectively depicts that samples were contaminated with Shiga-toxin-producing *Escherichia coli.* This infer that sun dried tomatoes could carry organisms with health hazard and consumers of sun dried tomatoes advised to properly processed this product before consumption. Although the tested antibiotics; Septrin, Chloramphenicol, Sparfloxaxin, Amoxicillin, Augumentin, Gentamycin, pefloxacin, Tarivid, Erythromycin and Streptomycin are commonly used for treatment of bacteria caused ailments in the study areas however, this study affirms that Ciprofloxacin could be a drug of choice for treatment of *E. coli* 0157:H7 infection.

Table 2. Coliforms, faecal coliforms and *E. coli* 0157:7 loads on sun dried tomatoes (n=250)

Sample source	Bacteria count		
	Coliforms (MPN/g)	Faecal coliforms (MPN/g)	*E. coli* 0157:H7 (Log_{10})
Hunkuyi drying site	920.0±5.50[a]	26.0±3.01[e]	1.18±0.05[d]
Samaru drying site	540.0±3.80[b]	34.0±2.22[e]	1.85±0.08[d]
Kafanchan market	220±2.10[c]	17.0±1.18[e]	1.30±0.02[d]
Central market	220.0±2.12[c]	14.0±1.01[e]	1.48±0.04[d]
Birnin Gwari	350.0±2.80[d]	21.0±2.20[e]	1.48±0.05[d]
p-value	0.03	0.91	0.91

Values are Mean ±S.E.M. and superscripts with different letters varied significantly

Table 3. Occurrence of *E. coli* 0157:H7 and faecal coliforms in Sun dried tomatoes (n=250)

Sample source	*E. coli* 0157:H7 (%)	Faecal coliforms (%)
Hunkuyi drying site	9.00	29.00
Samaru drying site	11.00	31.00
Kafanchan market	4.00	27.00
Central market	4.00	28.00
Birni-Gwari market	6.00	29.00

4. CONCLUSION

The mean bacterial counts on sundried tomatoes range between 2.3 x 10^2 – 5.6 x 10^8 cfu/g. Highest level of faecal coliforms and *E. coli* 0157:H7 contamination 34.0±2.22 MPN/g and Log_{10}1.85 respectively was observed on sundried tomatoes from Samaru. Occurrence of stx-1 (2.6%) and stx-2 (1.3%) suggest association of sundried tomatoes with certain strains of Shiga-toxin-producing *Escherichia coli* (STEC). Therefore, the traditional sun drying practice can be a vehicle for microbial contaminants. There is the need to prevent possible major public health threat to human consumers of sundried tomatoes through hygienic handling and improved sun drying practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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