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**Original Research Article**

# ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* O157:H7 FROM FRESH MILK AND FERMENTED MILK (NONO) IN BENIN CITY, EDO STATE, NIGERIA

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**ABSTRACT**

*This study was designed to investigate the occurrence of Escherichia coli O157:H7 in fresh and fermented milk (nono) in Benin City, Edo State, Nigeria using standard culture-based and polymerase chain reaction methods. A total of 42 milk samples which comprise of fresh milk (n=18) and fermented milk (n=24) were studied between March and May 2017. The heterotrophic cell count from fresh milk ranged between $4.00\times10^{9}$ and $3.39\times10^{10}$ cfu/ml while $4.50\times10^{9}$ and $3.65\times10^{10}$ cfu/ml were observed for the fermented milk. The E. coli O157:H7 cell count from fresh milk ranged from $3.00\times10^{4}$ to $3.12\times10^{6}$ cfu/ml while a range of $1.00\times10^{4}$ to $3.10\times10^{6}$ cfu/ml were observed for the fermented milk. Other E. coli cell count from fresh milk ranged from $1.00\times10^{4}$ to $1.36\times10^{6}$ cfu/ml while a range of $1.00\times10^{4}$ to $1.08\times10^{6}$ cfu/ml were observed for the fermented milk. Faecal coliforms cell counts from fresh milk ranged from $2.00\times10^{3}$ to $6.30\times10^{4}$ cfu/ml while a range of $1.00\times10^{3}$ to $2.76\times10^{5}$ cfu/ml were observed for the fermented milk samples. The population count difference in E. coli O157:H7, other E. coli and faecal coliforms from the fresh and fermented milk samples analysed were statistically significant ($p < 0.05$). Findings from this study revealed that both the fresh and fermented milk were positive for E. coli O157:H7 as well as other E. coli and faecal coliforms. In addition, the population count from the fresh and fermented milk samples exceeded the permissible limits of European Parliament and of the Council. Therefore, fresh and fermented milk could pose a potential health risk to consumers.*



## 1. INTRODUCTION

Milk is a significant source of nutrients to animals and humans. Milk meant to be consumed must be free from all pathogenic life-forms (Lye *et al.,* 2013). The contamination of milk by microorganisms may result in milk-borne illness to humans as well as spoilage of milk (Ombarak and Elbagory, 2017). A significant proportion of milk-borne epidemics of humans occur via milk contamination. Origin of microbial

contamination in milk includes primary contamination by microorganisms from the sick or infected lactating animal (Mansour *et al.,* 2013; Alam *et al.,* 2017). Secondary microbial contamination of milk occurs along the milk value chain which may include contamination during milking by milk handlers, unhygienic utensils, milking equipment and water supplies used in processing (Fagundes *et al.,* 2012; Suguna *et al.,* 2012). Others include recontamination of milk after processing as a result of the unhygienic conditions, improper handling and storage of milk during consumption (Alam *et al.,* 2017). Milk is referred to as a high-risk food as it is highly nutritious and serves as an ideal medium for microbial proliferation (Tasci, 2011). Common bacterial pathogens still of public health concern today in fresh milk and its derivatives include *Bacillus cereus, Yersinia enterocolitica, Listeria monocytogenes, Salmonella* spp., *Campylobacter jejunii* and *Escherichia coli* (Massa *et al.,* 1999; Laba and Udosek, 2013).

*Escherichia coli* O157: H7 has developed as a globally recognized zoonotic food borne pathogen, which results in serious illness such as haemolytic uraemic syndrome (HUS), haemorragic colitis and thrombotic thrombocytopenic purpura (TTP) in humans (Farougou *et al.,* 2012). It was first recognized as a food borne pathogen resulting from an epidemic of an unusual gastrointestinal illness in 1982 and was recovered from haemorrhagic colitis patients who previously consumed undercooked patties (Pal and Mahendra, 2016). Due to the low infective dose of *E. coli* O157:H7, severity of the disease symptoms, morbidity and mortality rate, it is considered a harmful threat in food safety (Greig *et al.,* 2010; Mosu *et al.,* 2013). Previous studies on *E. coli* O157:H7 from milk samples have been carried out in Sharkia Governorate, Egypt (Ahmed and Samer, 2017), Chittagong, Bangladesh (Alam *et al.,* 2017), Asosa Town, Western Ethiopia (Disassa *et al.,* 2017), and Finland (Jaakkonen *et al.,* 2017). In addition, *E. coli* O157:H7 and other *E. coli* have been characterized from food producing animals in Eastern Cape Province, South Africa (Msolo *et al.,* 2016), Benin City, Nigeria (Beshiru *et al.,* 2016), Hyogo Prefecture, Japan (Akiyama *et al.,* 2017), Belfast, United Kingdom (Stratakos *et al.,* 2016), ready-to-eat food products in Isfahan Province, Iran (Ranjbar *et al.,* 2017), surface water in Paris, France (Petit *et al.,* 2017), and from diarrheic patients in Mymensingh, Bangladesh (Islam *et al.,* 2016) and Benin City, Nigeria (Esumeh *et al.,* 2011). However, there is currently limited study on the isolation and identification of *E. coli* O157:H7 and other *E. coli* strains from "nono" and fresh milk in Benin City, Nigeria. Thus, this study was designed to determine the occurrence of *E. coli* O157:H7 and other *E. coli* from fermented milk (nono) and fresh milk in Benin Metropolis.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

The study was conducted on fresh milk and fermented milk samples purchased from local milk vendors in Benin City, between March and May 2017. On each sampling day, 3 to 6 fermented milk samples were randomly collected from milk vendors in sterile containers, while for the fresh milk samples, from healthy cows since the disease state of the cows were not ascertained. An average of 3 to 6 dairy cows was milked. All samples for the study were transported in a cooler containing ice to the Applied Microbial Processes & Environmental Health Research Group Laboratory, Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

### 2.2. Sample Processing and Cultivation Procedure

One (1) ml of each sample was diluted serially and 100 µl of the suspension with $10^{-8}$ dilution was spread plated unto nutrient agar (Lab M, Lancashire, United Kingdom). $10^{-4}$ was spread plated unto sorbitol MacConkey agar plates supplemented with cefixime and potassium tellurite (CT-SMAC) agar (Lab M, Lancashire, United Kingdom). $10^{-3}$ was spread plated unto m-FC agar (Merck, Germany). The inoculated plates (Nutrient agar and CT-SMAC) were incubated at 37 $^{o}$C for 18 to 24 h, while m-FC plates were incubated at 44.5 $^{o}$C for 18 to 24 h (Jaakkonen *et al.,* 2017).

### 2.3. Enumeration and Isolation Procedure

After incubation, colonies on Nutrient agar were enumerated for total heterotrophic bacteria cell counts. Blue colonies on m-FC agar were enumerated as faecal coliforms; beige to colourless colonies on CT-SMAC agar were enumerated as *E. coli* O157:H7 while pink colonies on the same CT-SMAC agar were enumerated as other *E. coli*. Distinct colonies (colourless to beige and pink) on CT-SMAC agar were sub-cultured streaking on a freshly prepared, sterile CT-SMAC agar and incubated at 37 ºC for 18 - 24 h. Thereafter, isolates were purified on Nutrient agar for 37ºC for 18 - 24 h. Purified isolates were stored on agar slants at 4 ºC until ready for use (Alam *et al.,* 2017).

### 2.4. DNA Extraction Procedure

DNA extraction from purified *E. coli* O157:H7 isolates was carried out by adopting the boiling method as previously described by Igbinosa et al. (2017). Briefly, purified isolates were inoculated into sterile tryptone soy broth (Merck, Germany) and incubated overnight. Thereafter, 2 ml from the overnight culture was transferred into 2 ml sterile Eppendorf tubes and centrifuged for 10 min at 11 000 r/min. Pellets obtained were washed twice using sterilized deionised water prior to re-suspending into 100 µl of sterilized deionized water. The mixture was transferred into the heating block and boiled at 100 °C for 10 min. The boiled cell lysate was centrifuged at 12 000 r/min for 5 min. The supernatant was thereafter transferred carefully into new sterile micro-centrifuge tubes and used as template DNA for PCR amplification.

### 2.5. PCR Amplification Assay

PCR amplification assay were carried out in a reaction mixture (25.0 µl) with master mix (12.5 µl), forward (0.50 µl) and reverse (0.50 µl) primers, nuclease free water (2.0 µl) and template DNA (5 µl). DNA templates of *E. coli* O157:H7 ATCC 35150 was used as a positive control while nuclease free water was used as a negative control in each PCR assay. PCR procedures were carried out using a primer set F-TACCATCGCAAAAGCAACTCC and R-GTCGG CAA CGTT AGTGATACC with amplicon size of 247 bp for $fliC_{H7}$; and primer set F-CTACAGGT GAAGGTGGAATGG and R-ATTCCTCTCTTTCCTCTGCGG with amplicon size of 327 bp for $rfbE_{O157}$ with the thermal cyclic conditions: initial denaturation for 5 min at 95 °C, denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s and extension at 72 °C for 90 s for a total of 35 cycles, and a final extension at 72 °C for 5 min; and the amplicons were held at 4°C (Wang *et al.,* 2002). Amplicons were electrophoresed with 1% agarose gel (Hispanagar, Spain) stained with ethidium bromide 0.5 mg/l (Merck, SA) at 100 V for 1 h in 0.5 × tris-acetate-ethylene diamine tetraacetic acid buffer (40 mmol/L tris-HCl, 20 mmol/L Na-acetate, 1 mmol/l ethylene diamine tetraacetic acid, pH 8.5) and viewed under a UV transilluminator (EBOX VX5, Vilber Lourmat, France).

### 2.6. Statistical Analysis

All data were analysed using the Statistical package SPSS version 21.0 and Microsoft Excel 2013. One Sample T-test, Paired T-test and Un-Paired T-test were used to analyse the data where a set or two set of variables were compared. In addition, one-way Analysis of Variance (ANOVA) was used to determine significant difference between multiple variables while Duncan multiple range test was used to determine significant difference between mean. *P*-values < 0.05 were considered statistically significant.

## 3. RESULTS AND DISCUSSION

The heterotrophic bacterial cell count from fresh milk in this study ranged from $2.86\times10^{10}$ to $3.05\times10^{10}$ cfu/ml in the month of March, from $4.00\times10^{9}$ to $1.20\times10^{10}$ cfu/ml in the month of April, from $4.10\times10^{9}$ to $3.39\times10^{10}$ cfu/ml in the month of May. For the fermented milk samples, the heterotrophic bacterial cell

count ranged from $2.40\times10^{10}$ to $2.84\times10^{10}$ cfu/ml in the month of March, from $4.50\times10^{9}$ to $3.60\times10^{10}$ in the month of April, from $2.56\times10^{10}$ to $3.65\times10^{10}$ cfu/ml in the month of May (Table 1). The least heterotrophic bacterial cell count for the fresh milk samples were observed in the month of April ($4.00\times10^{9}$ cfu/ml) while the highest was observed in the month of May ($3.39\times10^{10}$ cfu/ml). For the fermented milk samples, the least heterotrophic bacterial cell count was observed in the month of April ($4.50\times10^{9}$ cfu/ml) while the highest was observed in the month of May ($3.65\times10^{10}$ cfu/ml) (Table 1).

Table 1: Population count of total heterotrophic bacteria from the fresh and the fermented milk samples

| | March (cfu/ml) | | | *p*-value |
|---|---|---|---|---|
| | Min | Max | Mean ± SD | |
| Fresh milk | $2.86\times10^{10}$ | $3.05\times10^{10}$ | $2.94\times10^{10}\pm0.09^{c}$ | 0.000 |
| Fermented milk | $2.40\times10^{10}$ | $2.84\times10^{10}$ | $2.63\times10^{10}\pm0.02^{b}$ | 0.000 |
| *p*-value | | | 0.173 | |
| | April (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $4.00\times10^{9}$ | $1.20\times10^{10}$ | $7.65\times10^{9}\pm0.03^{a}$ | 0.000 |
| Fermented milk | $4.50\times10^{9}$ | $3.60\times10^{10}$ | $1.79\times10^{10}\pm0.13^{a}$ | 0.000 |
| *p*-value | | | 0.000 | |
| | May (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $4.10\times10^{9}$ | $3.39\times10^{10}$ | $2.14\times10^{10}\pm1.02^{b}$ | 0.000 |
| Fermented milk | $2.56\times10^{10}$ | $3.65\times10^{10}$ | $3.17\times10^{10}\pm0.35^{c}$ | 0.000 |
| *p*-value | | | 0.004 | |

Values represent the mean ± standard deviation of the bacterial enumerated from the total samples. Values which carry different alphabets across columns show significant difference ($p<0.05$). In addition, *p*-values <0.05 across rows are statistically significant

The emergence of *E. coli* O157:H7 serotype dates back to 1982 when it was first detected in an outbreak connected to Hamburgers (Igwe *et al.,* 2016). In this study, 18 fresh milk samples from dairy cows and 24 fermented milk samples (nono) sold for consumption were analysed. The total heterotrophic bacterial count of milk sample is an index of the herd health, quality of milk, efficacy of farm sanitation, milk handling, storage and transportation temperature (Zeinhom and Abdel-Latef, 2014). Previous studies have examined the total heterotrophic bacteria from fresh cow milk in the order as $< 10^{5}$, $12 \times 10^{6}$, $5.0 \times 10^{5}$, 4.5 $\log_{10}$, $1.3 \times 10^{6}$, $10^{6}$, 4.18 $\log_{10}$ and $10^{6}$-$10^{7}$ cfu/ml (Gran *et al.,* 2003; Chye *et al.,* 2004; Al-Tahiri, 2005; Godic-Torkar and Crole-Teger, 2008; Karami *et al.,* 2008; Shojaei and Yadollahi, 2008; Dan *et al.,* 2008; Franciosi *et al.,* 2009; Millogo *et al.,* 2010). The findings in this study were higher than those reported by the previous studies. This could be attributed to the high prevalence of unhygienic milking procedures or equipment, infected udders of the cows, the milk storage conditions and/or substandard microbiological quality of water used for cleaning utensils and animals as well. The cell count difference in bacterial isolates from the fresh and fermented milk samples analysed was statistically significant ($p < 0.05$) likewise the comparison between the difference in cell count of bacterial isolates from the fresh and fermented milk samples. This could be attributed to the fermented nature of nono which could alter bacteria proliferation within the fermented milk samples as a result of reduced or low pH. Laba and Udosek (2013) have reported that the most suitable pH for microbial proliferation in milk is within the pH range of 6.4 to 6.8. The heterotrophic bacterial count far surpassed the EC Regulation (No. 853, 2004) of the European Parliament and of the Council (European Commission, 2001) which pegged the permissible limit as $\leq 1.00\times10^{5}$ cfu/ml milk for the heterotrophic bacteria count (HBC) in cow's fresh milk.

The *E. coli* O157:H7 cell count from fresh milk in this study ranged from $2.75\times10^{6}$ to $3.12\times10^{6}$ cfu/ml in the month of March, from $1.00\times10^{5}$ to $1.60\times10^{6}$ cfu/ml in the month of April, from $3.0\times10^{4}$ to $1.41\times10^{6}$ cfu/ml in the month of May. For the fermented milk samples, the *E. coli* O157:H7 cell count ranged from $2.52\times10^{6}$ to $3.10\times10^{6}$ cfu/ml in the month of March, from $3.0\times10^{4}$ to $1.96\times10^{6}$ in the month of April, from $1.0\times10^{4}$ to $3.10\times10^{6}$ cfu/ml in the month of May (Table 2). The least *E. coli* O157:H7 cell count for the fresh milk samples were observed in the month of May ($3.0\times10^{4}$ cfu/ml), while the highest was observed in

the month of March ($2.75\times10^6$ cfu/ml). For the fermented milk samples, the least *E. coli* O157:H7 cell count was observed in the month of May ($1.0\times10^4$ cfu/ml) while the highest was observed in the month of March and May ($3.10\times10^6$ cfu/ml) (Table 2).

Table 2: Population count of *E. coli* O157:H7 isolates from the fresh and the fermented milk samples

| | March (cfu/ml) | | | *p*-value |
|---|---|---|---|---|
| | Min | Max | Mean ± SD | |
| Fresh milk | $2.75\times10^6$ | $3.12\times10^6$ | $2.97\times10^6\pm0.19^c$ | 0.000 |
| Fermented milk | $2.52\times10^6$ | $3.10\times10^6$ | $2.81\times10^6\pm0.29^c$ | 0.000 |
| *p*-value | | | 0.643 | |
| | April (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $1.00\times10^5$ | $1.60\times10^6$ | $8.91\times10^5\pm0.62^b$ | 0.000 |
| Fermented milk | $3.0\times10^4$ | $1.96\times10^6$ | $8.40\times10^5\pm0.59^a$ | 0.000 |
| *p*-value | | | 0.499 | |
| | May (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $3.0\times10^4$ | $1.41\times10^6$ | $3.51\times10^5\pm0.43^a$ | 0.000 |
| Fermented milk | $1.0\times10^4$ | $3.10\times10^6$ | $1.37\times10^6\pm1.41^b$ | 0.000 |
| *p*-value | | | 0.065 | |

Values represent the mean ± standard deviation of the *E. coli* O157:H7 enumerated from the total samples. Values which carry different alphabets across columns show significant difference ($p<0.05$). In addition, *p*-values <0.05 across rows are statistically significant

The mean log values ranging between 4.258 and 6.161 cfu/ml was obtained in the study carried out by Disassa et al. (2017). El-Asuoty et al. (2017) reported $59\times10^4\pm0.00$ cfu/ml from traditional yoghurt and $32\times10^4\pm0.08$ cfu/ml in probiotic yoghurt which was lower compared to those obtained in this study. The population cell counts difference in *E. coli* O157:H7 isolates from fresh and fermented milk samples analysed was statistically significant ($p < 0.05$) reflecting difference in bacterial contamination while the comparison between the differences in population cell counts of *E. coli* O157:H7 isolates from the fresh and the fermented fresh milk samples are statistically not significant ($p > 0.05$) for the months of March, April and May respectively, reflecting no variability in *E. coli* O157:H7 isolates. The resultant variation in microbiological quality from fresh milk in different studies may be attributed to different sample size, isolation and identification protocol, geographic area, farm size, population of animals on the farm and farm management practices (Lye *et al.,* 2013). According to Greig et al. (2010), dose of *E. coli* O157:H7 as low as 10 to 100 cfu is sufficient to cause infection with infectious dose for children ranging from 1 to 4 cfu (Duncan and Hackney, 1994; Lye *et al.,* 2013). Based on the risk assessment study using Rick Ranger, the estimated risk ranking for *E. coli* O157:H7 infection related to consumption of fresh milk is 73 cfu, indicating that there is risk of *E. coli* O157:H7 infection even though the prevalence detected is relatively low (Evrendilek and Richte, 2017).

Other *E. coli* cell count from fresh milk in this study ranged from $1.00\times10^4$ to $2.00\times10^4$ cfu/ml in the month of March, from $1.00\times10^4$ to $1.36\times10^6$ cfu/ml in the month of April, from $1.00\times10^4$ to $3.10\times10^5$ cfu/ml in the month of May. For the fermented milk samples, other *E. coli* cell count ranged from $6.00\times10^4$ to $6.00\times10^4$ cfu/ml in the month of March, from $4.00\times10^4$ to $1.08\times10^6$ in the month of April, from $1.00\times10^4$ to $8.30\times10^5$ cfu/ml in the month of May (Table 3). The least of other *E. coli* cell count for the fresh milk samples was observed in the month of March, April and May ($1.00\times10^4$ cfu/ml) while the highest was observed in the month of April ($1.36\times10^6$ cfu/ml). For the fermented milk samples, the least other *E. coli* cell count was observed in the month of May ($1.0\times10^4$ cfu/ml) while the highest was observed in the month of April ($1.08\times10^6$ cfu/ml) (Table 3).

*E. coli* is the most significant species of facultative anaerobe origin isolated in the gastrointestinal tract of human and animals and the most frequently encountered pathogen in the family "Enterobacteriaceae" (Beshiru *et al.,* 2016). Hence the occurrence of such microorganism in milk is indicative of faecal

contamination (Zeinhom and Abdel-Latef, 2014). The result from the mean population cell counts of other *E. coli* is higher than those obtained by Chye et al. (2004) with a population cell counts of $2.0 \times 10^4$ cfu/ml. Although worldwide importance of *E. coli* as an etiological agent for diarrheal illness has reduced drastically over the past 50 years succeeding the implementation of enhanced hygienic practices, it is still the significant cause of illness in the developing nations.

Table 3: Population count of other *E. coli* from the fresh and the fermented milk samples

| | March (cfu/ml) | | | *p*-value |
|---|---|---|---|---|
| | Min | Max | Mean ± SD | |
| Fresh milk | $1.00\times10^4$ | $2.00\times10^4$ | $1.50\times10^4\pm0.07^a$ | 0.000 |
| Fermented milk | $6.00\times10^4$ | $6.00\times10^4$ | $6.00\times10^4\pm0.00^a$ | 0.043 |
| *p*-value | | | 0.121 | |
| | April (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $1.00\times10^4$ | $1.36\times10^6$ | $4.18\times10^5\pm0.51^c$ | 0.000 |
| Fermented milk | $4.00\times10^4$ | $1.08\times10^6$ | $2.65\times10^5\pm0.40^b$ | 0.043 |
| *p*-value | | | 0.493 | |
| | May (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $1.00\times10^4$ | $3.10\times10^5$ | $1.05\times10^5\pm0.10^b$ | 0.000 |
| Fermented milk | $1.00\times10^4$ | $8.30\times10^5$ | $2.45\times10^5\pm0.39^b$ | 0.043 |
| *p*-value | | | 0.022 | |

Values represent the mean ± standard deviation of the other *E. coli* enumerated from the total samples. Values which carry different alphabets across columns show significant difference ($p<0.05$). In addition, *p*-values <0.05 across rows are statistically significant

The significant difference of *E. coli* in fresh and fermented milk may be attributed to the fact that fermented milk is mainly hawked by vendors after overnight fermentation while the fresh milk is usually obtained directly from dairy cows. Therefore, it will be liable to cross contamination via different processes as mixed fresh clean milk with unclean milk by hands of workers, containers of transportation or contaminated water used for cleaning utensils could be source of contamination. The presence of *E. coli* may not necessarily indicate a direct faecal contamination of milk but is an indicator of poor hygiene practices during milking and further handling of milk and presents potential hazard to consumers of such products. It could also be dangerous as the strain isolated may be either enteropathogenic or toxigenic, causing major public health menace (FAO/WHO, 2002).

Faecal coliforms cell count from fresh milk in this study ranged from $3.00\times10^3$ to $3.00\times10^5$ cfu/ml in the month of March, from $2.00\times10^3$ to $1.60\times10^4$ cfu/ml in the month of April, from $2.40\times10^4$ to $6.30\times10^4$ cfu/ml in the month of May. For the fermented milk samples, faecal coliforms cell count ranged from $2.00\times10^3$ to $2.00\times10^3$ cfu/ml in the month of March, from $2.00\times10^3$ to $1.20\times10^4$ in the month of April, from $1.00\times10^3$ to $2.76\times10^5$ cfu/ml in the month of May (Table 4). The least of faecal coliforms cell count for the fresh milk samples was observed in the month of April ($2.00\times10^3$ cfu/ml), while the highest was observed in the month of May ($2.40\times10^4$ cfu/ml). For the fermented milk samples, the least faecal coliforms cell count was observed in the month of May ($1.00\times10^3$ cfu/ml) while the highest was observed in the month of April ($1.20\times10^4$ cfu/ml) (Table 4).

The maximum standard set on coliform bacteria in fresh milk is $\leq 10$ cfu/ml per fresh milk. This standard is in conformation with both international and national public health and food safety regulation (FAO/WHO, 2002). However, this theory did not conform to the population cell counts observed as the total coliform count was way too high for all the samples examined. This may imply contamination from the grazing environment, as a consequence of their faeces, manure and soil, which enables easy dispersal of pathogens as the cows are often reared on a free range scale. Thus, contaminated water, poor herd hygiene, improperly washed milking bowl and unsanitary milking practices may have culminated in the elevated coliform cell counts in the fresh and fermented milk. The presence of faecal coliforms indicates

faecal contamination of the milk samples. The comparison between the total *E. coli* O157:H7 and other *E. coli* in the fresh milk revealed a minimum of $1.00\times10^4$ cfu/ml and a maximum of $1.36\times10^6$ cfu/ml with a mean population count of $2.18\times10^5 \pm 0.35$ cfu/ml for other *E. coli* while a minimum of $3.00\times10^4$ cfu/ml and a maximum of $3.12\times10^6$ cfu/ml with a mean population count of $9.67\times10^5\pm 0.24$ cfu/ml was observed for *E. coli* O157:H7. While for fermented milk, a minimum of $1.00\times10^4$ cfu/ml and a maximum of $1.08\times10^6$ cfu/ml with a mean population count of $2.39\times10^5 \pm 0.36$ cfu/ml for other *E. coli* while a minimum of $2.00\times10^4$ cfu/ml and a maximum of $3.71\times10^6$ cfu/ml with a mean population count of $1.35\times10^6 \pm 1.21$ cfu/ml was observed for *E. coli* O157:H7 (Table 5).

Table 4: Population count of faecal coliforms from the fresh and the fermented milk samples

| | March (cfu/ml) | | | *p*-value |
|---|---|---|---|---|
| | Min | Max | Mean ± SD | |
| Fresh milk | $3.00\times10^3$ | $5.00\times10^3$ | $4.00\times10^3\pm0.01^a$ | 0.001 |
| Fermented milk | $2.00\times10^3$ | $2.00\times10^3$ | $2.00\times10^3\pm0.00^a$ | 0.000 |
| *p*-value | | | 0.454 | |
| | April (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $2.00\times10^3$ | $1.60\times10^4$ | $6.00\times10^4\pm0.06^c$ | 0.001 |
| Fermented milk | $2.00\times10^3$ | $1.20\times10^4$ | $5.66\times10^3\pm0.05^b$ | 0.000 |
| *p*-value | | | 0.743 | |
| | May (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $2.40\times10^4$ | $6.30\times10^4$ | $4.43\times10^4\pm0.01^b$ | 0.001 |
| Fermented milk | $1.00\times10^3$ | $2.76\times10^5$ | $7.02\times10^4\pm1.37^c$ | 0.000 |
| *p*-value | | | 0.008 | |

Values represent the mean ± standard deviation of the faecal coliforms enumerated from the total samples. Values which carry different alphabets across columns show significant difference ($p<0.05$). In addition, *p*-values <0.05 across rows are statistically significant

Table 5: Comparison of the total population count of *E. coli* O157:H7 and other *E. coli*

| | *E. coli* (cfu/ml) | | | *p*-value |
|---|---|---|---|---|
| | Min | Max | Mean ± SD | |
| Fresh milk | $1.00\times10^4$ | $1.36\times10^6$ | $2.18\times10^5\pm0.35$ | 0.000 |
| Fermented milk | $1.00\times10^4$ | $1.08\times10^6$ | $2.39\times10^5\pm0.36$ | 0.000 |
| *p*-value | | | 0.289 | |
| | *E. coli* O157:H7 (cfu/ml) | | | |
| | Min | Max | Mean ± SD | |
| Fresh milk | $3.00\times10^4$ | $3.12\times10^6$ | $9.67\times10^5\pm0.24$ | 0.000 |
| Fermented milk | $2.00\times10^4$ | $3.71\times10^6$ | $1.35\times10^6\pm1.21$ | 0.000 |
| *p*-value | | | 0.323 | |

Values represent the mean ± standard deviation of the total population cell counts of *E. coli* O157:H7 and other *E. coli* enumerated from the total samples. The *p*- values <0.05 across columns and columns are statistically significant

This study assessed a total of 42 milk samples comprising 18 fresh milk samples and 24 fermented milk samples between March and May 2017. From the 42 milk samples analysed for the bacteriological status of *E. coli* O157:H7, all the samples harbour *E. coli* O157:H7. A total of 36 confirmed isolates were recovered from the fresh milk samples and 48 confirmed *E. coli* O157:H7 isolates were recovered from the fermented milk. Other *E. coli* isolates were also recovered from fresh milk (27) and fermented milk (20) respectively (Table 6). The population cell counts difference in the total *E. coli* O157:H7 and other *E. coli* from the fresh and fermented milk samples is statistically significant ($p < 0.05$), reflecting variability in contamination. The unclean or unsterilized teat can introduce microorganism into the fresh milk sample (Laba and Udosek, 2013). The herdsmen do not adopt disinfection procedure prior on the teats and udders before milking despite the muddy barnyard and dirty environment where the cow reside which unavoidably contaminate the milk and increase the population cell counts. The average values in this study indicate that the milk for consumers was of substandard quality as all milk samples obtained harboured *E. coli* O157:H7 and other *E. coli*. Laba and Udosek (2013) also reported that all samples harbour *E. coli* as

well as *E. coli* O157:H7. The occurrence of *E. coli* in the examined fresh buffalo and pasteurized cow milk samples by Ahmed and Samer (2017) were 66% and 30%, respectively. Alam et al. (2017) reported that *E. coli* from only six (18.2%) of the 33 positive samples yielded colourless colonies across the CT-SMAC, suggesting the probable presence of populations belonging to the serotype O157 and rest of the samples 27 (81.82%) produced coloured colony on CT-SMAC considering the probable presence of populations belonging to the serotype non-O157. Out of 380 fresh milk samples examined by Disassa et al. (2017), 129 (33.9%) and 11 (2.9%) were contaminated with *E. coli* and *E. coli* O157:H7, respectively. Hence, fresh and Nono milk should be considered as unsafe for consumption. Faecal pollution was confirmed by the isolation of faecal coliform and *E. coli* since *E. coli* are indicator organisms for faecal pollution. The high enumerated levels of *E. coli*, *E. coli* O157:H7 and faecal coliforms might be enough to justify that the milk samples were unsafe for human consumption since the bacteriological limits of fresh milk are not established in Nigeria.

Table 6: Prevalence of *E. coli* O157:H7 and other *E. coli* from the fresh and fermented milk samples

| Month of sample | No of samples analysed (*n*=42) | |
|---|---|---|
| | Fresh milk (*n*=18) | Fermented milk (*n*=24) |
| March | 3 | 3 |
| April | 6 | 9 |
| May | 9 | 12 |
| *p*-value | 0.074 | 0.094 |
| | No of *E. coli* isolates (*n*=47) | |
| | Fresh milk (*n*=27) | Fermented milk (*n*=20) |
| March | 3 | 2 |
| April | 11 | 14 |
| May | 13 | 4 |
| *p*-value | 0.098 | 0.214 |
| | No of *E. coli* O157:H7 isolates (*n*=84) | |
| | Fresh milk (*n*=36) | Fermented milk (*n*=48) |
| March | 6 | 6 |
| April | 12 | 18 |
| May | 18 | 24 |
| *p*-value | 0.074 | 0.094 |

## 4. CONCLUSION

Findings from the present study clearly indicated that the safety and quality of fresh and fermented milk was unsatisfactory. The presence of faecal coliform bacteria does not only denote poor hygiene but also pathogenic status. The pathogenic bacteria such as *E. coli* O157:H7 and other *E. coli* may pass through the milk to consumers. Fresh and fermented milk is of a special concern since these organisms can proliferate at variable conditions using the milk as a reservoir. It is therefore suggested that milk should often be tested for pathogens. When found positive, it should be withheld from human consumption. The production of safe and high-quality milk should be of primary importance to the farmer and the economy as well as the sustainability of the dairy industry in Nigeria.

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## 6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

## REFERENCES

Ahmed, W.F. and Samer, A. (2017). Detection of shiga toxin - producing *Escherichia coli* in raw and pasteurized milk. *Zagazig Veterinary Journal*, 45(1), pp. 47-54.

Akiyama, Y., Futai, H., Saito, E., Ogita, K., Sakae, H., Fukunaga, M., Tsuji, H., Chikahira, M. and Iguchi, A. (2017). Shiga toxin subtypes and virulence genes in *Escherichia coli* isolated from cattle. *Japanese Journal of Infectious Diseases*, 70, pp. 181–185.

Alam, K., Akther, S., Sarwar, N., Morshed, S. and Debnath, G.K. (2017). Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 isolated from raw milk marketed in Chittagong, Bangladesh. *Turkish Journal of Agriculture - Food Science and Technology*, 5(3), pp. 214-220.

Beshiru, A., Igbinosa, I.H. and Igbinosa, E.O. (2016). An investigation on antibiogram characteristics of *Escherichia coli* isolated from piggery farms in Benin City, Nigeria. *Annals of Science and Technology*, 1(1), pp. 8-12.

Chye, F.Y., Aminah, A. and Ayob, M.A. (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology*, 21(5), pp. 535-541.

Dan, S.D., Mihaiu, M., Rotaru, O. and Dalea, I. (2008). Evaluation of microbiological load and configuration of raw milk from collecting centers in CLUJ country. *Bulletin of Veterinary Medicine*, 65, pp. 346-352.

Disassa, N., Sibhat, B., Mengistu, S., Muktar, Y. and Belina, D. (2017). Prevalence and antimicrobial susceptibility pattern of *E. coli* O157:H7 isolated from traditionally marketed raw cow milk in and around Asosa Town, Western Ethiopia. *Veterinary Medicine International,* Article ID 7581531, 7 pages.

Duncan, S.E. and Hackney, C.R. (1994). Relevance of *Escherichia coli* O157:H7 to the dairy industry. *Dairy, Food Environ Sanitation*, 14, pp. 656-660.

El-Asuoty, M.S., Farag, H.F., Sabr, A.S. and El-Tedawey, F.A. (2017). Monitoring of antibacterial activity of traditional and probiotic supplemented yoghurt against *E. coli* and *S. aureus*. *Animal Health Research Journal*, 5(1), pp. 116-126.

Esumeh, F.I., Isibor, J.O. and Egbagbe, I.D.S. (2011). Screening for *Escherichia coli* O157:H7 in diarrheic patients in Benin City, Nigeria. *Journal of Microbiology and Biotechnology Research*, 1(4), pp. 1-4.

European Commission (2001). Overview of microbiological criteria for foodstuffs in community legislation in force. http://europa.eu.int/comm/food/fs/mr/mr_crit_en.pdf.

Evrendilek, G.A. and Richte, E.R. (2017). Concurrent detection of foodborne pathogens: Past efforts and recent trends. *Journal of Food Microbiology Safety and Hygiene*, 2(1), pp. 116-123.

Fagundes, H., Corassin, C.H., Tavolaro, P. and Oliveira, C.A.F. (2012). Milk hygienic practices and occurrence of *Staphylococcus aureus* and *Escherichia coli* O157:H7 in small-scale dairy farms in São Paulo, Brazil. *African Journal of Microbiology Research*, 6(28), pp. 5805-5808.

Food Agriculture Organization (FAO) and World Health Organization (WHO) (1997). General requirements (food hygiene). Codex Alimentarius, Food and Agriculture Organization, Rome.

Farougou, S., Sessou, P., Yehouenou, B. and Dossa, F. (2012). Microbiological quality of raw milk processed from cows raised under extensive system in the republic of Benin. *Research Journal of Microbiology*, 7(7), pp. 337-343.

Franciosi, E., Pecile, A., Cavazza, A. and Poznanski, E. (2009). Microbiological monitoring of raw milk from selected farm in the Trentingrana region. *Italian Journal of Animal Sciences*, 8, pp. 408-410.

Godic-Torkar, K. and Golc-Teger, S. (2008). The microbiological quality of raw milk after introducing the two days milk collecting system. *Acta Agric Slovenica*, 92, pp. 61-74.

Gran, H.M., Wetlesen, A., Mutukumiran, A.N., Rukure, G. and Narvhus, J.A. (2003). Occurence of pathogenic bacteria in raw milk, cultured pasteuized milk and naturally soured milk produced as small-scale daries in Zimbabwe. *Food Control*, 14, pp. 539-544.

Greig, J.D., Todd, E.C.D., Bartleson, C. and Michaels, B. (2010). Infective doses and pathogen carriage, USDA 2010 Food Safety Education Conference, pp. 19-20.

Igbinosa, I.H., Beshiru, A., Odjadjare, E.E., Ateba, C.N. and Igbinosa, E.O. (2017). Pathogenic potentials of *Aeromonas* species isolated from aquaculture and abattoir environments. *Microbial Pathogenesis*, 107, pp. 185-192.

Igwe, J.C., Onaolapo, J.A., Ehimidu, J.O., Bolaji, R.O., Tytler, A.B. and Ojiego, B.O. (2016). Antibiotic susceptibility profile of *E. coli* serotype O157:H7 in ABUTH, Zaria, Nigeria. *International Journal of Tropical Disease and Health*, 11(1), pp. 1-8.

Islam, M.M., Ahamed, S., Arafat, M.Y., Hasan, I., Rahman, M. and Nazir, K.H.M.N.H. (2016). Molecular detection and antibiogram of shiga toxin producing *Escherichia coli* (STEC) isolated from diarrheic children. *Bangladesh Journal of Veterinary Medicine*, 14(2), pp. 289-295.

Jaakkonen, A., Salmenlinna, S., Rimhanen-Finne, R., Lundstrom, H., Heinikainen, S., Hakkinen, M. and Hallanvuo, S. (2017). Severe outbreak of sorbitol-fermenting *Escherichia coli* O157 via unpasteurized milk and Farm visits, Finland 2012. *Zoonoses and Public Health* 64, pp. 468-475.

Karami, A.R., Dolgharisharf, J., Najafian, K., Khajeh, M. and Ghazani, M.H.M. (2008). Microbiological safety of raw milk in Tabriz, Iran. Journal of Animal and Veterinary Advances, 7, pp. 863-865.

Laba, S.A. and Udosek, C.E. (2013). Bacteriological quality of raw cow milk in Ilorin, North Central Nigeria. *Nature and Science*, 11, pp. 73-79.

Lye, Y.L., Afsah-Hejri, L., Chang, W.S., Loo, Y.Y., Puspanadan, S., Kuan, C.H., Goh, S.G., Shahril, N., Rukayadi, Y., Khatib, A., John, Y.H.T., Nishibuchi, M., Nakaguchi, Y. and Son, R. (2013). Risk of *Escherichia coli* O157:H7 transmission linked to the consumption of raw milk. *International Food Research Journal*, 20, pp. 1001-1005.

Mansour, M.A., Ayoub, M.A., Abd-El-Aal, S.F. and Mohammed, A.S. (2013). Prevalence of virulent *Escherichia coli* in cow milk at Sharkia Governorate, Egypt. *Zagazig Veterinary Journal*, 41, pp. 57-65.

Massa, S., Goffredo, E., Altieri, C. and Natola, K. (1999). Fate of *Escherichia coli* O157:H7 in unpasteurized milk stored at 8°C. *Letters in Applied Microbiology*, 28, pp. 89-92.

Millogo, V., Sjaunja, S., Ouedraogo, K. and Agenas, G.A.S. (2010). Raw milk hygiene at farms, processing units and local markets in Burkina Faso. *Food Control*, 21, pp. 1070-1074.

Mosu, S., Megersa, M., Muhie, Y., Gebremedin, D. and Keskes, S. (2013). Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia. *Complementary Journal of Food Science and Technology Research*, 1, pp. 1 -8.

Msolo, L., Igbinosa, E.O. and Okoh, A.I. (2016). Prevalence and antibiogram profiles of *Escherichia coli* O157:H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa. *Asian Pacific Journal of Tropical Diseases*, 6, pp. 990-995.

Ombarak, R.A. and Elbagory, A.M. (2017). Bacteriological quality and occurrence of some microbial pathogens in goat's and ewe's milk in Egypt. *International Food Research Journal*, 24, pp. 847-851.

Pal, M. and Mahendra, R. (2016). *Escherichia coli* 0517:H7: An emerging bacterial zoonotic food borne pathogen of global significance. *International Journal Interdisciplinary and Multidisciplinary Studies*, 4, pp. 1-4.

Petit, F., Clermont, O., Delannoy, S., Servais, P., Gourmelon, M., Fach, P., Fournier, M., Denamur, E. and Berthe, T. (2017). Change in the structure of *Escherichia coli* population and the pattern of virulence genes along a rural aquatic continuum. *Frontiers in Microbiology*, 8, pp. 609.

Ranjbar, R., Masoudimanesh, M., Dehkordi, F.S., Jonaidi-Jafari, N. and Rahimi, E. (2017). Shiga (Vero)-toxin producing *Escherichia coli* isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. *Antimicrobial Resistance and Infection Control*, 6(4), pp. 1-11.

Regulation (EC) (2004). No.853/2004 of the European Parliament and of the Council of 29. April 2004 laying down specific hygiene roles for food and animal origin. Official Journal of the European Communities, 1, pp. 22–82.

Shojaei, Z.A. and Yadollahi, A. (2008). Physicochemical and microbiological quality of raw, pasteurized and UHT milks in shops. *Asian Journal Scientific Research*, 1, pp. 532-538.

Stratakos, A.C., Linton, M., Millington, S. and Grant, I.R. (2016). A loop-mediated isothermal amplification method for rapid direct detection and differentiation of nonpathogenic and verocytotoxigenic *Escherichia coli* in beef and bovine faeces. *Journal of Applied Microbiology*, 122, pp. 817-828.

Suguna, M., Bhat, R. and Nadiah, W.W.A. (2012). Microbiological quality evaluation of goat milk collected from small scale dairy farms in Penang Island, Malaysia. *International Food Research Journal*, 19(3), pp. 1241-1245.

Tasci, F. (2011). Microbiological and chemical properties of raw milk consumed in Burdur. *Journal of Animal and Veterinary Advances*, 10(5), pp. 635-641.

Wang, G.H., Clark, C.G. and Rodgers, F.G. (2002). Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga-toxin family by multiplex PCR. *Journal of Clinical Microbiology*, 40(10), pp. 3613–3619.

Zeinhom, M.M.A. and Abdel-Latef, G.K. (2014). Public health risk of some milk-borne pathogens. *Beni-Suef University Journal of Basic and Applied Science*, 3(3), pp. 209-215.