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Occurrence and Seasonal Variation of *Escherichia coli* Isolated from Unpasteurised Raw Milk and its Products Sold in Abuja Metropolis, Nigeria

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A B S T R A C T

Raw milk and milk products are traditional staple diets that are popularly consumed by both rural and urban population of Northern Nigeria and many other parts of Africa. The aim of the study was to isolate *E. coli* from unpasteurised raw milk and milk products (Madara, Kindrimo, Nono and Manshanu) during the rainy and dry seasons in Abuja in order to ascertain the hygienic status of the dairy products. Three hundred unpasteurised raw milk and its products were collected in both rainy and dry seasons respectively. *Escherichia coli* was isolated by microbiological techniques and confirmed by Microbact™ (Oxiod™) GNB 24E System Identification Kit. Results showed that dry season had higher occurrence of *E. coli* (21, 7.0%) while occurrence during rainy season was (14, 4.7%). There was no statistically significant difference in occurrence of *E. coli* isolates during the two seasons ($p > 0.05$). These ready to eat dairy products frequently harbor coliforms that indicates poor hygiene and insanitary conditions possibly during processing and cause infections that are of great public health importance. Educating the milk handlers on the importance of personal hygiene and proper sanitary methods is necessary.

Introduction

Consumption of fresh produce is part of a healthy diet, but pathogen contamination of fresh produce has resulted in serious public health consequences (Jung *et al.*, 2014). Globally, the number of outbreaks and cases of foodborne illness associated with consumption of contaminated food continues to escalate (Teplitski *et al.*, 2011; Hoelzer *et al.*, 2012). Marketers and consumers of unpasteurised raw milk and their products have existed in many parts of the world. Unpasteurised raw milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population by consuming the raw milk and its products. Reasons that people may believe

that the raw milk and their products have beneficial dietary effects and additional nutritive value over the pasteurised one (Ali and Abdelgadir, 2011).

Raw milk and milk products of dairy cattle are known repository of food borne pathogens (Enabulele *et al.,* 2014). The presence of food borne pathogens in milk has been traced to direct contact with contaminated sources in the dairy farm environment particularly from water source, and excretion from the udder of an infected animal (Oliver *et al*., 2005). Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and utensils used for the storage of milk on farm or during transportation and unhygienic conditions. Nono, Kindrimo, Manshanu and Madara are local dairy products that are widely consumed as food especially in the northern part of Nigeria, and these have been reported to contain high nutritional values (Makut *et al.*, 2014). *Escherichia coli* which belongs to the Enterobacteriaceae family, is a Gram-negative, facultative anaerobic, non-sporulating bacteria. It is widely distributed in intestinal microbiota of humans and warm-blooded animals and in the environment, when contaminated with feaces (Nataro and Kaper, 1998; Smith *et al*., 2004).

Escherichia coli and other coliforms are microbes that can easily contaminate milk and milk products, which are often used as indicator organisms. The recovery and remuneration of *E. coli* is used as reliable indicator of feacal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard.

Most *E. coli* are harmless, but a substantial population is known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.*, 2004). Owning to the fact that these ready-to-eat dairy products are processed by 'too simple' a method and the knowledge on the microbial limit especially the coliforms is barely unknown. There are scanty reports on the microbiological quality of milk and its product in Nigeria. The aim of the study was to determine the presence of Enterobacteriaceae, *Escherichia coli* a coliform as an indicator of unsanitary practices in food during the two major seasons of the year.

Materials and Methods

Sample location

The study was carried out in Abuja, the Federal Capital Territory (FCT) located in the geographical Centre of Nigeria with a land area of 8, 000 square kilometres and lies between latitude 9° 10' north of the equator and longitude 7° 11' east. It is bounded North by Kaduna state (North), Niger state (West), Nasarawa state (East) and Kogi state (South) (Dawam, 2000). It is made up of six Area Councils namely, Abaji, AMAC (Abuja Municipal Area Council), Bwari, Gwagwalada, Kuje and Kwali. The study area experiences two weather conditions annually which are the rainy season and the dry season. The rainy season begins from April and ends in October and the dry season from November and ends in March (Abdulmalik *et al.,* 2013).

Sample collection

Three hundred (300) milk samples were randomly collected from the six Area Councils at various points in dry and rainy seasons respectively. Samples were aseptically collected in duplicates at point of sales in sterile plastic containers, labeled and transported in ice box to the laboratory for immediate analysis.

Microbiological analysis/ sample enrichment, selective and differential plating

Ten millilitres (10ml) of the unpasteurised raw milk samples were transferred into 90ml of modified Tryptic Soy broth (mTSBn) (oxoid) supplemented with 20mg/l novobiocin (oxoid) homogenized for 2minutes in a stomacher (Lab Blender 400, Seward Medical, London, UK) and then incubated at 37^0C for 18 hours as *E. coli* enrichment step. A loopful of the enriched broth was streaked on the plate of Eosin Methylene blue agar (EMB), (oxoid) and after overnight incubation at 37^0C. Suspected colonies of *E. coli* (greenish metallic sheen appearance with dark purple centres) were Gram stained, biochemically identified and confirmed.

Biochemical characterization of *Escherichia coli*

The presumptive Gram stained *E. coli* were subjected to conventional biochemical tests namely, Gelatin liquefaction, Nitrate reduction, Urease production, Oxidase, Indole-methylred-Voges-Proskauer, Catalase, Citrate Agar, and Sugar fermentation tests (Müller *et al*., 2003).

Confirmatory screening of presumptive *E. coli* MICROBACT™ GNB 24E system identification

Confirmatory screening was carried out on the presumptive Gram stained *E. coli* isolates using MICROBACT™24E. The isolates were subsequently confirmed using the commercially prepared biochemical test kits (Microbact Oxoid). The MICROBACT™ identification kits (Oxiod) were inoculated with the *E. coli* suspension, incubated at 37^0C for 18-24hours and results read as described by the manufacturer. The steps of the procedure were followed as prescribed by Cowen and Steel (1977), Balows *et al.* (1991).

Statistical Analysis

The data generated in this study was analyzed by Chi Square test using SPSS version 20.0. The value of ($p<0.05$) was considered statistically significant.

Results and Discussion

The percentage occurrence of *E. coli* during the dry season (Table 1), was (21, 7.0%). The highest percentage occurrence among the four milk types was Madara (10, 14.3%), followed by Kindrimo (6, 6.7%), Nono (3, 3.8%) and Manshanu (2, 3.3%). Though the percentage occurrence of *E. coli* in the milk samples differed during the dry season, there was no statistically significant difference (χ^2= 7.381, df= 3, P=0.061) in the occurrence of *E. coli* during the dry season. This indicates that dry season did not affect the growth of *Escherichia coli* at the period of this study.

Table 2 shows the percentage occurrence of *E. coli* during the rainy season (14, 4.67%). Madara had the highest percentage of occurrence (5, 6.02 %), followed by Kindrimo (4, 5.7%), Nono (3, 3.9%) and Manshanu (2, 2.9%). Though, there seemed to be varying percentages of occurrence of *E coli* in the different milk samples, there was no statistically significant difference (χ^2= 1.429, df= 3, P= 0.699) in the occurrence of *E. coli* during the rainy season, which indicates that rainy season did not affect the survival and growth of *Escherichia coli* in the milk products at the time of this study.

Figure 1 displays the overall distribution of *E. coli* in the two seasons (dry and rainy season), from the four milk products. Madara had the highest occurrence in the dry season (10,

14.3%) and highest in the rainy season (5, 6.0%) and total of (15, 9.8%) followed by Kindrimo (6, 6.7%) in the dry season and (4, 5.7%) in the rainy season with total of (10, 6.3%), Nono had (3, 3.8%) in the dry season and (3, 3.9%) in the rainy season with total of (6, 3.8%) while Manshanu had the lowest occurrence of *E. coli* (2, 2.9%) in the dry season and (2, 3.3%) in the rainy season with total of (4, 3.1%).

Unpasteurised raw milk and milk products are nutrient rich sources of food (Momtaz *et al*., 2012; Egwaikhide *et al*., 2014; Igwegbe *et al*., 2015) and has become a significant part of human diet (Igwegbe *et al*., 2015). The complex composition and water activity of raw milk (Soomro *et al.,* 2002), which makes it a favourable medium that supports the growth and survival of various microorganisms (Mohamed and El-Zubeir, 2007) either pathogenic or non-pathogenic strains which may result in desirable and undesirable products in the milk (Yagoub *et al.,* 2005; Ogbonna *et al.,* 2012) or may cause infections and intoxications in humans when consumed (Oliver *et al.,* 2005).

Several researchers have reported on the poor microbiological quality of unpasteurised raw milk, tracing the contamination of these milk and milk products to some factors such as unhygienic practices of the milkers/handlers (Soomro *et al.,* 2002; Abebe *et al.,* 2014), insanitary vessels/utensils for storage and temperature (Leedom, 2006; Campos *et al.,* 2009; Momtaz *et al.,* 2012), environment and infected milk producing animals(Momtaz *et al.,* 2012; Caine *et al.,* 2013; Jeyakumar and Lawrence, 2014), and contaminated water (Abdalla and El-Zubeir, 2006; Momba *et al.,* 2008). *Escherichia coli* is one of the most frequently encountered microorganisms in the food industry (Adeferekan *et al.,* 2014). Various disease outbreaks have been reported to be due to ingestion of food contaminated with pathogenic *E. coli* strains (Pradel *et al.,* 2000). Previous studies suggested that raw milk and various dairy products samples were considered to be the primary source of *E. coli* (Gundogan and Avci, 2014; Abebe *et al.,* 2014).The production of these milk products is based on traditional methods (bare hands) without any regard to the quality of raw materials used and the safe quality of the products (Soomro *et al.,* 2002; Campos *et al.,* 2009).

In developing countries, it is difficult to secure optimal food hygienic practices (Abebe *et al.,* 2014), and under such prevailing conditions microorganisms gain entry into the milk and its products. One of such microorganisms is *Escherichia coli. Escherichia coli* is an environmental pathogen (Caine *et al.,* 2013), a frequently contaminating organism, and is a reliable indicator of feacal contamination generally in insanitary conditions and poor hygiene of water, food, milk and other dairy products (Diliello,1982; Thaker *et al.,* 2012). The status of *Escherichia coli* as pathogen and its presence in food constitutes public health concern (Caine *et al.,* 2013).The recovery of *E. coli* from unpasteurised raw milk and milk products samples in this study agrees with reports of other researchers (Adesiyun *et al.,* 1997; Onono *et al.,* 2010; Ali and Abdelgadir, 2011; Rahimi, 2011; Cain *et al.,* 2013; Enabulele *et al.,* 2014; Gundogan and Avci, 2014; Makut *et al.,* 2014).

It also suggested that the methods of production, handling during processing, transportation and sales of these ready-to-eat food are entirely insanitary thereby exposing the milk samples to microbial contamination (Ali and Abeldagir, 2011). The nosedive in the aspect of sanitary practices could cause food borne diseases (Ijah *et al.,* 2002), especially among urban residents who drink fresh milk sold by the Fulani women (Okeke *et al.,* 2014). The presence of bacterial contamination of raw milk and milk products

especially by coliform, Enterobacteriaceae particularly *E. coli* have been evaluated and reported by several researchers (Soomro *et al.,* 2002; El-Zubeir and Ahmed 2007; Ali and Abdelgadir 2011; Momtaz *et al.,* 2012; Ogbonna *et al.,* 2012; Caine *et al.,* 2013; Abebe *et al.,* 2014; Vendramin *et al.,* 2014; Al-Zogibi *et al.,* 2015; Enem *et al.,* 2015; Mohammed and Abdullahi, 2015; Saba and Adzitey, 2015).

The presence of *E. coli* indicates that the control of raw material manufacturing processes and the final product is inefficient (Campos *et al.,* 2009). *Escherichia coli* is a frequently occurring microorganism in milk and milk products. This implies that the microbial contamination of these milk and its products is not far-fetched from the predisposing factors such as the insanitary conditions, unhygienic state of the milkers, insects and dusts (Momtaz *et al.,* 2012; Abebe *et al.,* 2014).

Several findings from different researchers showed high prevalence of *E. coli* in raw milk; Saba and Adzitey (2015) (49.3%) in Ghana; Abebe *et al.,* (2014) (26.6%) in Ethiopia; Ali and Abdelgadir (2011), (63%) in Sudan; in Turkey, Gundogan and Avci, (2014) (74%); in Brazil, Vendramin *et al.,* (2014) (53.5%); (Campos *et al.,* (2009) (79.2%); Meshref (2013) (52.6%) in Egypt; Thaker *et al.,* (2012) (30%) in India; Mailafia *et al.,* (2017) (27.3%) in Abuja, Nigeria; Caine *et al.,* 2014 (45%) in South Africa; Shah *et al.,* (2016) (33.3%) in Malaysia.

This is an indication that the presence of *E. coli* in raw milk provides evidence that unpasteurised raw milk could be a vehicle of transmission of food borne pathogens possibly carrying enterohaemorrhagic/ enteropathogenic and/or toxigenic microorganisms which could constitute a public health hazard (Ali and Abdelgadir, 2011).

Abebe *et al*. (2014) reported that raw milk is a vehicle and medium of growth for *E. coli.* The presence of *E. coli* implies a risk that other enteric pathogens may be present in the food samples (Meshref, 2013). According to Meshref (2013), raw milk is the basic material from which all dairy products are made. The diversity of microorganisms and the level of contamination in the raw material have a decisive effect on the quality and safety of the final product.

Several factors could be responsible for the contamination of raw milk with *E. coli* namely the use of contaminated utensils, use of contaminated water, milk contact surfaces, contamination of milk with feaces, improper storage conditions, unhygienic milking equipments, milking enviroment (El-Zubeir and Ahmed, 2007), dirty hands (Bagré*et al.,* 2014), infected animals (El-Zubeir *et al.,* 2006) and storage and transport equipment (Altalhi and Hassan, 2009; Bityqi *et al.,* 2011). Therefore, the implication of the presence of food borne pathogen in unpasteurised raw milk is that there is a high risk of ingestion of potentially harmful toxins and transmission of food-borne pathogens (Ali and Abdelgadir, 2011).

The result of this study showed higher occurrence of the *E. coli* in the dry season than during the rainy season. This finding corroborates with the reports of El- zubeir and Ahmed (2007) that higher bacterial counts are expected under tropical condition due to the fact that high temperatures enhances growth and multiplication of bacteria. Soomro *et al.,* (2002) in Pakistan reported that raw milk is easily contaminated due to high ambient summer temperatures.

Table.1 Frequency of occurrence of *E. coli i*solates from unpasteurised raw milk samples during dry season

Sample Type	No. Screened	No. Positive	% Positive
Madara	70	10	14.3
Kindrimo	90	6	6.7
Nono	80	3	3.8
Manshanu	60	2	3.3
Total	300	21	7.0

(χ^2= 7.381, df=3, P=0.061)

Table.2 Frequency of occurrence of *E. coli* isolates from unpasteurised raw milk samples during rainy season

Sample Type	No. Screened	No. Positive	% Positive
Madara	83	5	6.0
Kindrimo	70	4	5.7
Nono	77	3	3.9
Manshanu	70	2	2.9
Total	300	14	4.7

(χ^2= 1.429 df=3, P=0.699)

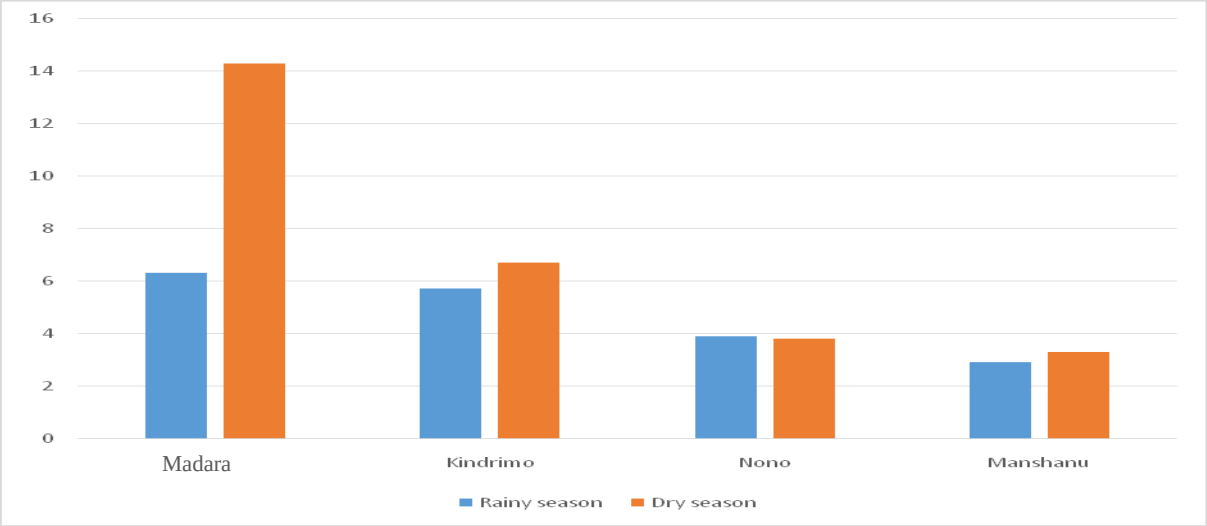

Fig.1 Relative percentage distribution of *E. coli* in rainy and dry seasons in unpasteurised raw milk samples

In conclusion, the study has shown that unpasteurised raw milk and its products are not without microbial contamination. As a developing country, perhaps in this part of the globe there are no established acceptable microbial limits in dairy products. With the growing concerns over the hygienic status of ready-to-eat food, the government should be proactive in monitoring food of bovine origin especially the dairy products that are consumed without necessarily cooking and ensuring effective food surveillance programs in order to monitor locally made food especially produced from animal origin that are readily available to the populace.

Recommendation

Preventive measures such as regular hand washing and sterilization of dairy equipment, utensils, milker's hands, animal udders, and eradication of diseased animals from the herd are highly recommended. The reduction of faecal contamination in milk production to its barest minimal load is the key to control this pathogen in dairy farms.

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