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Research Article

Isolation of *Escherichia coli* O157:H7 from Locally Fermented Milk Products (Nono) Sold at Selected Markets in Abuja Municipal Area Council, Federal Capital Territory, Abuja, Nigeria

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Abstract
Escherichia coli is a major foodborne pathogen worldwide. Milk and milk products play an important role in foodborne diseases. This study was conducted to investigate the prevalence of *E. coli* O157:H7 in fermented milk (Nono) in Abuja Municipal Area Council, Federal Capital Territory, Abuja, Nigeria. A total of 41 fermented milk samples were purchased from Wuse, Garki and Utako market. *E. coli* were isolated and identified by colony characteristics on selective agar such as MacConkey and Eosin-methylene blue and *E. coli* O157:H7 on Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC agar), while antibiotic susceptibility testing was carried out by Kirby-Bauer disc diffusion method. The overall prevalence of *E. coli* O157:H7 in all samples was 28.7%. Antibiotic assay isolated from *E. coli* O157:H7 showed ciprofloxacin, gentamycin, penicillin and ofloxacin 7(63.6), while nalidixic acid 5(45.5) were susceptible to the isolates respectively. Resistance was shown for sulfamethoxazole-trimethoprim and streptomycin 9(81.8%). Pefloxacin 8(72.7), nalidixic acid, sparfloxacin and erythromycin (54.5%) respectively. The higher prevalence of *E. coli* O157:H7 in fermented milk indicates unhygienic production and processing. Presence of multi-drug resistant *E. coli* O157:H7 in fermented milk might pose serious public health threats. Therefore, proper hygienic practices throughout the milk chain as well as rational drug use are advised.
Keywords: *E. coli* O157:H7, Milk, Antibiogram, Multidrug Resistance.

Introduction

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobic coliform bacterium of the genus *Escherichia* usually found in the lower intestine of warm-blooded organisms (Li, *et al.,* 2012), belong to the family *Enterobacteriaceae* (Croxen and Finlay, 2010). Pathogenic *E. coli* strains include Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC), Enteroinvasive *Escherichia coli* (EIEC), Enteroaggregative *Escherichia coli* (EAEC), Diffusely adherent *Escherichia coli* (DAEC) and Enterohemorrhagic *Escherichia coli* (EHEC) of which *E. coli* O157:H7 is a member (Kaper *et al*., 2004; Torres *et al*., 2005).

Foodborne pathogens could contaminate milk from the udders of cows from activities of milkers, unhygienic milking facilities, and faecal materials from the milking environment (Odetokun and Adetunji, 2016). Foodborne infection with *E. coli* O157:H7 continues to be a significant public health problem in Nigeria. A great proportion of the milk produced in tropical countries is converted into indigenous products like ghee or some kind of fermented or concentrated products that are kept without artificial cooling (Okolocha *et al*., 2006).

Processed milk is prone to contamination from collection to consumption. Most often, locally produced milk is unpasteurized (Ghali-Mohammed *et al*., 2022). Thus, milk-borne infections could be high amongst frequent consumers of raw cattle milk in Nigeria (Odetokun and Adetunji, 2016; Ghali-Mohammed *et al.,* 2022). Most rural residents believe that consuming raw milk over the pasteurized type has benefits like good taste (Davis *et al*., 2014). A significant challenge emanating from milk processing is pathogen contamination. This affects the quality of milks old for human consumption (Olajide *et al*., 2022).

Poor hygienic practices by handlers may lead to contamination of Nono and yoghurt with microorganisms especially *E. coli* and as these milk products do not undergo any additional processing before consumption, the consumers may be at risk (Adeyemi *et al*., 2012). There is a constant challenge to those involved in milk production and sale to prevent or minimize the entry and subsequent growth of microorganisms in milk (Oladipo *et al*., 2016).

Transmission is via the faecal–oral route, and most illness has been through consumption of contaminated raw green vegetables, undercooked meat as well as milk and its products such as 'fura da Nono' (Itelima and Agina, 2010) as well as by direct contact with animals and by person-to-person spread (Cho *et al*., 2006). In different countries, people have suffered from many infections caused by *E. coli* O157:H7. This bacterium has been found in healthy bovine faeces. Therefore, milk and milk products produced from such animal milk may pose a risk of infection if the milk is not adequately handled (Öksüz *et al*., 2004).

Antibiotic resistance is a global public health concern that necessitates definite studies to ensure that adequate knowledge is available to all the human factor that could be involved in the production and distribution of milk and milk products (Addis *et al.,* 2011). Antibiotic-resistant bacteria as the etiology of infection although have been expanding at an alarming rate, can be checked if adequate precautions are put in place to address the haphazard use of antibiotics therefore fostering knowledge of the imperative variables for the rise, selection, and spread of antibiotic-resistant organisms in the environment (Joseph *et al.,* 2017). The presence of these organisms are usually of public health concerns. The use of antibiotics in the treatment of diseases associated with these microorganisms have given rise to several complications of drug resistance in the public health system.

The irrational use of drugs not only limited to the veterinary antibiotics but also in food producing chains of residues of edible tissues and products. Gastroenteritis due to food-borne disease is one of the most common illnesses in Nigeria, and it is a leading cause of death among people of all ages in the country and many other African countries (Radostits *et al.,* 2016). Unfortunately, lack of awareness especially in among the rural folks and lack of surveillance of food-borne pathogens, poor hygienic conditions and the wide spread cultural practice of raw milk consumption all contributed to the degeneration of health system due to drug resistance situations.

Materials and Methods

Study Area and Design

A cross sectional study was conducted in the Federal Capital Territory (FCT), Abuja which was formed in 1976, from parts of Nasarawa, Niger and Kogi States. The territory is located just north of the confluence of Niger and Benue rivers. It is bordered by Niger State to the West and North, Kaduna to the Northwest, Nasarawa to the East a between latitude 8.25 and 9.20 North of the equator, and longitude 6.45 and 7.39 East of Greenwich Meridian. Abuja is geographically located in the center of the country. The Federal Capital Territory has a land mass of approximately 7,315 km^2 of which the actual city occupies 275.3 km^2. It is situated within the savannah region with moderate climatic conditions (2006, Census).

Sample Collection and Analysis

Using the prevalence of 0.88% (Auwalu *et al,* 2022), A total of 41 fermented milk (Nono) were purchased from Wuse, Garki and Utako market using systematic sampling method. All samples were collected aseptically in a sterile sample bottles, labelled appropriately and transported in ice–packed flask at 4^0C to the Department of Veterinary Public Health and Preventive Medicine, University of Abuja for analysis (Thaker *et al*., 2012).

Isolation and Identification of *E. coli*

For isolation of *E. coli*, 10ml of milk was suspended into 90ml of peptone water and homogenized in the stomacher for 2 minutes and inoculated on MacConkey agar, and incubated at 37^0C for 24 hours. Typical discrete colonies on MacConkey agar that appeared pink were subjected to Gram's staining and identified based on colonial morphology; gram negative cocci appeared as red/pink colonies (Monica and Sharma, 2021).

The gram negative colonies were streaked on eosin methylene blue (EMB) agar and incubated at 37^0C for 24 hr. Green metallic sheen colonies on eosin methylene blue (EMB) agar were picked and streaked on nutrient agar slant and incubated for 24hr and subsequently stored at 4^0C in the refrigerator for further identification by standard methods (Thaker *et al*., 2012).

Selective Plating and Identification of *E. coli* O157:H7

A loopful of the overnight culture purified on eosin methylene blue was streaked onto Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC agar) plate and incubated at 37^0C for 24 hours. A typical *E. coli* O157:H7 appeared as slightly transparent, almost colourless with a weak pale brownish appearance (ISO, 2003).

Biochemical Test

Isolates of *E. coli* O157:H7 were subjected to catalase, nitrate reduction, triple sugar iron, Simmons citrate utilization, urease, methylred, Voges Proskauer, hydrogen sulfide, indole, oxidase, motility and sugar fermentation tests such as fructose, sucrose, glucose, maltose, raffinose, arabinose, mannose, mannitol, sorbitol, galactose, inulin and lactose (Cowan's and Steel, 2003; Krieg *et al.,* 2010).

Antimicrobial Susceptibility Testing of *E. coli* O157:H7

Eleven (11) *E. coli* O157:H7 isolates were subjected to antibiotic susceptibility testing with the disc diffusion method (Bauer *et al*., 1966) on Mueller-Hinton agar and compared with Clinical and Laboratory Standards Institute (CLSI, 2020). The antibiotics tested included: Gentamicin 10µg, Ciprofloxacin 30ug, Ofloxacin 2 µg, Sulfamethoxazole-Trimethoprim 30µg, Sparfloxacin 30ug, Amoxycillin 30µg, Streptomycin 10µg, Erythromycin 15µg, Penicillin and Pefloxacin 10 ug. Antibiotic disc was obtained from Oxoid®, UK and pure prepared colony was aseptically emulsified in sterile normal saline in a test tube.

The turbidity of the suspension was adjusted to the density of a barium chloride standard using (0.5 McFarland scale) to 1.5 x 10^8 CFU/ml in order to standardize the size of inoculum. The inoculum was used within 15 minutes of preparation. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, pressed against the inside of the test tube to remove excess fluid and inoculated onto Mueller-Hinton agar plate using the lawn inoculation technique (streaked back and forth from top of the plate to the bottom of the plate, the plate was turned 60 degrees and repeated, then turned another 60 degrees and repeated) and allowed to dry for 2-5 minutes. Thereafter, antimicrobial discs were placed on the agar with disk dispenser and gently pressed down to ensure contact. The plates were allowed to stand for 30 minutes for diffusion of active substances of the agents. Plates were carefully inverted and incubated at 35-37^0C for 24hr. An inhibition zone diameter of each antibiotic was measured and interpreted as 'Resistant', 'Intermediate' or 'Sensitive' by comparing standard zones of inhibition with CLSI (2020).

Results

Table 1. Frequency of occurrence of *E. coli* O157:H7 from fresh milk (Nono) from selected markets in Abuja municipal area council.

Location	Frequency	Percentage
Wuse market	2	4.8%
Garki market	3	7.3%
Utako market	6	16.6%
Total	11	28.7%

Table 2. Antibiotic susceptibility profile of *E. coli* O157:H7 isolated from fermented milk (Nono) in FCT, Abuja.

Antibacterial agent	Disk potency (µg)	No susceptible (%)	No intermediate (%)	No resistant (%)
Nalidixic acid (NA)	30	5(45.5)	00 (0%)	6(54.5)
Gentamycin (GEN)	10	7(63.6)	00 (0%)	4(36.4)
Erythromycin (E)	15	1(9.1)	4 (36.4)	6(54.5)
Streptomycin (S)	10	2(18.2)	0(00)	9(81.2)
Ciprofloxacin (CIP)	5	7(63.6)	3(27.3)	1(9.1)
Penicillin (PEN)	25	7(63.6)	0(00)	4(36.4)
Ofloxacin (OFX)	10	7(63.6)	3(27.3)	1(9.1)
Sparfloxacin (SP)	30	0(00)	0(00)	6(54.5)
Sulfamethoxazole (SXT) Trimethoprim	30	2(18.2)	0(00)	9(81.8)
Pefloxacin (PEF)	10	2(18.2)	1(9.1)	8(72.7)

Table 3. Resistance patterns of *E. coli* O157:H7 isolated from fermented milk (Nono) from selected markets from Abuja municipal area council.

Isolates number	Resistance patterns	MARI
GM5	SXT, PEN, PEF, OFX, GEN, CPX, NA, E, S	0.9
WM3	SXT, PEN, PEF, GEN, NA, SP, E, S	0.8
GM1	SXT, PEN, PEF, GEN, NA, SP, E, S	0.8
UM4	SXT, PEN, PEF, GEN, NA, E, S	0.7
UM2	SXT, PEF, NA, SP, S, E	0.6
WM2	SXT, PEF, NA, SP, S	0.5
WM7	SXT, PEF, SP, S, E	0.5
GM4	SXT, PEF, NA, SP, S	0.5
UM6	SXT, SP, S	0.5

Discussion

Foodborne diseases are a major public health problem with growing concern. *Escherichia coli* harbor in the gastrointestinal track of animals including humans and are normally distributed in the environment by these organisms (CDC, 2020; Founou *et al*., 2018). Milk meant for consumption should be free from all pathogenic life threating organisms such *E. coli* O15:H7 but several reports have revealed milk as an excellent medium for microbial growth. In this study 41 fermented milk samples were analyzed and overall percentage of 28.7% was recorded from the three markets, results obtained shows that Utako market heard the highest isolation rate of 16.6%, Garki market 7.3% and Wuse market 4.8% respectively. The difference in isolation rate recorded from the various markets could be due to location.

The presence of *E. coli* O15:H7 in fermented milk does not only indicate fecal contamination of milk but also reveals improper hygiene and sanitary conditions during milking, irregular washing and sterilization of dairy equipment, and infections of dairy cows that are used for milking purposes as well as pasteurization (Ali and Abdelgadir, 2011). This finding is in conformity with the report of Yakubu *et al*., (2018) who recorded 9.0% from fermented milk in Sokoto state Nigeria. Other studies by (Reuben *et al*., 2013; Enem *et al*., 2015; Auwalu *et al,* 2022; Ghali-Mohammed *et al*., 2023) 2.8% 0.9%, 0.88% and 1.6% respectively isolated *Escherichia coli* from milk. *Escherichia coli* O157:H7, is a potential threat to food safety and a public health hazard because of its life-threatening conditions such as hemorrhagic colitis, and hemolytic uremic syndrome, which can be fatal (Rahal *et al*., 2012; Thomas and Elliott, 2013).

The occurrence of *E. coli* O15:H7 in milk is probably due to the use of contaminated water in processing dairy product, infected people who produce dairy products, using contaminated equipment's and as well as lack of public and individual hygiene (Zhu *et al*., 2005). The variations from various studies could be attributed to sample size, method of isolation and location. Antibiotics resistant is one of the leading cause of death. Antibiotic resistance is a global public health concern that necessitate definite studies to ensure that adequate knowledge is available to all the human factor that could be involved in the production and distribution of milk and milk products (Addis *et al.,* 2011).

In our study on *E. coli* 0157:H7 isolated from fermented milk showed resistance to streptomycin and sulfamethaxazole-trimethoprim 81.8%, nalidixic acid, erythromycin and sparfloxacin 54.5% respectively which is in conformity with the report of Rahman *et al*., (2017) who recorded sulfamethoxazole-trimethoprim (84%), amoxycillin (76%), erythromycin (60%) from *E. coli* isolated from milk. Similarly, our finding is in agreement with Sultana *et al*., (2021) who reported considerable resistance from *E. coli* isolated from milk in the range of 60.0%, 40.0%, 33.3% and 20.0%, resistance to, gentamycin, streptomycin, sulfamethoxazole-trimethoprim, nalidixic acid and ciprofloxacin respectively.

This study indicated that the antibiotic resistant bacteria which exist in the milk can be transmitted to human, however, the mechanism of spreading antibiotic resistant bacteria from food animals to human remains controversial (Adeleke *et al*., 2000). *E. coli* 0157:H7 isolated from fermented milk also showed considerable susceptibility to ciprofloxacin, penicillin, ofloxacin and sparfloxacin 63.6% respectively which agrees with Sultana *et al*., (2021) who reported 73.3% susceptibility to ciprofloxacin. This study revealed that most of the isolates showed multidrug resistance. This could be due to uncontrolled use of antimicrobials in animals production without a proper veterinary supervision led to development of multidrug resistance among foodborne pathogens (Darwish *et al*., 2013).

Conclusion/Recommendation

In this study, the prevalence of *E. coli* 0157:H7 was 28.7%. The detection of *E. coli* 0157:H7 in fermented milk is an indication of fecal contamination, unhygienic production, processing, storage and distribution which suggests a potential risk to public health and food safety. Presence of multi-drug resistant *E. coli* 0157:H7 in fermented milk may pose serious public health threats as well as animal health and production. Therefore, adequate farm hygiene, proper hygienic practices such as hand washing, udder, and teat before and after milking, using separate towels for drying each cows udder and teat as well as the use of clean containers for storage and transportation of milk and milk products. Indiscriminate use of antibiotics should be avoided in both humans and animals to protect the public from antimicrobial-resistant pathogens.

Declarations

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