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Occurrence and Antimicrobial Susceptibility Profile of *Escherichia coli* Isolates from Vended Milk in University of Abuja Community, Federal Capital Territory, Nigeria

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Abstract

Purpose: This study was conducted to establish occurrence and antimicrobial susceptibility profile of *Escherichia coli* (*E. coli*) isolates from cow milk (Nono) sold within University of Abuja campuses.

Methodology: Fifty (50) samples of "nono" milk were randomly collected from vendors in mini and main campus of University of Abuja from August to October 2021. All samples were analyzed using pour and surface plating methods. Presumptive *E. coli* isolates on Eosin methylene blue agar were characterized using preliminary and standard biochemical identification methods such as microbial plate count, Gram staining, oxidase, indole, methyl red, voges-proskauer, citrate and catalase tests. Antimicrobial susceptibility testing was conducted on positive isolates using the disc diffusion method.

Findings: Out of the 50 samples of nono milk cultured, thirty (30) yielded growth for *Escherichia coli* which appeared as greenish metallic sheen on EMB agar plate and Gram-negative short rods on microscopic examination, representing an overall prevalence of 60%. The overall viable count of all samples was extremely high, with 8.7x10^7cfu/ml recorded as the highest colony forming unit per milliliter (CFU/ml) and 1.5×10^7cfu/ml recorded the lowest colony forming unit per milliliter (CFU/ml). All isolates were indole positive, methyl red positive, oxidase negative, citrate negative, catalase positive and voges-proskauer negative. *E. coli* isolates in this study were susceptible to sparfloxacin (66.7%), gentamicin (66.7%), ciprofloxacin (60%), streptomycin (60%), and tarivid (53.3%) and resistant to Septrin (73.3%), Augumentin (60%), Pefloxacin (66.7%), chloramphenicol (60%) and amoxicillin (53.3%). In conclusion, this study showed that the degree of contamination of *E. coli* in vended milk in the study area is higher than the maximum permissible bacteria count recommended by Codex Alimentarius standard for fermented milk products which is indicative of potential hazard to consumers.

Recommendation: Public enlightenment on hygienic handling of milk during milking and especially vending by milk vendors is hereby recommended. Use of cool thermos for milk hawking is also advocated.

Keywords: *Escherichia coli isolates, "nono" milk, antimicrobial susceptibility testing, University of Abuja.*

Introduction

Milk is a fluid secreted by the mammary gland of mammals for the nourishment of their young. Raw milk is composed of water, fats, proteins, carbohydrates, minerals and vitamins that favors growth of both harmless and pathogenic bacteria when suitable temperature exists (Uzoagu *et al.*, 2020). This complex dairy product can be easily contaminated with enteric bacteria such as *E. coli*, Shigella, Salmonella and a host of other non-enteric bacteria which can reduced its quality and poses serious public health threat when ingested (Maikai & Madaki, 2018).

"Nono" is a fermented cow milk locally produced and sold in Nigeria by Hausa/Fulani women. It is believed to be highly nutritious and mostly consumed in the norther part of Nigeria. The drinking of locally fermented nono milk has been linked to milk-borne illness such as salmonellosis, shigellosis, cholera, tuberculosis and traveler's diarrhea (Jayarao *et al.*, 2006). Consumption of "nono" has a potential health hazard, owing to the fact the milk used for its production in Nigeria is usually drawn mechanically by herders whose hygiene and health status are unknown. More worrisome is the fact that nono is usually consumed without having been cooked, therefore it is likely to transmit milk-borne diseases that are life threatening.

Though *E. coli* which is part of the normal floral of the intestinal tract of animals and human is harmless, however some strains are pathogenic. They acquire the ability to cause gastroenteritis through the production of enterotoxins that is plasmid coded (Tortora *et al.*, 1986). Pathogenic *E. coli* strains are categorized into six pathotypes. Most of the pathotypes are associated with diarrhoea and collectively are referred to as diarrheagenic *E. coli*. Shiga toxin-producing *E. coli* (STEC) is one of the most common cause associated with foodborne outbreaks. Others are the Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely Adherent *E. coli* (DAEC) (Jafari *et al.*, 2012; Mailafia *et al.*, 2017).

Coliforms are used as indicator organisms to determine the bacteriological quality of milk and its products, its count is often used as a definite index of fecal contamination considering the fact that they are part of the normal floral of the intestine of humans and animals (Maikai et al., 2018). Coliforms such as E. coli and fermentative bacteria (Lactobacillus fermenti, Streotococcus lacti, Streptococcus cremonis) are part of the normal floral of raw milk (Tortora et al., 1986). The Codex Alimentarius Standards require that there are fewer than 10 coliforms per milliliter of milk, pasteurized grade. A milk is expected to have a standard plate count of fewer than 20,000 bacteria and not more than 10 coliforms per milliliter (Tortora et al., 1986). Food and water safety is ensured through determination of feacal coliforms. This study was undertaken to determine the occurrence and degree of E. coli contamination in vended "nono" within the University of Abuja.

Materials and Method

The Study Area

The study was conducted in University of Abuja campuses; the mini and main campus. The mini campus is located in Gwagwalada area council about 54 kilometers from the city centre while the main campus which is the permanent site is located along Abuja- Lokoja express way, few kilometers from the Nnamdi Azikiwe International Airport. Gwagwalada is one of the six area councils of the Federal Capital Territory, Abuja, Nigeria. It has an area of 1,043km^2 and a population of 157,770 as at the 2006 National population census (Anon, 2011).

Sample Collection

Fifty samples of "nono" were randomly collected from vendors in mini and main campus, of University of Abuja. 30 samples were collected from the mini campus at different retailed outlets such as mini campus gate, angle 90, behind boys' hostel, school field area, Gwagwalada market, and University of Abuja Teaching Hospital which were designated as A, B, C, D, E and F. While 20 samples were obtained from main campus vended at different locations such as convocation ground, Adikwu market, shuttle stand and main campus gate were designated as G, H, I and J respectively. Five (5) samples were obtained from each of the ten (10) different locations within the university community, and on each occasion, about 15 mls of the nono samples were purchased into sterile containers, placed in a cool thermos and transported to the laboratory of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, for immediate processing.

Microbial Analysis

Total bacteria plate counts were determined and expressed in colony forming unit per milliliter of sample (cfu/ml) using standard plate count as describe by (Olatunji *et al*,, 2012). 1ml of the "nono" milk samples was inoculated into test tubes containing 9ml of buffered peptone water and then incubated at 37 °C for 24hours for the purpose of enrichment. Ten-fold serial dilutions were done in sterile normal saline, and 1 ml of the serially diluted milk was inoculated onto the surface of already prepared plates of Nutrient agar and MacConkey agar (Oxoid, UK), using the spread plate technique, and then incubated at 37°C for 24hours for Quantitative analysis. The total bacteria plate counts of each sample was enumerated and calculated as: total number of colonies in a plate multiply by the dilution factor of the sample, and then expressed as the colony forming unit per milliliter (cfu/ml).

Isolation of *E. coli*

A loop full of the inoculated peptone water was inoculated into 9 mls of MacConkey broth and then incubated at 37°C for 24hours for selective enrichment. Selective plating was carried out by inoculating a loop full of the MacConkey broth on already prepared plates of MacConkey agar and Eosin Methylene Blue agar (Oxoid, UK), and then incubated at 37°C for 24hours. On MacConkey agar, colonies were differentiated into lactose and non-lactose fermenters based on the colors shown on the plates with yellow or pale white color signifying non lactose fermenters and pink colonies lactose fermenters. While presumptive *E. coli* isolates appeared as greenish metallic sheen on Eosin Methylene Blue agar. Distinct colonies (2-3) were picked and sub cultured on nutrient agar slants, then incubated at 37ºC for 24 hours to obtain pure culture and for biochemical characterization.

Characterization and Identification of *E. coli*

Characterization of *E. coli* isolates was carried out based on colonial morphology, Gram staining technique, idole test, methyl red and voges Proskauer reaction, citrate utilization (IMVIC), triple sugar iron and catalase tests as described by Moses et al. (2010) with some modifications. *E. coli* isolates were identified by comparing their characteristics with those of known taxa using the scheme of Cowan and Steel (2002).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of *E. coli* isolates were evaluated using the disk diffusion method prescribed by Kirby- Bauer *et al* (1966) and in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2018). The antibiotics used were Sparfloxacin (10µg), Ciprofloxacin (30µg), Septrin (30µg), Pefloxacin (30µg), Tarivid (10µg), Chloramphenicol (30µg), Augumentin (10µg), Amoxicillin (30µg), Streptomycin (30µg), Gentamicin (30µg). An overnight culture of each pure isolates was prepared on nutrient broth and incubated at 37 °C for 18 h. The turbidity of the broth was adjusted to McFarland standard of 0.5. The inoculum was then spread on already prepared plates of Mueller Hinton's agar (Oxoid, UK) and left standing for 1-2 minutes. Using forcep, antibiotics multi-discs (Mast diagnostics) were aseptically placed on the inoculated plates and then incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured to the nearest millimeter using a transparent ruler and the values were recorded and interpreted as sensitive, intermediate and resistant according to CLSI, 2018 guidelines.

Data Analysis

Statistical Package for Social Sciences (SPSS version 26) was used for data analysis. Simple descriptive statistics such as frequency, Arithmetic mean, percentages and tables were used to express the rate of occurrence of *E. coli*

Results

In this study, total bacteria plate counts of $8.7x10^7$ cfu/ml was recorded as the highest plate count, while $1.5x\ 10^7$ cfu/ml was the lowest plate count from all sampling locations as shown in table 1.

Table 1: Total bacteria plate counts of nono milk sold at University of Abuja, FCT

Location	No of samples	Viable counts	Mean Total plate counts	(CFU/ml)
Minicampus gate A	5	$1944x10^4$	$7.0x10^7$	$1008x10^5$
Angle 90 B	5	$1200x10^4$	$3.6x10^7$	$480x\ 10^5$
Boys hostel C	5	$456x10^4$	$1.5x10^7$	$216x10^5$
School field D	5	$1728x10^4$	$8.7x10^7$	$1448x10^5$
Gwagwalada market E	5	$1992x10^4$	$8.\ 7X10^7$	$1272X10^5$
UATH F	5	$1226X10^4$	$7.2X10^7$	$1226X10^5$
Convocation ground G	5	$1742X10^4$	$6.7X10^7$	$1010X10^5$
Adikwu market H	5	$1326X10^4$	$7.8X10^7$	$1326X10^5$
Shuttle stand I	5	$1248X10^4$	$4.9X10^7$	$728X10^4$
Main gate J	5	$1896X10^4$	$6.3x10^7$	$912x10^5$

Out of the 50 milk samples analyzed, 30 were positive for *Escherichia coli*, representing an overall prevalence of 60%. Sites I, G, H and J in the main campus had the highest prevalence while site B and site F in the mini campus of the University of Abuja had the least prevalence as shown in table 2.

Table 2: Distribution of *E. coli* from milk sold at University of Abuja, FCT

Locations	No samples	No positive	Prevalence (%)
Mini campus gate A	5	3	60
Angle 90 B	5	1	20
Boy's hostel C	5	2	40
School field D	5	1	20
Gwagwalada market E	5	4	80
University of Abuja teaching hospital F	5	1	20
Convocation ground G	5	5	100
Adikwu market H	5	4	80
Shuttle stand I	5	5	100
Main campus gate J	5	4	80
Total	**50**	**30**	**60**

E. coli isolates in this study were susceptible to sparfloxacin (66.7%), gentamicin (66.7%), ciprofloxacin (60%), streptomycin (60%), and tarivid (53.3%) and resistant to Septrin (73.3%), Augumentin (60%), Pefloxacin (66.7%), chloramphenicol (60%) and amoxicillin (53.3%) as shown in table 3.

Table 3: *In- vitro* antibiotics susceptibility testing of *Escherichia coli* isolates obtained from nono sold in University of Abuja, FCT. N= 30

Antibiotics	No of isolates Susceptible (%)	No of isolates Resistant (%)
Tarivid (10µg)	16(53.3)	14(46.6)
Septrin (30µg)	8(26.6)	22 (73.3)
Chloramphenicol(30µg)	12(40)	18(60)
Sparfloxacin(10µg)	20(66.7)	10(33)
Ciprofloxacin(10µg)	18(60)	12 (40)
Amoxicillin(30µg)	14(46.6)	16(53.3)
Augmentin(10µg)	18(60)	12(40)
Gentamicin(10µg)	20(66.7)	10(33.3)
Pefloxacin(10µg)	10(33.3)	20(66.7)
Streptomycin(30µg)	18(60)	12(40)

N = no of *E. coli* isolates tested, figures outside brackets = no of isolates susceptible or resistant, figures within brackets = percentage (%) susceptible or resistant.

Discussion

In this study, the total viable bacteria count recorded in all the samples exceeded the maximum permissible bacteria count recommended by Codex Alimentarius Standards (CAS) for fermented milk products. CAS stipulated that the total aerobic plate count for milk meant for consumption should not exceed 5.0 $\log_{10}$ cfu/ml, which aligns with the European Commission Standard and the Standard Organization of Nigeria (SON) with a benchmark aerobic plate count of 50, 000/ml (SON, 1996). This finding shows that ready to consumed nono milk sold in the study area is highly contaminated with bacteria organisms, thus a potential health hazard to its consumers. The high degree of contamination recorded in this study may be attributed to unhygienic practices during or after milking of cows, personal hygiene of the milk handlers and or largely due to environmental factors such as high temperature of the study area, which favors bacteria growth and multiplication (Echioda-Ogbole *et al.*, 2017). This is also in line with the report of Omotosho *et al.* (2013) which stated that retailed milk products like nono stands a high chance of contamination because of unhygienic environment and hot weather conditions.

In this study, *E. coli* were isolated and identify phenotypically based on cultural morphology on Eosin methylene blue agar (EMB) which yielded greenish metallic sheen colonies and pink coloured colonies on MacConkey agar (Oxoid, UK). Presumptive colonies on EMB and MacConkey were confirmed positive following Gram staining showing rod shaped bacteria. Biochemically, *E. coli* isolates were indole positive, methyl red positive, oxidase negative, citrate negative, catalase positive and voges proskauer negative. The limitation of this study was the inability to characterize other bacteria species isolated from the analyzed samples, because they were not within the scope of this study.

The isolation rate of *E. coli* from vended milk in the study area was found to be relatively high, 100% prevalence was recorded in site G and I as shown in table2. The overall prevalence of 60% recorded in this study is higher than the 18.2% prevalence reported by Mailafia *et al.* (2016) and 12.3% reported by Uzoaga *et al.* (2020). The high isolation rate in this study may be attributed to exogenous contaminations from unhygienic practices of milk handlers in the study area. Also, since nono is an unpasteurized milk product, the source of the microbes could have originated from the animal, through dirty skin or mastitic udder. Olatunji (2009) emphasized the importance of washing the udder and milkers' hands before milking in other to avoid the dangers of microbial contamination of milk.

Antibiotic susceptibility testing showed vary degree of sensitivity and resistance to the antimicrobials tested in this study. The *E. coli* isolates in this study showed relatively high level of resistance to most of the antimicrobial agents tested, 73.3% resistance to septrin, 66.7% resistance to pefloxacin, 60% resistance to chloramphenicol and 60% resistance to augmentin was recorded in this study. This finding is of public health significance considering the risk of acquiring antimicrobial resistant *E. coli* and other pathogenic organisms via the consumption of nono and other milk products in Nigeria (Uzuaga *et al.*, 2020). This varied resistance of *E. coli* isolates in nono within the study could also lead to Multidrug resistant (MDR) *E. coli*, which is currently of growing public health interest, because they decrease treatment options in both human and veterinary medicine.

Conclusion

This study reports high occurrence and degree of contamination of *E. coli* in ready to consume "nono" milk in the study area, higher than the maximum permissible bacteria count recommended by Codex Alimentarius standard for fermented milk products.

Recommendation

Public enlightenment on the potential health hazard of indiscriminate "nono" consumption is hereby recommended. In addition, there is also a need to minimize rampant use and misuse of different antibiotics in food animals to curtail the possible emergence of resistant genes in animals which could be transferred to the human population as advocated (Mailafia *et al.*, 2017).

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