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Original article

ANTIBIOTICS SUSCEPTIBILITY PROFILE OF *Escherichia coli* FROM FERMENTED MILK (*Nono*) SOLD IN LAPAI, NIGER STATE, NIGERIA.

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ABSTRACT

The antibiotics susceptibility profile of *Escherichia coli* from fermented cow milk (*Nono*) was investigated. *Nono* samples were collected from the Main and Badeggi markets twice a month (four times) in April and May 2021. The pour plate technique was used to determine the bacteria count in Nono. Identification of bacteria isolates from the sample was carried out by subjecting the isolates to different biochemical tests. Kirby Bauer disc diffusion method was used to determine the sensitivity of the isolates to the antibiotics (Tarivid, Reflacine, Ciprofloxacin, Streptomycin, Augmentin, Gentamycin, Ceporex, Nalidixic acid, Septrin, and Ampicillin) used in the study. Mean bacteria count ranged from 4.2 x 10^4 to 2.9 x 10^6cfu/ml. From the four samplings carried out across the two (2) markets in Lapai metropolis, the highest mean bacterial count of 2.9 x 10^6 cfu/ml at the dilution factor of 10^{-5} occurred at the Badeggi market, while the least colony count of 4.2 x 10^4 cfu/ml at the dilution factor of 10^{-3} also occurred at Badeggi market. The incidence of bacteria isolates indicated that the highest population was *Escherichia coli* 4(33%), while the least population of bacteria was observed in *Pseudomonas aeruginosa* and *Bacillus cereus* 1(8.3%). The percentage susceptibility of bacteria isolates to the antibiotics indicated that the most active antibiotics were Ceporax (100%) and Streptomycin (100%), and the most resisted antibiotics was ampicillin (100%). *E. coli* was the most susceptible bacteria isolate to the multiple antibiotics, while *P. aeruginosa* showed resistance to most antibiotics used. The study revealed high resistance to most antibiotics by some bacteria isolated in this study, which might be likely due to the indiscriminate use of antibiotics to treat livestock.

Keywords: Antibiotics, Bacteria, *Escherichia coli,* fermented milk

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INTRODUCTION

Fermented cow milk (*Nono*) is an opaque white milky coloured liquid food drink got from the fermented raw milk of cows [1]. Nono contains amino acids, calcium, phosphorus, and vitamin A, B, E, and B complex. Predominantly, *nono* is prepared and hawked by nomadic Fulani/Hausa cattle herdsmen/women, who control over 80% of Nigeria's cattle production [1]. Consumption of *nono* was limited to Hausa /Fulani indigenous and some other tribes in the northern part of Nigeria. Milk is a significant source of nutrients for animals and humans. Fresh cow milk is collected in the morning hours in calabashes, sieved, and left to ferment for a period of 24 to 48 hours under the ambient temperature (28 $\pm$2 ^{o}C). The fermentation of milk is spontaneous with its own natural bacteria. The curd separates from the whey, is removed and used in the preparation of local cheese or butter, while the milk (whey) is left to ferment further for a few hours thereby converting it to yogurt [2]. Fermentation span varies from one producer to another resulting in products of variable quality and stability. The finished product, *nono* is sold in beautiful calabashes along with Fura (dough of boiled ground millet mixed with a host of other ingredients and spices) as 'Fura da Nono'. Milk meant to be consumed must be free from all pathogenic life forms. The contamination of milk by microorganisms may result in milk-borne illness in humans, as well as spoilage of the milk. 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, while the 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 [3]. Others include recontamination of milk after processing as a result of the unhygienic conditions and improper handling and storage of milk during consumption [4]. Milk is referred to as a high-risk food as it is highly nutritious and serves as an ideal medium for microbial proliferation. Common bacterial pathogens still of public health concern today in fresh milk and its derivatives include *Bacillus cereus*, *Yersinia enterocolitica, Listeria monocytogenes*, *Salmonella* sp, *Campylobacter jejunii,* and *Escherichia coli*. *Escherichia coli* has developed as a globally recognized zoonotic foodborne pathogen, which results in serious illnesses such as hemolytic uremic syndrome (HUS), hemorrhagic colitis, and thrombotic thrombocytopenic purpura (TTP) in humans [5]. It was first recognized as a food-borne pathogen resulting from an epidemic of unusual gastrointestinal illness in 198, and was recovered from hemorrhagic colitis patients who previously consumed undercooked patties [6]. These virulent strains are responsible for diarrheal infections worldwide, as well as neonatal meningitis, septicemia, and urinary tract infections (UTIs). Previous studies on *E. coli* from milk samples have been carried out in Asosa Town, Western Ethiopia [7]. In addition, *E. coli* and other *E. coli* have been characterized from food producing

animals in Benin City, Nigeria [8], Hyogo Prefecture, Japan [9]. The study is aimed at determining the antibiotics susceptibility profile of *Escherichia coli* isolated from fermented cow milk.

METHODOLOGY

Study area

Lapai is a Local Government area of Niger state, North Central Nigeria, adjoining Federal Capital Territory. Its headquarters are in the town of Lapai, Lapai is located between longitudes 4°27'30" to 13°60'95" North and 2°60'60" to 14°89'44" East.

Sample collection

Nono samples were collected twice in a month from the hawkers in the two markets of Lapai metropolis, namely, Main market and Baddegi market in April and May, 2021. The samples were collected in conical flask (250ml), covered and labeled appropriately, and placed in a cooler that was supported the by ice packs, and transported to Microbiology Department Laboratory of Ibrahim Badamasi Babangida university, Lapai, Nigeria where it was stored in the refrigerator before further analysis (within 24hours).

Determination of Bacteria population of *Nono*

A five-fold serial dilution of the Nono sample was made up to 10^{-5}. One (1) mL of each sample (*Nono*) was withdrawn aseptically from the stock (100mL), using a sterile pipette and transferred gently into a test tube containing 9ml of distilled water. The dilution was done up to 10^{-5}. Subsequently, one (1) mL each from dilutions 10^{-3} and 10^{-5} were aseptically taken and plated on Nutrient agar (NA) using the pour plate method. The plates were incubated at 37°C for 24 hours in the Microbiology Laboratory of Ibrahim Badamasi Babangida University, Lapai, Nigeria. Distinct colonies found on the plates after incubation were counted using a colony counter and records were taken accordingly [10].

Isolation of *Escherichia coli*

Pure cultures were obtained by streaking distinct colonies from the plates on to another freshly prepared Nutrient agar plates and incubated at 37ºC for 24 hours, before sub culturing onto EMB agar plates using the streak method, the plates were also incubated at 37°C for 24 hours [10] to observe the colony morphology. Gram stain procedure was carried out on the distinct colonies from the EMB agar plates according to the methods described by [10] to determine the size, shape and arrangement of bacteria. The isolates that appeared pink or red colored with the characteristic rod shape and arranged in singles or pairs were suspected to be *Escherichia coli.*

Characterization and identification of *Escherichia coli*

Sub- cultured, young isolates were subjected to different biochemical tests after the gram stain procedure for the purpose of identification. These biochemical tests includes, Catalase, Indole, Methyl red, Voges proskauer, Citrate utilization, Oxidase, Urease and Sugar fermentation tests according to the methods described by [10].

Antibiotics Susceptibility Testing of *Escherichia coli* isolated from *Nono*

The isolates were tested for antimicrobial susceptibility using the agar disk diffusion

method on Mueller-Hinton agar (Cheesbrough, 2006). The following antibiotics (Oxoid) were used: Tarivid (10µg), Reflacine (10µg), Ciprofloxacin (10µg), Streptomycin (30µg), Augmentin (30µg), Gentamycin (30µg), Ceporex (10µg), Nalidixic acid (30µg), Septrin (30µg) and Ampicillin (30µg).The identified isolates were uniformly streaked on Muller-Hinton agar plate and the antibiotic impregnated discs were placed onto the inoculated plates using sterile forceps. The plates were incubated at 37^{o}C for 24hours, after which clear zones of inhibition for each antibiotics were measured using transparent ruler. The results were interpreted using the Clinical and Laboratory Standards Institute [11].

RESULTS

Total viable mean colony counts of bacteria from fermented milk (Nono) sold in Lapai Metropolis is shown in Table 1. From the four samplings carried out across the two (2) markets in Lapai metropolis, twice in a month from April to May, 2021, the highest mean bacterial count of 2.9 x 10^{6} cfu/ml at the dilution factor of 10^{-5} was obtained at Badeggi market, while the least colony count of 4.2 x 10^{4} cfu/ml at the dilution factor of 10^{-3} was also obtained at Badeggi market.

Table 1:
Total viable mean colony counts of bacteria from fermented milk (Nono) sold in Lapai

Sample	Dilution factor	No of colony(s)	Population of bacteria (cfu/ml)
Main market	10^{-3}	49	4.9 x 10^{4}
	10^{-5}	26	2.6 x 10^{6}
Badeggi market	10^{-3}	42	4.2 x10^{4}
	10^{-5}	29	2.9 x 10^{6}

The incidence of bacteria isolates indicated the highest percentage ocurrence to be *E. coli,* 4(33%), while the least percentage ocurence was observed in *Pseudomonas aeruginosa* and *Bacillus cereus* with 1(8.3%) isolate each. *Klebsiella* sp, *S. aureus* and *Proteus* sp have equal percentage occurrence, with 2(16%) isolates each (Table 2).

Table 2: Incidence of bacteria in fermented cow milk (*Nono*) samples across the markets in Lapai.

Bacteria	No of isolate (s)	Percentage occurrence (%)
Escherichia coli	4	33.3
Klebsiella sp	2	16.7
Pseudomonas aeruginosa	1	8.3
Proteus sp	2	16.7
Bacillus cereus	1	8.3
Staphylococcus aureus	2	16.7
Total	**12**	**100**

Tarivid, Ciprofloxacin, Septrin and Septromycin antibiotics were all active against the bacterial isolates. This is evident from the fact that all the bacterial isolates were one hundred percent (100%) susceptible to these antibiotics. *Pseudomonas aeruginosa* showed one hundred (100%) resistance to reflacin, gentamicin and augmentin. This same characteristic was exhibited by *Bacillus cereus* that also resisted nalidix acid, gentamicin and ampicilin by one hundred percent (100%), while *Klebsiella* sp showed one hundred (100%) resistance to reflacin and ampicilin (Table 3).

Table 3: Percentage susceptibility of bacteria isolates to the antibiotics.

Bacteria Isolates	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
Klebsiella sp	100(0)	100(0)	100(0)	0(100)	100(0)	100(0)	100(0)	100(0)	100(0)	0(100)
Escherichia coli	100(0)	100(0)	75(25)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	75(25)
Proteus sp	100(0)	100(0)	100(0)	100(0)	100(0)	50(50)	100(0)	100(0)	100(0)	100(0)
Pseudomonas aeruginosa	100(0)	100(0)	100(0)	0(100)	0(100)	0(100)	100(0)	100(0)	100(0)	100(0)
Staphylococcus aureus	50(50)	100(0)	0(100)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)
Bacillus cereus	100(0)	100(0)	0(100)	100(0)	0(100)	100(0)	100(0)	100(0)	100(0)	0(100)

Figures in the parenthesis represent the percentage of resistance to the antibiotics by the bacteria isolates.

Key: CEP =ceporex (10gμ), OFX =tarivid (10μg), NA= nalidix acid (30μg), PEF =reflacin (10μg), CN=gentamicin (30μg), AU = augmentin (10μg), CPX =ciprofloxacin (10μg), SXT =septrin (10μg), S = septromycin (30μg), PN = ampicillin (30μg).

DISCUSSION

The mean population of bacteria is on the high side at the dilution factors of 10^{-3} and 10^{-5}that ranged from $4.2x10^4$ cfu/ml to 2.9 x 10^6 cfu/ml across the two markets in Lapai metropolis. This is expected to be so, owing to the factor of exposure of the fermented milk to the atmosphere. Also, at the various stages of the milk preparation; from the animal floor house, unhygienic nature of the farmers, not washing their hands before milking, milking of sick animals, using unclean water to wash hands and equipment/utensils, type of storage containers used, milk storage duration under room temperature, and the exposure of the final product of the fermented milk to the atmosphere when it is been hawked; all of these contribute greatly to the population of bacteria in the fermented milk as recorded in this study. This finding is similar to the result of

[12] and [13], where a high bacterial load was also recorded in a related study. The high bacteria population in this study however negates the maximum recommended level of bacteria population given by the East African Community (EAC) of 2.0 x 10^6cfu/ml standards. The mean viable count recorded in this study is also higher than the result of [14], [15], where a lower bacterial count was recorded in a study of bacterial species isolated from fermented milk. The targeted bacteria in this study are *Escherichia coli*; this explains why Eosine methylene blue agar was used in the isolation procedure. *Escherichia coli* predominates other bacteria species isolated in the fermented milk. This notwithstanding, other bacteria could grow accidentally on this medium; others may have grown possibly as contaminants from the handlers of the fermented milk during processing. Other reasons that could be advanced for the growth of other bacterial species aside *Escherichia coli* includes, lactating of sick mammals, careless handling procedure during Nono production and traditional milking method. The presence of *Staphylococcus aureus* in Nono could be due to poor hygiene practices during milk collection or poor preservation, unclean hand of the vendors and utensils for Nono collection. However, the isolation of enteric pathogens in the fermented Nono could be a route of transmission of human diseases following their consumption. The isolation of *Escherichia coli* as the predominant bacteria in this study is in agreement with the findings of [14], where *Escherichia coli* also account for the most populated bacteria among other bacterial species, but contrary to the work of [13], where other bacterial species predominate, and not *E. coli*. *Escherichia coli* isolation could be a result of fecal contamination of the water used for the processing of raw milk [16]. The isolated bacteria showed high resistance to some antibiotics but susceptible to most antibiotics used. The most active antibiotics to the bacteria isolates were Ceprofloxacin, Septrin and Septromycin, while the resisted antibiotics were Ampicillin and Nalidix acid. *E. coli* was highly susceptible to ciprofloxacin, septrin, septromycin, reflacin and augmentin, but was resistant to ampicillin. Resistance shown to most antibiotics in this study could be as a result of indiscriminate use of these antibiotics on the animals when presumed to be sick, leading to the development of resistant bacteria strains. The development of antimicrobial resistance by bacteria to these drugs can pose a major challenge in both human and animal medicines used. However, the bacteria species were susceptible to some antibiotics used in the

study, this is as a result of the efficacy of these drugs, and less of bacteria exposure to them, since they are not indiscriminately applied or used. The findings in this study are in agreement with the outcome of the work carried out by [17], where *Pseudomonas aeruginosa* and *Bacillus cereus* showed resistance to some antibiotics tested, which includes gentamycin, reflacin and nalidix acid, but were susceptible to tarivid and ceporex. This is in disagreement with the findings of [18], where *Pseudomonas aeruginosa* and *Bacillus cereus* were susceptible gentamycin, reflacin and nalidix acid. Other studies have reported a number of resistant bacteria to commonly used antibiotics in livestock production in Tanzania [17].

CONCLUSION

The most predominant bacteria isolated from fermented nono sample sold in Lapai were *E. coli* and the least bacteria isolates were *P.aeruginosa* and *B. cereus*.

The most active antibiotics against the bacteria isolates were streptomycin, ceprofloxacin, tarivid, and ceporex, while the most resisted antibiotics by the bacteria isolates were ampicillin, nalidix acid, and gentamycin.

The study observed high resistance shown by some bacteria to commonly used antibiotics in livestock.

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