

Risk of Bacterial infection from selected Fermented Milk Products in Abakaliki Metropolis

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ABSTRACT

Background: This study was aimed at evaluating the risk of bacterial infection from fermented fura de nunu milk product consumed in Abakaliki metropolis.

Method: Twenty five (25) samples of nunu and fura de nunu each were purchased from different vendors at Hausa Quarters' Market in Abakaliki metropolis. Bacteria were isolated using differential and selective media and identified using standard biochemical tests. Molecular identification was performed by amplification of 16S rDNA. Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion method.

Result: A total of 90 bacterial isolates from fura de nunu (56) and nunu (34) samples were isolated. The total bacteria count and mean bacteria colony forming units were significantly higher ($P \leq 0.05$) in fura de nunu (57 ± 26.5), (11.4 ± 5.3) than in nunu (24 ± 14.1), (4.8 ± 2.8) respectively. All the bacteria isolates were resistant to cefotaxime, meropenem, amoxicillin-clavulanic acid and susceptible to imipenem and gentamicin. Eighty five (94%) of the bacteria isolates were resistant to ceftriazone while (6%) showed intermediate zone of inhibition. About 58% of the bacteria isolates were resistant to sulphamethoxazole/trimethoprim, while 42% of the isolates showed susceptibility with multidrug resistance observed in over 90% of the isolates. Out of 58 isolates used, 64% and 26% exhibited hemolytic and hemagglutination properties.

Conclusion: It is evident that fura de nunu may be a source of different bacterial infections capable of causing food-borne illnesses, food intoxication and gastroenteritis.

Keywords: Fura de nunu, antibiotic susceptibility, multidrug resistance, hemolysis, hemagglutination.

INTRODUCTION

The processing of fura (fermented millet) involves de-hulling and washing of the millet grains followed by wet milling into dough which is fermented over a period of about 12-18 h. The fermented millet dough is molded into large balls (about 10 cm in diameter), cooked, pounded into a sticky cohesive mass and finally molded again

into smaller balls for marketing. The mixture of fermented milk and cooked spiced fermented millet known as 'fura de nunu' is almost a complete food with milk serving as a source of protein while the cooked spiced millet provides energy [1]. Fura de nunu production like many other indigenous fermented foods traditionally

relies on spontaneous fermentation initiated by natural microorganisms' natural starters [2]. Depending on its preservation and process-line, microorganisms other than [lactic acid](#) bacteria could be found in the drink [3]. *Fura de nunu* however, does not appeal to majority of the people because of the apparent unhygienic conditions in which it is prepared, and also its short shelf-life [4]. The sources of contamination include disease-causing organisms (pathogens) shedding in milk, infected udder and/or teats, animal skin, fecal soiling of the udder, contaminated milking and storage equipment and water used for cleanliness. Other bacterial sources are from air, milkers, handlers and from water used for adulteration by unscrupulous and unfaithful workers/sellers who may be contaminated and may cause additional health problems [5]. Hence, there are general skepticism about the preparation processes of this product as well as the proper observation of personal/environmental hygiene [3]. Different potential pathogenic bacteria such as *Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., *Campylobacter* sp., *Pseudomonas* sp., etc have been isolated from *nunu* and raw milk products indicating the possibility of these product serving as source of microbial food poisoning [4, 6,]. This work therefore aimed at evaluating the risk of bacterial infection from consumption of *fura de nunu* in Abakaliki Metropolis, Ebonyi State, Nigeria.

2.0 METHODOLOGY

2.1 Sample collection

This study was conducted within Abakaliki metropolis, the capital city of Ebonyi state in Eastern part of Nigeria. Samples of *nunu* and *fura de nunu* were purchased from Hausa quarters with high population of Hausa and Fulani tribes. Twenty five (25) samples each of *nunu* and *fura de nunu* were collected from different vendors. The samples were transported directly to the laboratory and used within 6 hrs in a sterile large screw capped bottles. For sterility of the collection process, face mask, hand gloves, washed hands and sterile capped bottles was ensured.

2.2 Bacterial isolation

The Pour Plate method adopted has been described earlier [3]. In brief, 9 ml of distilled water was dispensed in 10 sterile test tubes. Using a sterile pipette, 1 ml of the *nunu* and *fura de nunu*

samples were transferred into 9ml of distilled water and mixed. Ten-fold serial dilution of the inoculums from 10^{-1} to 10^{-10} was done into sterile distilled water solution using disposable sterile pipettes tips. After the incubation period, bacterial colonies on the culture plates were counted using the electronic colony counter. Sterile loop full of discrete colonies from the incubated nutrient agar dishes were streaked on different media. The well-isolated colonies were selected and used for gram staining and further biochemical tests.

2.3 Molecular identification of isolates

Genomic DNA was extracted using *Quick-DNA*TM Miniprep Kit (Zymo Research) following the manufacturer's instructions. The DNA concentrations were measured in ng/ μ L using Nanodrop instrument (Colibri Spectrometer, Berthold Detection System, Germany). DNA quality was analyzed by electrophoresis in 1% agarose gels in TBE buffer at 100V. The universal primers (Forward primer, 27F 5'-AGAGTTTGATCMTGGCTCAG -3' and reverse primer, 1525R 5'-AAGGAGGTGWTCCARCCGCA-3') were used for the amplification of the 16S rDNA gene fragment. Amplifications were done by initial denaturation at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 second, annealing temperature of primers at 56°C for 45 second and extension at 72°C for 45 minute. The final extension was conducted at 72°C for 7 minutes. Purified PCR amplicons were Sanger-sequenced using a Genetic Analyser 3130 xl sequencer from Applied Biosystems and BigDye terminator version 3.1 cycle sequencing kit. The amplified 16S rDNA genes sequence were used to query National Center for Biotechnology Information (NCBI) database followed by Basic Local Alignment Search Tool (BLAST) analysis.

2.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Disk Diffusion Method according to the Clinical and Laboratory Standards Institute guidelines (CLSI) [7] on Muller-Hinton (MH) agar. The antimicrobial discs used (Oxoid Ltd, UK) and their corresponding concentrations in brackets were as follows; sulphamethoxazole/trimethoprim (25 μ g) gentamycin (30 μ g), ciprofloxacin (5 μ g), amoxicillin/Clavulanic acid (30 μ g), nalidixic acid (30 μ g), imipenem (10 μ g), ceftriaxone (30 μ g), penicilin G (10 μ g),

meropenem (10 µg) and cefotaxime (30 µg).

2.5 Hemagglutination and hemolytic activity

The isolates were sub-cultured in 5 mL of brain-heart infusion broth and incubated overnight at 37°C. The cultures were centrifuged to sediment the bacteria and the supernatant discarded. The bacterial cells were suspended in PBS to a concentration of 5×10^{10} cells per mL. Chilled plates were taken and 25 ml of chilled bacterial suspension and 25 ml of 3% concentration of chilled RBC were added to separate wells and incubated at 4°C for one hour and hemagglutination were observed as a complete even sheet of agglutinated RBCs. Positive tests were graded as 1+, 2+, 3+ or 4+ depending upon the clumping pattern when viewed. For hemolytic activity, 200 µl of culture were distributed into each well of a microtitre plate and an equal volume of 1% concentration of washed human RBC were added and the plates covered by a cellophane tape and incubated at 37°C for 24 hours and observed for hemolysis [8].

3.0 RESULTS

Bacterial colony counts

From Table 1, the total bacteria count was higher

in *fura de nunu*, 1421 than in *nunu*, 604. The mean bacteria count was significantly higher ($P \leq 0.05$) in *fura de nunu* (57 ± 26.5) than in *nunu* (24 ± 14.1). The colony forming units (CFU) ranged from 3.0×10^5 to 18.0×10^5 cfu/mL. In *nunu*, the highest CFU was 15.6×10^5 cfu/mL while the lowest was 3.0×10^5 cfu/mL while in *fura de nunu* the highest CFU was 18.0×10^5 cfu/mL while the lowest was 3.8×10^5 cfu/mL.

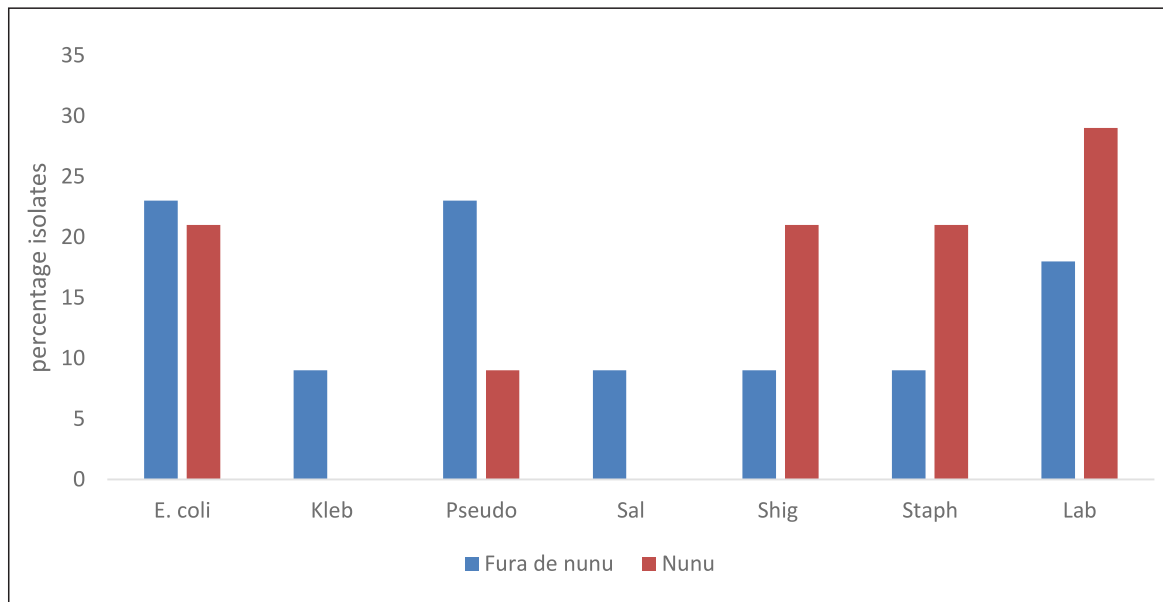
Bacterial Isolates

From Figure 1, a total of 90 bacterial isolates from *fura de nunu* (56) and *nunu* (34) samples were isolated. Seven genera of bacteria (*Escherichia*, *Staphylococcus*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Shigella* and *Lactic acid bacteria*) were isolated from *fura de nunu*, while five genera (*Escherichia*, *Staphylococcus*, *Pseudomonas*, *Shigella*, *Lactic acid bacteria*) were isolated from *nunu* which means that *Klebsiella* and *Salmonella* were not isolated from *Nunu*. In *fura de nunu*, the highest isolates were *E. coli* and *Pseudomonas*, 13 (23%) each followed by *Lactic acid bacteria*, 10 (18%). In *nunu*, the highest isolates were *Lactic acid bacteria*, 10 (29%) followed by *E. coli*, *Shigella* and *Staphylococcus*, 7 (21%).

Table 1: Bacterial colony counts (cfu x 10⁵/ml) for *nunu* and *fura de nunu*

Colony	<i>Nunu</i> (n=25)	<i>Fura de nunu</i> (n=25)
1.Total counts		
Total Bacteria	604	1421
Mean bacteria count	24±14.10	57±26.50
2.Colony forming units (cfu x 10⁵/mL)		
Total bacteria cfu	121	284
Mean bacteria cfu	4.8±2.80	11.4±5.30
Range	3 - 15.6	3.8 -18

Values expressed as mean ± SD



Key: *kleb*: *Klebsiella* spp. *Pseudo*: *Pseudomonas* spp. *Sal*: *Salmonella* spp., *Shig*: *Shigella* spp., *taph*: *Staphylococcus* spp., *Lab*: *Lactic acid bacteria*, *E. coli*: *Escherichia coli*.

Figure 1: Bacterial Isolates from Fura de nunu and Nunu (n =90) Antibiogram

Table 2: Antibigram of the bacteria isolates (n=90)

Antibiotics	Resistant(R) (%)	Intermediate(I) (%)	Subsceptible(S) (%)
Ceftriazone	85 (94)	5 (6)	0 (0)
Cefotaxime	90 (100)	0 (0)	0 (0)
Ciprofloxacin	28 (31)	35 (38)	28 (31)
Nalidixic acid	70 (78)	20 (22)	0 (0)
Amoxycilin–clavulanic acid	90 (100)	0 (0)	0 (0)
Penicillin G	85 (94)	0 (0)	5 (6)
Imipenem	0 (0)	0 (0)	90 (100)
Meropenem	90 (100)	0 (0)	0 (0)
Gentamicin	0 (0)	0 (0)	90 (100)
Sulphamethoxazole/ Trimethoxazone	53 (58)	0 (0)	
Total	65.5%	6.6%	27.9%

Table 3: Antimicrobial resistance pattern of isolates from fura de nunu (n=56)

Drugs	<i>E. coli</i> (n=13) (%)	<i>Pseud</i> (n=13) (%)	<i>Shig</i> (n=5) (%)	<i>LAB</i> (n=10) (%)	<i>Sal</i> (n=5) (%)	<i>Staph</i> (n=5) (%)	<i>Kleb</i> (n=5) (%)	Total (n=56) (%)
CRO	13 (100)	13 (100)	5 (100)	10 (100)	5 (100)	5 (100)	3 (60)	54 (96)
CN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
STX	5 (38)	8 (62)	3 (60)	3 (30)	5 (100)	3 (60)	5 (100)	32 (57)
MEM	13 (100)	13 (100)	5 (100)	10 (100)	5 (100)	5 (100)	5 (100)	56 (100)
IMP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P	13 (100)	13 (100)	3 (60)	10 (100)	5 (100)	5 (100)	5 (100)	54 (96)
AML	13 (100)	13 (100)	5 (100)	10 (100)	5 (100)	5 (100)	5 (100)	56 (100)
CIP	5 (38)	0 (0)	3 (60)	3 (30)	3 (60)	0 (0)	3 (60)	17 (30)
CTX	13 (100)	13 (100)	5 (100)	10 (100)	5 (100)	5 (100)	5 (100)	56 (100)
NA	13 (100)	13 (100)	3 (60)	10 (100)	5 (100)	5 (100)	3 (60)	52 (93)

Key: Ciprofloxacin: (CIP), Ceftriaxone: (CRO), Sulphamethoxazole: (STX), Cefotaxime: (CTX), Meropenem: (MEM), Penicilin G: (P), Nalidixic acid: (NA), Amoxicilin –clavulanic acid: (AML), Gentamicin: (CN), Imipenem: (IPM).

Table 4: Antimicrobial resistance pattern of isolates from nunu (n=34)

Antibiotics	<i>E. coli</i> (n=7) (%)	<i>Pseud</i> (n=3) (%)	<i>Shig</i> (n=7) (%)	<i>LAB</i> (n=10) (%)	<i>Staph</i> (n=7) (%)	Total (n=34) (%)
CRO	7 (100)	3 (100)	5 (71)	10 (100)	7 (100)	32 (94)
CN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
STX	7 (100)	0 (0)	5 (71)	7 (70)	2 (29)	21 (62)
MEM	7 (100)	3 (100)	7 (100)	10 (100)	7 (100)	34 (100)
IMP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P	7 (100)	3 (100)	7 (100)	10 (100)	7 (100)	34 (100)
AMC	7 (100)	3 (100)	7 (100)	10 (100)	7 (100)	34 (100)
CIP	2 (29)	0 (0)	5 (71)	2 (20)	0 (0)	9 (26)
CTX	7 (100)	3 (100)	7 (100)	10 (100)	7 (100)	34 (100)
NA	7 (100)	3 (100)	2 (29)	5 (50)	5 (71)	34 (100)

Key: Ciprofloxacin: (CIP), Ceftriaxone: (CRO), Sulphamethoxazole: (STX), Cefotaxime: (CTX), Meropenem: (MEM), Penicilin G: (P), Nalidixic acid: (NA), Amoxicilin –clavulanic acid: (AML), Gentamicin: (CN), Imipenem: (IPM).

Table 6: Hemagglutination and hemolytic properties of the isolates (n=58)

Isolates	Hemolysis		Hemagglutination			
	positive	negative	high	moderate	low	negative
<i>E. coli</i> (n=20)	13 [67%]	7 [33%]	7 [35%]	7 [35%]	0 [0%]	6 [30%]
<i>Pseudo</i> (n=16)	8 [50%]	8 [50%]	5 [33%]	3 [17%]	8 [50%]	0 [0%]
<i>Shig</i> (n=12)	8 [67%]	4 [33%]	0 [0%]	0 [0%]	4 [33%]	8 [67%]
<i>Salm</i> (n=5)	3 [60%]	2 [40%]	3 [60%]	0 [0%]	2 [40%]	0 [0%]
<i>Kleb</i> (n=5)	5 [100%]	0 [0%]	0 [0%]	0 [0%]	3 [60%]	2 [40%]
Total	37 [64%]	21 [36%]	15 [26%]	10 [17%]	17 [29%]	16 [28%]

As seen in Table 2, all the bacteria isolates were resistant to cefotaxime, meropenem, amoxicillin – clavulanic acid while susceptible to imipenem and gentamicin. Eighty five (94%) of the bacteria isolates were resistant to ceftriazone antibiotics while five (6%) showed intermediate zone of inhibition. With ciprofloxacin, twenty eight (31%) of the isolated bacteria were susceptible, thirty five (38%) were intermediate and twenty eight (31%) showed resistance. Fifty three (58%) of the bacteria isolates were resistant to sulphamethoxazole/ trimethoprim, while thirty eight (42%) of the isolates showed susceptibility. Penicillin G (P) showed resistance to eighty five (94%) of the isolated bacteria, while five (6%) showed significant susceptibility to the antibiotics drug. Seventy (78%) of the isolates were resistant to nalidixic acid while twenty (22%) were intermediates.

Antimicrobial resistance patterns

Table 3 shows the antimicrobial resistant pattern of the fifty six bacteria isolates from the twenty five *nunu* samples. All the *Salmonella* isolates showed 100% resistance to ceftriazone, sulphamethoxazole/trimethoprim, meropenem, penicillin G, amoxicillin-clavulanic acid, cefotaxime and nalidixic acid except for ciprofloxacin with 60% resistance. All the bacteria isolates of *E. coli*, *Pseudomonas*, *Lactic acidbacteria*, and *Staphylococcus* showed 100% resistance to ceftriazone, meropenem, penicillin G, amoxicillin- clavulanic acid, cefotaxime and nalidixic acid. While *E. coli* showed 38% resistance to sulphamethoxazole/trimethoprim

and ciprofloxacin, *Pseudomonas* showed 62% resistance to sulphamethoxazole/trimethoprim. All the *Lactic acid bacteria* isolates showed both 30% resistance to sulphamethoxazole/trimethoprim and ciprofloxacin while *Staphylococcus* showed 60% resistance to sulphamethoxazole/trimethoprim. *Shigella* showed 60% resistance to sulphamethoxazole/trimethoprim, ciprofloxacin, penicillin G, nalidixic acid and 100% resistance to ceftriazone, meropenem, penicillin G, amoxicillin-clavulanic acid and cefotaxime, while *Klebsiella* showed 60% resistance to ceftriazone, ciprofloxacin, nalidixic acid and 100% resistance to sulphamethoxazole/trimethoprim, penicillin G, meropenem, amoxicillin- clavulanic acid and cefotaxime.

Table 4 shows the antimicrobial resistant pattern of fourteen thirty four bacteria isolates from twenty five *nunu* samples. All the isolates showed 100% resistance to meropenem, penicillin G, amoxicillin-clav and cefotaxime. *E. coli*, *Pseudomonas*, *Lactic acid bacteria*, *Staphylococcus* showed 100% resistance to ceftriazone, meropenem, penicillin G, amoxicillin-clav, cefotaxime. *Shigella* showed 71% resistance to sulphamethoxazole/ trimethoprim, ceftriazone and ciprofloxacin; 29% for nalidixic acid and 100% resistance to meropenem, penicillin G, amoxicillin and cefotaxime. *Lactic acid bacteria* showed 50% resistance for nalidixic acid, 20% for ciprofloxacin, 70% for sulphamethoxazole/trimethoprim antibiotics and 100% resistance for ceftriazone,

meropenem, penicillin G, amoxicillin-clav, cefotaxime. *Staphylococcus* isolates showed 29% resistance for sulphamethoxazole/ trimethoprim and 71% resistance to nalidixic acid and 100% resistance to the following antibiotics ceftriaxone, meropenem, penicillin G, amoxicillin and cefotaxime.

Hemagglutination and hemolytic properties of the isolates

Fifty eight isolates representing typical *Enterobacteriaceae* were tested for their ability to clump and lyse red blood cells (table 6). Generally, higher numbers [64%] of the isolates were positive for hemolysis while 36% were negative. Highest numbers of the isolates had the lowest hemagglutination property [29%], followed by those without hemagglutination property [28%], then those with high property [15%] and moderate hemagglutination property [10]. Among the isolates, *E. coli* had the highest number of hemolytic, 13 [67%] and hemagglutination 7[35%] properties followed by *Pseudomonas* 8 [50%] and 5 [33%] respectively.

4.0 Discussion

TBC for both *fura de nunu* and *nunu* ranged from 3.0×10^5 to 18.0×10^5 cfu/ml which was higher than the maximum recommended level of 2.0×10^5 cfu/ml as given by East Africa Community (EAC) standards (EAS 67:2007), suggesting unfitness for human consumption. Our TBC results were in agreement with the reports from the western part of Nigeria [9] and elsewhere, Tanzania [10, 11, 12], Jordan [13], India [14], Ghana [15] and Nigeria [16]. Some of the organisms isolated from this study have been found to be abundant at milking area hence can predispose the milk to contamination [17]. In this study, *E. coli* and *Pseudomonas* isolates were higher in *fura de nunu* than in *nunu*. This is different from the results obtained from Nassarawa *et al.*, (2020) and Eka (1996) where *staphylococcus sp.* isolates was found to be predominantly higher in *fura de nunu* than in *nunu* [18, 19]. We reported a higher *Staphylococcus aureus* isolates in *nunu* than *fura de nunu*, similar to the result obtained by Eka and Ohaba (1996) [19]. Contamination with *S. aureus* might have originated via contact with hands or other body parts [20]. The presence of *Salmonella sp.* in *nunu* as reported by Junaidu *et al.*, (2011) and Ukaefu, *et al.*, (2019) disagrees with our

result, as *salmonella* was only isolated from *fura de nunu* [21, 17]. *Salmonella* is one of the most etiological agents implicated in several outbreaks associated with the consumption of raw milk and milk products [22, 23].

Lactobacillus sp. isolates in this study were predominantly high in both *fura de nunu* and *nunu* samples, similar to those obtained elsewhere [17, 3, 9]. Some lactic acid bacteria cause spoilage of dairy products when conditions for their growth become unfavorable [24]. This study showed that *Pseudomonas sp* isolated from *fura de nunu* was predominantly higher than that of *nunu*. Isolation of *Pseudomonas* from *nunu* has also been reported by Otoikhian, (2012), which further stated that *Pseudomonas* species were most likely from soil [25]. The growth and multiplication of microorganism in milk conditions is further aggravated by the tropical hot climate [26].

The possible reason for the high prevalence of bacterial resistance seen in this study could be due to indiscriminate use of antibiotics linked to uncontrolled availability of antibiotics in livestock markets [27, 28,]. Multi-antibiotic resistance [29, 30] was observed in 90% of bacteria isolates. All the *Salmonella* isolates showed 100% resistance to ceftriaxone, sulphamethoxazole/trimethoprim, meropenem, penicillin G, amoxicillin- clavulanic acid, cefotaxime and nalidixic acid. Similar to the work done by Raji and Jiya (2019), *Salmonella spp* recorded 100% resistance to amoxicillin/clavulanic acid. The study further reported that gentamicin was 100% resistance to *Salmonella sp.* which disagrees with our result where *salmonella* was 100% susceptible to gentamicin [31]. *Staphylococcus* isolates showed 100% resistance to amoxicillin-clavulanic acid which agrees with the work done in Sokoto, Nigeria by Raji and Jiya (2019), where *Staphylococcus aureus* recorded 60% resistance to amoxicillin/clavulanic acid [31]. Resistance to amoxicillin/clavulanic acid has great health implications as these are antibiotics of choice in the treatment of bacterial infections [32]. All the bacteria isolates of *E. coli*, *Pseudomonas*, *Lactic acid bacteria* and *Staphylococcus* from *fura de nunu* and *nunu* in this study showed 100% resistance to ceftriaxone, meropenem, penicillin G, amoxicillin-clavulanic acid, cefotaxime and nalidixic acid while *E. coli* showed 40% resistance to sulphamethoxazole/trimethoprim and

ciprofloxacin. This is in contrast with the result obtained by Talukder and Ahmed, (2016), who reported that all the *Escherichia coli* isolates were 100% resistant to ciprofloxacin, nalidixic acid, amoxicillin-clavulanic acid, gentamicin, sulphamethoxazole/trimethoprim antibiotics [33]. *Pseudomonas* showed 60% resistance to sulphamethoxazole/trimethoprim, 100% resistance to penicillin G, similar to the research in Nigeria [34, 35]. This study showed that *Shigella* isolates were 50 – 70% resistant to sulphamethoxazole/trimethoprim, ciprofloxacin, penicillin G, nalidixic acid and 100% resistance to ceftriaxone, meropenem, penicillin G, amoxicillin-clavulanic acid and cefotaxime which agrees with the work done in Dhaka [36].

The ability of variety of bacteria to cause hemagglutination (HA) and hemolysis of erythrocyte are considered virulence factors, because these activities were associated with binding capacity of bacterial cells to epithelial cell surfaces [37]. Correlation between HA, adhesion and bacterial pathogenicity for many intestinal bacterial pathogens have been reported [38]. Our result is similar to the studies done elsewhere where *Pseudomonas aeruginosa* strains and *E. coli* isolates from milk products produced hemolysis and hemagglutination on bovine blood agar and caused lysis of sheep erythrocytes [8]. In this study, all the *Klebsiella* isolates and half of the *salmonella* isolates analyzed for hemolysis were positive. This is similar to the work done by El-Sukhon (2003) and Gundogan *et al.*, (2010) [39, 40].

Conclusively, this present study has shown that *fura de nunu* had higher bacteria growth count than that of *nunu*. This may be due to introduction of more organisms by the presence of *fura* in the milk product as a result of the poor handling of *fura* during processing and storage. The presence of pathogenic bacteria with hemagglutination and hemolytic properties isolated raises a public health concern about safety of milk to consumers. Routine assessment of *fura de nunu* microbial quality produced by small-scale producers and consumed by public has to be mandatory in order to safeguard the public from milk-borne zoonotic infections which may radiate through consumption of unsafe milk and milk products; hence more food safety education should be given to producers, handlers and consumers.

FUNDING

The authors declare that no funds, grants, or other

support were received during the preparation of this manuscript

COMPETING INTEREST

The authors have no relevant financial or non-financial interests to disclose

AUTHOR'S CONTRIBUTIONS

All authors contributed to the study conception. Study design and writing were performed by David CN, Okonkwo E and David EE. Material preparation was performed by Obasi DO, Ogunwa SC and Kanu S. Data collection was performed by Onyemuche TN and Ezennaya CF. Data analysis was performed by Ejeje NJ and Obeten UN. All authors commented on the previous versions of the manuscript. All authors have read and approved the final article

REFERENCE

1. Owusu-Kwarteng, J., Tano-Debrah, K., Glover, R. & Akabanda, F. (2010). Process characteristics and microbiology of fura produced in Ghana. *Nature and Science*, 8: 41-51.
2. Jespersen, L., Halm, M., Kpodo, K. & Jakobsen, M. (1994). Significance of yeasts and moulds occurring in maize dough fermentation for 'kenkey' production. *International journal of food microbiology*, 24: 239-248.
3. Anyanwu, N. (2019). Microbiological and comparative analysis of indigenous and semi-industrial fermented milk drinks (Fura Da Nono and Fura Da Yoghurt) sold in Nigeria's capital. *International Journal of Bioassays*, 8: 5716-5723.
4. Eruteya, O. C. & Eze, C. O. (2017). Proximate Composition and Antibiotic Resistant *Listeria* Species in Fura-de-nunu Sold in Port Harcourt. *Journal of Advances in Biology & Biotechnology*, 1-7.
5. Swai, E. & Schoonman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pacific journal of tropical biomedicine*, 1: 217-222.
6. Otoikhian, C. (2012). Bio-chemical

- evaluation of local milk product (fura-de-nunu). *International Journal of Engineering Science and Technology*, 1: 1-10.
7. Jenkins, S. G. & Schuetz, A. N (2012). Current concepts in laboratory testing to guide antimicrobial therapy. *Mayo Clinic Proceedings*, Elsevier, 290-308.
 8. Rajkumar, H., Devaki, R. & Kandi, V. (2016). Comparison of hemagglutination and hemolytic activity of various bacterial clinical isolates against different human blood groups. *Cureus*, 8.
 9. Adebessin, A., Amusa, N. & Fagade, S. (2001). Microbiological quality of locally fermented milk (nono) and fermented milk-cereal mixture (fura da nono) drink in Bauchi, a Nigerian city. *Journal of food Technology in Africa*, 6 (3): 87-89.
 10. Shija, F. (2013). Assessment of milk handling practices and bacterial contaminations along the dairy value chain in Lushoto and Handeni districts, Tanzania. MSc thesis, Sokoine University of Agriculture. Morogoro, Tanzania: Sokoine University of Agriculture
 11. Kivaria, F., Noordhuizen, J. & Kapaga, A. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Tropical Animal Health and Production*, 38: 185-194.
 12. Karimuribo, E. D., Kusiluka, L. J., Mdegela, R. H., Kapaga, A. M., Sindato, C. & Kambarage, D. M. (2005). Studies on mastitis, milk quality and health risks associated with consumption of milk from pastoral herds in Dodoma and Morogoro regions, Tanzania. *Journal of Veterinary Science*, 6: 213-221.
 13. Riadh, A.-T. (2005). A comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies. *Pakistan Journal of Nutrition*, 4: 345-348.
 14. Parekh, T. & Subhash, R. (2008). Molecular and bacteriological examination of milk from different milch animals with special reference to coliforms. *Current Research in Bacteriology*, 1: 56-63.
 15. Addo, K., Mensah, G., Aning, K., Nartey, N., Nipah, G., Bonsu, C., Akyeh, M. & Smits, H. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Tropical Medicine & International Health*, 16: 227-232.
 16. Oduori, D.O., Kwoba, E., Thomas, L., Grace, D. and Mutua, F. (2022). Assessment of foodborne disease hazards in beverages consumed in Nigeria: A systematic literature review. *Foodborne Pathogens and Disease* 19(1): 1–18.
 17. Ukaefu, C.C., Braide, W and Mike-Anosike, E. E. (2019). Molecular Studies of Prevalent Bacteria Isolated from Unpasteurized Traditional Fermented Dairy Products Sold in Parts of Imo State. *Food & Nutrition Journal*, 4 (5): 207.
 18. Nassarawa, S. S. & Sulaiman, S. A. (2020). Microbiological and Chemical Evaluation of Traditional Fermented Skimmed Milk (nono) in Kano State Nigeria. *International Journal of Food Sciences*, 3: 33-46.
 19. Eka, O. & ohaba, J. (1977). Microbiological examination of Fulani milk (nono) and butter (manshanu). *Nigerian Journal of Science*, 11: 113-122.
 20. Apha, A. (1992). WPCF (American public Health association, American waterworks association, water pollution control federation)(1992) standard methods for the examination of water and wastewater. *Stand. Methods Exam. Water Wastewater*, 17.
 21. Junaidu, A., salihu, M., MambuwaL, F., Magaji, A. & Jaafaru, S. (2011). Prevalence of mastitis in lactating cows in some selected commercial dairy farms in Sokoto metropolis. *Advances in Applied Science Research*, 2: 290-4.
 22. De Buyser, M.-L., Dufour, B., Maire, M. &

- Lafarge, V. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *International journal of food microbiology*, 67: 1-17.
23. Oliver, S. P., Murinda, S. E. & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. *Foodborne pathogens and disease*, 8: 337-355.
24. Akabanda, F., Owusu-Kwarteng, J., Glover, R. & Tano-Debrah, K. (2010). Microbiological characteristics of Ghanaian traditional fermented milk product, Nunu. *Nature and Science*, 8: 178-187.
25. Wiri, ThankgodBariyaa., Osumenya, Inimeya Thompson and Gbode, YenorLekia (2020). Public Health Implications of Locally Femented Milk (Nono) and Antibiotic Susceptibility Testing of *Pseudomonas Aeruginosa* Isolated From The Product in Borokiri, Rivers State Nigeria. *International Journal of Research and Innovation in Applied Science*, 5 (3): 2454-6194.
26. Pourhassan, M. & Taravat, N. A. (2011). The spatial distribution of bacteria pathogens in raw milk consumption on Malayer City, Iran. *Shiraz E medical Journal*, 12 (1): 2-10.
27. Katakweba, A., Mtambo, M., Olsen, J. E. & Muhairwa, A. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development*, 24: 170.
28. David, E. E., Igwenyi, I.O., Iroha, I.R., David, C.N., et al (2021). [Trends in Empirical Treatment of Hospitalized Children with Acute Gastroenteritis in Nigeria](#). *Recent Advances in Anti-Infective Drug Discovery*, 16 (3): 237-244
29. David E.E., Yameen M.A., Igwenyi I., Okafor A.C., Obeten U.N., Obasi D.O., Ezeilo U.R., Emeribole M.N., David C.N (2021). Multidrug resistance and biofilm formation among diarrheal *Escherichia coli* pathotypes isolated from the stools of children with acute diarrhea. *Infection and Immunity*, 11(5): 958-964.
30. David E.E., Yameen M.A., Igwenyi I., Okafor A.C., Obeten U.N., Obasi D.O., Ezeilo U.R. & David C.N (2020). The frequency of virulent genes and antimicrobial resistance patterns of diarrheagenic *Escherichia coli* isolated from stools of children presenting with diarrhea in a tertiary hospital in Abakaliki, Nigeria. *International Journal of One Health*, 6(2): 147-152.
31. Raji, M. & Jiya, M. (2019). Research Article Evaluation of Pathogenic Bacteria in Packaged Milk Products Sold in Sokoto Metropolis, Nigeria. *Asian Journal of Applied Sciences*, 12 (2): 85-90.
32. Uddin, M. A., Motazzim-Ul-Haque, H. M. & Noor, R. (2011). Isolation and identification of pathogenic *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus* spp. in raw milk samples collected from different areas of Dhaka City, Bangladesh. *Stamford Journal of Microbiology*, 1: 19-23.
33. Talukder, M. & Ahmed, H. M. (2016). Determination of antibiotics sensitivity profiles of bacteria isolated from raw milk. *Asian Journal of Medical and Biological Research*, 2: 396-401.
34. Okpalugo, J., Ibrahim, K., Izebe, K. & Inyang, U. (2008). Aspects of microbial quality of some milk products in Abuja Nigeria. *Tropical Journal of Pharmaceutical Research*, 7: 1169-1177.
35. Oladipo, I., Tona, G., Akinlabi, E. & Bosede, O. (2016). Bacteriological quality of raw cow's milk from different dairy farms in Ogbomoso, Nigeria. *International Journal of Advanced Research in Biological Sciences*, 3.

36. Biva, I. J., Ndi, C. P., Semple, S. J. & Griesser, H. J. (2019). Antibacterial Performance of Terpenoids from the Australian Plant *Eremophila lucida*. *Antibiotics*, 8: 63.
37. Hogan, J.S., Todhunter, D.A., Smith, K.L. and Schoenberger, P. S. (1990). hemagglutination and hemolysis by *Escherichia coli* isolated from Bovine intramammary infections. *Journal Dairy Science*, 73: 3126-3131.
38. Klemm, P. and Schembri, M.A.S. (2000). Bacterial adhesions: function and structure. *International Journal of Medical Microbiology*, 290: 27-35
39. El-Sukhon S. N. Identification and characterization of klebsiellae isolated from milk and milk products in Jordan (2003). *Food Microbiology*, 20: 225-230.
40. Gundogan N., Citak S and Yalcin E (2010). Virulence Properties of Extended Spectrum b-Lactamase-Producing *Klebsiella* Species in Meat Samples. *Journal of Food Protection*, 74 (4): 559–564.