

RESEARCH ARTICLE

Prevalence of *Salmonella* Species in Locally Fermented Milk (Nono) in Gambari Market, Ilorin East Local Government, Kwara State, Nigeria

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Abstract

Although milk is highly nutritious, it is also an excellent substrate for microorganisms' growth like *Salmonella* which is one of the most common causes of foodborne disease worldwide. This research aimed to isolate *Salmonella* spp. from nono sold in Gambari market, Ilorin, Kwara State, Nigeria, test the isolated *Salmonella* spp. to ten antimicrobial agents and evaluate the risk factors associated with the production and retailing processes of nono in the study area. These were achieved by collecting 113 nono samples and analysing them using conventional isolation methods, confirming the suspected isolates using Microbact GNB 24E. Disc diffusion method for antimicrobial sensitivity test and structured questionnaires were also used. Six (5.3%) *Salmonella* spp. were isolated. All (100%) the *Salmonella* isolates were resistant to amoxicillin, ampicillin and penicillin. Three (50%) of the isolates had resistance breakpoints of 14mm or less to colistin. Five resistance patterns were recorded among the isolates. Eighty percent of the respondents did not wash their hands before milking, while 70% didn't clean the udder before milking. Self-administration of antibiotics to milking cows by 85% of respondents was also recorded. The isolation of *Salmonella*, including multiple-antimicrobial-resistant species, in nono in the study area could be an indication of contamination.

Keywords

Salmonella isolation, Nono, Gambari, Ilorin, Nigeria

Introduction

Salmonella is a rod-shaped gram-negative facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*. Nono is a refreshing milk beverage mostly associated with the Fulani tribe widely consumed in many African countries including Nigeria. Salmonellosis is regarded as one of the most severe bacterial zoonotic diseases affecting humans and animals [1]. It is a

digestive system infection caused by *Salmonella* species which affects both humans and animals and millions of illnesses worldwide were reported. It is clinically characterized by one or more of the three major syndromes; septicaemia, and acute and chronic enteritis [2].

Salmonella is a rod-shaped gram-negative facultative anaerobic bacterium belonging to the family of *Enterobacteriaceae* [1]. It is a major

inhabitant of the gastrointestinal tract and is seen as one of the most widely recognized reasons for food-borne infections all over the world [3,4]. The genus *Salmonella* is classified into two species, *Salmonella enterica* and *S. bongori*. *S. enterica* can be further classified into six subspecies based on their genomic relatedness and biochemical properties. The subspecies are denoted with roman numerals: I; *S. enterica* subsp. *enterica*, II; *S. enterica* subsp. *salamae*, IIIa; *S. enterica* subsp. *arizonae*, IIIb; *S. enterica* subsp. *diarizonae*, IV; *S. enterica* subsp. *houtenae*, and VI; *S. enterica* subsp. *indica* [5]. *Salmonella* possesses three major antigens: H or flagella antigen, O or somatic antigen and Vi or surface antigen [5, 6]. *Salmonella* species are chemoorganotrophic which means that members of this genus may metabolize nutrients through both respiratory and fermentative routes [6].

Antimicrobial-resistant *Salmonella* has been reported to be on the increase [2]. Its presence in locally fermented commercial milk could be a source of concern to epidemiologists and health workers. This may be a threat to the effective prevention and treatment of the diseases caused by *Salmonella* [2].

Salmonellosis is a major public health concern in many countries [2]. It can be transmitted by both direct and indirect contact with infected animals [7]. Salmonellosis is also spread through the consumption of contaminated food, water or contact with an environment contaminated with *Salmonella* spp. Watery diarrhoea, fever, headache, abdominal pain, nausea, vomiting and loss of appetite are common symptoms that appear between 6 and 72 hours after *Salmonella* infection [2].

Nono is a refreshing fermented beverage made locally from fresh cow milk. It is popular in many African countries, including Nigeria and is most commonly associated with the Fulani tribe [8, 9]. This dairy product is usually sold in local markets and streets in certain towns, but it can also be purchased within walking distance of most Fulani settlements. Depending on the quantity purchased, it can be sold in previously used plastic bottles and nylons [9]. Sanitary standards are critical in the milk processing industry to reduce the risk of foodborne illnesses [10]. Traditionally, Nono is made by hand-milking cows into big calabashes (Kworia) in contaminated environments with subsequent skimming to remove the top cream, which creates Fulani's butter, a by-product of the process [11]. The collected fresh milk is then exposed to the sun in an open atmosphere for up to two hours, after which previously fermented milk is added to serve as a source of starter culture. A large amount of water is usually added to it and the mixture is stirred with a T-shaped stick until it reaches the desired consistency [9, 12].

This study aimed to isolate *Salmonella* spp. from nono obtained from Gambari market at the Ilorin East Local Government Area, Ilorin, Kwara State, Nigeria, and determine the antimicrobial resistance patterns and the risk factors involved in the spread and transmission of the organism. This was achieved using conventional methods of *Salmonella* isolation. Confirmation of presumptive isolates was done with Microbact GNB 24E. Antimicrobial sensitivity test was accomplished using disc diffusion method while risk factors were assessed with the aid of the information collected from questionnaire administered to milk-sellers in the study area.

Materials and methods

Study area

Gambari market is situated in the Ilorin East Local Government Area of Kwara state, Nigeria. Ilorin East which is cited at Oke-Oyi (latitude 8° 34' 57" N and longitude 4° 42' 58" E) is sixteen kilometres from Ilorin, the Kwara state capital [13]. It was selected because it had the highest number of nono sellers in Ilorin, Kwara state.

Sample collection and analyses

A total of 113 nono samples were purchased in clean, dry and sterile polythene bags from nono-traders in the study area and transported in insulated containers with ice packs to the Food Safety Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ilorin for processing. Sample analysis was done as described by Oludairo *et al.* [14] and carried out within 1-4 hours of sample collection. Pure smooth, colourless colonies with the characteristic blackish centres of *Salmonella* were then subjected to biochemical identification. Confirmation of suspected *Salmonella* isolates was done using Microbact GNB 24E according to manufacturer's instruction. Organisms with percent probabilities of over 80 were stored for further tests [15].

Antibiotic Sensitivity Test

Disc diffusion method was used for antibiotic sensitivity test of the isolates to detect their resistance to ten antimicrobial agents namely: amoxicillin, ampicillin, chloramphenicol, gentamicin, colistin sulphate, penicillin G, neomycin, oxytetracycline, sulphamethoxazole, ciprofloxacin. This was done as described by Oludairo *et al.* [16] and the zones of inhibition were measured using a

transparent ruler and recorded [2]. The recorded results were compared to the Clinical and Laboratory Standards Institute (CLSI) [17] standards to determine the sensitivity of the organisms to the antibiotics used.

Results

Out of 113 locally fermented milk (nono) samples, 6 *Salmonella* species were isolated resulting in a prevalence of 5.3%. Samples 72B, 83A, 107A, 107B, 110A, and 110B were urease positive, indole negative, Voges Praker negative, and Simmons citrate negative. Samples 72B and 83A tested methyl red negative, and 107A, 107B, 110A, and 110B were methyl red positive (Table 1). The 6 isolates gave Microbact GNB 24E probability score of over 70% and above.

The antibiotic susceptibility test showed that all the *Salmonella* isolates (100%) were resistant to amoxicillin, ampicillin and penicillin G. All the isolates (100%) were also sensitive to chloramphenicol and sulfamethoxazole (Table 2).

Two (2) *Salmonella* isolates (33.33%) were resistant to oxytetracycline and ciprofloxacin, 1 *Salmonella* isolate was resistant to neomycin and chloramphenicol (16.67%) while no *Salmonella* isolate (0%) was resistant to sulfamethoxazole and gentamicin (Table 3).

Five (5) antimicrobial resistance patterns were recorded in this study (Table 4). While AML-AMP-P-OT-CIP was reported in 2 *Salmonella* isolates, the other four resistant patterns (16.67%) occurred in the remaining four isolates (Table 4).

The results of the administered questionnaire indicated that 16 (80%) nono sellers do not wash their hands

before milking their cows, 10% of the respondents do not wash their equipment and milking wares before and after usage

Eighty per cent (80%) of the respondents (nono sellers) to the questionnaire do not wash their hands

before milking their cows. Seventy per cent (70%) of the respondents do not clean the udder of their cows before they milk them while 85% indicated they administer drugs/ antimicrobials to the animals periodically (Table 5).

Table 1: Results of the biochemical test performed on 6 *Salmonella spp.* isolated in nono in Gambari Market, Ilorin, Kwara state.

S/N	Sample number	Biochemical test					Prevalence
		Urease	Indole	Methyl red	VogesPra keur	Simmons citrate	
1	72B	+	-	-	-	-	5.3% ($\frac{6}{113} \times 100$)
2	83A	+	-	-	-	-	
3	107A	+	-	+	-	-	
4	107B	+	-	+	-	-	
5	110A	+	-	+	-	-	
6	110B	+	-	+	-	-	

Table 2: Zones of inhibition measurement for the antibiotic sensitivity test for the *Salmonella spp.* isolated from nono collected at Gambari Market in Ilorin, Kwara state.

S/N	Sample number	Antimicrobial agents/zone of inhibition measurement (mm)									
		AML (10 µg)	AMP (10 µg)	C (30 µg)	CN (30 µg)	CT (10 µg)	P (10 µg)	N (30 µg)	OT (30 µg)	SXT (25 µg)	CIP (5 µg)
1	72B	6 (R)	6 (R)	16 (S)	20 (S)	19 (S)*	6 (R)	15 (S)	6 (R)	25 (S)	27 (R)
2	83A	6 (R)	6 (R)	20 (S)	22 (S)	15 (S)*	6 (R)	15 (S)	10 (R)	26 (S)	30 (R)
3	107A	10 (R)	6 (R)	28 (S)	19 (S)	13 (R)*	6 (R)	14 (R)	18 (S)	28 (S)	37 (S)
4	107B	10 (R)	6 (R)	27 (S)	20 (S)	14 (R)*	6 (R)	16 (S)	18 (S)	30 (S)	36 (S)
5	110A	11 (R)	6 (R)	30 (S)	20 (S)	15 (S)*	6 (R)	17 (S)	20 (S)	30 (S)	40 (S)
6	110B	15 (R)	16 (R)	27 (S)	19 (S)	11 (R)*	6 (R)	17 (S)	16 (S)	21 (S)	35 (S)

*For colistin, CLSI disc diffusion break point was not available.

Key: AML-Amoxicillin, AMP-Ampicillin, C-Chloramphenicol, CN-Gentamicin, CT-Colistin sulphate, P-Penicillin G, N-Neomycin, OT-Oxytetracycline, CIP-Ciprofloxacin, SXT-Sulfamethoxazole

Table 3: Numbers and Percentages of the *Salmonella* isolates in nono samples collected from Gambari market, Ilorin, Kwara state that were resistant to the antimicrobial discs.

S/N	Antimicrobial Agent (μg)	Number (%) of <i>Salmonella</i> isolates resistant
1	Amoxicillin (10)	6 (100)
2	Ampicillin (10)	6 (100)
3	Chloramphenicol (30)	1 (16.67)
4	Gentamicin (30)	0 (0)
5	Colistin Sulphate (10)	*
6	Penicillin G (10)	6 (100)
7	Neomycin (30)	1 (16.67)
8	Oxytetracycline (30)	2 (33.33)
9	Sulfamethoxazole (25)	0 (0)
10	Ciprofloxacin (5)	2 (33.33)

*CLSI disc diffusion break point was not available

Table 4: Antibiotic Resistance Patterns of *Salmonella* isolates in the test carried out.

S/N	Resistance Pattern	Frequency (%)
1	AML-AMP-P-OT-CIP	2 (33.33)
2	AML-AMP-CT-P-N	1 (16.67)
3	AML-AMP-CT-P	1 (16.67)
4	AML-AMP-P	1 (16.67)
5	AML-AMP-CT-P	1 (16.67)
TOTAL		6 (100)

Key: AML-Amoxicillin, AMP-Ampicillin, CT-Colistin sulphate, P-Penicillin G, N-Neomycin, OT- Oxytetracycline, CIP-Ciprofloxacin

Table 5: Results of questionnaire administered to nono sellers in Gambari market, Ilorin, Kwara state

S/N	Question	Yes (%)	No (%)	No answer (%)	Total (%)
1	Do you wash your hands before milking the cow?	4 (20)	16 (80)	0 (0)	20 (100)
2	Do you wash your equipment before and after use?	16 (80)	2 (10)	2 (10)	20 (100)
3	Do you clean the udder of the cow before milking?	6 (30)	14 (70)	0 (0)	20 (100)
4	Do you administer drugs (antibiotics) to your cows?	17 (85)	3 (15)	0 (0)	20 (100)

Discussion

The 6 *Salmonella* species isolated from ready-to-drink nono could be a result of contamination. This could be due to unhygienic practices during the milking of cows and the process of preparation up to the point of sale of the milk produced by the vendors. It could also be due to poor sanitary conditions of the milkers' hands, clothing and the environment [18]. There could be various points of contamination in nono preparations which include starter cultures, cow udder, utensils/equipment and the water used in processing the milk [19]. The presence of flies in the environment where fermented milk was sold could be a potential source of *Salmonella* spp. [20]. *Salmonella* is a pathogen that could originate from the animals themselves.

The prevalence of *Salmonella* reported in this study is of public health importance, since the presence of one *Salmonella* species can lead to serious public health hazards. The World Health Organization (WHO) and Codex Alimentarius Commission recommended the recall of food items from the market in the event of isolation of one *Salmonella* specie from ready-to-eat food.

The prevalence of *Salmonella* in nono in this study is higher compared to Tamba *et al.* [19] who reported a prevalence of 3.4% in Zaria, Nigeria. It is also higher than Karshima *et al.* [20] who reported a prevalence of 0.8 % in nono in Plateau state, Nigeria. The prevalence of *Salmonella* in nono in this study is however lower than the 24% reported by Okonkwo *et al.* [21] in Maiduguri metropolis. These variations in prevalence could be due to the differences in locations and the level of hygienic practices of nono sellers. These variations could also be an indication that milk

products like nono can only be fit for consumption if processed under hygienic conditions and preserved.

The 100% antibiotic resistance of all *Salmonella* species isolated in this study to amoxicillin, ampicillin and penicillin G is in tandem with the level of antimicrobial resistance in the study of Tamba *et al.* [19]. The probable reason for antimicrobial resistance may be inappropriate use of antibiotics by farmers and animal feed producers in preventing or treating infectious diseases in their animals [19]. This could lead to mutations from susceptible bacteria to new resistant bacteria through gene transfer. It could also lead to prolonged treatment and additional costs of diagnostic testing on animals [19]. The antimicrobial resistance patterns reported in this study could be an indication of different modes of action of antimicrobial resistance of the *Salmonella* species isolated from nono in the study.

The questionnaire carried out indicated that the sellers did not wash their hands before milking, some usually administer drugs to their animals without observing the recommended withdrawal periods, and they did not clean the udders of their animals before milking. These were indications of the poor hygienic practice of the sellers. The environment where nono was sold was observed to appear clean in the morning which may be attributed to the fact that the market sellers swept the environment every morning before beginning their sales. In the afternoon, the environment was however slightly dirty as nylon wrappers in which the nono was being sold, were littered on the floor. Although most of the nono sellers appeared clean, however only a few rinsed their utensils such as spoons and cups after each sale. The nono sellers also sometimes purchased some quantity of nono from their peers whenever the

quantity they brought for sale from their various places was not enough. This however gives room for cross-bacteria contamination because the containers/utensils of the person from which the nono was purchased may be contaminated with bacteria.

Isolation of antimicrobial resistant *Salmonella* in this study could be due to unhygienic practices during the production process and is also of public health importance. Encouraging good hygienic practices, discouraging self-prescription/administration of antibiotics to milking cows by milkers, handlers and herdsmen will enhance the production of wholesome milk for consumption by the public in the study area.

Conclusion

The presence of antimicrobial resistant *Salmonella* spp. in nono which is highly cherished and consumed locally by many is a source of public health concern. It poses considerable health risk to consumers and is a serious threat to food safety, especially locally processed foods in the study area because nono is consumed without further processing. Particular attention should therefore be given to the microbiological safety of these products because their direct consumption may cause health hazards to the consumers.

Recommendations

Based on the findings of this research, it can be recommended that nono retailing environments should be kept clean to avoid contamination of the product. The use of an old portion of previously fermented nono as a starter should be discouraged as they could be a possible source of contaminating organisms. The milkers should practice personal hygiene by wearing clean clothing and washing

their hands regularly with soap and water before milking the animals. Public health education is needed so that the environment where the animals are kept are clean and also to ensure the udder of the cow are wiped before milking with clean cloth, warm water and edible disinfectant. Government agencies like National Agency for Food and Drug Administration and Control (NAFDAC) should put in place policies and measures that would prevent the indiscriminate use of antibiotics by animal owners. Nono sellers and Fulani herdsmen should be educated on the need to call a veterinary doctor to treat their animals instead of administering antimicrobials to their animals themselves. More awareness programs and outreach programs to rural settlements should be put in place.

Conflict of Interest

The authors declare no conflict of interest.

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الملخص العربي

انتشار أنواع السالمونيلا في الحليب المخمر محلياً (نونو) في سوق جمباري ، الحكومة المحلية شرق إيلورين ، ولاية كوارا ، نيجيريا

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1 قسم الصحة العامة البيطرية والطب الوقائي ، جامعة إيلورين ، نيجيريا

2 قسم الأحياء الدقيقة البيطرية ، جامعة إيلورين ، نيجيريا

3 قسم الصحة العامة البيطرية والطب الوقائي ، جامعة مايكل أوكبارا للزراعة ، أوموديك ، نيجيريا

على الرغم من أن الحليب مغذي للغاية ، إلا أنه يمثل أيضًا ركلة ممتازة لنمو الكائنات الحية الدقيقة مثل السالمونيلا التي تعد أحد أكثر الأسباب شيوعًا للأمراض المنقولة عن طريق الأغذية في جميع أنحاء العالم. يهدف هذا البحث إلى عزل *Salmonella* spp. من nono المباع في سوق جامباري ، إيلورين ، ولاية كوارا ، نيجيريا ، اختبار السالمونيلا المعزولة. إلى عشرة عوامل مضادة للميكروبات وتقييم عوامل الخطر المرتبطة بعمليات الإنتاج والبيع بالتجزئة لـ nono في منطقة الدراسة. تم تحقيق ذلك من خلال جمع 113 عينة nono وتحليلها باستخدام طرق العزل التقليدية وتأكيد العزلات المشتبه بها باستخدام Microbact GNB 24E. كما تم استخدام طريقة نشر القرص لاختبار حساسية مضادات الميكروبات والاستبيانات المنظمة. ستة (5.3%) *Salmonella* spp. تم عزلهم. جميع عزلات السالمونيلا (100%) كانت مقاومة للأموكسيسيلين والأمبيسلين والبنسلين. ثلاثة (50%) من العزلات لديها نقاط مقاومة 14 مم أو أقل للكوليستين. تم تسجيل خمسة أنماط مقاومة بين العزلات. ثمانون بالمائة من المستطلعين لم يغسلوا أيديهم قبل الحلب ، بينما 70% لم ينظفوا الضرع قبل الحلب. كما تم تسجيل إعطاء المضادات الحيوية للأبقار الحلوب من قبل 85% من المستجيبين. قد يكون عزل السالمونيلا ، بما في ذلك الأنواع المقاومة لمضادات الميكروبات المتعددة والذي يعتبر مؤشر التلوث على في منطقة الدراسة