

Risk Factors Associated with the Occurrence of *Salmonella* spp. Isolated From Locally Fermented Cattle Milk (Nono) in Gambari Market, Ilorin, Nigeria

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ABSTRACT

Several factors contribute to the occurrence of *Salmonella* in locally fermented milk (nono) from cattle in many parts of Africa. This study aimed to assess the risk factors associated with the occurrence of the organism in nono from Gambari Market, Ilorin, Nigeria. A cross-sectional study with a systematic random sampling method was used to administer a questionnaire to 35% of the 113 nono sellers from which samples were collected to isolate *Salmonella*. Out of the respondents who were all females (100%), 80% did not wash their hands before milking their cattle, the same percentage did not have a formal education ($P < 0.05$), while more (15%) younger respondents (≤ 25 years) engaged in hand washing compared to older (> 25 years) ones, 5%. Although 80% of the respondents reported they wash their milking equipment before and after use, 70%, 100%, 45% and 100% did not observe other hygiene practices like cleaning milked cow udder before milking, boiling of milking during product processing, cleaning of the milking cattle holding areas, daily and using disinfectants in the milking areas ($P < 0.05$) respectively. Ninety-five per cent of the respondents reported keeping the dairy cattle in well-ventilated areas, while 85% and 95% self-administered antibiotics to their cattle ($P < 0.05$) and had their cattle mingle with other cattle, respectively. The general hygienic practices (GHP) and environmental hygiene (GEH) levels of the respondents in the study area were poor. The government and all stakeholders should conduct extensive health education for nono sellers to achieve improved public health.

Keywords: Risk factors, *Salmonella*, Occurrence, Nono

INTRODUCTION

Nono is a refreshing locally fermented milk made from fresh milk of cattle in many African countries, including

Nigeria (Omola et al., 2019). Nigeria is reported to require 1.7 million metric tonnes of milk annually (FAO, 2025). The demand for livestock products like

nono is predicted to double by 2050, mainly due to an increase in population (Imohimi, 2025).

Salmonella infection is regarded as one of the most severe zoonotic bacterial diseases affecting humans and animals (Abd El-Ghany, 2020). It is a digestive system infection caused by *Salmonella* species, and causes millions of illnesses worldwide. It is clinically characterised by one or more of the three major syndromes: septicaemia, acute and chronic enteritis (Musa et al., 2017).

The hyper-nutritious nature of milk, especially raw and unpasteurized milk, makes it a preferred substrate for the growth of foodborne disease (FBD)-causing microorganisms worldwide, which compromises the quality of the product due to the contaminant's degradative activities (Loor-Giller et al., 2025). A prevalence of 37.5% (225/600) for *Salmonella enterica* in raw milk was recorded in a study in Ecuador (Loor-Giller et al., 2025), while 5.3%, 6.5% and 6.7% were reported in studies involving milk in Ilorin, Abuja and Zaria, Nigeria, respectively (Oludairo et al., 2023a; Okon et al., 2022; Esonu et al., 2021). The contamination of milk by *Salmonella* spp. is particularly concerning in communities where raw, unpasteurized or improperly pasteurised milk, like nono is generously consumed because of its potential to cause FBDs that may lead to loss of active time, unproductivity, death and economic losses. Therefore, in the case of the isolation of one *Salmonella* spp. in food, the Codex Alimentarius Commission and the World Health Organisation (WHO) recommended the non-consumption and withdrawal of such food item because of the public health implications (CAC, 2016).

The risk factors associated with the spread of *Salmonella* in nono are the factors that increase the possibility of the organism's contamination and its spread in fermented milk. The factors range from ignorance/inept knowledge of the prevalence of the disease/microorganism, milkers' unhygienic practices, milk processing in an unhygienic environment, use of contaminated milk processing materials/equipment, non-washing of hands/udder/milking equipment before and after milking and non-use of disinfectants in the milking cleaning process (Karshima et al., 2013).

This study aimed to assess the risk factors in the occurrence and spread of *Salmonella* spp. isolated from cattle in Gambari Market, Ilorin, Nigeria. Gambari Market has been where the largest congregation of nono sellers in Ilorin was and a major retail and wholesale point of nono in Ilorin (Oludairo et al., 2023a).

MATERIALS AND METHODS

The study area was Gambari Market, Ilorin, Kwara State, Nigeria, with coordinates 8°30'3"N 4°33'6"E. The market has the largest presence of nono retailers in Ilorin, a transit city in North-Central Nigeria that links the Northern and Southern parts of the country. The 113 nono sellers from which milk samples were collected for the isolation of six multidrug-resistant (MDR) *Salmonella* spp., as described by Oludairo et al. (2023a), were used for this study.

A cross-sectional study design was adopted to determine the risk factors associated with the occurrence of *Salmonella* spp. and its contamination of nono. This was achieved by the administration of questionnaires

(Appendix 1) to all the nono sellers from which samples were collected for *Salmonella* isolation, which made up 35% of the 113 nono traders. From the point of entrance of the market, selection of the 4th, 6th, 11th, 16th and 20th nono seller was made using a systematic random sampling method. This was done during the four months, eight rounds of sample collection in which 113 nono samples were collected. Questionnaires were therefore administered to five nono sellers at each round of collection to make a total of forty respondents. Analyses and presentation of the filled questionnaire were done using Microsoft Excel Office Mondo 2016 version 2405 and tables.

RESULTS

The demographic data obtained from the respondents in the study revealed that all respondents (100%) were females, 60% were 25 years old or younger, and 95% of them had no formal Western education (Table 1).

The majority (80%) of the respondents in this study did not wash their hands before milking their cattle, while 80% and 70% did not wash the milking equipment nor clean the cow's udder before milking, respectively. Out of the respondents, 85% self-administered antibiotics to their animals, 100% did not boil milk in the course of processing nono, did not keep milked cattle in

covered structures and did not use disinfectants in the milking areas. However, 95%, 55% and 95% of the respondents kept milked cattle in well-ventilated areas, cleaned the milking areas daily and had their milked cattle mingle with cattle from other places, different from their ranches, respectively (Table 2).

Out of the respondents that were ≤ 25 years old, 45% and 35% did not wash their hands before milking a cow and clean the udder of the cow before milking, respectively, while 60% did not boil milk during processing, keep milked cattle in covered cowhouses and nor employ the use of disinfectants ($P < 0.05$) in the cattle keeping and milking areas. In addition, 50%, 50%, 90%, 35%, and 92.5% of the respondents washed their equipment before/after use, self-administered antibiotics to their cows, kept cattle in well-ventilated areas, cleaned the cattle holding areas daily, and had their milked cattle mingle with those from other sources, respectively (Table 3).

The majority, 80% and 95% of the respondents in this study with no formal education, did not wash their hands before milking and did not use disinfectants in cleaning the milking areas, respectively ($P < 0.05$). Eighty-five per cent of the respondents, however, self-administered antibiotics to their cattle ($P < 0.05$) (Table 4).

Table 1: Demographic data of Respondents to the questionnaire administered to nono sellers in Gambari Market, Ilorin, Kwara State, to assess the risk factors associated with *Salmonella* occurrence

Variable	Frequency (%)
Sex	
Male	0 (0)
Female	40 (100)
Age	
≤25	24 (60)
>25	16 (40)
Educational level	
None	38 (95)
Primary School	2 (5)
Secondary School	0 (0)
Higher Degrees	0 (0)

Table 2: Result of questionnaire administered to nono sellers in Gambari market, Ilorin, Kwara State to assess the risk factors associated with *Salmonella* occurrence based on sex

S/N	Question	Yes (%)	No (%)	No answer (%)	Total (%)
		Female*			
1.	Do you wash your hands before milking the cow?	8 (20)	32 (80)	0 (0)	40 (100)
2.	Do you wash your equipment before and after use?	32 (80)	4 (10)	4 (10)**	
3.	Do you clean the udder of the cow before milking?	12 (30)	28 (70)	0 (0)	
4.	Do you administer drugs (antibiotics) to your cows?	34 (85)	6 (15)	0 (0)	
5.	Do you boil/heat milk while processing nono?	0 (0)	40 (100)	0 (0)	
6.	Are your cattle kept in closed/covered cowhouses?	0 (0)	40 (100)	0 (0)	
7.	Is your cattle holding/milking area well ventilated?	38 (95)	2 (5)	0 (0)	
8.	Do you clean your cattle holding/milking areas daily?	22 (55)	18 (45)	0 (0)	
9.	Do you use disinfectants in your cattle holding/milking areas?	0 (0)	40 (100)	0 (0)	
10.	Do your cattle mingle with other cattle from other places?	38 (95)	2 (5)	0 (0)	

#GHP/GEH=46%

* All 40 (100%) respondents were female. ** Response was not given to the question by respondents, # GHP/GEH 460/1000*100=46%

Table 3: Respondents' response to the questionnaire administered to nono sellers in Gambari Market, Ilorin, Kwara State, to assess the risk factors associated with *Salmonella* occurrence based on age

S/N	Question	Response			
		Age (years)			
		≤25		>25	
		Yes (%)	No (%)	Yes (%)	No (%)
1	Do you wash your hands before milking the cow?	6 (15)	18 (45)	2 (5)	14 (35)
2	Do you wash your equipment before and after use?	20 (50)	2 (5)	12 (30)	2 (5)*
3	Do you clean the udder of the cow before milking?	10 (25)	14 (35)	2 (5)	14 (35)
4	Do you administer drugs (antibiotics) to your cows?	20 (50)	4 (10)	14 (35)	2 (5)
5	Do you boil/heat milk while processing nono?	0 (0)	24 (60)	0 (0)	16 (40)
6	Are your cattle kept in closed/covered cowhouses?	0 (0)	24 (60)	0 (0)	16 (40)
7	Is your cattle holding/milking area well ventilated?	36 (90)	2 (5)	2 (5)	0 (0)
8	Do you clean your cattle holding/milking areas daily?	14 (35)	10 (25)	10 (25)	6 (15)
9	Do you use disinfectants in your cattle holding/milking areas?	0 (0)	24 (60)	16 (40)	0 (0)***
10	Do your cattle mingle with other cattle from other places?	37 (92.5)	1 (2.5)	1 (2.5)	1 (2.5)

* No response was received from 4 (10%) of the respondents.

*** P value (Fisher's exact test) < 0.05

Table 4: Respondents' response to the questionnaire administered to nono sellers in Gambari Market, Ilorin, Kwara State, to assess the risk factors associated with *Salmonella* occurrence based on educational level

S/N	Question	Response							
		Educational level							
		None		Primary school		Secondary School		Higher Education	
		Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
1	Do you wash your hands before milking the cow?	6 (15)	32 (80)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)***
2	Do you wash your equipment before and after use?	30 (75)	4 (10)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3	Do you clean the udder of the cow before milking?	10 (25)	28 (70)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	Do you administer drugs (antibiotics) to your cows?	34 (85)	4 (10)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)***
5	Do you boil/heat milk while processing nono?	0 (0)	38 (95)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)
6	Are your cattle kept in closed/covered cowhouses?	0 (0)	38 (5)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)

7	Is your cattle holding/milking area well ventilated?	36 (90)	2 (5)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
8	Do you clean your cattle holding/milking areas daily?	20 (50)	18 (45)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
9	Do you use disinfectants in your cattle holding/milking areas?	0 (0)	38 (95)	0 (0)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)***
10	Do your cattle mingle with other cattle from other places?	37 (92.5)	1 (2.5)	1 (2.5)	1 (2.5)	0 (0)	0 (0)	0 (0)	0 (0)

* No response was received from 4 (10%) of the respondents

*** P value (Fisher's exact test) < 0.05

DISCUSSION

No male nono milk seller was identified in the milk market in this study, which suggests that milk sales are a female-dominated occupation in the study area. Having all the local nono sellers in the study area as females showed that the men in the milkers' families were not involved in the retail of nono (Cinjel and Weinoh, 2024). Other family members might have been involved in different stages of milk production. The older and younger men usually shepherd the cattle to pasture during the day, when the women will be in the market engaged in the sales of nono (Kariuki et al., 2024). This is in agreement with the study of Omola et al. 2019 who reported the majority of traders of the locally fermented milk to be females.

The application of General Hygienic Practices (GHP) and General Environmental Hygiene (GEH) procedures could help in the reduction of the contamination of milk by *Salmonella*.

Poor personal and cow hygiene reported in this study may be due to low formal and health education of the respondents in the area. Washing of handlers' hands and milking equipment before and after the milking process, as well as cleaning the udders of the cows to be milked, are

important actions to take in the nono production process to ensure the production of wholesome milk for the public (Hristov, 2023). It has been reported that unwashed hands can transfer bacteria to clean milk. Proper hand washing with soap before, during and after milking, wearing clean clothes and ensuring standard personal hygienic practices will enhance the production of safe nono for the public (AI, 2023b). In addition, ensuring good cow hygiene, such as regular removal of dirt, urine and cow dung from cow holding and milking areas, trimming of the hair around the tail and udders, washing the teats and cleaning of the surroundings with warm water and disinfectants will improve GEH (Bello et al., 2020). Poor milkers and milking cows' hygiene during the production of milk products was also reported by Bello et al. (2020) and Dafur et al. (2018) in Zaria and Mangu, Nigeria, respectively.

Heat is not routinely and traditionally applied to milk during the production of nono, which may explain why all respondents indicated that heat was not applied during the production process. The application of heat to milk in the process of pasteurisation is, however, a proven means of mitigating the food safety risk associated with the consumption of milk. The most

commonly used method has been the High Temperature Short Time (HTST) pasteurisation in which milk is heated for 15 seconds at 72°C, since the boiling point of milk is about 100.4°C (AI, 2023a). Milk in the study area is known to be retailed as unpasteurised milk products without a cold chain and lacking regulatory control (Owusu-Kwarteng et al., 2020). The traditional preparation of nono is documented by Omola et al. (2019).

The poor sanitary milking environment and equipment could have been a source of *Salmonella* contamination of nono, since a clean milking environment is important in the production of wholesome milk. The facility's layout and design are expected to enhance cleanliness, easy flow of water, and proper ventilation (to reduce humidity and carbon contaminants) (AI, 2023b). The report of the unsanitary environment in this study is in tandem with that of Bello et al. (2020), who reported unhygienic cow holding and milking environments in Samaru, Zaria, Nigeria (Bello et al., 2020).

Self-administration of unprescribed antibiotics to milking cows recorded in this study may have been a means to prevent or treat diseases, especially mastitis (FAO, 1989; Chaudhari et al., 2023). The continual use of sublethal doses may result in the development of AMR-*Salmonella* in cows, which may contaminate milk, leading to AMR-resistant *Salmonella* infections in humans and animals (Oludairo et al., 2023b). In addition, it may result in the risk of the introduction of antibiotic residue in milk (Salam et al., 2023; AI, 2023b). Indiscriminate, unprescribed administration of antibiotics should be avoided in milking cows, and if they are

used, the withdrawal time should be strictly adhered to before the milk from such animals is collected and added to the bulk milk (FAO, 1989; Alemu, 2022). The contamination of milk by AMR-*Salmonella* in various parts of the world has been reported by several scholars (Esonu et al., 2021; Okon et al., 2022; Oludairo et al., 2023a; Mugabe et al., 2025; Kanat and Gulel, 2025).

Younger respondents generally paid less attention to the risk factors of the spread of *Salmonella*, which include washing of milkers' hands and the cow udder. Older participants may tend to adhere to preventive health measures in milk processing because of maturity and better understanding due to age. Older categories of respondents probably had more experience in self-prescription and administration of drugs, especially antibiotics, to cattle, the knowledge of which might have been passed down to them by previous generations that had been in the business of trying to save their cattle for longer periods (Olasoju et al., 2021). Often, because antimicrobial sensitivity tests were not carried out, drugs used may not be effective, which may further predispose *Salmonella* to the development of AMR (Mushayija et al., 2021).

CONCLUSION

The poor disposition to GHP, GEH and the risk factors involved in the process involved in the production of wholesome nono from fork to farm is of high public health concern.

There is a need to engage nono sellers, cattle herders and stakeholders in training on GHP, GEH, proper milk/food processing techniques and food safety tips like hand washing before and after milking the cow, thorough cleaning of

the udder of the cow before milking and washing of milking equipment and utensils before and after use to reduce the risk of contamination of nono meant for human consumption. Milkers and milk handlers should be encouraged to observe good personal hygiene, ranging from the use of hand gloves, aprons and facemasks during the collection and processing of fresh milk to nono. Cattle owners and herders should be encouraged to engage the services of animal health professionals in the treatment of their animals, especially the prescription and administration of antimicrobial agents. To prevent the development of AMR, antimicrobial sensitivity tests should be performed to ensure that the preparations the organisms are sensitive to are the ones that are administered.

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Appendix 1

Questionnaire to Assess Risk Factors associated with *Salmonella* spp. Isolated from locally fermented cattle milk (nono) in Gambari Market, Ilorin, Nigeria

Dear Participants,

We are conducting this study to elicit information on risk factors associated with the occurrence and spread of *Salmonella* spp. in nono from cattle in Ilorin, Nigeria. Data generated will be used for research purposes only, towards the prevention of salmonellosis in humans and animals.

The data collected will be treated with utmost confidentiality. The questionnaire will take about 10 minutes of your time to complete.

Thank you, Sir/Ma,

Name of Field Surveyor:

Telephone number:

Location/LGA: Ilorin East

Coordinate: Long: 8°30'3"N Lat: 4°33'6"E

SECTION A: DEMOGRAPHIC PROFILE

1. Gender: Male ☐ Female ☐
2. Age (years) ≤25 ☐ >25 ☐
3. Educational level
 - None ☐
 - Primary Sch ☐
 - Secondary Sch ☐
 - None ☐

SECTION B: RISK FACTORS ASSOCIATED WITH THE OCCURRENCE OF SALMONELLA SPP. IN NONO FROM CATTLE IN GAMBARI MARKET, ILORIN, NIGERIA

4. Do you wash your hands before milking the cow? Yes ☐ No ☐
5. Do you wash your equipment before and after use? Yes ☐ No ☐
6. Do you clean the udder of the cow before milking? Yes ☐ No ☐
7. Do you administer drugs (antibiotics) to your cows? Yes ☐ No ☐
8. Do you boil/heat milk while processing nono? Yes ☐ No ☐
9. Are your cattle kept in closed/covered cowhouses? Yes ☐ No ☐
10. Is your cattle holding/milking area well ventilated? Yes ☐ No ☐
11. Do you clean your cattle holding/milking areas daily? Yes ☐ No ☐
12. Do you use disinfectants in your cattle holding/milking areas? Yes ☐ No ☐
13. Do your cattle mingle with other cattle from other places? Yes ☐ No ☐