



Research Article

Isolation of *Salmonellae* from Fermented Milk (Nono) and the Knowledge Attitude and Practices of Consumers and Sellers of Nono in Gwagwalada, FCT, Abuja, Nigeria

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ABSTRACT

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Milk and milk products play an important role in food borne diseases. This study was carried out to determine *Salmonella* from Nono. Thirty samples of locally fermented milk products (Nono) were obtained from eight locations namely; Paikon kore, Kutunku, Phase 2 quarters, Teaching hospital, Dukpa road, Permanent site, Giri, Gwagwalada market all in Gwagwalada area council, F.C.T Abuja. The milk samples were cultured for *Salmonella* spp. Isolates were phenotypically and biochemically identified to genus level. Ten percent (10%) of the samples were contaminated with *Salmonella* spp. Antibiogram of the isolates revealed 100% susceptibility to ciprofloxacin, amoxicillin and tarivid, with (66.66%) to sparfloxacin. *Salmonella* spp showed (100%) resistance to commonly used antibiotics like septrin, augmentin, streptomycin, gentamicin and pefloxacin, and variable resistance to chloramphenicol (66.66%) and sparfloxacin (33.33%) was recorded in some of the isolates. All resistant isolates had a multiple antibiotics resistance index of 0.6 (100%). A total of 102 study participants were interviewed on the knowledge attitude and practices of salmonellosis in milk. There were variations in responses among the respondents in the study area. The important prominent response observed were the level of informal education 33.3%, primary education 18.6%, secondary education 16.7% and 31.3% had tertiary education. From this survey 14.7% of the respondents had no knowledge of *Salmonella* and 6.9% were not aware that *Salmonella* can be transmitted via milk. Based on the risk factor, 100% of the cattle handlers milk the cows with their bare hands. 52.9% use stream water to process the milk. Conclusion, the general sanitary condition of the milking environment, utensils, and personnel hygienic practices should be standard. Multidrug-resistant strains of *Salmonella* in food of animal origin is of public health concern. Hence, implementation of *Salmonella* prevention and control strategies from farm production to consumption is crucial.

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INTRODUCTION

Milk and milk products are highly consumed because of their rich nutrients. It is therefore very important avenue for the transmission of many disease-causing microorganisms (Oloso *et al.*, 2018). Fermented milk is an important diet in many parts of Nigeria and the world at large. The rural populace depends so greatly on milk and its products for both as food and as well as source of employment especially the women who often add value to the products (Rozenberg *et al.*, 2016). The safety of dairy products and by-products with respect to food-borne disease is a great concern worldwide. Raw milk may contain pathogenic microorganisms, and it may occasionally play a great role in the transmission of these pathogenic microorganisms to humans (Vahedi *et al.*, 2011). These microorganisms are indicators arising from handling milk or milking till consumption and also determine the quality of the milk (Elmoslemayn *et al.*, 2010; Shunda *et al.*, 2013). Foodborne bacterial diseases are a major problem to the public health (Forshell and Wierup, 2016).

Foodborne illnesses cause considerable morbidity, mortality, and economic losses globally. The World Health Organization (WHO) estimates approximately 2 billion illnesses resulting in over 1 million deaths caused by 22 major foodborne pathogens (Kirk *et al.*, 2015). The foodborne illness has been estimated to be approximately 48 million cases, 128,000 hospitalizations, 3,000 deaths annually (Scallan *et al.*, 2011), and about 72 billion dollars in economic losses (Scharff, 2012)

The bacteria are commonly found in environment, water, plant, animals and animal products including milk, meat, cheese and yoghurt examples are *Campylobacter*, *Listeria*, *Salmonella* (CDC, 2018). *Salmonella* is a foodborne pathogen that belongs to the family Enterobacteriaceae. Salmonellosis is one of the most common food-borne bacterial disease worldwide (Forshell and Wierup, 2006). The presence of pathogenic bacteria which may be associated with spoilage of milk such as *Salmonella* spp, *Brucella* spp, *Mycobacterium bovis*, *Listeria monocytogenes* and *Campylobacter jejuni* can render raw and processed milk unsafe for human consumption (Nanu *et al.*, 2007).

Salmonella species belong to Gram negative, rod shaped, facultative intracellular bacteria that potentially infect a wide variety of hosts. *Salmonella* is comprised of two species, *Salmonella bongori* and *Salmonella enterica* (Guibourdenche *et al.*, 2000). Depending on the bacterial outer membrane somatic 'O' antigen, and flagellar 'H' antigen over 2700 different serovars of *Salmonella* has been characterized (Collazo and Galan, 1997). Out of these 2700 serovars, nearly 1500 belong to the *Salmonella enterica* subsp. *enterica* (Bäumler *et al.*, 1998). *Salmonella enterica* subsp. *enterica* are the most common pathogenic and zoonotic bacteria causing different forms of salmonellosis in human and animals (Forshell and Wierup, 2016). *Salmonella* is transmitted to human either through the fecal-oral route or through consumption of contaminated food (milk, eggs, and meats) and cause either typhoidal or non-typhoidal salmonellosis. In addition, milk is a potential source of multiple drug resistance, and is a potential public health concern. Antimicrobial resistance is one of the biggest global public health challenges (Zewdu *et al.*, 2009). In the present time, AMR both in human and veterinary medicine has reached alarming levels in most parts of the world and has been recognized as a significant emerging threat to global public health and food security (Chuanchuen *et al.*, 2014). However, the likelihood that milk and milk products may act as a vehicle for antibiotic-resistant bacterial genes has become a concern to the food industry and a public health issue (Cenci-Goga *et al.*, 2004). This study was carried out to determine the prevalence of *Salmonella* in nono, and the knowledge, attitude and practice of nono sellers in Gwagwalada area council of the FCT, Abuja, Nigeria.

MATERIALS AND METHODS

Study area and design

A cross sectional study was conducted in the Federal Capital Territory (FCT), Abuja which was formed in 1976, from parts of Nasarawa, Niger and Kogi States. The territory is located just north of the confluence of Niger and Benue rivers. It is bordered by Niger State to the West and North, Kaduna to the Northwest, Nasarawa to the East and lies between latitude 8.25 and 9.20 North of the equator and longitude 6.45 and 7.39

East of Greenwich Meridian. Abuja is geographically located in the center of the country. The Federal Capital Territory has a land mass of approximately 7,315 km² of which the actual city occupies 275.3km². It is situated within the savannah region with moderate climatic conditions (2006, census).

Sampling collection

A total of 30 milk samples were collected based on the 8.7% prevalence of Karshima *et al.*, (2013). Samples were purchased in a sterile sample bags from eight different locations namely Paikon kore, Kutunku, Phase 2 quarters, Gwagwalada market, Dukpa road, Giri, Teaching Hospital and University of Abuja Permanent site within Gwagwalada area council proportionately using simple random sampling method and transported in cool chain to the Laboratory of Veterinary Public Health and Preventive Medicine University of Abuja for analyses.

Isolation and identification of *Salmonella*

The isolation of *Salmonella* was carried out using a modified standard procedure derived from the Bacteriological Analytical Manual (BAM) by Andrew *et al.*, (2011). Non-selective enrichment was conducted by inoculating the milk sample directly into the sterile buffered peptone water and incubated at 37°C for 24 hr. Using a sterile Pasteur pipette 1 ml of pre-enriched sample was inoculated into 10 ml of tetrathionate broth and incubated at 37°C for 24 h for selective enrichment. A loopful of the overnight broth were streaked directly on

MacConkey agar, Brilliant -green agar (BGA) and Salmonella-Shigella (SS) agar plates and incubated at 37°C for 24 hours. Suspected colonies from the selective agar plates were purified and subjected to Gram's staining, catalase test, triple sugar iron (TSI), Urea (U), indole (I), methyl red (MR), Voges proskauer (VP), Citrate utilization (CIT), motility (M) and sugar fermentation using mannitol, glucose and sucrose.

Antibiotic susceptibility testing

A total of 3 positive isolates were tested against ten (10) antibiotics using the method of Baur *et al.*, 1966 and by comparing with the Clinical and Laboratory Standards Institute (CLSL, 2020).

Questionnaire Administration

The Nono sellers and consumers in Gwagwalada area council were interviewed by means of a structured closed ended questionnaire to ascertain the knowledge and risk factors that are associated with both personal and general hygiene practices towards salmonellosis in Nono sold for human consumption. The questionnaire was pre-tested for validity and clarity prior to administration to respondents. The questions were put in a simple, concise and specific manner to prevent ambiguity.

RESULTS

The laboratory Results showed that of 30 samples tested in the laboratory using morphological, cultural, and biochemical reactions, 3 (10%) samples were positive and 27 (90%) samples were negative for *Salmonella* species.

Table 1: Presumptive isolates of *Salmonella* collected from Nono in Gwagwalada area council, F.C.T. Abuja

Locations	Number. of samples collected	No. (%) of positive samples	No. (%) of negative samples
Paikon kore	8	0 (0)	8 (100)
Phase 2 quarters	5	1 (20)	4 (80)
Gwagwalada market	4	0 (0)	4 (100)
Kutunku	4	1 (25)	3 (75)
Giri	2	0 (0)	2 (100)
Permanent site	3	0 (0)	3 (100)
Teaching hospital	2	0 (0)	2 (100)
Dukpa road	2	1 (50)	1 (100)
Total	30	3 (10)	27 (90)

Table 2 shows the percentage distribution of *Salmonella* spp. among milk samples purchased from 8 locations in Gwagwalada FCT, Abuja.

Table 2: Frequency of occurrence of *Salmonella* in Nono from Gwagwalada area council, F.C.T. Abuja

Bacterial isolates	Location	Frequency	Percentage (%)
<i>Salmonella</i> spp. K4	Kutunku	1	25%
<i>Salmonella</i> spp. D2	Dukpa road	1	50%
<i>Salmonella</i> spp. Q4	Phase 2 quarters	1	20%

Antibiotic Susceptibility of Isolates

All the isolates showed 100% susceptibility to ciprofloxacin, amoxicillin and tarivid with 66.7% susceptibility to sparfloxacine. The antibiotic susceptibility test showed that *Salmonella* spp. were 100% susceptible to

ciprofloxacin, amoxicillin and tarivid, while the isolates showed 66.7% susceptibility to sparfloxacine. The isolates were 100% resistant to septrin, augmentin, gentamicin, streptomycin and pefloxacin, and 66.7% to chloramphenicol, 33.3% to sparfloxacine respectively.

Table 3: Antibiogram of *Salmonella* spp. isolates from nono purchased from Gwagwalada area council, F.C.T, Abuja.

Antibacterial agent	Disc potency (ug)	Susceptible no (%)	Intermediate no (%)	Resistant no (%)
Septrin (SXT)	30	0 (0)	0(0)	3 (100)
Chloramphenicol (CH)	30	0 (0)	1 (33.3)	2 (66.7)
Sparfloxacine (SP)	10	2 (66.7)	0 (0)	1 (33.3)
Ciprofloxacin (CXP)	30	3 (100)	0 (0)	0 (0)
Amoxicillin (AM)	30	3 (100)	0 (0)	0 (0)
Augmentin (AU)	10	0 (0)	0 (0)	3 (100)
Gentamicin (CN)	30	0 (0)	0 (0)	3 (100)
Pefloxacin (PEF)	30	0 (0)	0 (0)	3 (100)
Tarivid (OFX)	10	3 (100)	0 (0)	0 (0)
Streptomycin (S)	30	0 (0)	0 (0)	3 (100)

Key: no= number, %= percentage. SXT= Septrin, AU=Augmentin, CN=Gentamicin, S=Streptomycin, CH=Chloramphenicol, PEF= Pefloxacin, SP=Sparfloxacine, CXP=Ciprofloxacin, AM=Amoxicillin, OFX=Tarivid,

Table 4: Shows the resistance patterns of the *Salmonella* spp. to the antibiotics.

Table 4: Resistance Patterns of *Salmonella* species from nono in Gwagwalada area council, F.C.T Abuja.

Isolates number	Resistance patterns	MARI
K4	SXT, AU, CN, S, SP, PEF	0.6
D2	SXT, AU, CN, S, CH, PEF	0.6
Q4	SXT, AU, CN, S, CH, PEF	0.6

Keys: SXT= Septrin, AU=Augmentin, CN=Gentamicin, S=Streptomycin, CH=Chloramphenicol, PEF= Pefloxacin, SP= Sparfloxacine, MARI= Multiple antibiotics resistance indices.

Table 5: Shows the characteristics of participants captured under demographic information. 40 (39.2%) of the 102 respondents were male and 62 (60.8%) were female.

According to Educational qualifications, 33 (33.3%) had no educational qualifications, 19 (18.6%) attended primary school; 17 (16.7%) attended secondary school and 32 (31.3%)

attended tertiary schools which included any studies above secondary education ($p < 0.05$). Nono sellers constituted 51 (50%) of the respondents; consumers constituted 51 (50%) of

the respondents. ($p < 0.05$). The Population of married respondents 56 (54.9%) and that of single respondents is 46 (45.1%).

Table 5: Demographic Information

Characteristics n=102	Frequency	Percentage (%)
Gender		
Male	40	39.2
Female	62	60.8
Educational status		
Informal	34	33.3
Primary	19	18.6
Secondary	17	16.7
Tertiary	32	31.3
Study designation		
Seller	51	50.0
Consumer	51	50.0
Marital status		
Single	56	54.9
Married	46	45.1

Table 6: Shows the knowledge and risk factors among the respondents, about 14.7% of the respondents were aware of *Salmonella* spp. while 85.3% are unaware of *Salmonella* spp. and about 6.9% of them are aware that *Salmonella* can be transmitted through consumption of contaminated milk and 93.1% were unaware. Hundred (100 %) of the respondents milk their

cows with their bare-hands. About 52.9 % of the respondents use stream water to process their milk. Milk from sick animals 5.9 %. While 39.2% do not wash the udder before milking. On the other hand, 13.7% do not wash their hands before milking, and 27.5 % of the respondents stated that they practice self-medication.

Table 6: Knowledge and Risk Factors associated with consumers with milk sellers of Nono

Knowledge	NQA	Frequency (n)		Percentage (%)	
		Yes	No	Yes	No
Have you heard of Salmonella	102	15	87	14.7	85.3
Can Salmonella be transmitted through milk	102	7	95	6.9	93.1
Risk factors					
Milk the cow with bare-hands	51	51	0	100	0
Use stream waster to process the milk	51	27	24	52.9	47.1
Forward milk from sick animals to the market	51	3	48	5.9	94.1
Wash udder before milking	51	31	20	60.8	39.2
Wash hands before milking	51	44	7	86.3	13.7
Practice self-medication	102	28	74	27.5	72.5

Key: NQA= Number of questionnaires administered

Figure 2: shows the pie chart illustrating the awareness of consumers and sellers of nono about *Salmonella* spp. 85% of the respondents do not know about *Salmonella* while as 15% have the knowledge of *Salmonella* spp.

Figure 3: Shows the pie chart illustrating awareness of respondents on *Salmonella* transmission through milk in Gwagwalada area council of the F.C.T, Abuja.

Figure 4: Shows the bar chart illustrating the sources of water used by sellers to process milk

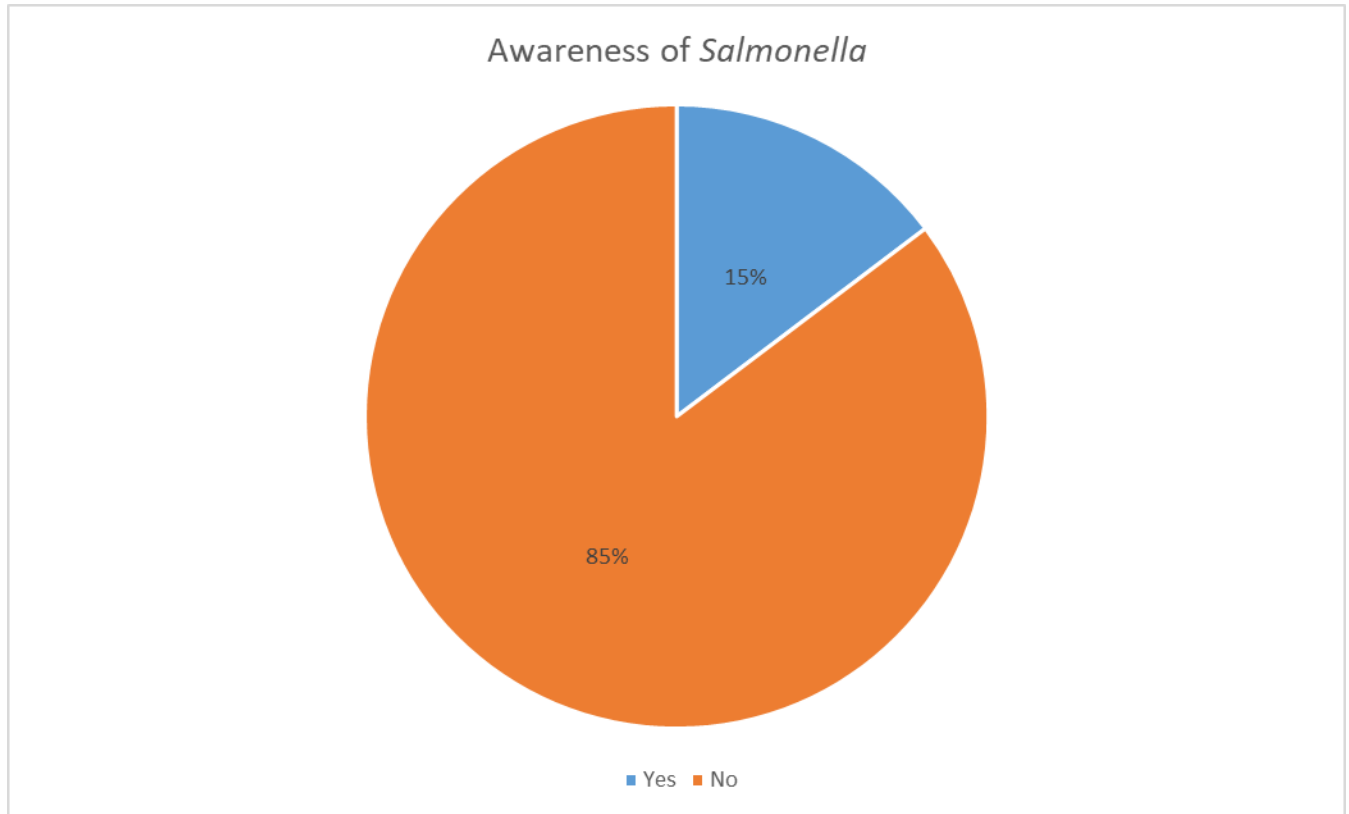


Figure 2; Awareness of *Salmonella* among Nono sellers and consumers in Gwagwalada area council F.C.T, Abuja.

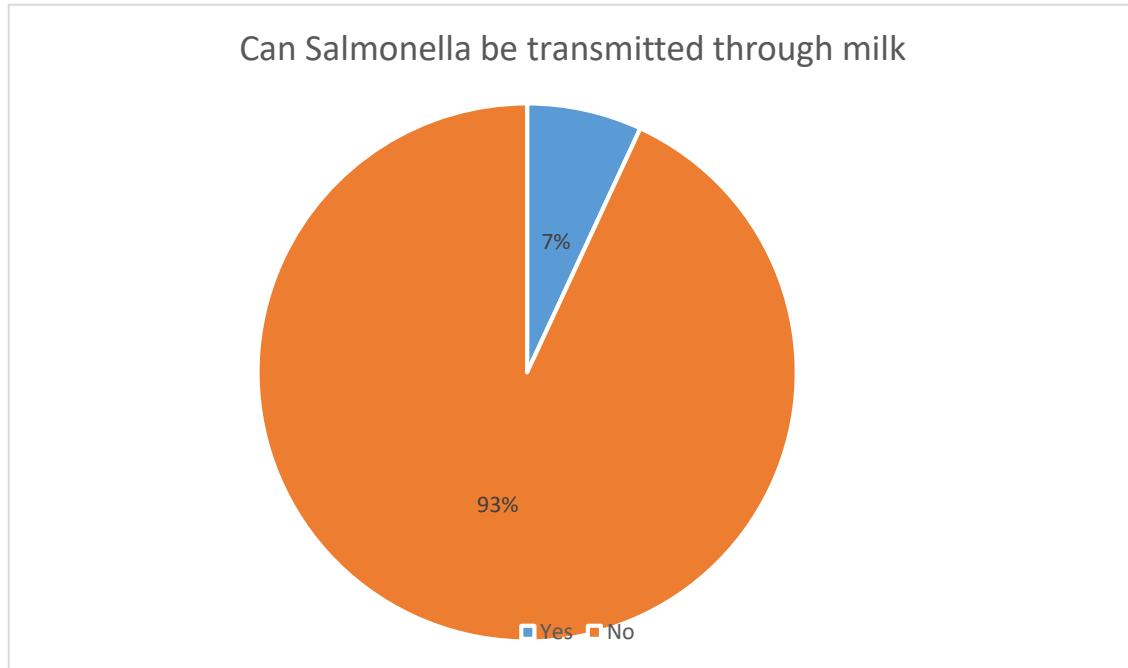


Figure 3: Awareness of *Salmonella* transmission through milk among sellers and consumers of Nono in Gwagwalada area council F.C.T, Abuja.

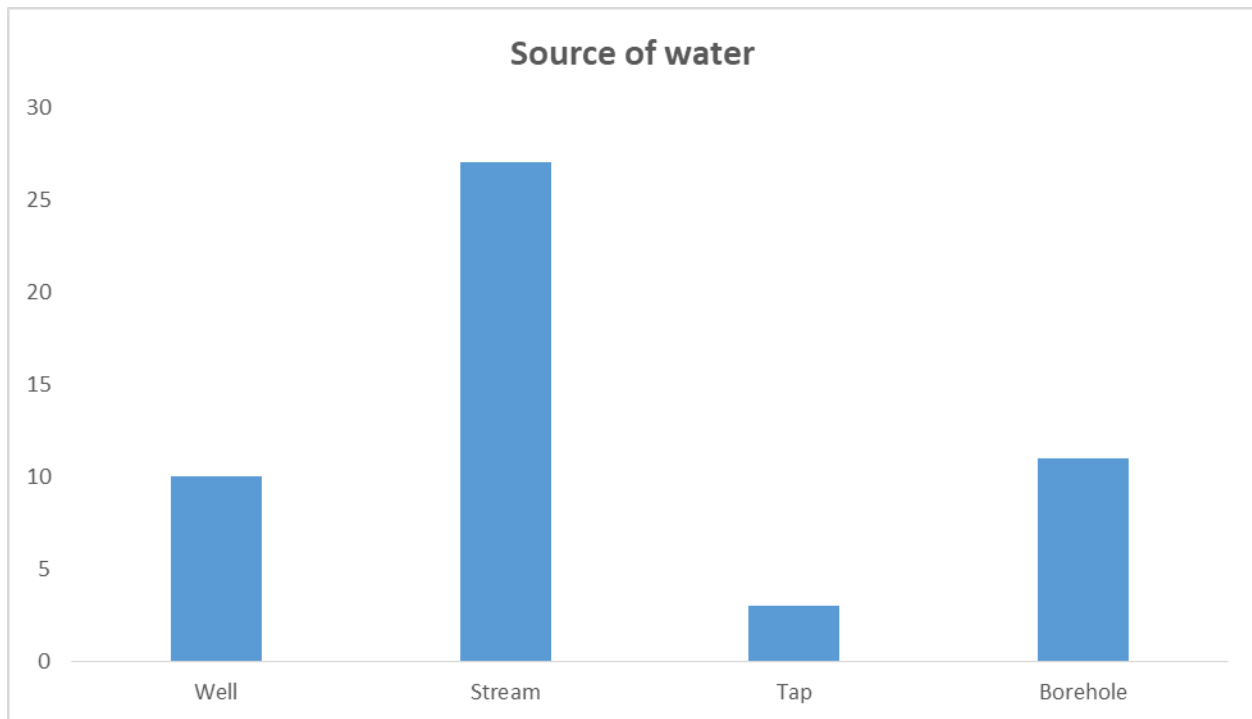


Figure 4: Sources of water used by sellers to process milk.

DISCUSSION

In the tropics, where the environmental conditions are favorable for the growth and multiplication of spoilage bacteria, milk and milk products are particularly perishable goods. The food products under research are healthy for human welfare and have been successful in eliminating hunger in many areas where the animals used for production are located. *Salmonella* can grow and multiply well in raw milk due to its high nutritional value and conducive environment. These microbes can contaminate milk in a variety of ways, which could produce a variety of outcomes. While some microbes may promote fermentation, others might result in food spoilage. In addition, some microbes support health while others induce disease (Garbaj *et al.*, 2022). *Salmonella* are viewed as markers of both fecal contamination and of poor hygienic and sanitary procedures during milking and subsequent handling at all stages of processing (Okon *et al.*, 2022). Out of the 30 samples analyzed, 3 (10%) were *Salmonella* species. The results obtained shows that Dukpa road had the highest frequency with 50% followed by Kutunku 25% and Phase 2 quarters 20%. Public health implication of *Salmonella* includes enteric fever, gastroenteritis, bacteraemia and other extraintestinal complications, and chronic carrier state (Sheorey & Darby, 2008).

The overall prevalence of 10% revealed by this study is higher than the 0.0% reported by Mohamed and El-Zubeir, (2007) and Mhone *et al.*, (2012) in Sudan and Zimbabwe respectively. Ogbonna *et al.*, (2011) reported 24% isolation rate of *Salmonella* species from Maiduguri while Karshima *et al.*, (2013) in Plateau state reported 8.7% and Tamba *et al.*, (2016) in Zaria reported 3.4% which is lower than the report from this present study. The variations can be attributed to the differences in geographical and environmental conditions of study area, hygienic and management practices, number of samples collected and the water utilized in processing the milk. Type of agricultural practices on farms may also cause variations in levels of contamination of the product with *Salmonella* spp. Similarity Okon *et al.*, (2022) from Kuje Area council of Abuja recorded a prevalence rate of 6.5% from milk.

The difference in isolation rate could be to human activities as well as water source, farming system, milking equipment and technique, ecology, length of milk transportation and hygienic conditions.

The presence of *Salmonella* spp. in fermented milk is not strange since they are pathogens that could originate from the animals themselves and are capable of being transmitted from the animal before milk preparation or could have come through cross contamination (Ogbonna *et al.*, 2011). Milk contamination by microorganisms is multifactorial and can come from sources like the air, feed, soil, faeces, grasses and the cow milking. Other possible sources of milk contamination include; utensils and water utilized in the collection and processing of milk (Coorevit *et al.*, 2008)

Not all Nono sent to the market by peddlers are sold the same day and in most cases such unsold ones may return back to the market without attention to preservation or safety. Thus pathogenic organisms that might have gained access into the product are able to multiply and/or produce harmful metabolites (Ogbonna *et al.*, 2011). The 10% prevalence established in this study is of public health importance, since the presence of one *Salmonella* spp. can lead to recall of food items from the market following the WHO standard (Codex Alimentarius Commission, 2016). The primary reason for this high prevalence of *Salmonella* spp. are possibly due to clothing, the poor sanitary state of the milker's hands and presence of flies in the milk collection site, introduction of contaminated utensils, it could also be due to river water usually used and ice block added to the milk so as to increase the quantity of the milk.

Antibiotic resistance patterns of *Salmonella* isolates from this study showed resistance to multiple antibiotics. *Salmonella* isolates from nono milk were 100% resistant to septrin, chloramphenicol, augmentin, gentamicin and streptomycin with 33.3% resistance to sparfloxacin. Such resistant phenotypes indicate antibiotic failure should these antibiotics be used to treat infection caused by *Salmonella* spp. This present finding is in agreement with the report of Uzeh and Imafidon, (2022) who recorded 100% resistance to augmentin and streptomycin from *Salmonella* isolates from dairy products in

Lagos state, Nigeria. This study differs from the work of Okon *et al.*, (2022) who reported 100% susceptible to gentamicin to isolates of *Salmonella*. Additionally, multiple drug resistance patterns were observed in this study. Impact of antibiotic resistance may result in severe illness, disability, long hospital stays, increase healthcare cost, treatment failure and even death.

The abuse and improper use of antibiotics by herd owners and animal health specialists, who are typically looking to maximize their profits at the expense of human life, may be to blame for the existence of antimicrobial resistance. In the nation, medications like gentamicin and chloramphenicol are frequently utilized for treating and preventing disease in both human and animal health management (Onohuean and Igere, 2022). There has been some direct correlation between the misuse of these drugs and high incidence of drug resistance. Understanding the emergence of resistant infections in people, animals, and the environment requires collaboration within sectors under the One Health Concept (Singh *et al.*, 2018). Antibiotic susceptibility testing of *Salmonella* isolates showed 100% susceptible to ciprofloxacin, amoxicillin, tarivid, and 66.7% susceptibility to sparfloxacin which is in conformity with the reports of (Singh *et al.*, 2018; Musa *et al.*, 2017) that isolates of *Salmonella* were susceptible to (56.5%), amoxicillin (56.2) and tarivid (57%) ciprofloxacin (40%) and tarivid (86.6%) respectively.

The resistance profile indicates indiscriminate use of antimicrobial agent in the environment covered by this study. The bacteria could also acquire inherent factors from the environment that could generate resistance. There is therefore need for public health concern and government to employ stringent measures and regulations in the control of antibiotics use in animal treatment. Report on infectious diseases in 2007 by the World Health Organization (WHO) declared that antibiotic resistance poses a severe threat to human health and that the problem is growing rapidly in a global scale. All isolates had multiple antibiotic resistance indices (MARI) of 0.6, which suggest area of high and indiscriminate usage of antibiotics (Furtula *et*

al., 2013). In addition it could be due to continued administration of antibiotics repeatedly against infections that appear non-responsive to the normal dose given earlier (Ezenduka *et al.*, 2011). This multiple antibiotic resistance indices (MARI) finding is similar to that described by Tamba *et al.*, (2016). According to Kollef and Micek, (2005), the major clinical strategies for antibiotic resistance prevention are infection control, infection-specific prevention protocols, and antibiotic management strategies. Antibiotic can be control through, antimicrobial stewardship programs (Owens, 2008), Education such as public awareness is a key element of stewardship and there are ample indications of knowledge gaps (Canton and Bryan, 2012), hygiene and disinfection such as regular hand washing, cooking utensils and surfaces because contamination could result from hands of food handlers and healthcare workers either directly from contact with patients or indirectly from touching contaminated environmental surfaces (Klevens *et al.*, 2002; Weber *et al.*, 2010). Avoiding the use of antibiotic in veterinary medicine as growth promoters (Anderson *et al.*, 2003; Anthony *et al.*, 2001; Cabello *et al.*, 2006). Vaccination and infection prevention strategies as well as the ban on over the counter sale of antimicrobials (CDC, 2004; National Policy for Containment of Antimicrobial Resistance, 2011). Surveillance programmes can help control antibiotic usage (Masterton, 2008).

Response from Nono seller and consumers within Gwagwalada metropolis, Abuja to the structured questionnaire to ascertain the knowledge and analysis of risk factors that are concerned with both personal and general hygiene practices towards salmonellosis in the Nono traded for human consumption. A total of 102 participant were issued the questionnaire which include personal data such as gender, educational status, study designation and marital status. From the survey, 39.2% were males while 60.8% were females. In the level of education, 33.3% had informal education, 18.6% had primary education, 16.7% only had secondary education and 31.3% had tertiary education. The selling and consumption of nono in the study area cut across all cadre of

education level. The study designation was done according sellers and consumers which we had 50.0% for each respectively. 54.9% were single, while 45.1% were married on our study.

On the knowledge, risk factor and level of awareness of *Salmonella* in nono, 14.7% have no knowledge of *Salmonella* bacteria and 6.9% are not aware *Salmonella* can be transmitted via milk. Based on the risk factors, 100% of the cattle handlers milk the cow with their bare hands. 52.9% use stream water to process the milk, while 5.9% said to forward milk from sick animals to the market. 60.8% wash the udder before milking, 86.3% wash hands before milking while 27.5% practice self-medication. This survey and findings further gives reasons for possible high contamination vended milk within Gwagwalada metropolis and this calls for public health concern.

CONCLUSION AND RECOMMENDATION

In conclusion, this research has detected *Salmonella* species from fermented milk traded

for human consumption with the prevalence of 10% from Gwagwalada area council in FCT, Abuja. Ciprofloxacin, amoxicillin, tarivid are 100% effective to all the isolates and septrin, chloramphenicol, augmentin, gentamicin were 100% resisted by the isolates. This study therefore recommend public health education to milk processors and Nono sellers on proper personal hygiene. Discourage the sale of Nono in open calabash and processing of Nono using water from the river or stream, and also recommend that there should be regulations and enforcement of existing legislation prohibiting access to indiscriminate antimicrobial usage.

CONFLICT OF INTEREST

Authors declare that the there was no conflict of interest.

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