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OCCURENCE AND ANTIBIOGRAM OF *SALMONELLA* SPP. IN RAW AND FERMENTED MILK IN ZARIA AND ENVIRONS

Z. Tamba[1]*, M. Bello[2] and M. A. Raji[3]

[1]Department of Veterinary Public Health and Preventive Medicine, [2]Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria; [3]Department Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Kwara State, Nigeria

ABSTRACT

Salmonellosis is an important food-borne disease affecting both humans and animals. The aim of this study was to determine the occurrence and antibiogram of *Salmonella* species from raw and fermented milk. The results showed that out of the 350 milk samples examined 14 (4.0%) were positive for salmonella. The prevalence of *Salmonella* is higher in raw milk (4.6%) than in fermented milk (3.4%) with no statistical difference (P>0.05) between them. The prevalence of both raw (75.0%) and fermented milk (83.33%) is significantly higher (P<0.05) in SabonGari LGA than in Giwa LGA with 25.0% and 16.67% respectively. No salmonella was isolated from the all the 50 raw and 78 fermented milk examined from Zaria LGA. The antibiotic susceptibility test showed that ciprofloxacin and gentamicin were 100% susceptible, while chloramphenicol, kanamycin, nalidixic acid showed 93% susceptible each. Others are streptomycin and tetracycline (64.3%), amoxicillin (21.4%), ampicillin and erythromycin (14.3%) and lincomycin (0%). This connotes that all the *Salmonella* isolates were 100% resistance to lincomycin, 85.7% to erythromycin, 85.7% to ampicillin and 78.6% to amoxicillin. The antibiotic resistance pattern of 14 *Salmonella* isolates from raw and fermented milk showed 9 different resistance patterns of the11 antimicrobial agents used. The Minimum Inhibitory Concentration (MIC) test showed that only 1(7.1%) of the isolate was sensitive to Amoxicillin with < 0.12 while the remaining 9 (98.9%) were resistant to the two antibiotics used (AML and E) with >256.

Keywords: Antibiogram, *Salmonella* species, milk, minimum inhibitory concentration (MIC), Zaria, Nigeria

INTRODUCTION

Milk is a fresh, clean and normal mammary secretion obtained by milking of one or more dairy animals for nourishment of their young (Rani and Maheshwari, 2012). In dairy animals, cow milk is the most consumed milk worldwide and serves as good source of animal proteins, fats, vitamins and minerals to the human body (Muehlhoff *et al*., 2013). Food and Agricultural Organization (FAO) of the United Nations estimated that 85% of all milk world-wide was produced from cows (Gerosa and Skoet, 2013). Despite rich in nutrients, fresh milk and its fermented products constitute a good medium for the multiplication of various bacteria, and hence serve as a medium for the transmission of milk borne diseases mostly salmonellosis, brucellosis, tuberculosis, shigellosis and staphylococcosis (Gerosa and Skoet, 2013).

Pathogenic bacteria in raw milk have been a major factor for public health concern. The main sources of contamination are the dairy cattle handlers and dairy equipment (Zeinhom and Latef, 2014). Consumption of raw milk is considered to be the main cause of several outbreaks of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Grant *et al*., 1995) and posed the greatest human health problems in various countries (Lejeune and Rajala-Schultz, 2009). However, raw cow milk form the basis for most commonly sold local milk products in Nigeria. Some milk products in Nigeria include locally fermented skimmed milk known as "Nono", full creamed milk, "Kindirmo" local butter, "Man Shanu" and cheese, "Wara". *Salmonella* is a Gram negative bacterium, rod shaped, aerobic or facultative anaerobic, belonging to the family *Enterobacteriaceae*. It is a major pathogenic bacteria' inhabiting the intestinal tract of humans and animals (Holt *et al*., 1994). *Salmonella* spp are important sources of bacterial contamination of the environment and the food chain (Ponce *et al*., 2008), and are the leading causes of acute gastroenteritis in several countries (Soltan *et al*., 2009). Several studies in Nigeria and other parts of the world have documented the occurrence and prevalence of *Salmonella* in foods and milk in particular. For instance Karshima *et al*. (2013) had a prevalence of 6.4% from raw milk and 0.8% from fermented milk. Olatunji *et al*. (2012), also had a prevalence of 8.7% from raw milk.

*Corresponding e-mail address: salehzee1@gmail.com

In Africa, Nyenje and Ndip (2013) in their study also recorded 43% *Salmonella* from farms in raw milk and in the United States, Mead *et al*. (1999) documented different food borne pathogens of which Salmonella caused 31% of food related deaths, followed by Listera with 28%, Campylobacter 5% and *Escherichia coli* 3%. This study is therefore, aimed at isolating and identifying *Salmonella* species from raw and locally fermented milk ("Nono") in Zaria, Nigeria.

MATERIALS AND METHODS

Sample collection

The study was carried out in Zaria, Kaduna State which is located within latitudes 11^0 $07^{'}$, 11^0 12'N and longitudes 07^0 41'E, in the Northern region of Nigeria. A total of 350 samples were collected from 3 local Government areas (LGA) of Zaria and Environs (174 raw and 176 fermented milk samples) namely Giwa, Sabon-Gari, and Zaria. Herds of cattle were visited during milking time, where 10ml of raw milk samples were collected directly from milking cows using convenient sampling technique and placed into sterile sample bottles. Fermented milk (nono) samples were collected from 10 Fulani women at different retail points in the same locations into the sterile sample bottles. All the samples collected were placed on ice and transported to the Bacterial Zoonoses Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for analysis.

Identification of the *Salmonella* spp

Bacterial isolation, identification and biochemical tests (indole, methyl-red, Voges-Proskauer, citrate (IMViC), motility and triple sugar iron, TSI) were carried out using standard procedures described elsewhere (Cheesbrough, 2002). Pure cultures obtained from nutrient agar slants were subjected to biochemical tests. All the isolates were characterized using indole, methyl-red, Voges-Proskauer, citrate, motility, H_2S, sugar fermentation tests as described by Quinn *et al*. (2002). The isolates were further characterized using Microbact 12E (Oxoid, Basingstoke, England).

Sensitivity to antimicrobials

The susceptibility of all the isolates were tested to the following antimicrobials such as chloramphenicol (30µg), ciprofloxacin (5µg), kanamycin (30µg), nalidixic acid (30µg) gentamicin (10µg), lincomycin (10µg), tetracycline (10µg), ampicillin (l0µg), amoxicillin (l0µg), streptomycin (10µg) and erythromycin (15 µg) using the disk diffusion method described by CLSI (2011). Two colonies of the overnight culture were inoculated into 5ml tryptone soya broth (Oxoid, UK) and incubated at 37^0C until the turbidity approximated 0.5 Mac Farmland's standard was obtained (CLSI, 2011). Mueller-Hinton agar plates were prepared and used according to manufactures instruction's sterile swabs were dipped into the broth culture with the excess broth drained by pressing on the inner side of the tube and used to streak the Mueller-Hinton agar until the entire surface was streaked. The plates were allowed to dry at room temperature for 10min and the antimicrobial disc dispensed on the plates using the multiple disc dispenser (Oxoid, UK). The petridishes were then inverted and incubated at 37^0C for 18hrs, after which the zones of inhibition were measured to the nearest millimetre and interpreted based on interpretation of zone diameter of test culture provided by CLSI (2011).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of erythromycin and amoxycillin against the 10 isolates was carried out at the National Food Institute Kemitovet, Technical University of Denmark, Denmark according to recommendations of CLSI (2011).

Statistical analysis

The occurrence of *Salmonella* species in raw and fermented milk were calculated using simple percentages and Chi square to test for association and values of $P < 0.05$ was considered statistically significant. The results were analysed using Statistical Package for Social Sciences (SPSS 20.0).

RESULTS AND DISCUSSION

The results showed that prevalence of *Salmonella* is higher in raw milk (4.6%) than in fermented milk (3.4%) with no statistical difference ($P>0.05$) between them (Table 1).The prevalence of both raw (75.0%) and

fermented milk (83.33%) is significantly higher (P<0.05) in Sabon Gari LGA than in Giwa LGA with 25.0% and 16.67% respectively and no *Salmonella* was isolated from the all the 50 raw and 78 fermented milk examined from Zaria LGA.

The antibiotic susceptibility test showed that ciprofloxacin and gentamicin were 100% susceptible, while chloramphenicol, kanamycin, nalidixic acid showed 93% susceptible each. Others are streptomycin and tetracycline, 64.3%, amoxicillin (21.4%), ampicillin and erythromycin (14.3%) and lincomycin (0%) (Table 2). This connotes that all the isolates were 100% resistance to lincomycin, 85.71% to erythromycin, 85.71% to ampicillin and 78.57% to amoxicillin (Table 2).

Table 1. Prevalence of *Salmonella* spp from raw and fermented milk based on LGA

LGA	Raw milk		Fermented milk	
	No. of samples examined	No. (%) of *Salmonella*	No. of samples examined	No. (%) of *Salmonella*
S/Gari	74	6 (75.0)	68	5 (83.33)
Zaria	50	0 (0.0)	78	0 (0.00)
Giwa	50	2 (25.0)	30	1(16.67)
Total	174	8 (4.6)	176	6 (3.4)

Table 2. Susceptibility of *Salmonella* isolates to antimicrobial agents from raw and fermented milk samples

S/No	Antimicrobial	No. of *Salmonella* susceptible	% of *Salmonella* susceptible
1	Chloramphenicol	13	93.0
2	Ciprofloxacin	14	100.0
3	Kanamycin	13	93.0
4	Nalidixic Acid	13	93.0
5	Gentamicin	14	100.0
6	Ampicillin	2	14.3
7	Amoxicillin	3	21.4
8	Streptomycin	9	64.3
9	Lincomycin	0	0.0
10	Erythromycin	2	14.3
11	Tetracycline	9	64.3

Table 3. Minimum inhibitory concentration evaluation on *Salmonella* species

S/No	Isolate no	Amoxicillin	Erythromycin
1	*RSK 41	S	R
2	*RSKn 57	R	R
3	*RSm 76	R	R
4	*RSg 77	R	R
5	*RSg 79	R	R
6	*RZg 110	R	R
7	*RZg 121	R	R
8	Fsm 1	R	R
9	Fkw 50	R	R
10	FKw 5	R	R

Sm = Samaru, Sk = Shika, Skn = ShikaNapri, Sg = SabonGari, Zg = Zango, Kw = Kwangila, *R = Raw milk, F = Fermented milk; S = sensitive, R = resistance, >256 = resistant, < 256 = sensitive

The Minimum Inhibitory Concentration (MIC) test showed that only 1(7.1%) of the isolate was sensitive to

amoxicillin with < 0.12 while the remaining 9 (98.9%) were resistant to the two antibiotics used (AML and E) with >256 (Table 3).

This study established the presence of *Salmonella arizonae* which are members of sub-species IIIa with overall prevalence of 4.0. Raw milk had 4.6% prevalence while fermented milk had 3.4% prevalence. The difference in prevalence between raw and fermented milk could be due to low p^H and effect of fermentation in fermented milk because fermented milk is not a suitable environment for the majority of spoilage bacteria. Karshima *et al*. (2013) also had a higher prevalence of 6.4% from raw milk and 0.8% for fermented milk. Mhone *et al*. (2012) carried out a study in Zimbabwe from selected farms on raw and processed cow milk and out of all the samples collected, 0% *Salmonella* spp. was reported. This result could be due to good management practices or small sample size or method of isolation used in their studies.

The 4% prevalence established in this work is of public health importance, since the presence of one *Salmonella* species can lead to recall of food items from the market following the WHO standard (Codex Alimentarius Commission). Moreso, the reason for this high prevalence of *Salmonella* spp. in Sabon Gari LGA is probably due to the poor sanitary conditions of the milkers' hands, clothings and it could also be due to the presence of flies in the environment where the milk samples were collected, another probable reason could be the addition of baoba seeds, river or stream water, ice block to the milk so as to increase the quantity of the milk.

The antibiotic susceptibility test showed that isolates in raw and fermented milk are resistant to commonly used antibiotics and there was also resistance to multiple antimicrobials, including lincomycin, ampicillin, erythromycin, amoxicillin and tetracycline and showed sensitivity that is (0%) resistance to gentamicin and ciprofloxacin. The probable reason of multidrug resistance with 100% resistance on lincomycin, erythromycin, ampicillin and amoxicillin may be due to the indiscriminate use of antibiotics by farmers and the vets in treating certain diseases of their animals. This finding is similar to that described by Chen *et al*. (2004).

Based on the minimum inhibitory concentration evaluation, amoxicillin and erythromycin strips were used. Only 1 *Salmonella* isolate had 0.12 zone of inhibition to amoxicillin strips while 9 isolates had zones of inhibition > 256 on both antibiotic used. This could be due th the presence of resistance gene but does not necessarily lead to treatment failure, because the level of expression may be too low. More so, the B-lactams showed high resistance while the floroquinolones and aminoglucoside shows 0% resistance, this agrees with the work of Tafida *et al*. (2013) who also carried out antibiotics study on *Salmonella* isolates in retail beef and related meat product in Zaria, reported low prevalence of resistance to fluoroquinolones (ciprofloxacin) and aminoglycoside (Gentamicin) but high prevalence of resistance to macrolide (erythromycin) and amantadine (amoxicillin). Also, antibiotic resistance shown by 90% of the *Salmonella arizonae* indicates indiscriminate use of antibiotics by both the veterinarians and the animal owners and calls for corrective measures.

In conclusion, this research has established the presence of *Salmonella* species with an overall prevalence of 4.0% in the milk samples, with raw milk recording 4.6% prevalence and locally fermented milk (Nono) recording 3.4% prevalence in Zaria and environs. The results are not in agreement with the Codex Alimentarius Commission of the World Health Organization (WHO) which states "that milk for human consumption must be free of *Salmonella"*. This study therefore recommended that milker's should practice personal hygiene by wearing clean clothing and washing their hands regularly with soap and water before milking the animals. There should be public health education to the fulani herds men on the need to practice personal hygiene by washing their hands regularly with soap and water before milking the animals and to wear clean clothing and also to keep the environment where the animals are kept clean.

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