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Prevalence and Antibiotic sensitivity pattern of *Salmonella* isolates from milk products and water reservoirs in Maiduguri, North-Eastern Nigeria

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Abstract: *The study was conducted to determine the presence of Salmonella in retail milk products and water reservoirs in Maiduguri, Nigeria. A total of 150 samples were collected using convenient sampling technique. One hundred samples; fifty each of full cream milk or Kindirmo (n=50) and Skimmed milk or Nono (n=50) were collected from Bulumkutu and Monday market retail sellers. Furthermore, of the fifty samples obtained from the two different locations, twenty five samples each of kindirmo and nono were collected. The samples were aseptically kept in sterile plastic bags. Additionally, fifty water samples were collected from reservoirs within the University campus. They were processed according to standard bacteriological protocols followed by Gram's staining and biochemical test; Triple sugar iron test, citrate and urease. The Salmonella isolates were further subjected to ten different antibiotics to determine their sensitivity. The overall prevalence of Salmonella in milk samples was found to be 10.00%, while the total prevalence of Salmonella from water sample was 40.00%. Of the fifteen isolates tested, resistance to Amoxicillin, Ceftriaxone and Erythromycin was 100.00%, Gentamicin had 80.00% and Cotrimoxazole was 53.33%, whereas Gentamycin, Ceftriaxone and Amoxicillin displayed 100.00% sensitivity. Moderate sensitivity of 53.33% to Streptomycin and Pefloxacin, 46.67% to Ciprofloxacin and 86.67% sensitivity to Ofloxacin was found in this study. Antibiotic resistance is associated with frequent usage both in livestock and humans as they are commonly available. Adequate sanitary measures should be ensured in milk processing and use of water reservoirs. Antibiotics should be used based on their antibiogram pattern. Prudent use of antibiotics is essential and its continuous use as growth promoters should be discouraged, as this may result to failure in the treatment of Salmonella-associated diseases due to resistance. Disease surveillance programmes should be established as a means for curtailing salmonellosis.*

Keywords: *Salmonella, prevalence, milk, water reservoir, antibiotics, susceptibility, Nigeria*

I. Introduction

Salmonella are widely distributed in nature and survive well in a variety of foods and contamination can occur at multiple steps along the food chain (Pui *et al.*, 2011). The non-typhoidal *Salmonella* serovars are predominantly associated with food of animal origin such as milk, eggs, poultry, beef and pork (Kaushik *et al.*, 2014). The infection dose of *Salmonella* can be as low as 15 to 20 cells, depending on the age and health of the susceptible host (Zaki *et al.*, 2009). It causes inflammation of the gastrointestinal tract and in some cases, if the immune response is not sufficiently powerful and treatment is not instituted, can become systemic and cause more serious conditions throughout the body. The bacteria cause infection by invading the epithelial cells of the small intestine and macrophages. Infants, elderly and immunocompromised individuals are at high risk of the disease (Ricci-Tam, 2008). *Salmonella* has more than 2,500 different serovars and are considered pathogenic to humans (Kemal, 2014).

Salmonellosis is an infection of the digestive system caused by *Salmonella* serotypes and characterized clinically by one or more of the three major syndromes; septicaemic, acute and chronic enteritis, which infect both humans and animals with millions of illness, reported worldwide (Kemal, 2014). Food borne salmonellosis constitutes a major health problem in many countries (Bayu *et al*., 2013).

Salmonellosis is transmitted by ingestion of contaminated food, water or contact with environment infected with *Salmonella* organisms. Symptoms usually develop between 6-72 hours post–infection, which includes watery diarrhea, fever, headache, abdominal pain, nausea, vomiting and loss of appetite (WHO, 2013). Intestinal salmonellosis typically resolves in five to seven days and does not require treatment with antibiotics. When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy, such as Ciprofloxacin and Ceftriaxone in adult and children may be lifesaving (White *et al.*, 2001). The prevention and control of microbial diseases such as salmonellosis with prior isolation, identification and characterization of that particular etiological agent may be needful (Mondal *et al.*, 2008). It is best prevented by good hygiene, avoiding

unpasteurized milk or products made from it (CFSPH, 2006). Drinking of clean, filtered and boiled water is recommended to avoid infection with *Salmonella* species.

*Salmonell*a is one of the most common causes of food borne diarrheal disease worldwide, a zoonosis which can be transmitted through contaminated food such as milk (Wegener *et al*., 2003). Most herdsmen and some dairy farms do not disinfect the teats and udders prior to milking and there could be the possibility of contamination at critical control points in the course of processing. Salmonellosis remained a significant cause of economic loss in farm animals, due to expenses which are incurred from clinical disease including treatment of clinical cases, cleaning and disinfection and cost of prevention and control (Kemal, 2014). Therefore, heavy economic losses occur due to morbidity, mortality and reduce production (Mondal *et al*., 2008). Great microbial risks are associated with ingestion of water contaminated with human or animal faeces (Cabral, 2010). Polluted or contaminated sources of drinking water carry a number of pathogens that causes diarrhoea and typhoid fever (Nagpal *et al.,* 2011). Water borne infection is prevalent where general hygiene and environmental sanitation are poor and where there is shortage of protected water supply (Demena *et al*., 2003).

The research was aimed to identify and provide information on *Salmonella* in milk products sold at retail outlets and water from reservoirs in Maiduguri, which may be pathogenic to animals and humans.

II. Materials And Methods

2.1 Study Area

This study was conducted in Maiduguri metropolis, Borno State located in North-Eastern Nigeria.

2.2 Study Design and Sample Collection

The study design was a cross sectional study. A total of 150 samples were collected using convenient sampling technique (Portney and Watkins, 2007). Of the one hundred samples; fifty each of full cream milk or Kindirmo (n=50) and Skimmed milk or Nono (n=50) were aseptically sampled from Bulumkutu and Monday market retail sellers. Furthermore, of the fifty samples obtained from the two different locations, twenty five samples each of kindirmo and nono were collected. The samples were aseptically kept in sterile plastic bags. Additionally, fifty water samples (n=50) from fifty different water reservoirs were collected in sterile sample bottles from a University campus. All the samples were labelled properly and immediately transported on ice packs in a Coleman® box to the Veterinary Microbiology laboratory, Faculty of Veterinary Medicine, University of Maiduguri where they were immediately processed.

2.3 Sample Processing

2.3.1 Processing of milk samples

One milliliter (1ml) of each of the fifty samples of kindirmo (n= 25) and nono (n= 25) from Bulumkutu retail market were inoculated into 10 ml of Selenite F broth and incubated at 37ºC for 24 hours. Similarly, samples from Monday market comprising of full cream milk (kindirmo = 25) and skimmed milk (nono = 25) were inoculated into Rappaport Vassiliadis broth (Oxoid-CMO669) and then incubated at 37ºC for 24 hours. Loopful of the broth cultures were subcultured onto Xylose lysine desoxycholate (XLD) (ISO, 2002) and incubated for 24-48 hours at 37ºC to observe for the typical growth indicative of *Salmonella*.

All samples that exhibited characteristic colonies of *Salmonella* were subcultured onto Nutrient agar slants and then stored at 4^{o}C until required.

2.3.2 Gram Staining

Pure colonies of *Salmonella* isolates were picked up from XLD plates with bacteriological loop, smeared onto separates clean glass slides with a drop of distilled water and fixed by gentle heating. Crystal violet was applied on each smear to stain for 1 minute and then washed with water. Then Lugol's iodine (as mordant) was applied for 1 minute and again washed with water. This was followed by the addition of 95% alcohol which serves as decolourizer and allowed to stand for a period of 10 seconds. After rinsing with water, safranin was added as a counter stain and then washed with water after 30 seconds. The preparation was air dried and examined under the microscope with high power objective (x100) using oil immersion (Mondal *et al.,* 2008).

2.4 Biochemical Characterization

Isolated organisms with supporting growth characteristics of *Salmonella* were subjected to various biochemical tests. Biochemical tests such as Triple sugar iron (TSI), Simmons citrate and urease were used. The isolates of *Salmonella* were subcultured onto the biochemical media, incubated at 37ºC for 24-48 hours to check for phenotypic changes within the media (Global Salm-Surv, 2003).

2.5 Antibiogram of *Salmonella* Isolates

The sensitivity of the *Salmonella* isolates to ten different antimicrobial agents was performed through disc diffusion method to determine the drug sensitivity pattern. The antibiotics used were Ciprofloxacin (10 µg), Streptomycin (10 µg), Pefloxacin (5 µg), Cotrimoxazole (25 µg), Amoxycillin (25 µg), Ofloxacin (5 µg), Ceptriaxone (30 µg), Gentamycin (10 µg), Chloramphenicol (30 µg) and Erythromycin (5 µg) obtained from Fondiscs®. The *Salmonella* isolates were subcultured into Mueller-Hinton broth (Oxoid-CM0405) and then incubated at 37ºC for 24 hours. Serial dilution was performed in the ratio of 2:4 of the broth and distilled water to reduce the degree of the turbidity of the broth culture. Then 1 ml was dispensed onto Mueller-Hinton agar and the excess discarded. Antibiotic discs were applied aseptically to the surface of the plate with the help of sterile needle. This was incubated at 37ºC for 24 hours under aerobic condition to observe for zones of inhibition, which were measured with the aid of a ruler (Mondal *et al*., 2008; Kaushik *et al.,* 2014).

2.6 Data Analyses

Data were summarized into tables using descriptive statistics. Statistical Analysis System (SAS) version 9.3 was used to determine the Fisher's Exact Test for association between *Salmonella* and sources of samples. The $P < 0.05$ was considered significant.

III. Results

3.1 Distribution of *Salmonella* in Milk Samples Collected from Retail Markets in Maiduguri Metropolis

A total of one hundred samples of milk products; full cream milk (kindirmo) and skimmed milk (nono) collected from Bulumkutu and Maiduguri Monday market were analyzed. The number of samples from each location was 25, out of which the number of positive samples was found to be 10. Higher prevalence of 24.00% was obtained for *Salmonella* from samples of skimmed milk (nono) in Maiduguri Monday market, followed by kindirmo that had 16.00% from the same location. No positive samples were obtained from Bulumkutu. The overall prevalence of *Salmonella* in milk samples was found to be 10.00% (Table 1).

The *Salmonella* isolates on XLD exhibited red colonies with black centres. The discrete colonies are indicated with arrows (Figure 1).

3.2 Distribution of *Salmonella* in Samples Collected from Water Reservoirs on University Campus

Fifty samples collected from six categorized sampling locations; namely hostels, faculties, departments, worship centres, lecture halls and others were analyzed to determine the prevalence of *Salmonella*. All the locations had one positive isolate for *Salmonella* (RA2, RB2, RBOT, RMG2, RMG3, REM, RFA, RFD, RFE, RFSC, RFV2, RHD, RMCM, RS1, RS2, RCG1, RCG2, RCM, RLG, RNF). The number of positive samples for *Salmonella* was 20 with the highest number (8) identified in reservoirs sampled from faculties, while the lowest value was zero from lecture hall reservoirs. The highest prevalence of 75.00% occurred in worship centres, followed by 61.45% for faculties, 50.00% for others and the lowest prevalence of 0.00% was obtained from reservoirs/dams in the premises of/or close to the lecture halls. The total prevalence of *Salmonella* from water sample was found to be 40.00% (Table 2).

3.3 Antibiotic Sensitivity Test for *Salmonella* isolates

The resistance and sensitivity pattern were displayed by fifteen *Salmonella* isolates to ten different antibiotic agents. Amoxicillin, Ceftriaxone and Erythromycin had the highest resistance, 15 (100.00%) to *Salmonella* isolates, followed by Gentamycin, 12 (80.00%), Cotrimoxazole, 8 (53.33%), Chloramphenical, 7 (46.67) and Pefloxacin, 7 (46.67), Streptomycin, 4 (26.67) and Ofloxacin, 2 (13.33%) and Ciprofloxacin, 2 (13.33%). The highest intermediate value was 8 (53.33%) for Streptomycin and Pefloxacin, while the lowest, 0 (0.00) were found in Amoxicillin, Ofloxacin, Ceftriaxone and Erythromycin. The *Salmonella* isolates were highly sensitive to Ofloxacin, 13 (86.67%) and none to Amoxicillin, Ceftriaxone, Pefloxacin and Eryhromycin (Table 3).

IV. Discussion

Pathogenic organism such as *Salmonella* has been a major concern to the public all over the world. The fact that milk contains a lot of nutrients made it havens for growth and development of *Salmonella* species (Ademola and Effiong, 2013). The microbial contamination of milk is multifactorial, originating from sources like feed, faeces, grasses and milking cow itself. Other possible causes include; unsterilized teats, utensils and unsafe water used in milk processing (Karshima *et al.,* 2013). Several reports have documented the prevalence and distribution of *Salmonella* in milk, but there is paucity of information in the study area.

The prevalence of *Salmonella* observed in milk products was 10.00%. This is in agreement with the findings of Rastegar *et al.* (2013) who reported a prevalence of 11% in Iran, but lower than the 20% reported by Tadesse and Dabassa (2012) in Ethiopia and higher than 8.7% reported by Karshima *et al.* (2013) in Kanam

local government area of Plateau State, Nigeria. These variations may be explained by the differences in management system of dairy farms, method of milking, hygienic practices and availability of potable water. This indicated that milk from Monday market can pose potential risk to public health when consumed or used in production of dairy products such as cheese, yoghurt and ice cream without being subjected to sufficient heat treatment. The negative result recorded by samples process from Bulumkutu may not be unconnected with the cleanliness of the source of water used for milk processing, proper pasteurization and maintenance of good hygiene.

Prevalence of 40.00% from water was recorded in this study which is higher than 16.12% reported by Carvalho *et al.* (2013) in Brazil. Consumption of such contaminated water without boiling can predispose individuals to *Salmonella* infection, more especially the immunocompromised or very young/old individuals. The presence of *Salmonella* in water reported in this study is not likely to originate from the water itself, but may possibly be associated with contamination from the user of those sources of water.

The antibiotic sensitivity test of fifteen *Salmonella* isolates to ten commonly used antibiotics revealed 100.00% resistance to Amoxicillin, Ceftriaxone and Erythromycin, while Gentamicin had 80.00% and Cotrimoxazole was 53.33%. This finding is in consonance with the 100.00% resistance to Erythromycin reported by Nesa *et al.* (2011), but higher than 75.00% to Amoxicillin. Additionally, Agada *et al.* (2014) reported lower resistance of 69.4% to Ceftriaxone than the one found in this study. However, the finding of the present study is in contrast with the report of Tesfaw *et al.* (2013) where 100.00% sensitivity to each of these antibiotics; Gentamycin, Ceftriaxone and Amoxicillin were reported. Moderate sensitivity of 53.33% to Streptomycin and Pefloxacin, 46.67% to Ciprofloxacin, and 86.67% sensitivity to Ofloxacin were found in this study. The 86.67% sensitivity to Ofloxacin concurs with 87% reported by Falegan and Akere (2014). Antibiotic resistance is associated with sub therapeutic doses and frequent usage both in livestock and public health as they are relatively cheaper and commonly available. The effectiveness of Ofloxacin might be due to the difference in frequency of usage among the available antibiotics, nature of the drug and interaction with the bacteria as opined by Tesfaw *et al.* (2013).

V. Conclusion

This study has shown that the presence of *Salmonella* in milk may be attributed to unhygienic practices in milk processing, use of unsafe water, suboptimal pasteurization, inappropriate transportation and storage facilities and milking with bare hands. This poses a serious risk of *Salmonella* infection to consumers. Contaminated water for either drinking or food processing which may result from inappropriate treatment or recontamination is of public health concern. Therefore, consumption of such milk and water increases the risk of acquiring zoonotic diseases. The study revealed high sensitivity to Ofloxacin, moderate reaction to Pefloxacin and Streptomycin, but high resistance were observed to Amoxicillin, Ceftriaxone and Erythromycin.

Conflict of Interest The authors declare that there is no conflict of interest.

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Table 1: Distribution of *Salmonella* in Milk Samples collected from Retail Markets in Maiduguri Metropolis

Sampling Location	Sample Identity	Milk Type	Total no. Collected	Number Positive	Prevalence (%)
Bulumkutu	BK	Full cream milk(kindirmo)	25	0	0.00
Bulumkutu	BN	Skimmed milk (nono)	25	0	0.00
Maiduguri Monday Market	MMK	Full cream milk(kindirmo)	25	4	16.00
Maiduguri Monday Market	MMN	Skimmed milk (nono)	25	6	24.00
Total			100	10	10.00

Fisher's Exact Test: P< 0.5000 was not statistically significant

Table 2: Distribution of *Salmonella* in Samples Collected from Water Reservoirs on University Campus

Location	Total no. Collected	Positive *Salmonella* with Respect to Specific Location	Number Positive	Prevalence (%)
Hostels	18	RA2, RB2, RBOT, RMG2, RMG3	5	27.78
Faculties	13	REM, RFA, RFD, RFE, RFSC, RFV2, RHD, RMCM,	8	61.54
Departments	9	RS1, RS2	2	22.22
Worship Centres	4	RCG1, RCG2, RCM	3	75.00
Lecture halls	2	Nil	Nil	Nil
Others	4	RLG, RNF	2	50.00
Total	50		20	40.00

Fisher Exact Test: P< 0.0167 was statistically significant.

Table 3: Antibiotic Sensitivity Test for *Salmonella* isolates

Antibiotic Agents	**Resistance (%)**	**Intermediate (%)**	**Sensitive (%)**
Amoxicillin	15 (100.00)	0 (0.00)	0 (0.00)
Ofloxacin	2 (13.33)	0 (0.00)	13 (86.67)
Streptomycin	4 (26.67)	8 (53.33)	3 (20.00)
Chloramphenicol	7 (46.67)	3 (20.00)	5 (33.33)
Ceftriaxone	15 (100.00)	0 (0.00)	0 (0.00)
Gentamicin	12 (80.00)	1 (6.67)	2 (13.33)
Pefloxacin	7 (46.67)	8 (53.33)	0 (0.00)
Cotrimoxazole	8 (53.33)	5 (33.33)	2 (13.33)
Ciprofloxacin	2 (13.33)	7 (46.67)	6 (40.00)
Erythromycin	15 (100.00)	0 (0.00)	0 (0.00)

Figure 1: *Salmonella* isolates on XLD appearing as Pink-red colonies with black centres