



Prevalence and Antibiotic Susceptibility of *Salmonella enterica* isolated from Cow Milk, Milk Products and Hands of Sellers in the Tamale Metropolis of Ghana

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ABSTRACT: *Salmonella enterica* are among the foodborne pathogens of importance due to their involvement in a number of foodborne outbreaks, illnesses and occasionally death. This study was carried out in the Tamale metropolis of Ghana to investigate the prevalence and antibiotic resistance of *Salmonella enterica* in cow milk, cow milk products and hands of cow milk sellers. The conventional method and the disc diffusion method were used for the isolation and antibiotic resistance of *Salmonella enterica*, respectively. The overall prevalence of *Salmonella enterica* was 7.3% (22/300). *Salmonella enterica* were detected in 24.0% (12/50) of raw 'wagashie', 8.0% (4/50) of fried 'wagashie', 6.0% (3/50) of cow milk, 4.0% (2/50) of left hand swab of milk sellers, and 2.0% (1/50) of right hand swab of milk sellers. *Salmonella enterica* was not isolated from 'brukina'. The prevalence of *Salmonella enterica* did not differ ($P = 0.193$) among fried 'wagashie', cow milk, hand swab of milk sellers and 'brukina' samples. However, the prevalence of *Salmonella enterica* in raw 'wagashie' was significantly higher ($P = 0.000$) than the other samples examined. Resistant to erythromycin (86.0%) was most frequently detected. The *Salmonella enterica* isolates were highly susceptible to ciprofloxacin (100.0%), chloramphenicol (91.0%), ceftriaxone (91.0%), sulphamethoxazole/trimethoprim (91.0%), tetracycline (86.0%) and ampicillin (86.0%). Of the 22 *Salmonella enterica* isolates, 14 were resistant to only one antibiotic, 4 were resistant to two antibiotics and 2 were resistant to three antibiotics. Two *Salmonella enterica* isolates were resistant to none of the antibiotics. This study revealed that some milk products and other samples were contaminated with *Salmonella enterica*. The isolated *Salmonella enterica* were susceptible to most of the antibiotics examined but were highly resistant to erythromycin.

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Milk and milk products constitute important nutritional components of human diets and play prominent roles in human nutrition (Medhammar *et al.*, 2012). Bacterial contamination of milk and its products can occur from within the udder, outside the udder and from milk handling equipment (Oliver *et al.*, 2005; Adzitey *et al.*, 2018). Cow health, milking procedures, equipment sanitation and environment such as water and personnel can influence the level of microbial contamination of raw milk (Altug and Bayrak, 2003; Adzitey *et al.*, 2016a). There have been outbreaks of milk-borne diseases in humans, with pathogens such as *Salmonella spp.*, *S. aureus*, *E. coli*, and *Yersinia spp.* being implicated (Yagoub *et al.*, 2005).

Salmonella is a rod-shaped, Gram-negative, non-spore forming, catalase positive, oxidase negative and predominantly motile bacteria possessing peritrichous flagella (Yan *et al.*, 2003). Its members are generally small enterobacteria with a diameter ranging from

0.7µm to 1.5µm and a length of 2.0µm to 5.0µm (Yan *et al.*, 2003). *Salmonella* grows optimally at a temperature of 35 to 37°C, pH of 6.5 to 7.5 and water activity of 0.84 to 0.94 (Yan *et al.*, 2003). *Salmonella* is one of the main causes of foodborne illnesses in developing as well as in developed countries (Adams and Moss, 2008; Scallan *et al.*, 2011). *Salmonella* causes gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting (Hensel, 2004). Because *Salmonella* is shed in the animal's faeces, there is a risk of the pathogens contaminating milk and milk products.

Outbreaks of milk-borne diseases have occurred despite pasteurization caused either by improper pasteurization or recontamination thereby posing some risks to consumers (DeSilva *et al.*, 2001). Milk and milk products can be contaminated with pathogens that are resistant to several antibiotics. Hleba *et al.* (2015) reported that it is currently impossible to effectively exclude antibiotic resistant strains from

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milk products. Thus, exposure of milk to antibiotics is one of the pathways for transfer of antibiotic resistant pathogens to humans. Yet, data on the prevalence and antibiotic susceptibility of *Salmonella* in milk and milk products in Ghana are inadequate. To be able to create more awareness of *Salmonella* infections in the country, there is the need to undertake studies on prevalence and antibiotic susceptibility of *Salmonella enterica* in foods for human consumption.

MATERIALS AND METHODS

Study area: This study was conducted in the Tamale metropolis. Tamale is the capital town of the Northern region of Ghana. The Metropolis has a total estimated land size of 646.90180sqkm (Ghana Statistical Service (GSS), 2010). Geographically, the city lies between latitude 9°16 and 9°34 North and longitudes 0°36 and 0°57 West (GSS, 2010). It has a population of about 2,479,461 and a growth rate of 2.9% (GSS, 2010). Tamale has annual rainfall of 1100mm and mean temperature range of 28°C to 43°C (GSS, 2010).

Samples examined: Three hundred milk, milk products and hands of milk/milk product sellers were randomly sampled in the Tamale metropolis. The samples included; cow milk, raw 'wagashie', fried 'wagashie', 'brukina', and palm swabs of the hands of milk sellers. 'Wagashie' is a traditional West African cottage cheese normally produced by the semi-nomadic Fulani. 'Brukina' or 'Fura' is a milk product made mainly from millet that has undergone fermentation. 'Wagashie' and 'brukina' are delicacies for many Ghanaians. Fifty samples of each item were collected. They were analysed for the presence of *Salmonella enterica*. Isolated *Salmonella enterica* were subjected to antimicrobial susceptibility test to determine their resistances.

Isolation and identification of *Salmonella enterica*: *Salmonella enterica* were isolated and identified according to the procedures in the Bacteriological Analytical Manual of USA-FDA (Andrews *et al.*, 2015). Briefly, samples were pre-enriched in buffered peptone water (BPW) and incubated at 37°C for 18 - 24hours. These were the ratios for the samples; 10ml of milk sample: 90ml of BPW, 10g of 'wagashie' (fried and raw): 90ml of BPW, 10ml of 'brukina': 90ml of BPW, and 1 swab of hand (left and right, 10cm²): 10ml of BPW. After pre-enrichment, 0.1ml portion was transferred to 10ml Rappaport Vassilidis (RV) broth and 1ml portion into 9ml selenite cysteine (SC) broth and incubated at 42°C and 37°C, respectively for 24-48hours. Enriched samples were streaked on xylose lysine deoxycholate (XLD) and Brilliant Green Agar (BGA) with an inoculating loop. The plates (XLD and BGA) were incubated at 37°C for

24 to 48 hours. Presumptive *Salmonella* colonies were picked and purified on tryptic soy agar. Confirmation and identification was carried out using Gram stain, triple sugar iron agar (TSI), lysine iron agar (LIA) and *Salmonella* latex agglutination test kits. All media and kits used were purchased from Oxoid, UK.

Antimicrobial susceptibility test: The disk diffusion method was used to determine the antibiotic susceptibility of 22 *Salmonella enterica* isolates against 8 antimicrobial agents (ampicillin (Amp) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (Cip) 5µg, ceftriaxone (Cro) 30µg, gentamicin (Cn) 10µg, erythromycin (E) 15µg, sulphamethoxazole/trimethoprim (Sxt) 22 µg and tetracycline (Te) 30 µg, all purchased from Oxoid, UK).

Pure *Salmonella enterica* isolates were grown in tryptic soy broth (TSB) (Oxoid, UK) at 37°C for 16 to 24 hours and the concentration adjusted using sterile TSB until 0.5 McFarland turbidity was attained. Approximately, one hundred microliters of the culture were spread plated on Mueller Hinton agar (Oxoid, UK). The antibiotic disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16 to 24 hours and the results were interpreted as resistance, susceptibility and intermediate according to the Clinical and Laboratory Standards Institution guidelines (Clinical and Laboratory Standards Institute (CLSI), 2008).

Statistical analysis: The data obtained was analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Version 17. Test for statistical difference was done using wald chi-square at 5% significance level.

RESULTS AND DISCUSSION

Prevalence of *Salmonella* in cow milk, cow milk products and hand samples: The distribution of the *Salmonella enterica* in the cow milk, 'wagashie' (raw and fried), hand of cow milk sellers (left and right hands) and 'brukina' samples analyzed in the Tamale metropolis of Ghana is showed in Table 1. Out of the 300 samples examined, 22 (7.3%) were positive for *Salmonella enterica*. The prevalence of *Salmonella enterica* was 24.0% (12/50), 8.0% (4/50), 6.0% (3/50), 4.0% (2/50), 2.0% (1/50) and 0.0% (0/50) for raw 'wagashie', fried 'wagashie', cow milk, left hand swab of milk sellers, right hand swab of milk sellers and 'brukina', respectively. The prevalence of *Salmonella* in raw 'wagashie' was significantly higher (P = 0.000) than the rests of the samples examined. There was no significant difference (P = 0.193) in the prevalence of

Salmonella enterica between fried 'wagashie', cow milk, hand swab of milk sellers and 'brukina'. This study examined the prevalence and antibiotic resistance of *Salmonella* in cow milk, cow milk products and hand samples of cow milk sellers. The overall prevalence of *Salmonella enterica* in the milk and its related samples was 7.3%. Raw 'wagashie' was

the most contaminated source, followed by fried 'wagashie', cow milk, left hand and right hand. *Salmonella enterica* were not detected in 'brukina'. The presence of *Salmonella* in the raw 'wagashie' was significantly higher ($P = 0.000$) than the rest of the samples examined.

Table 1: Distribution of *Salmonella enterica* in milk and its related samples

| Type of sample | Number of samples tested | Number of samples positive/negative | Percentage prevalence |
|------------------|--------------------------|-------------------------------------|-----------------------|
| Raw 'wagashie' | 50 | 12 | 24.0 |
| Fried 'wagashie' | 50 | 4 | 8.0 |
| Cow milk | 50 | 3 | 6.0 |
| Left hand | 50 | 2 | 4.0 |
| Right hand | 50 | 1 | 2.0 |
| 'Burkina' | 50 | 0 | 0.0 |
| Total | 300 | 22 | 7.3 |

Contamination of raw 'wagashie' (24.0%) by *Salmonella enterica* was also statistically higher ($P = 0.000$) than fried 'wagashie' (8.0%); thus frying 'wagashie' likely reduced the level of *Salmonella* contamination. Also cow milk (6.0%), which are normally pasteurized for sale at the Tamale metropolis involved the use of heat during processing, and had lower contamination rate as compared to raw 'wagashie'. Lower contamination of hand samples by *Salmonella enterica* suggests that the hands are not the primary source of this foodborne pathogen but might have been cross contaminated. Hands of the sellers can also cause cross contamination of different milk products sold in this metropolis. The absence of *Salmonella enterica* in 'brukina' may be attributed to the cold temperature (about 4°C) they are kept for sale. Mhone *et al.* (2012) examined 120 raw cow milk and 20 processed cow milk from smallholder dairy farms in Zimbabwe and found no *Salmonella enterica*. Similarly, to this study, *Salmonella enterica* were not found in 'brukina' but were found in the other samples examined. Karns *et al.* (2005) also examined 854 bulk tank milk from dairies across the United States and found that 100 (11.7%) and 22(2.6%) were positive for *Salmonella enterica* by real time PCR assay and the conventional culture techniques, respectively. In Addis Ababa, 2.1% (2/91) of cow milk samples were contaminated with *Salmonella enterica* (Tefaw *et al.*, 2013). This study found an average prevalence of 7.3%. A number of factors including the study area, isolation method, number and type of samples examined and the overall hygienic conditions employed in the handling of milk and its related samples contribute to differences in the prevalence rate.

Milk and milk products in the Tamale Metropolis are normally processed without adherence to strict hygienic practices and are sold locally (Adzitey *et al.*,

2018). The contamination of the pasteurized milk, milk products and hands by *Salmonella enterica* attests to the fact that strict hygienic practices were not followed in their preparation. The possible sources of *Salmonella enterica* contamination may be at the farm, during processing and/or selling points. It was observed that farmers/milkers use ordinary or unboiled water without detergents to wash equipment, udder and their hands before and after milking at the farms milk samples were collected. Also milking was done on some lactating cows that were dirty. Bonfoh *et al.* (2003) reported that well-constructed herd structure, milking and pre-storage conditions are important determinants of the quality and safety of raw milk. It was also observed in this study that majority of the milk sellers in the market operated very close to main streets without shades exposing the milk and milk products to dust and other contaminants. Unhygienic practices observed among some of the milk sellers included wearing unclean clothes, allowing other people to sell milk to consumers, use of dirty/uncleaned hands to sell and drinking from cups used to sell milk. These practices coupled with the environment under which milk and milk products are sold are potential sources of contamination. The presence of *Salmonella enterica* in the milk, milk products and hands of milk sellers is very disturbing since majority of people in the northern region and the country as a whole consume these products.

Table 2. Percent *Salmonella enterica* isolates from cow milk, and other samples that are resistant to different antibiotics

| Antimicrobial | Distribution of antibiotic resistance in <i>Salmonella</i> spp. | | |
|---------------------------|-----------------------------------------------------------------|-------|-------|
| | R (%) | I (%) | S (%) |
| Ampicillin (Amp) 30 µg | 9.0 | 5.0 | 86.0 |
| Chloramphenicol (C) 30 µg | 5.0 | 5.0 | 91.0 |
| Ciprofloxacin (Cip) 5 µg | 0.0 | 0.0 | 100.0 |
| Ceftriaxone (Cro) 30 µg | 0.0 | 9.0 | 91.0 |
| Gentamicin (Cn) 10 µg | 14.0 | 18.0 | 68.0 |
| Erythromycin (E) 15 µg | 86.0 | 9.0 | 5.0 |
| Sxt 22 µg | 0.0 | 9.0 | 91.0 |
| Tetracycline (Te) 30 µg | 14.0 | 0.0 | 86.0 |

Key: R=resistant, I=intermediate resistant, S= susceptibility, Sxt= Suphamethoxazole/trimethoprim

Salmonella is known to cause Salmonellosis with symptoms such as nausea, vomiting and diarrhea (Adams and Moss, 2008). Additionally, complications associated with Salmonellosis include septicemia, reactive arthritis and even death (Adams and Moss, 2008; Scallan *et al.*, 2011).

Antibiotic susceptibility of Salmonella isolated from cow milk, cow milk products and hand samples: The antibiotic resistance of the *Salmonella enterica* is presented in Table 2. A high percentage (86.0%) of the *Salmonella enterica* were resistant to erythromycin. Susceptibility to ciprofloxacin (100.0%), ceftriaxone (91.0%), chloramphenicol (91.0%), sulphamethoxazole/trimethoprim (91.0%), tetracycline (86.0%) and ampicillin (86.0%) was also high. The 22 *Salmonella enterica* isolates exhibited minor intermediate resistances to all the antibiotics except ciprofloxacin and tetracycline. The antibiotic resistance pattern revealed that 14 isolates were resistant to only one antibiotic, 4 isolates were resistant to two antibiotics and 2 isolates were resistant to three antibiotics (Table 3). Two *Salmonella enterica* isolates were resistant to none of the antibiotics. Resistant to only erythromycin was most common.

Table 3: Contaminated samples with *Salmonella enterica* and corresponding antimicrobial resistance

| No. | Sample | Antibiotic resistant pattern | Number of antibiotics |
|-----|------------------|------------------------------|-----------------------|
| 1 | Raw 'wagashie' | - | 0 |
| 2 | Fried 'wagashie' | - | 0 |
| 3 | Left hand | E | 1 |
| 4 | Left hand | E | 1 |
| 5 | Right hand | E | 1 |
| 6 | Raw 'wagashie' | E | 1 |
| 7 | Raw 'wagashie' | E | 1 |
| 8 | Raw 'wagashie' | E | 1 |
| 9 | Fried 'wagashie' | E | 1 |
| 10 | Fried 'wagashie' | E | 1 |
| 11 | Fried 'wagashie' | E | 1 |
| 12 | Fried 'wagashie' | E | 1 |
| 13 | Fried 'wagashie' | E | 1 |
| 14 | Fried 'wagashie' | E | 1 |
| 15 | Fried 'wagashie' | E | 1 |
| 16 | Fried 'wagashie' | E | 1 |
| 17 | Cow milk | CTe | 2 |
| 18 | Cow milk | ETe | 2 |
| 19 | Fried 'wagashie' | ECn | 2 |
| 20 | Fried 'wagashie' | ECn | 2 |
| 21 | Cow milk | EAmPte | 3 |
| 22 | Fried 'wagashie' | EAmPCn | 3 |

Key: Erythromycin (E), Tetracycline (Te), Ampicillin (Amp), Gentamycin (Cn), and chloramphenicol(C)

Antibiotics are widely used for the treatment of bacterial infections. Although their use has received much attention and cautions, their use for therapeutic and other purposes cannot be totally eliminated (Adzitey, 2015). In this study, most of the *Salmonella enterica* isolates of milk, milk product and seller's

hands were susceptible to the 8 different antibiotics examined; some of the isolates were resistant and others exhibited intermediate resistant. All the *Salmonella enterica* isolates were susceptible to quinolones (ciprofloxacin). Similarly, Tesfaw *et al.* (2013) found that all *Salmonella enterica* isolates from milk and milk products were susceptible to ciprofloxacin. Susceptibility $\geq 68.0\%$ occurred for aminoglycosides (gentamycin), penicillins (ampicillin), tetracycline, chloramphenicol, cephalosporins (ceftriaxone) and sulfonamides (sulphamethoxazole/trimethoprim). Intermediate resistant occurred for all the antibiotics except ciprofloxacin and tetracycline. *Salmonella enterica* isolates that exhibited intermediate resistances have the tendency to become resistant (Adzitey *et al.*, 2015). Resistant to macrolides (erythromycin) was high in the current study. Tesfaw *et al.* (2013) reported that 83.3%, 50.0%, and 16.7% of milk and milk product *Salmonella enterica* isolates were resistant to tetracycline, ampicillin, and chloramphenicol, respectively. *Salmonella enterica* isolates from cow milk were resistant to ampicillin (100%), tetracycline (50%), gentamycin (33.3%), and chloramphenicol 16.7% (Addis *et al.*, 2011). In this current study, lower resistances (5.00% to 14.0%) to these antibiotics (tetracycline, ampicillin, gentamycin and chloramphenicol) were observed.

Of the 22 *Salmonella enterica* isolates, 4 were resistant to two antibiotics, 2 were resistant to three antibiotics, 14 were resistant to one antibiotic and 2 were resistant to none of the antibiotics. Thus 9.09% (2/22) of the *Salmonella enterica* isolates were multidrug resistant.

The 2 *Salmonella enterica* isolates that were multidrug resistance exhibited resistant patterns; EAmpTe (erythromycin-ampicillin-tetracycline) and EAmpCn (erythromycin-ampicillin-gentamicin). The resistant patterns CTe (chloramphenicol-tetracycline), ETe (erythromycin-tetracycline) and ECn (erythromycin-gentamicin) were exhibited by an isolate each. Multidrug resistant isolates are dangerous and difficult to control when they are involved in infection compared to non-multidrug resistant isolates and *Salmonella enterica* isolates resistant to more than one antibiotic have been reported by other investigators (Foley and Lynne, 2008; Tesfaw *et al.*, 2013; Abike *et al.*, 2015; Adzitey *et al.*, 2016b; Raja and Adzitey, 2017). Tesfaw *et al.* (2013) found that milk and milk product *Salmonella enterica* isolates were resistant to TeAmp (tetracycline-ampicillin) and TeAmpAmo (tetracycline-ampicillin-amoxicillin).

They also reported that 50.0% of the *Salmonella enterica* isolates were multiple antibiotic resistant

while the rest were resistant to a single antibiotic. The reasons for the development of resistance among foodborne pathogens including *Salmonella enterica* have been linked to the indiscriminate use of antibiotics for therapeutic and growth purposes in animals (Forshell and Wierup, 2006).

Conclusions: The present study revealed that some milk and milk products were contaminated with *Salmonella enterica*. Raw 'wagashie' was the most contaminated source, followed by fried 'wagashie', cow milk, left hand and right hand. *Salmonella enterica* was not found in 'burkina'. Resistance of *Salmonella enterica* to some of the antimicrobial drugs except ciprofloxacin may be attributed to the use of these antimicrobials for therapeutic purposes in cattle production in the Tamale metropolis.

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