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# Prevalence of resistant *Salmonella* spp. isolated from raw meat and liver of cattle in the Wa Municipality of Ghana

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**Abstract.** This study was carried out to determine the prevalence of resistant *Salmonella* spp. isolated from meat (beef) and liver of cattle in the Wa, Municipality of Ghana. Isolation of *Salmonella* spp. was done according to the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the CLSI guidelines. Antibiotic residues determination was carried out using Premi®Test Kit. Liver samples 32%(16/50) were more contaminated with *Salmonella* spp. as compared to beef samples 30% (15/50). The *Salmonella* spp. were highly resistant to teicoplanin (96.77%) but susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), suphamethoxazole/ trimethoprim (100%), amoxycillin/clavulanic acid (93.55%), ceftriaxone (93.55%) and gentamicin (83.87%). The *Salmonella* spp. exhibited 5 antibiotic resistant patterns with the pattern (Tec) being the commonest. Multiple antibiotic index ranged from 0.11 to 0.33. A total of 35 samples (20 beef and 15 liver samples) were examined for antibiotic residues, and 7 (20%) were positive for antibiotic residues; 6 (17%) were found in the liver while 1 (3%) was in the beef. This study revealed that salmonellae are present in beef and liver samples examined and showed high resistance to teicoplanin. Some of the beef and kidney samples harbored antibiotic residues.
**Keywords – a**ntibiotics, cattle, drug residues, raw meat, *Salmonella* spp.

## 1. Introduction

Foodborne disease outbreaks continue to be an important public health problem globally and most of the food safety hazards are caused by foods of animal sources [1]. Bhandare et al. [2] also reported that contaminated raw meat has been identified as one of the main sources of foodborne illnesses. Among the reasons for which meat and meat products are consumed includes their high protein contents, available vitamins, minerals, lipids and savuory sensation. However, due to its high nutrient content which supports the growth of microorganisms, it is classified among the most perishable staple foods [3]. Adeyemo [4] indicated that there are two sources for which microorganisms get into meat that is either from the hide of the animals or from the abattoirs where the animals are slaughtered and processed. Food-borne pathogens such as *Staphylococcus* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Bacillus* spp. and *Listeria*

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*monocytogenes* have been isolated from meats in Ghana [5,6,7, 8] some of which have been subjected to antimicrobial susceptibility test [9]

According to Fratamico et al. [10] most illnesses and deaths in the developing countries are caused by foodborne diseases and these cost billions of dollars in medical care and social expenditures. Globally, *Salmonell*a spp. are one of the pathogens that lead millions of cases of enteric diseases, thousands of hospitalizations and deaths each year [11,12]. CDC [13] reported that *Salmonella* spp. causes an estimated 1.4 million cases of foodborne illnesses and more than 500 deaths per year in the US. This pathogen is also responsible for collective food poisoning with approximately 65% of cases in France [14].

For past twenty years, the occurrence of antibiotic resistant *Salmonella* spp. has become a serious health challenge in the globe. The frequent use of antimicrobial agents in food animal production and the routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases has contributed to the occurrence of *Salmonella* spp. with decreased susceptibility to drugs [15]. The objectives of this study were to determine the prevalence and antibiotic resistance of *Salmonella* spp. isolated from raw meat and liver of cattle in the Wa Municipality of Ghana. The study also aimed at determining the presence of antibiotic residues in the meat and liver samples.

## 2. Materials and methods

### *2.1. Sample collection and preparation*

A total of 100 swab samples (50 beeves and 50 livers) were randomly collected from the Wa, Abattoir and examined for the presence of *Salmonella* spp. Additionally, 35 samples (20 beeves and 15 livers) were randomly collected for antibiotic residue test. The swabs and beef/liver samples kept in an ice chest containing ice block and transported to the Spanish Laboratory of University for Development Studies, Nyankpala Campus. Microbial analysis was carried out immediately on arrival.

### *2.2. Isolation and confirmation of Salmonella* spp.

#### *2.2.1. Non selective pre-enrichment step*

To allow recovery and growth of any stressed organism, each swab was placed in 10ml buffered peptone water and incubated at a temperature of 37°C for 18-24 hours.

#### *2.2.2. Selective enrichment step*

Two types of media were used for selective enrichment, Rappaport-Vassiliadis and Selenite broths to encourage growth of *Salmonella* spp. while inhibiting other microorganisms. After non selective pre-enrichment, 0.1ml of aliquant was transferred into Rappaport Vassiliadis (RV) broth and inoculated at 42°C for 18-24 hours while 1ml was transferred to Selenite broth and incubated at 37 °C for 18 to 24 hours.

#### *2.2.3. Isolation step*

Selective enrichment media were streaked unto two selective solid agars (Xylose Lysine Deoxycholate and Brilliant Green) containing one or more agents that inhibit non-Salmonella organisms. Xylose Lysine Deoxycholate and Brilliant Green Agar plates were incubated at 37°C for 18-24 hours. Colonies having a slightly transparent zone of reddish/ pink color with or without a black center on Xylose Lysine Deoxycholate plates and grey to reddish/pink and slightly convex colonies that caused the color of the medium to be red/pink in Brilliant Green Agar plates were suspected as Salmonella. The suspected colonies from both Xylose Lysine Deoxycholate and Brilliant Green Agar agars were plated onto nutrient agar plates for biochemical confirmation.

*2.2.4 Confirmation step*

Presumptive Salmonella spp. were purified on Trypticase Soya Agar and confirmed using Gram Stain, growth in Lysine Iron Agar, Triple sugar iron agar and Salmonella latex agglutination test kit. All media and reagents used were purchased from Oxoid, UK.

*2.3 Antimicrobial susceptibility test*

Antimicrobial susceptibility of *Salmonella* isolates was checked against some commonly used antibiotics such as Amoxycillin/clavulanic acid (30µg), Azithromycin (15µg), Ceftriaxone (30ug), Chloramphenicol (30ug), Ciprofloxacin (5ug), Gentamicin (10ug), Teicoplanin (30 µg), Tetracycline (30ug) and Suphamethoxazole/trimethoprim using Kirby-Bauer antibiotic discs diffusion method. The results interpreted as sensitive, intermediate, or resistance according to Clinical and Laboratory Standards Institute guidelines (CLSI) [16].

*2.4 Procedure for antibiotic residues testing*

Premi®Test Kit for determination of drug residues in meat was used for qualitative screening of antibiotics by following the manufacturer's instructions. Approximately 2cm$^2$ of raw meat and kidney were used. The samples were frozen and thawed to obtain their extract/juice. 100µl extract/juice were pipetted onto the agar in the ampoule of the test kit and incubated at 37°C for 20 minutes for a pre-diffusion. The extract/juice was carefully flushed out by washing the test tubes twice with demineralized water. The test ampoule was closed with foil to avoid evaporation during incubation. The test ampoules were incubated in water bath at 64.0 °C until negative control change colour from purple to yellow. Color change in the negative control was observed within 3 hours. After the negative control has changed color the results were read using color chart. If there is no antibiotic residue in the extracted solution, the bacteria grow and the acidic pH causes the medium to change from purple to yellow color and this is marked as "negative. If antibiotics are present in the extracted solution, they would kill the bacteria; the whole or part of the tube remains purple and this is marked as "positive for antibiotic residue".

## 3. Result and discussion

*3.1 The prevalence of Salmonella* spp*. in meat and kidney of cattle*

The prevalence of *Salmonella* spp. in meat and liver of cattle in the Wa, Municipality of Ghana is shown in Table 1.

**Table 1**. Distribution of *Salmonella* spp. in liver and meat of cattle in the Wa, Municipality.

| Source of meat | Number of samples examined | Number of positive samples | % Prevalence |
|---|---|---|---|
| Liver | 50 | 16 | 32 |
| Muscle | 50 | 15 | 30 |
| Total | 100 | 31 | 24 |

*Salmonella* spp. were isolated from liver 32% (16/50) and beef 30%(15/50) samples. This means that the liver and beef samples were contaminated with *Salmonella* spp. Contamination of meat samples happens when maximum care is not taken during slaughtering and dressing of animals. The contamination of various meat types such as beef [5,6,7, 8], guinea fowl meat [9], and pork [8] by *Salmonella* spp. in Ghana have been reported. Adzitey [6] reported an overall prevalence of *Salmonella* spp. in beef samples to be 31% (22/70) in the Tamale Metropolis. The prevalence of *Salmonella* spp. was significantly higher in beef 75% (45/60) than that of table (60%), knife (60%) and apron (33%) samples collected from the Techiman Municipality [9]. Anachinaba et al. [8] found *Salmonella* spp. in beef samples sold at the Bolgatanga

Municipality. In Nigeria, Adesiji et al. [17] did not find *Salmonella* spp. in retail raw chicken, beef and goat meat. They found that 8% (6) of pork was positive for *Salmonella* spp.

*3.2 Antibiotic resistance of Salmonella* spp. *in meat and liver of cattle*

The antimicrobial susceptibility of the *Salmonella* spp. is shown in Table 2. The *Salmonella* spp. were mostly resistant to teicoplanin (96.77%). They were susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), suphamethoxazole/trimethoprim (100%), amoxycillin/clavulanic acid (93.55%), ceftriaxone (93.55%) and gentamicin (83.87%). Danikuu (2004) reported that *Salmonella* spp. isolated from farm animals in the Kumasi Metropolis, Ghana were all resistant to tetracycline but susceptible to ciprofloxacin, which agrees with the current study. An intermediate resistance of 12.90% was found for azithromycin. Intermediate resistance means that those isolates are not clearly resistant or susceptible, and such isolates have the tendency to easily become resistant [9].

**Table 2.** Percentage antibiotic resistance of Salmonella spp. isolated from cattle liver and meat.

| Antimicrobial | R (%) | I (%) | S (%) |
|---|---|---|---|
| Amoxycillin/clavulanic acid 30µg (AMC) | 3.23 | 3.23 | 93.55 |
| Azithromycin 15µg (AZM) | 29.03 | 12.90 | 58.06 |
| Ceftriaxone 30ug (CRO) | 0.00 | 6.45 | 93.55 |
| Chloramphenicol 30ug (C) | 0.00 | 0.00 | 100.00 |
| Ciprofloxacin 5ug (CIP) | 0.00 | 0.00 | 100.00 |
| Gentamicin10ug (CN) | 12.90 | 3.23 | 83.87 |
| Teicoplanin 30 µg (TEC) | 96.77 | 0.00 | 3.23 |
| Tetracycline 30ug TE | 0.00 | 0.00 | 100.00 |
| Suphamethoxazole/trimethoprim (SXT) | 0.00 | 0.00 | 100.00 |
| Overall | 17.77 | 2.87 | 81.36 |

S, susceptible; I, Intermediate; R, resistant

The antimicrobial resistance profile and MAR index of the *Salmonella* spp. isolates is showed in Table 3. The *Salmonella* spp. exhibited 5 antibiotic resistant patterns and the resistant pattern Tec (teicoplanin, MAR index = 0.11) was the most common. The resistant to 3 different antibiotics AmcAzmTec (MAR index= 0.33), and AzmTecCn (MAR index= 0.33) were observed in 1 and 3 isolates respectively. Thus 12.9% (4/31) of the *Salmonella* spp. exhibited multidrug resistant.

**Table 3.** Antibiotic resistance profile and multiple antibiotic resistance index of individual *Salmonella* species.

| Code | Source | No. of Antibiotics | Antibiotic resistant profile | MAR index |
|---|---|---|---|---|
| L18 | Liver | 3 | AmcAzmTec | 0.33 |
| M8 | Muscle | 2 | AzmCn | 0.22 |
| eL15 | Liver | 2 | AzmTec | 0.22 |
| M9 | Muscle | 2 | AzmTec | 0.22 |
| M36-2 | Muscle | 2 | AzmTec | 0.22 |
| M36-3 | Muscle | 2 | AzmTec | 0.22 |

| | | | | |
|---|---|---|---|---|
| L14 | Liver | 3 | AzmTecCn | 0.33 |
| M11 | Muscle | 3 | AzmTecCn | 0.33 |
| M28-2 | Muscle | 3 | AzmTecCn | 0.33 |
| L1 | Liver | 1 | Tec | 0.11 |
| L2 | Liver | 1 | Tec | 0.11 |
| L7 | Liver | 1 | Tec | 0.11 |
| L16 | Liver | 1 | Tec | 0.11 |
| L20 | Liver | 1 | Tec | 0.11 |
| L35 | Liver | 1 | Tec | 0.11 |
| L36-1 | Liver | 1 | Tec | 0.11 |
| L36-2 | Liver | 1 | Tec | 0.11 |
| L42-1 | Liver | 1 | Tec | 0.11 |
| L42-2 | Liver | 1 | Tec | 0.11 |
| L43 | Liver | 1 | Tec | 0.11 |
| L49-1 | Liver | 1 | Tec | 0.11 |
| L49-2 | Liver | 1 | Tec | 0.11 |
| M4 | Muscle | 1 | Tec | 0.11 |
| M6 | Muscle | 1 | Tec | 0.11 |
| M21 | Muscle | 1 | Tec | 0.11 |
| M23 | Muscle | 1 | Tec | 0.11 |
| M27 | Muscle | 1 | Tec | 0.11 |
| M28-1 | Muscle | 1 | Tec | 0.11 |
| M36-1 | Muscle | 1 | Tec | 0.11 |
| M48-1 | Muscle | 1 | Tec | 0.11 |
| M48-2 | Muscle | 1 | Tec | 0.11 |

In this study, 16.13% (4/31) of the *Salmonella* spp. were resistant to two antibiotics, while 70.97% were resistant to one. [9] found that the resistant pattern EVa was the commonest and was exhibited by 9 different *Salmonella* spp. isolated from beef in Techiman. They also found that some of the *Salmonella* spp. were resistant to 3 or more antibiotics and exhibited multidrug resistance. The increasing multi-drug resistance constitutes a potential source of transmission of resistant strains to humans and of public health concern.

*3.3 Antibiotic residues in meat and liver of cattle*

Table 4 shows the prevalence of antibiotic residues in cattle liver and meat samples. From Table 4, the prevalence of antibiotic residues in liver 17% (6/15) was higher than muscle 3% (1/35). In [19] reported that beef samples collected from selected markets in Kumasi, Ghana contained drug residues. Babapour et al. [20] screened 500 samples of beef and mutton collected from Iran for drug residues and reported a prevalence rate of 22.8% and 14% for beef and mutton, respectively. In Pakistan, 38.33% of beef samples were observed to be contaminated with antibiotic residues [16]. They observed that the presence of antibiotic residues in beef samples was higher at Karachi (48.33%) followed by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas (33.34%) and Larkana (31.67%).

**Table 4.** Prevalence of antibiotic residues in cattle liver and meat.

| Source of meat | Samples | Positive | % Prevalence |
|---|---|---|---|
| Liver | 15 | 6 | 17 |
| Muscle | 20 | 1 | 3 |
| Total | 35 | 7 | 20 |

The use of antimicrobials in animal production is mainly responsible for the dissemination of antibiotic resistance bacteria isolates. To minimise antimicrobial use, a multifaceted integrated approach is recommended [21].

## 4. Conclusion

It is concluded that some liver and meat of cattle from the Wa, Municipality of Ghana are contaminated with *Salmonella* spp. which are resistant to some antibiotics. Kidney and meat samples also do harbor antibiotic residues.

## Reference

[1] Maripandi A, Al-Salamah A. 2010. Multiple antibiotic resistance and plasmid profiles of S. enteritidis isolated from retail chicken meats. *Am J Food Technol*. 5:260-268.
[2] Bhandare S, Sherikarv A, Paturkar A, Waskar V, Zende, RJ. 2007. A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control*. 18:854—68.
[3] Huda N, Shen YL, Huey R, Ahmad, A. Mardiah 2010. Evaluation of physico-chemical properties of Malaysian commercial beef meatballs. *Am J Food Technol,*. 5:13-21.
[4] Adeyemo OK 2002. Unhygienic operation of a city abattoir in South Western Nigeria: Environmental Implication. *African J Environ Appl Microbiol*. 4(1):23-28.
[5] Soyiri IN, Agbogli HK, Dongdem JT. 2008. A Pilot microbial assessment of beef in the Ashaima Market, a suburb of Accra Ghana. *African J Food Agric Nutr Dev*. 8(1):91-103.
[6] Adzitey F. 2015. Prevalence of Escherichia coli and Salmonella spp. in beef samples sold at Tamale Metropolis, Ghana. *Int J Meat Sci*. 5(1):8-13.
[7] Adzitey F, Teye GA, Anachinaba I. 2015. Microbial quality of fresh and smoked guinea fowl meat sold in the Bolgatanga Municipality, Ghana. Asian. *J Poult Sci*. 9:165-171.
[8] Anachinaba IA, Adzitey F, Teye GA. 2015. Assessment of the microbial quality of locally produced meat (beef and pork) in Bolgatanga Municipal of Ghana. *Internet J Food Safety, 17: 1-6.*
[9] Adzitey F, Nsoah JK, Teye G.2015. Prevalence and antibiotic susceptibility of Salmonella species isolated from beef and its related samples in Techiman Municipality of Ghana. *Turkish J Agric - Food Sci Technol*. 3(8):644-650.
[10] Fratamico P, Bhunia A, Smith J. 2005. Food borne pathogens in microbiology and molecular biology. Wymondham,. *Norfolk, UK Caister Acad Press*. 270—5.
[11] CDC. 2009. Third Annual 2009 National Conference on Health Communication, Marketing, and Media. In: *Third Annual 2009 National Conference on Health Communication, Marketing, and Media*.
[12] Hur J, Jawale C, Lee JH. Antimicrobial resistance of Salmonella isolated from food animals: A review: *Food Res Int*. 2011;45(2):819 – 830.
[13] CDC. 2006. *Surveillance for Foodborne Disease Outbreaks - United States,*. Vol 58.
[14] Haeghebaert S, Sulem P, Deroudille L, et al. Two outbreaks of Salmonella Enteritidis phage type 8

linked to the consumption of Cantal cheese made with raw milk, France, 2001. *Eurosurveillance*. 2003;8:151–156.

[15] Angulo F, Johnson K, Tauxe R, Cohen M. 2000. Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. *Microb Drug Resist*. 6(1):77-83.

[16] Mangsi As, Khaskheli M, Soomro A, Muhammad H, Shah G. 2014. Antibiotic Residues Detection In Raw Beef Meat Sold For Human Consumption In Sindh,. *Pakistan Int J Res Applied, Nat Soc Sci*. 2(7):15-20.

[17] Adesiji YO, Alli OT, Adekanle MA, Jolayemi JB. 2011. Prevalence of Arcobacter, Escherichia coli, Staphylococcus aureus and Salmonella species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone J Biomed*. 3:8-12.

[18] Danikuu M. 2004. Isolation and serotyping of Salmonella from slaughtered food animals in the Kumasi Metropolis. Available at: http://ir.knust.edu.gh/handle/123456789/1936 [Accessed 25 December, 2014].

[19] Abavelim DA 2014. Determination of Antibiotics Residues in Beef and Mutton from some Selected Markets in Kumasi -Ghana..

[20] Babapour J, Fartashmehr L, Azami A. 2012. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World. *Appl Sci J*. 19(10):1495-1500.

[21] EFSA. European Food Safety Authority and European Centre for Disease Prevention and Control (ECDC) 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J*. 14:4634-4865.