



Phenotypic Resistance of *Salmonella enterica* to Antibiotics in Imported Meats in Tema Metropolis, Ghana

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ABSTRACT

Salmonella enterica (*S. enterica*) causes certain foodborne diseases and poses public health concerns when it becomes antibiotic-resistant. The present study aimed to determine the resistance of *S. enterica* to antibiotics from imported meats sold in the Tema Metropolis, Ghana. A total of 300 beef, chicken, and pork samples randomly selected from markets of the Metropolis were tested for *S. enterica*. The disc diffusion method was used to determine the antibiotic resistance in 60 *S. enterica* isolates. The current results revealed that 40% of imported chicken, 38% of imported pork, and 29% of imported beef were positive for *S. enterica*. The *S. enterica* isolated from imported meat sources was 36.7% resistant to tetracycline but susceptible ($\geq 68\%$) to imipenem, ciprofloxacin, tetracycline, chloramphenicol, trimethoprim, gentamicin, and ceftriaxone. The multiple antibiotic resistance (MAR) index fell within the range of 0.1 to 0.6, and 12 different patterns were observed. The present study revealed the contamination of *S. enterica* in some imported meat sold in Tema Metropolis, Ghana. Avoidance of cross-contamination and adequate thermal treatment of meats before eating is essential.

Keywords: Antibiotic resistance, Meat, Prevalence, *Salmonella enterica*

INTRODUCTION

Worldwide, meat is an essential source of protein for human populations. According to [Ahmad et al. \(2018\)](#), meat also contains some minerals, such as zinc, selenium, iron, and phosphorus, and vitamins, including vitamins A and B-complex. Ghana's meat production gross value reached approximately 150.88 million dollars, a net decrease of -53,266,00 USD (-26.09%) during previous years ([Diversitytimes, 2022](#)). Approximately 48,047 tonnes of bovine meat and 287,930 tonnes of poultry meat were imported into Ghana in 2020 ([Knoema, 2021](#)). Furthermore, importing edible meat offal in 2019 was recorded to be 78.4 million dollars, corresponding to about 484 million cedis ([Sasu, 2012](#)). Meat is an important part of the daily diet of most Ghanaians ([Nkegbe et al., 2013](#)). [Nkegbe et al. \(2013\)](#) added that chicken is the most consumed, followed by beef, chevon, grasscutter, pork, and mutton.

Despite the high nutrient composition of meats and their consumption, they serve as potential sources of microbial contaminants ([El Hanafi et al., 2023](#); [Adzitey et al., 2024](#); [Monten et al., 2024](#)). In particular, *Salmonella enterica* (*S. enterica*) has been found in chicken, pork, and beef ([Zhao et al., 2001](#); [Meyer et al., 2010](#); [Ekli et al., 2020](#)). Additionally, *S. enterica* has been reported to cause foodborne diseases in humans ([CDC, 2021](#)), causing 273 hospitalizations, 1,135 disease, and two deaths in people who consumed the animal meats ([CDC, 2021](#)).

Antibiotics, including tetracycline and amoxicillin, are sometimes used for the treatment of diseased animals, as prophylactics, and as growth promoters ([Chattopadhyay, 2014](#)). Antibiotics are also used for treating infections in humans that might be caused by consuming meat contaminated by bacteria such as *S. enterica* ([CDC, 2021](#)). The development of resistance by bacteria to antibiotics is partly due to the use of antibiotics in human treatments and animal production ([Katakweba et al., 2012](#)). Bacteria can develop resistance to three or more antibiotics ([Yang et al., 2019](#)), a phenomenon described as multidrug resistance. Bacteria that are resistant to multiple medicines are difficult to treat when they cause infections ([Adzitey et al., 2020](#)). *Salmonella enterica* isolated from meat sources have been reported to be resistant to antibiotics, such as gentamicin, tetracycline, chloramphenicol, amoxicillin, and ceftriaxone ([Thai et al., 2012](#); [Ejo et al., 2016](#)).

Tema Metropolis in Ghana is one of the country's busiest urban centers, with a population from different countries and parts of Ghana. Therefore, active marketing and consuming meat are unavoidable. However, limited studies are

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available on the existence of bacteria in sellable meat in Ghana. Therefore, the present study aimed to investigate the prevalence of *S. enterica* and their resistance to antibiotics in imported meats sold at Tema's markets, Ghana.

MATERIALS AND METHODS

Ethical approval

The study was conducted in accordance with the approved ethical procedures of the University for Development Studies ethics committee, as referenced by the UDS/RB/099/22.

Study area

The present study was carried out in Tema Metropolis, which lies within latitude 5°38'32' north and longitude 0°0'9' west (Tema Metropolitan Assembly [TMA], 2021). The Metropolis is known as Harbor City because the largest seaport and numerous industries are located there (TMA, 2021), and has a size of 565 km² (Ghana Administrative Division, 2021). Maps of Ghana and Tema Metropolis are shown in Figure 1.

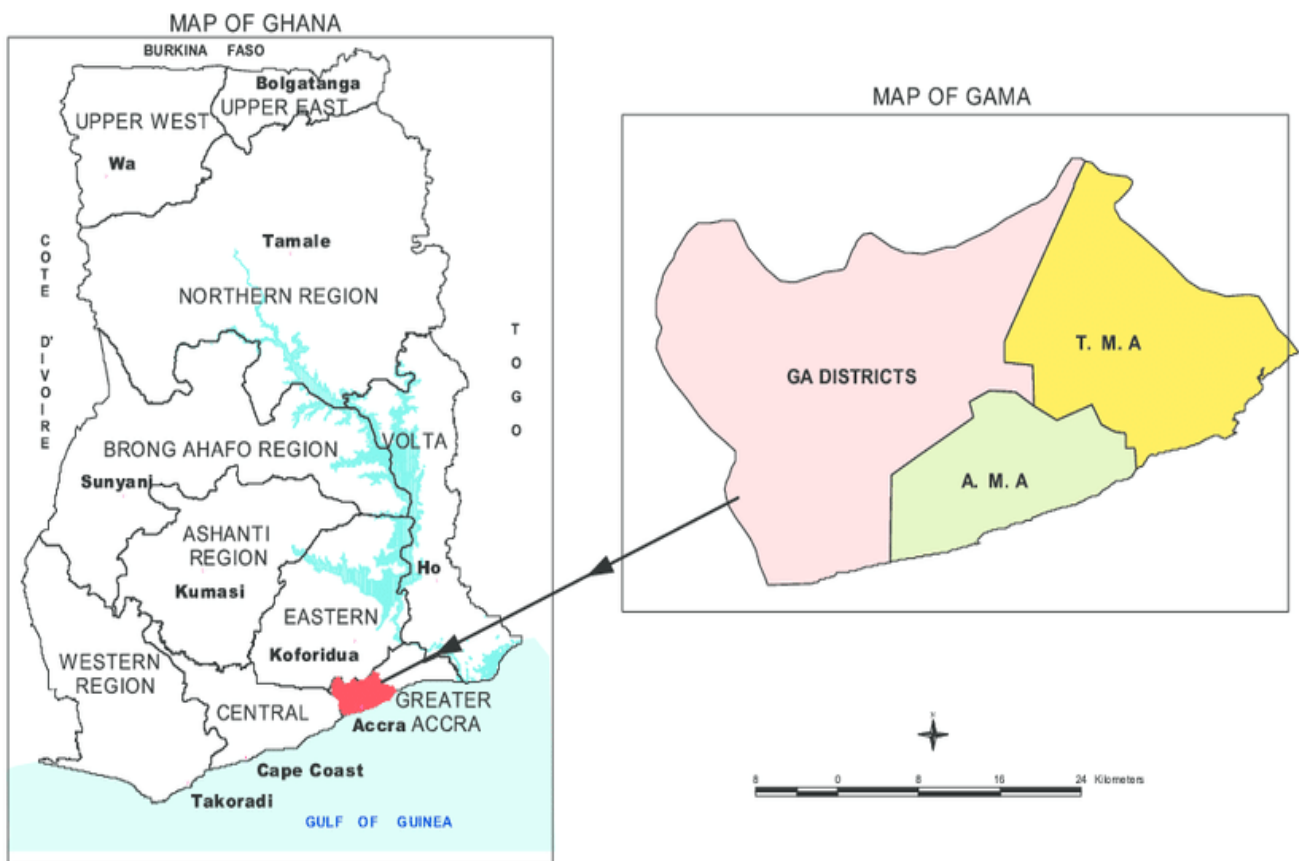


Figure 1. The map of Ghana, the Greater Accra region, and the Tema Metropolis (T.M.A). Source: TMA (2021)

Sample collection

Three hundred samples of imported chicken, pork, and beef (100 each) were randomly collected from markets in Tema Metropolis and analyzed for *S. enterica*. Sampling was carried out from April 2022 to September 2022. Fifty samples were collected each month from five different vendors. Ten samples were collected from each vendor. Collected samples were transported on ice to the Spanish Laboratory of the University for Development Studies for microbiological analysis.

Salmonella enterica microbiological analysis

The analysis for *S. enterica* was performed according to Wallace and Hammack (2013). The samples (10 g) were transferred into 90 mL buffered peptone water and incubated overnight at 37°C. Thereafter, the mixture was transferred into 10 mL of selenite cysteine and incubated at 37°C for 24 hours. In parallel, 0.1 mL was transferred into 10 mL of Rappaport-Vassiliadis broth (Oxoid Limited, UK) and incubated at 41°C for 48 hours. Incubated samples were plated onto brilliant green and xylose, lysine, and deoxycholate agars (Oxoid Limited, UK), and incubated overnight at 37°C.

Potential *S. enterica* isolates were streaked on trypticase soy agar (Oxoid Limited, UK) and incubated overnight at 37°C for purification. The isolates were then identified using Gram stain, growth on Simon citrate agar, triple sugar iron, lysine iron, and *Salmonella* latex agglutination test kit (Oxoid Limited, UK).

Confirmation of *Salmonella* using the polymerase chain reaction

The polymerase chain reaction (PCR) was carried out using the method of Upadhyay et al. (2010). Firstly, DNA was extracted from *Salmonella* cultures by lysing at 99°C for 10 minutes using 30 µl RNase/DNase-free water in a universal thermal cycler (VWR Prelab, UK). The 20 µl PCR reaction was made up of 10 µM each of primers (invA-F GTGAAATTATCGCCACGTTCTGGGCAA and invA-R TCATCGCACCGTCAAAGGAACC), 22 mM NH₄Cl, 20 mM Tris-HCl (pH 8.9 at 25°C), 0.2 mM dNTPs, 0.25U One Taq® DNA polymerase (New England Biolabs® Inc), 1.8 mM MgCl₂, 0.06% IGEPAL® CA-630, 5% glycerol, 0.05% Tween-20, xylene Cyanol FF, and Tartrazine, and 2 µl lysate as template. The temperature cycles used were 94°C for five minutes, 40 cycles of 94°C for 40 seconds, 64°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for seven minutes.

The PCR products were separated using 2% agarose and stained with 2.5 µl ethidium bromide. The PCR products (7 µl) were mixed with the loading dye (1 µl), and the mixture was subjected to gel electrophoresis for 30 minutes at 80V. The gel was then viewed under a UV transilluminator from Cleaver Scientific Company, UK.

Antibiotic susceptibility of *Salmonella enterica*

The antibiotic susceptibility test was carried out according to the Bauer et al. (1966) method against nine antibiotics, including ceftriaxone 30 µg (Cro), amoxicillin 30 µg (A), tetracycline 30 µg (T), chloramphenicol 30 µg (C), azithromycin 15 µg (Ath), gentamicin 10 µg (Gm), imipenem 10 µg (Imi), ciprofloxacin 5 µg (Cip), and trimethoprim 2.5 µg (Tm), purchased from MAST Group Limited, UK. *Salmonella enterica* colonies were transferred into trypticase soy (TS) broth (Oxoid Limited, UK) and incubated for 15 hours at 37°C. Sterile TS broth was used to adjust the turbidity to 0.5 McFarland Standard solution and transferred into Muller-Hinton (MH) agar (Oxoid Limited, UK). Four or five antibiotic discs were placed on MH agar and incubated for 24 hours at 37°C. Inhibition zones were measured, and the results were compared to the values of Clinical and Laboratory Standard Institute (CLSI, 2017). The multiple antibiotic resistance (MAR) index was calculated using the formula a/b, where a represents the number of antibiotics to which a particular isolate was resistant and b the total number of antibiotics examined (Krumperman, 1983).

Statistical analysis

Data on the prevalence of *S. enterica* were statistically computed using a generalized linear model of the Statistical Package for the Social Sciences version 20. Significant differences were recorded at 5% using the Wald chi-square.

RESULTS AND DISCUSSION

Prevalence of *Salmonella enterica*

Figure 2 depicts the prevalence of *S. enterica* in the imported chicken, beef, and pork samples collected from Tema Metropolis, Ghana. *Salmonella enterica* was isolated from 40.0% of the imported chicken, 38.0% of the pork, and 29.0% of the beef samples. Significant differences were not observed among the chicken, beef, and pork samples ($p > 0.05$). *Salmonella enterica* isolates were confirmed using PCR (Upadhyay et al., 2010). The bacteria yielded a band size of approximately 284 bp, confirming that the isolates were *S. enterica* (Figure 3).

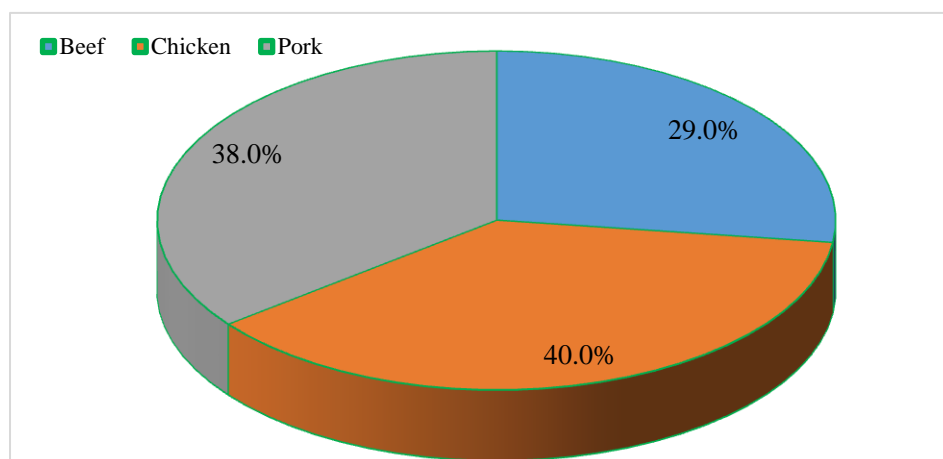


Figure 2. Prevalence of *Salmonella enterica* in imported beef, chicken, and pork samples obtained from Tema Metropolis, Ghana

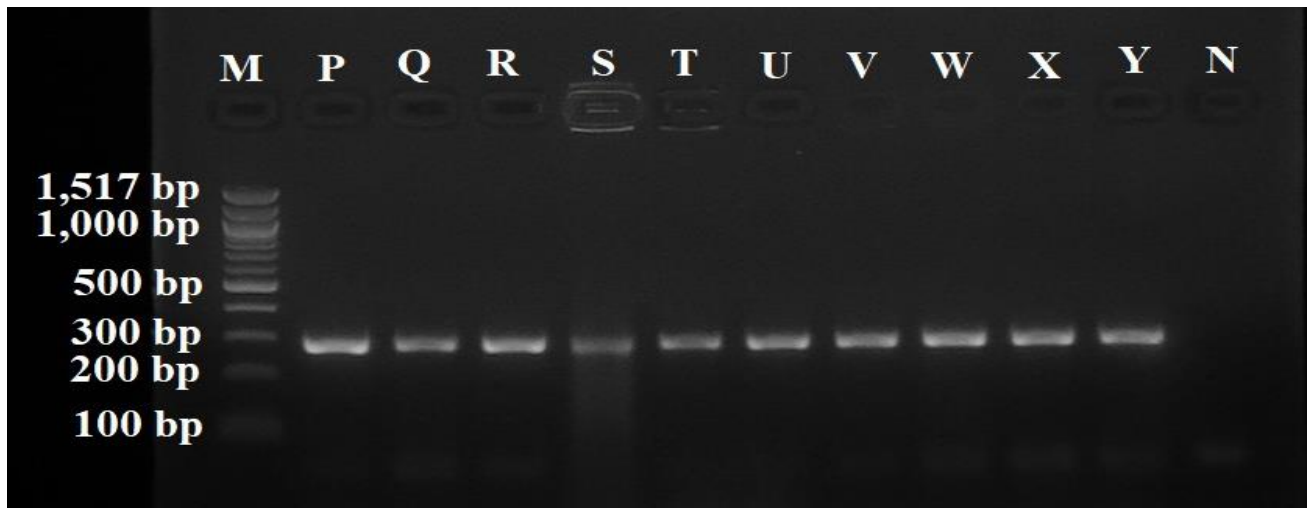


Figure 3. The bands of *Salmonella enterica* isolated from imported meats obtained from Tema Metropolis, Ghana. M: 100bp DNA ladder (New England Biolabs), P: Positive control (*Salmonella enterica* ATCC 14028), Q to Y: *Salmonella enterica* isolates (~284 bp), and N: Negative control (No DNA/Template).

In the present study, *S. enterica* were found in the imported chicken, pork, and beef samples obtained from the Tema Metropolis, Ghana. The contamination of the meat could be from the origin country, since not all meat samples were tested microbiologically at the ports of Ghana before being allowed into the country or during handling in Ghana. The contamination of different types of meat in different countries has been reported. In Southern Germany, Vidas' system for *Salmonella* identification found 1.8% of pork and 0.6% of beef to be positive for *Salmonella*, while the culture method found 1.1% of pork and 0.1% of beef to be positive for *Salmonella* (Meyer et al., 2010). The higher prevalence of *Salmonella* in pork samples compared to beef samples aligns with the present results. The presence of *S. enterica* in beef and pork during the present study was higher than that of Meyer et al. (2010). Poultry meat (29.3%) and beef (16.0%) samples from butcher shops and delicatessens in Bolu, Western Turkey, were found to be positive for *Salmonella* species using the cultural method (Arslan and Eyi, 2010). Additionally, the prevalence of *S. enterica* in beef samples was higher in the current study than that of Arslan and Eyi (2010). However, the prevalence of *S. enterica* in chicken meat was lower than that reported by Arslan and Eyi (2010). In the USA, 4.2% of chicken, 3.3% of pork, and 1.9% of beef meat were identified to be contaminated with *Salmonella* using the cultural method (Zhao et al., 2001), which were all lower than the findings of the present study. In Gondar, Ethiopia, Ejo et al. (2016) examined 50 raw meats using the cultural method and found 12.0% of the samples to be positive for *Salmonella*. While Yang et al. (2019) reported a higher prevalence in pork (37.3%) than in beef (16.1%) in China, the present study found a lower overall prevalence in pork but not in beef. Differences in the rate of contamination of meats could be attributed to the differences in the country or city of isolation, observance of standard and hygienic practices in animal and meat handling, the type of analyzed meat, and the isolation method employed (Zhao et al., 2001; Meyer et al., 2010).

Resistance of *Salmonella enterica* isolates

The resistance of *S. enterica* isolated from meat samples is presented in Table 1. The *S. enterica* isolates were 36.7% resistant to azithromycin and 20.0% resistant to amoxicillin. The *S. enterica* isolates were highly susceptible to imipenem (100.0%), ciprofloxacin (96.7%), tetracycline (96.7%), chloramphenicol (95.0%), trimethoprim (81.7%), gentamicin (70.0%), and ceftriaxone (68.3%). Furthermore, *S. enterica* exhibited relatively higher intermediate resistance to amoxicillin (48.3%) and azithromycin (36.7%).

Table 1. Resistance of *Salmonella enterica* isolated from imported chicken, beef, and pork meats in Tema, Ghana

Antibiotics	Susceptibility (%)	Intermediate resistance (%)	Resistant (%)
Amoxicillin 30µg (A)	31.7	48.3	20.0
Azithromycin 15µg (Ath)	26.7	36.7	36.7
Ceftriaxone 30µg (Cro)	68.3	26.7	5.0
Chloramphenicol 30µg (C)	95.0	0.0	5.0
Ciprofloxacin 5µg (Cip)	96.7	3.3	0.0
Gentamicin 10µg (Gm)	70.0	13.3	16.7
Imipenem 10µg (Imi)	100.0	0.0	0.0
Tetracycline 30µg (T)	96.7	0.0	3.3
Trimethoprim 2.5µg (Tm)	81.7	15.0	3.3
Overall	74.1	15.9	10.0

Multiple antibiotic resistance index and antibiotic resistance profile

The MAR index and antibiotic resistance profile of each *S. enterica* from the chicken, beef, and pork samples are shown in Table 2. The MAR index was in the range of 0.1 to 0.6, and 12 different resistance patterns occurred. Resistance to azithromycin alone was the most common pattern, which was observed in 12 isolates. The resistance to five, four, and three different antibiotics were 3.0%, 3.0% and 12.7%, respectively. Furthermore, 27 *S. enterica* isolates were not resistant to any of the antibiotics tested.

Table 2. Multiple antibiotic resistance index and antibiotic resistance pattern of individual *Salmonella enterica* isolated from imported chicken, beef, and pork meats in Tema, Ghana

Source	Code	Number of antibiotics	Antibiotic-resistant profile	MAR index
Foreign Beef	BLB4	5	Cro-A-C-Tm-T	0.6
Foreign Beef	CB4	4	A-C-Gm-T	0.4
Foreign Pork	BP5	3	Tm-Gm-Ath	0.3
Foreign Beef	BLB4	3	Cro-A-Ath	0.3
Foreign Chicken	CC5	3	A-Gm-Ath	0.3
Foreign Beef	UB1	3	A-C-Ath	0.3
Foreign Beef	CB10	2	Cro-Ath	0.2
Foreign Chicken	BC3	2	A-Gm	0.2
Foreign Chicken	UC8	2	A-Gm	0.2
Foreign Beef	BB4	2	A-Ath	0.2
Foreign Chicken	CC8	2	A-Ath	0.2
Foreign Pork	UP7	2	A-Ath	0.2
Foreign Beef	UB6	1	Gm	0.1
Foreign Chicken	BC7	1	Gm	0.1
Foreign Chicken	BLC6	1	Gm	0.1
Foreign Pork	UP2	1	Gm	0.1
Foreign Pork	UP9	1	Gm	0.1
Foreign Beef	BB2	1	Ath	0.1
Foreign Beef	BB5	1	Ath	0.1
Foreign Beef	BLB6	1	Ath	0.1
Foreign Chicken	BC1	1	Ath	0.1
Foreign Chicken	BC6	1	Ath	0.1
Foreign Chicken	CC9	1	Ath	0.1
Foreign Chicken	UC1	1	Ath	0.1
Foreign Chicken	UC10	1	Ath	0.1
Foreign Pork	BP10	1	Ath	0.1
Foreign Pork	BP8	1	Ath	0.1
Foreign Pork	CP2	1	Ath	0.1
Foreign Pork	CP3	1	Ath	0.1
Foreign Pork	CP4	1	Ath	0.1
Foreign Pork	NP5	1	Ath	0.1
Foreign Beef	CB7	1	A	0.1
Foreign Chicken	BC10	1	A	0.1

MAR: Multiple antibiotic resistance, Ceftriaxone 30µg (Cro), Tetracycline 30µg (T), Azithromycin 15µg (Ath), Chloramphenicol 30µg (C), Amoxicillin 30µg (A), Ciprofloxacin 5µg (Cip), Imipenem 10µg (Imi), Gentamicin 10µg (Gm), Trimethoprim 2.5 µg (Tm).

Using antibiotics in animal production and non-adherence to withdrawal periods before slaughtering the animals are the main factors driving the deposition of antibiotic residues in meat (Thai et al., 2012; Adzitey et al., 2013). Several studies have shown that *Salmonella* from different meat sources exhibited some level of resistance to commonly used antibiotics (Ejo et al., 2016; Yang et al., 2019; Aduah et al., 2023). Outbreaks resulting from antibiotic-resistant bacteria, including *Salmonella* species, are of public health concern, especially when consumed via meat (CDC, 2021). In contrast to the high resistance rates reported in US meat samples to tetracycline (80%), ceftriaxone (16%), and chloramphenicol (16%; White et al., 2001), the isolates in the current study exhibited lower resistance to these antibiotics. Resistance to gentamicin, however, was higher than the 4% previously reported. However, the current findings on ciprofloxacin and those of White et al. (2001) were similar. In China, Yang et al. (2019) examined meat samples for *Salmonella* and

reported resistance, intermediate resistance and susceptibility to ceftriaxone (5.5%, 0.9% and 93.6%), chloramphenicol (30.3%, 7.8% and 61.9%), ciprofloxacin (16.1%, 34.9% and 49.1%), gentamicin (15.1%, 5.0% and 9.8%), imipenem (0.9%, 2.8% and 96.3%) and tetracycline (65.6%, 5.5% and 28.9%), respectively, which were comparable to the present results. In contrast to the study conducted by Ejo et al. (2016) in Ethiopia, who reported 100% resistance to tetracycline and 33.3% to gentamicin with no resistance to amoxicillin or ceftriaxone, the present study found a distinct profile, with lower resistance to tetracycline and gentamicin, coupled with emerging resistance to amoxicillin and ceftriaxone. While Thai et al. (2012) documented considerable resistance to tetracycline (58.5%), chloramphenicol (37.3%), and trimethoprim (34.0%) in Vietnam, the resistance levels for these antibiotics were low during the present study. The present findings confirmed that *Salmonella* resistance to one or more antibiotics, including multidrug resistance, is a persistent global issue, as reported in several studies (Arslan and Eyi, 2010; Thai et al., 2012; Ejo et al., 2016; Yang et al., 2019).

CONCLUSION

Salmonella enterica was detected in imported beef, poultry, and pork samples from the Tema Metropolis, Ghana, with imported chicken being the most frequently contaminated source. *Salmonella* isolates demonstrated antimicrobial resistance, most notably to azithromycin and amoxicillin. One *Salmonella enterica* isolated from imported beef was resistant to five different antibiotics. Thus, imported meats sold at Tema Metropolis can be a potential source for transferring *S. enterica* resistant to some antibiotics. Further studies on the genetic diversity between *S. enterica* isolates from imported and local meats are recommended.

DECLARATIONS

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Authors' contributions

Frederick Adzitey conceived the idea, provided funds, was involved in data collection, and wrote the first draft. Innocent Allan Anachinaba was involved in data collection, provided funds, and data analysis. Agus Susilo conceived the idea, was involved in funding, and proofreading. Nurul Huda also conceived the idea, secured funding, and reviewed. All authors have reviewed and approved the final edition of the manuscript for publication.

Competing interests

The authors declared that they have no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors explicitly and transparently disclose that no AI tool was used in the whole of the article.

Availability of data and materials

The data obtained in the present study are available from the corresponding author upon reasonable request.

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