



Prevalence and Antibiotic Susceptibility Pattern of *Salmonella* Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author ARA designed the study, performed the laboratory experiments, performed the statistical analysis, and drafted it. Author CKSS provided the media and laboratory equipment for the work and corrected the drafted manuscript. Author SWK assisted the laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research was carried out to determine the prevalence of *Salmonella* species from smoked fish sold in the three major markets in Tamale Metropolis and examined the isolates resistance patterns to various antibiotics.

Study Design: The study was in two parts which include administering questionnaires and collecting samples. The second part was the laboratory analysis to detect *Salmonella* species from the collected samples.

Place and Duration of Study: The study was carried out in the Spanish laboratory (microbiology section) of the Department of Biotechnology, Faculty of Biosciences, of the University for Development Studies.

Methodology: A total of one hundred samples were examined. The samples were enriched on buffered peptone water and inoculated on Modified Semi-Solid Rappaport Vassiliadis. Xylose Lysine Deoxycholate was used to identify the bacteria, and Simons Citrate agar was used for the biochemical test. The antibiotic test was done by using the Kirby-Bauer disc diffusion test.

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Results: After the laboratory analysis, 67 (67%) out of the 100 samples were confirmed to be positive for *Salmonella* species of which Cut fish (11.9%), Redfish (11.9%), Mudfish (11.9%), and Chale fish (11.9%) recorded the highest contamination, and among the three markets, Central market had the highest fish contamination. The resistance patterns of the isolates to the various antibiotics used were; Ciprofloxacin (2.98%), Ceftriaxone (34.32%), Ampicillin (83.58%), Doxycycline (88.05%), Amoxicillin/Clavulanic acid (91.04%), and Oxytetracycline (92.53%).
Conclusion: This study revealed that smoked fish sold in the Tamale Metropolis contained *Salmonella* positive that can cause food poisoning and other gastrointestinal problems.

Keywords: Salmonella; smoked fish; antibiotics; susceptibility.

1. INTRODUCTION

The world's per capita fish from outcome growth of aquaculture fisheries supply intended for human consumption is about 50%. It has become an important food commodity worldwide, one of the popular dietary options due to its nutritional characteristics [1,2]. In Africa, one of the foods that provide nutrients in its proper proportion of protein to humanity is fish, representing 82% of animal protein intake [3]. Ghanaians consume 75% of fish harvested since they consider it the best animal protein source [4]. Ghanaians prefer the intake of traditional fish processing to any other method because of its good flavor, taste, and affordability and is believed to be the best preservation [5,6]. There is no supervision or quality assurance on the equipment and the buildings used to process smoked fish in Ghana. Since there is no quality control, the fish is smoked in an open space or unstructured housing, calling for pathogenic microorganisms [7]. Since fish is a suitable medium for pathogenic organisms such as *Salmonella* species, its safety must be considered before meeting the standard requirements [8,9]. In Ghana, smoked fish could be infected with *Salmonella* species due to improper handling of fish and filthy places where fish processing and smoking occur [10,11]. The contamination of fish occurs during processing, handling, storing, and inadequate heat application. However, these ways of fish contamination could contribute to *Salmonella* species and could foodborne disease in Ghana [12,13].

Moreover, old newspapers, cement papers, and polyethylene bags being used for wrapping or packaging the smoked fish during storage and handling of fish have also contributed to the contamination of *Salmonella* species [14]. Sardines smoked fish were contaminated, having a total bacterial count to be (2,547 cfu) and a fungal count to be (1,023 cfu) in Kenya (Mombasa) [15].

Salmonella species were prevalent in smoked fish sold in the Benin Metropolis (Nigeria) [16]. Contrary to these findings, there is no adequate data on *Salmonella* species prevalence level in smoked fish sold in most Ghanaian markets.

The challenges confront now of which Ghana is not exempted from is poor hygienic states where the fish is harvested and where the fish is being sold [17,18]. Therefore, the objectives of this study were to assess the prevalence rate and antibiotic susceptibility pattern of *Salmonella* species in smoked fish sold in the Tamale Metropolis (Ghana).

2. MATERIALS AND METHODS

2.1 Sample Collection

One hundred (100) samples were randomly purchased from smoked fish sellers at three (3) different markets within the Tamale metropolis. All samples were wrapped with a black polythene bag (actually, this is the standard procedure they practice in the markets) and labeled according to the type of fish and the market in which the samples were bought. The samples were purchased with a questionnaire. The samples were then kept in the ice chest containing ice block to keep the temperature of 4 °C. The samples were then transported to the Spanish laboratory complex of the University for Development Studies, Nyankpala campus for bacteriological studies immediately after transportation.

2.2 Media Preparation

The media were obtained from Oxoid company and were prepared according to the Manufacturer's direction or protocol.

2.2.1 Preparation of buffered peptone water

Twenty grams was added to one (1) liter of sterile water and mixed gently in a round-bottom

flask. The medium was sterilized by autoclaving at 121 °C for 15 minutes. It was then allowed to cool and stored in a fridge.

2.2.2 Sample preparation/ pre-enrichment

Samples were brought out from the ice chest after transportation to the microbiology laboratory. Twenty-five grams (25 g) of each smoked fish sample were weighed with a sterile bag using an electronic balance scale. The sample was suspended into 225 ml of buffered peptone water and then homogenized gently with hand and incubated at the temperature of 42 °C for 18-24 hours.

2.2.3 Preparation, identification, and isolation on modified semi-solid rappaport vassiliadis (MSRV) medium

The MSRV medium was prepared by suspending 15.8 g in 500 ml of sterile water and boiled with frequent agitation. It was then allowed to cool under the lamina flow chamber and then poured into sterilized petri dishes to air dry at room temperature. After that, 0.1 ml of pre-enriched broth was pipetted, and three drops were allocated on the MSRV media plates. The inoculated MSRV media were incubated at a temperature of 42 °C for 18-24 hours with an upright side. After the incubation, the halo growth around the inoculation area were suspected to be *Salmonella* species.

2.2.4 Preparation of Xylose Lysine Deoxycholate (XLD) medium

Fifty-three grams (53 g) of XLD medium were measured and suspended in 1 liter of distilled water and heated with frequent agitation. It was then allowed to cool at room temperature and poured into sterilized petri dishes to solidify under the lamina flow chamber.

2.2.5 Identification and isolation on XLD medium

The suspected *Salmonella* colonies on the MSRV media were scooped using a loop full of inoculum and streaked on Xylose Lysine Deoxycholate (XLD) agar plates, incubated at 37 °C for 18-24 hours. The suspected *Salmonella* colonies on the XLD were found to be red colonies with black centers, which indicated the presence of *Salmonella* species. This is due to lysine, which helped the *Salmonella* grouping appear different from the other non-pathogens because the *Salmonella* fermented the Xylose.

2.2.6 Biochemical identification of *Salmonella* species

The suspected *Salmonella* colonies found on XLD agar plates were confirmed biochemically using Simmons citrate agar plates and incubated for 18-24 hours at 37 °C.

The positive result gave a blue color change, indicating a growth of bacteria (*Salmonella*). Because the bacteria use citrate as their primary source of carbon, that results in alkalinity for metabolism reaction. Alternately, the negative result gave no growth of bacteria (*Salmonella*) and remained the same as the green color of the medium used.

2.2.7 Identification of cultures on nutrient agar

The suspected *Salmonella* colonies on XLD were scooped using a loop full of inoculum, streaked on a nutrient agar plate, and incubated for 24 hours at 37 °C in an inverted position. Pure *Salmonella* colonies were obtained, which was colorless and transparent, and it was stored for antibiotics susceptibility test.

2.3 Antibiotic Susceptibility Test

The antibiotics susceptibility test was done by using Kirby-Bauer disc diffusion methods. Their free zones diameter of inhibition was measured in millimeters for each of the antibiotics. The diameter of their inhibition was expressed as resistance (R), susceptible (S), or intermediate (I). Compared with European Union Committee on Antibiotics Susceptibility Testing (EUCAST) breakpoint 2019, Clinical and Laboratory Standards Institute (CLSI) breakpoint 2018, and French breakpoint for antibiotic susceptibility testing.

3. RESULTS AND DISCUSSION

3.1 Study Population Characteristics

The survey observed that all the sellers were female, representing (100%). The most age range engaged in selling is the ages of 21-30 (38%). Furthermore, for educational level, apart from (4%) of the respondents who had tertiary education and 7% who had senior high school education. The majority of the respondents (41%) had no formal education. The rest had an elementary education and junior high school education, representing 10% and 38%, respectively.

3.2 Qualitative data- Detection of *Salmonella* species.

One hundred samples (100) were randomly selected from three (3) markets: Central market, Aboabo market, and Lamashegu market across Tamale metropolis.

Out of one hundred samples collected, forty (40) samples were collected from the Central Market, thirty (30) were collected each from Aboabo and Lamashegu markets.

Table 1. Gender of smoked fish sellers

Gender	Number	Percentage (%)
Male	0	0
Female	100	100

With one hundred (100) samples collected, 67 % were confirmed positive on Simmons citrate agar medium.

Of the forty (40) samples that were collected from the Central market, thirty-one (31) were contaminated with a percentage of (46 %). For Aboabo and Lamashegu markets, the number of contaminations was twenty (20) and sixteen (16), representing 30 % and 24 %, respectively. The various types of smoked fish collected from these three (3) markets were Salmon fish, Herring fish,

Horse mackle fish, Mudfish, Chale fish, Redfish, Catfish, Doctor Fish, Tuna fish, and Mullet fish. These ten (10) types of smoked fish were repeated ten (10) times each. The number of contaminations that were confirmed from these ten types of fishes are 6 (8.9%), 5 (7.4%), 7 (10.4%), 8 (11.9%), 8 (11.9%), 8 (11.9%), 8 (11.9%), 6 (8.9%), 4 (5.9%) and 7 (10.4%) respectively.

Table 2. Age of smoked fish sellers

Age group	Number	Percentage (%)
10-20	6	6
21-30	38	38
31-40	22	22
41-50	27	27
60+	7	7

Table 3. Educational background of smoked fish sellers

Educational level	Number	Percentage (%)
No formal school	41	41
Elementary school	10	10
Junior high school	38	38
Senior high school	7	7
Tertiary	4	4

Smoked fish contamination in markets

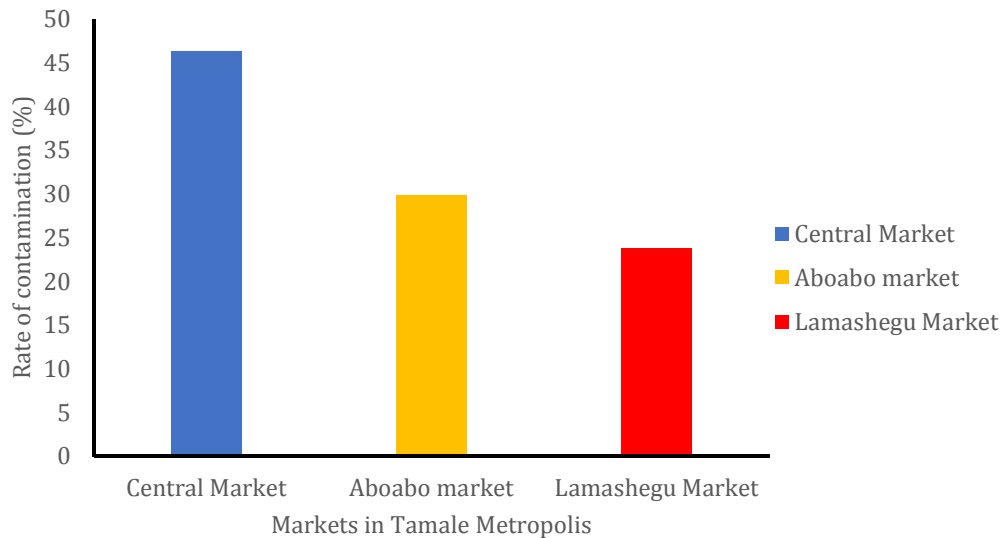


Fig. 1. Show the comparison of contamination of fish samples from different markets

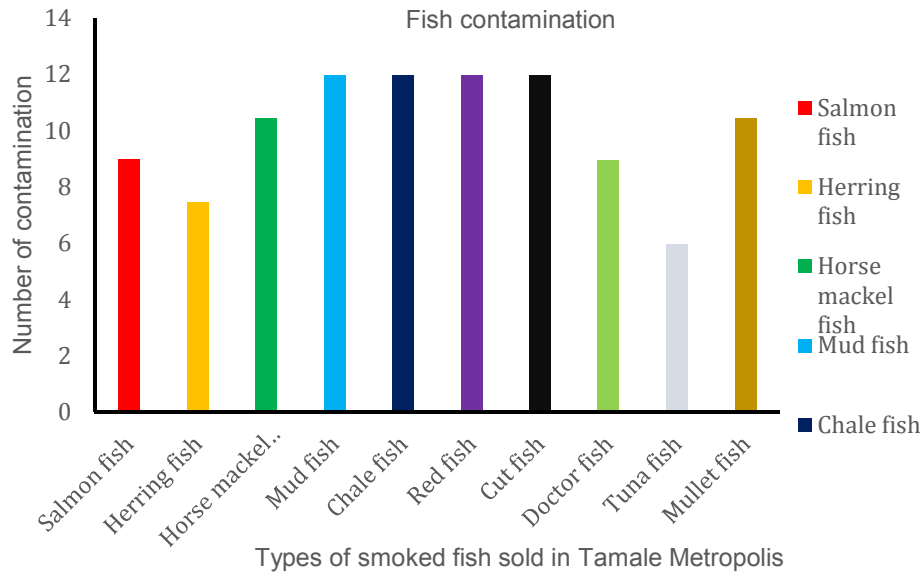


Fig. 2. The comparison of various types of smoked fish sold at Tamale Metropolis contaminated with *Salmonella*

This study found out that, the overall prevalence of *Salmonella* species in some smoked fish from three (3) markets across the Tamale Metropolis was relatively high (67 %). This result confirms with [19] report, which recorded 67 % contamination of fish samples with *Salmonella* spp. This contamination was because markets within the Tamale Metropolis are not in a good state to sell fish. This contamination could cause foodborne diseases, of which consumers could be at risk to public health care due to the bacterial being resistant to antibiotics [20]. The prevalence of *Salmonella* species in smoked fish in Akwa Ibom State (Nigeria) confirmed this research result. This finding agrees with [21], which reported a prevalence of *Salmonella* Typhimurium and *Salmonella* Paratyphi from horse mackel with their respective percentages of 1.83 % and 1.52 %. The findings from this study also agree with [22] report, which recorded 20 % and 6.7 % of *Salmonella* Rubislam and *Salmonella* Senegal from Mullet fish samples and 13.3 % of *Salmonella* Senegal from Catfish. This result is also similar to the work done by [23] which reported that, 65 % and 30 % of *Salmonella* species were present in fish sold at the local market and that of supper shop, respectively. [24] indicated that, fish sold in local markets are not of high standard quality for consumption.

Again, works done by other researchers also recorded a lower prevalence of *Salmonella*

species in smoked fish compared to the results from this study; [25] recorded 4 %, [26] reported 1.8 %, [27] also recorded 3.2 % of *Salmonella* species. [16] Recorded 20 % of *Salmonella* species from Benin Metropolis (Nigeria). All these results from the research could pose a high risk of sickness. The result in this study is higher than several types of research conducted on the prevalence of *Salmonella* species. Contamination of street and market vended smoked fish with *Salmonella* species was reported higher in other research, with an incidence rate of 83 % as compiled by [28], 100 % in three wholesale markets, 100 % in three retail shops said by [29].

3.3 Mode of Storage and Handling

From the survey, we observed that the type of storage practice they adopted most is to smoke the fish again after market and stored in the kitchen or storeroom, which recorded (42 %). The handling practice was touching the fish with the bare hand, which recorded the highest percentage (63 %).

SMK= Smoke again after market and store in the kitchen, SMF= Smoke aftermarket, and store in the fridge, WPK= Wrapped with paper and store at the kitchen, WPF= Wrapped with paper and store in the fridge, TBH= Touch with bear hand, TCH= Touch with covered hand, NT= Not touch In developing countries, the open place is where

smoked fish are purchased for consumption, and this has led to microbial contamination which has resulted to public health problems [30]. Fish serves as the passageway for microorganisms. Some of the significant ways that could contaminate fish are; physical damage, storage, and handling, which causes the fish to smell due to dirt and pathogenic microorganisms [31].

However, transportation, storage, and handling procedures are the activities that cause contamination in the supply food chain [32]. This study observed that, vendors in Tamale Metropolis employ the various procedures of handling, storing, and transporting smoked fish from one town or region to another had contributed to microbial contamination. However, the equipment and unhygienic standards adopted by fish handlers could contaminate such products during those activities, and the overcrowding of smoked fish in baskets or containers could lead to contamination. This is because cross-contamination could occur from one fish to another, as indicated by [33] that, fish contamination is due to fish handling, transportation, and cross-contamination.

The study had few market sellers (5 %) that smoke the remaining fish aftermarket and store in the fridge, while 23 % also wrap the fish with paper and stored in the fridge but result from [34] and [35] report that, a suitable temperature associated with storage, appropriate processing steps, and the proper freezing system can prevent contamination. *Salmonella* species from wrapped smoked fish could result from the paper or polyethylene bags that are being used. This finding is following the work conducted by [36] which reported that, the availability of old newspaper, cement papers, and polyethylene bags used for wrapping or packaging the smoked fish during storage and handling of fish contribute to the prevalence of *Salmonella* species. The

presence of *Salmonella* species could be a result of the unhygienic condition under which the smoked fish is handled and kept. However, the study also revealed that moisture content and pH encourage microbial load [37]. Findings of this study revealed that, the most practicing storage activities by smoked fish sellers (42 %) after selling are; the fish is being smoke for some minutes and stored in their kitchen or storeroom while 30 % of them wrapped the fish with papers and store them in their storeroom or kitchen. These practices resulted in the contamination of smoked fish because it was openly exposed to the environment that is being stored. Also, the covering of smoked fish with paper could generate heat that would influence the growth of bacteria. Results from [38] and [21] indicated that, microbial contamination had been found in smoked fish due to unhygienic practices during processing, packaging, storage, poor environmental condition, and handling activities practiced by sellers and buyers contribute to the contamination of fishes. However, this study recorded a higher percentage (63 %) of buyers touching the fish with their bare hand without disinfecting their hand, and this could cause cross-contamination from the buyers.

Moreover, the study recorded few buyers (7 %) that covered their hand before touching the fish, and 30 % of buyers do not touch the smoked fish. Therefore this study showed that, smoked fish sold at Tamale Metropolis poses serious health problems to buyers and sellers as there is a high prevalence of *Salmonella* species. Since these smoked fish can be eaten raw without further processing, the study advocates the need to adopt good storage, handling, transportation, and proper hygienic practice for smoked fish to follow the European Union Regulation on seafood products.

Table 4. The detailed result of the mode of storage and handling

Mode of Storage & Handling	Number of contaminations	Percentage (%)
A. Storage		
SMK	30	44.77
SMF	5	7.46
WPK	20	29.85
WPF	12	17.91
Total	67	100
B. Handling		
TBH	40	59.70
TCH	7	10.44
NT	20	29.85
Total	67	100

3.4 The Resistance Levels of the Antibiotics

For the 67 positive *Salmonella* species, 62 were resistant to Oxytetracycline, four were susceptible, and one intermediate, which represents (92.53 %), (5.97 %) and (1.49 %) respectively. Out of 67 isolates, 56 (83.58 %), 11 (16.41 %) were resistant and susceptible to ampicillin. Sixty-one (61) (91.04 %) out of the total isolates were resistant to amoxicillin, and only 6 (8.95 %) were susceptible. Fifty-nine (59) (88.05 %) were also resistance to doxycycline of which 8 (11.94 %) were susceptible. Twenty-three (23) (34.32 %) out of 67 were resistance to ceftriaxone with 38 (56.71 %) being susceptible and 6 (8.95 %) intermediate. Interestingly, only 2 (2.98 %) out of 67 were resistant to ciprofloxacin, and 65 (97.01 %) were susceptible.

3.5 Multidrug Resistance Levels of the Isolates

All the isolates resistant to more than two antibiotics were grouped into three or more antibiotics (multidrug resistance). From the result, 61 out 67 were resistant to three or more

antibiotics, representing (91.04 %) of multidrug resistance. The details of the group were as follows; out of 61, 9 (14.75 %) were resistant to three antibiotics, 32 (52.45 %) were resistant to four antibiotics, 19 (31.14 %) were also resistant to five antibiotics, and only 1 (1.63 %) were resistance to six antibiotics.

Salmonella species are important pathogens of global concern. The emergence of antibiotic-resistant strains is mainly due to the indiscriminate use of antibiotics in animal feeds as growth promoters, animal disease treatment, and therapeutic agents for treating humans [39,40].

Following the trends in the antibiotic's susceptibility pattern of *Salmonella* species, this research showed few percentages (32.84 %) of the isolates were susceptible to all the six antibiotics tested. In comparison, 65.42 % were resistant, and only 1.74 % were intermediate to all the antibiotics. This study recorded the least resistance of ciprofloxacin with 2.98 %, followed by ceftriaxone with 34.32 %, ampicillin 83.58 %, doxycycline 88.05 %, amoxicillin/ Clavulanic acid 91.04 %, and Oxytetracycline being the highest resistance with the percentage of 92.53 %.

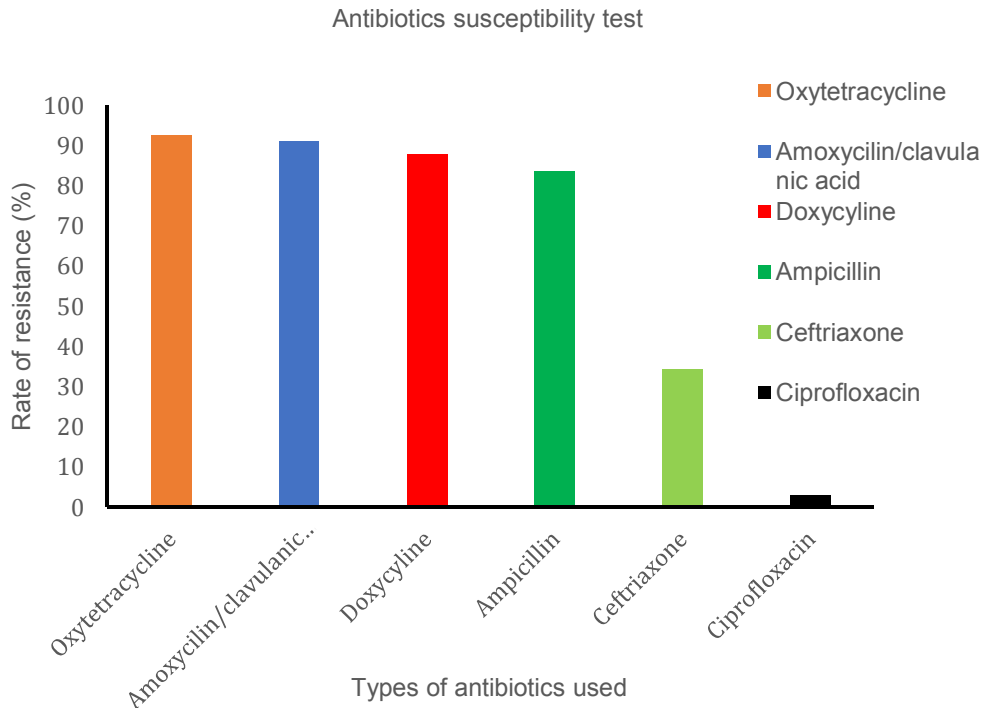


Fig. 3. Percentages of resistance levels to the various antibiotics

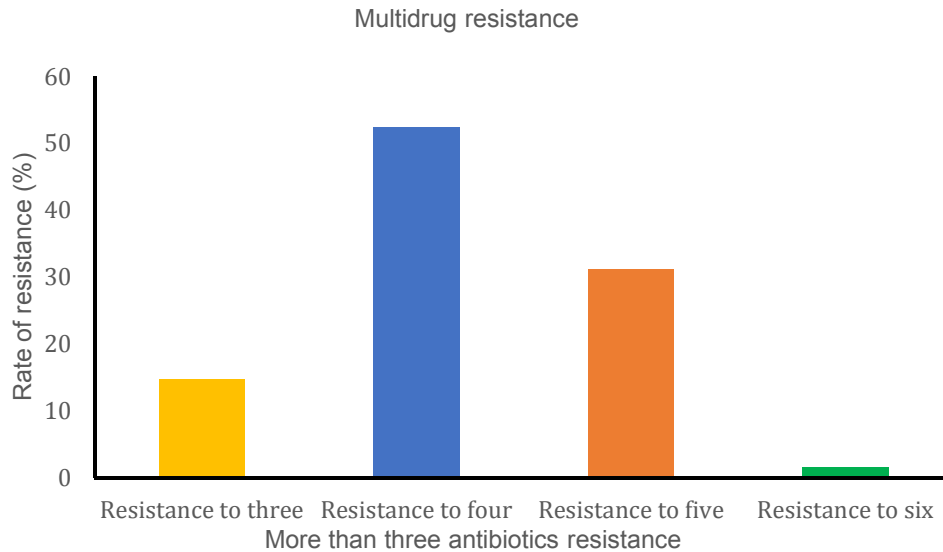


Fig. 4. Percentages of isolates that were resistant to three or more antibiotics

This result means that, there is a high proportion of the isolates being resistant to the first-line antibiotics such as amoxicillin/ Clavulanic acid, ampicillin, and tetracycline groups such as Oxytetracycline and doxycycline, and this could be a result of too much exposure and misuse of this antibiotics in aquaculture fisheries. This result is similar to previous work done by [41], who recorded high resistance to ampicillin [75 %] and other first-line antibiotics in Kenya.

Previously, [42] also recorded high resistance to ampicillin (72 %), chloramphenicol (72 %), and cotrimoxazole (70 %). Contrary to the current research work done by [43] reported that, *Salmonella* species show an increased susceptibility rate of ampicillin, cotrimoxazole, and chloramphenicol with 97.6 %, 98.8 %, and 98.8 %, respectively. This is because, it could be due to not being used for a long time for treatment. [44] Reported 100% resistance of *Salmonella* species to ciprofloxacin and 27% resistant to ceftazidime from food in Malaysia, which is far above what this study recorded (2.98%). This result is also above this study's result. Though this research recorded low resistance of fluoroquinolones and cephalosporins, but researchers are still worry about *Salmonella* isolates showing resistance or reduced sensitivity to ciprofloxacin and ceftriaxone [42]. This research result contradicts [45], who recorded no resistance rate of ceftriaxone, ciprofloxacin, and ceftazidime of *Salmonella* species isolated from food vendors in

Jirapa in the Upper West region of Ghana. Similarly, previous results of [16] recorded 97.06% susceptibility to ciprofloxacin and 0% resistance to *Salmonella* species from drinking water and recorded 70.59% susceptibility to ceftriaxone and 11.76% resistance rate and indicated that care must be taken in order not to lose their sensitivity.

For the result of multidrug resistance, 91.04% were recorded as multidrug resistance. Resistant to four antibiotics recorded the highest. This result follows research work with 100% multidrug resistance of the *Salmonella* isolates tested in Malawi [14]. Other works also reported 70% of multidrug-resistant [41]. This research result contradicts with what [46] had in their result. They recorded (0%) multidrug-resistant of *Salmonella* strains in their studies in Nepal. These multidrug-resistant patterns could pose a severe threat to the treatment of invasion *Salmonella* infection.

4. CONCLUSION

The prevalence rate of *Salmonella* species in smoked fish sold in the Tamale Metropolis is 67%.

Antibiotics that were susceptible to *Salmonella* isolates from smoked fish were ciprofloxacin with a rate of (97%). Oxytetracycline recorded the highest resistance rate (92.53%) of the antibiotics that were used. The multidrug-resistant rate was recorded to be (91%).

The majority of the smoked fish vendors were in the age range from 21-30, and most vendors had no formal education, which may also contribute to the level of contamination.

To avoid contamination, smoked fish sellers and buyers ought to handle, process, and store fish under hygienic conditions.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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