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Research Article

Prevalence of Multi-antibiotics resistance Salmonella in Fish from selected lakes in Bishoftu Town, Central Ethiopia

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Abstract

Antimicrobial-resistant *Salmonella* spp. are important foodborne pathogens capable of causing disease in humans and animals .Thus, a cross-sectional study was conducted from November 2024 to June 2025 to determine the prevalence and antimicrobial susceptibility of *Salmonella* spp. isolated from fish harvested from selected lakes (Kilole, Hora, and Cheleleka) in Bishoftu, Central Ethiopia.. Systematic random sampling was used to collect 280 samples comprising skin (n=70), gills (n=70), muscle (n=70), and intestinal contents (n=70). Samples were processed using buffered peptone water and Rappaport–Vassiliadis enrichment, followed by culture on Xylose Lysine Deoxycholate agar and biochemical identification. Antimicrobial susceptibility was determined using the Kirby–Bauer disk diffusion method against nine antimicrobial agents. Data were analyzed using descriptive statistics and regression analysis in STATA; statistical significance was set at $P<0.05$. Prevalence differed significantly among lakes ($P=0.042$), with 7.5% in Kilole, 10.3% in Hora, and 25.0% in Cheleleka. By sample type, *Salmonella* was detected in skin (7.1%), gills (10.0%), muscle (17.1%), and intestinal contents (4.3%), with no significant difference among sample types. All isolates tested for antimicrobial susceptibility showed multidrug resistance, with resistance to three or more antimicrobial agents. Resistance was highest to tetracycline and streptomycin, each reported at 100%, followed by cefotaxime at 59.2%. The detection of *Salmonella* spp. in fish from all three-study lakes and the high level of multidrug resistance indicate a potential food safety and public health concern. Improved water-quality management, hygienic fish handling, antimicrobial stewardship, and continued surveillance of antimicrobial-resistant *Salmonella* are warranted.

Keywords: Salmonella; fish; antimicrobial resistance; multidrug resistance; lakes; Bishoftu; Ethiopia

1. Introduction

Fish are highly nutritious foods that provide high-quality protein, essential fatty acids, vitamins (particularly vitamins D and B2), and important minerals such as iron, zinc, and phosphorus (1, 11). Globally, aquaculture has become an increasingly important source of fish, contributing approximately 50% of the total fish and fishery products supplied for human consumption (12). In Ethiopia, the annual fish production potential is estimated at more than 51,000 tons. Despite this considerable resource, the fisheries sector contributes only marginally to the national economy due to both natural and human-induced constraints, including inadequate management practices and a shortage of trained personnel (14).

Salmonella is a genus of Gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria belonging to the family Enterobacteriaceae.

These facultative intracellular pathogens measure approximately 2–3 µm and infect both humans and animals. The genus comprises two species, Salmonella enterica and Salmonella bongori, both of which are associated with infectious diseases. Clinically, three major syndromes characterize salmonellosis: septicemia, acute enteritis, and chronic enteritis (7). Among these, non-typhoidal S. enterica is a leading cause of foodborne illness, producing clinical manifestations such as gastroenteritis, bacteremia, and, less commonly, typhoid fever. Gastroenteritis typically presents with abdominal pain, nausea, vomiting, diarrhea, and headache (3). In recent years, the emergence of antimicrobial-resistant Salmonella strains has become a significant public health concern, contributing to increased morbidity, mortality, and treatment costs associated with salmonellosis (5).

The increasing interaction between humans and aquatic animals, particularly fish that may harbor infectious agents, represents an important public health concern. Many bacterial and parasitic pathogens associated with fish are zoonotic and can be transmitted to humans through the consumption of contaminated fish or direct contact with infected fish and contaminated water via wounds or skin abrasions (7). Rapid and accurate detection of bacterial pathogens is essential for ensuring food safety and monitoring contamination in the food supply chain. Although microbial contamination does not always result in disease, environmental stressors can disrupt the balance between pathogens and their hosts, increasing the risk of infection (6). In Africa, surveillance of salmonellosis remains challenging because many laboratories lack the diagnostic capacity required to identify both typhoidal and non-typhoidal Salmonella infections (13).

In Bishoftu Town, fish harvested from local lakes are at risk of contamination due to unrestricted access of livestock and wildlife to the lakes, inflow of domestic drainage, improper waste disposal, including poultry carcasses, and flood runoff from surrounding areas. Furthermore, fish processing is commonly performed under traditional and unhygienic conditions, with gutting and filleting carried out on bare ground and limited attention to food safety. These conditions may increase the risk of contamination with zoonotic pathogens and the emergence of antimicrobial-resistant bacteria. Therefore, this study was conducted to determine the prevalence of multidrug-resistant Salmonella in fish production systems in Bishoftu Town, Central Ethiopia.

2. Materials and Methods

2.1 Study Area

The study was conducted in Bishoftu City Administration, Central Ethiopia, located approximately 45 km southeast of Addis Ababa. The area lies between 38°51′–39°04′ E longitude and 8°46′–8°59′ N latitude, covering about 1,750 km². Approximately 90% of the area consists of plain highlands at elevations ranging from 1,600 to 2,000 m above sea level. Bishoftu has a subtropical climate with a mean annual rainfall of about 860 mm. The main rainy season extends from June to September, followed by a dry period that is occasionally interrupted by short rains during February and March. The annual temperature ranges from 8°C to 28°C. The lakes in and around Bishoftu support several commercially important fish species belonging to the families Cyprinidae, Clariidae, and Cichlidae. The identified species include Cyprinus carpio, Labeobarbus intermedius, Clarias gariepinus, Oreochromis niloticus, and Tilapia zillii. The estimated mean annual fish catch was 2.3, 2.8, 1.1, and 1.6 tons per gear type for Lakes Kilole, Hora Arsedi, Koriftu, and Babogaya, respectively (10)..

2.2 Study Design and Sample Collection

A cross-sectional study was conducted from November 2024 to June 2025 to determine the prevalence of Salmonella and associated risk factors in fish production in Bishoftu City Administration, Central Ethiopia. Three lakes were purposively selected based on their fish production, the number of fishery operators, and their importance in the local fish value chain. Fish were selected using simple random sampling from harvesting nets. Freshly captured fish were handled individually using sterile arm-length gloves to prevent cross-contamination. The specimens were immediately transported under a cold chain to the Veterinary Public Health Laboratory, College of Veterinary Medicine and Agriculture, Addis Ababa University, for bacteriological analysis. 70 fish were examined, including 43 from Lake Kilole, 22 from Lake Hora, and 5 from Lake Cheleleka. From each fish, four samples—skin, gill, muscle, and intestinal contents were aseptically collected, resulting in 280 samples (70 skin, 70 gill, 70 muscle, and 70 intestinal samples). All specimens were cultured using selective media for the isolation and identification of Salmonella following conventional bacteriological procedures. Confirmed Salmonella isolates were subsequently subjected to antimicrobial susceptibility testing to determine their antibiotic resistance profiles and assess multidrug resistance patterns.

2.3 Laboratory Examination

2.3.1 Isolation and identification of Salmonella

As soon as arriving to the laboratory, each fish was dissected and sampled for skins, muscle intestinal content and gills A 25 mg of fish tissue samples were put into a sterile plastic bag and 225 ml of buffered peptone water was added and then homogenized using a stomacher (Salm.2010). Isolation of Salmonella on the enrichment and isolation media followed the ISO 6579-1:2017 technique (ISO-6579 ,2002). In belief, the homogenized combined sample was enriched with enrichment media and incubated for 24 hrs. at 37 °C. After 24 hrs of incubation, 0.1 ml of the pre- enrichment was transferred with a pipette to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth and incubated at 41.5°C for 24 hrs. Then loop full of the sample was inoculated on XLD agar selective media. The appearance of black per plate was suspected as Salmonella and taken for further biochemical test for confirmation of Salmonella. Those colonies suspected Salmonella on XLD was sub-cultured on Nutrient agar and subjected for Biochemical characterization of Salmonella by using the citrate test, TSI agar, Urea agar, methyl red and Voges Proskauer, Indole test, catalase test, oxidase test, and motility test. Triple sugar iron (TSI) agar, mannitol, urea, ornithine decarboxylase and lysine decarboxylase were conducted to confirm Salmonella suspected colonies.

2.3.2 Antimicrobial Susceptibility Test

Twenty-six (26) isolates were subjected to nine (9) antibiotic discs for their antibiotic susceptibility tests by using Kirby–Bauer disk diffusion techniques according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayn, 2023). Nine panels of antimicrobial such as: ampicillin (AMP) 10 µg, chloramphenicol (C) 30 µg, Ciprofloxacin (CIP) 5 µg, Cefotaxime (CTX) 30 µg, Azithromycin (AZM) 30 µg, Ceftrazidim (CAZ)10 µg, streptomycin (S) 10 µg, Nitrofurantoin (F)10 µg, and tetracycline (TE) 30 µg.were selected s its available at the time of the study in the laboratory According to WHO 2011, to Mueller-Hinton agar (MHA). The medium was prepared and bacteria colony was

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inoculated in 5ml of 0.85 sterile saline water turbidity of bacterial inoculums measured using densitometer to 0.5 McFarland then the bacterial suspension were spreader on the plates of the Muller-Hinton agar and the disc of the antibiotics were placed on plates by using Disc dispenser and press gently by thumb forceps, the plates were incubated at 37 ºC for 18 hours, Finally, the results were obtained by measuring the diameter (mm)of the zone of inhibition, by using digital caliper and the bacteria was interpreted as susceptible, intermediate or resistant to the antimicrobial agents and record the zone size of the test and control strains in the computer using standard used for salmonella species.

2.4 Data Management and Analysis

Data obtained during the sample collection and laboratory work was entered Microsoft Excel, cleaned, organized and sorted. Data was then coded and imported into STATA software for data analysis. Descriptive analysis was done to generate frequencies and percentages for an outcome variable from the independent variables: lakes sample types and different antimicrobial discs, A Logistic regression data analysis was used for initial analysis to determine differences in the proportions of positive results among lakes and sample types. Significant statistical difference was considered at P <0.05.

3. Results

3.1 Prevalence of Salmonella Isolates

From the total samples (280) observed in the current study Salmonella was recovered and identified from 27 (9.6%) samples. The isolation rate of Salmonella spp. was 13(7.5%) from kilole, 9(10.3%) from hora and, 5(25%) from cheleleka lakes. The Chi-square analysis of the result indicates that there was statistically significant difference between the lakes from which the samples were collected (P-value =0.042) (Table 1). The result indicated that the prevalence of Salmonella recovered from the different organs of the fish was five (7.1%) in skin, 7(10%) in gill, 12(17.1%) and 3(4.3) in tissue samples. As it is depicted in Table 1, there was no statistical difference between the prevalence of Salmonella isolate between four organs (P-value > 0.05).

Table 1: Association of Salmonella prevalence in fish with the risk factors

Factors	Category	Tested	Positive (%)	$\chi 2$	p-value
Lakes	Kilole	172	13(7.5)	6.36	0.042
	Hora	88	9(10.3)		
	Chaleleka	20	5(25.0)		
Sample Sources	Skin	70	5(7.1)	7.34	0.062
	Gills	70	7(10.0)		
	Intestine	70	12(17.1)		
	Tissue	70	3(4.3)		

4.2 Antibiotic Resistance

Antibiotic susceptibility testing was performed for 26 Salmonella isolates for selected antibiotic drugs that are commonly used in animal production and human health. The Antibiotic susceptibility test of the current study revealed that all Salmonella isolates examined were resistant or intermediately resistant to two or more antimicrobials. All Salmonella isolates (100%) were resistant to Tetracycline (TE) and Streptomycin (S). Similarly, 59.2% of the isolates were resistant to Cefotaxime (CTX). The current finding showed that most of the isolates (51.8%) were found intermediately resistant to Ciprofloxacin (CIP), while 53.8% of the isolates were susceptible to Azithromycin (AZT) (Table 2).

Table 2: Drug susceptibility profile of all isolates to the antimicrobials used (N=13)

Antibiotic	Number of isolates	Susceptibility profiles (%)		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (AMP)	26	37%	14.8	48.2%
Tetracycline (TE)	26	100	0	0
Ceftrazidim (CAZ)	26	92.5%	7.5	0
Streptomycin (S)	26	100	0	0
Cefotaxime(CTX)	26	59.2%	18.5	22.3
Chloramphenicol (C)	26		11.1	74.1
Nitrofurantoin (F)	26	14.8% 40%	14.8	45.2%

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Ciprofloxacin (CIP)	26	11%	51.8	37.2
Azithromycin(AZT)	26	18.5%	22.2	59.3

4.3 Multi-drug Resistance

The multi-drug-resistant features of Salmonella isolates are shown in Table 4. Of all 27 Salmonella isolates subjected to nine (9) Antibiotic susceptibility, all isolates were resistant to three (3) or more antimicrobials. Multi-drug resistant Salmonella isolates showed 12 different resistant patterns for nine antibiotic agents tested. Out of 26 multidrug-resistant isolates, 3(11.5%) isolates exhibited resistance to three antibiotics. Whereas, 6(23%) of the isolates were resistant to four antibiotics. Antibiogram profile also indicated that 8 (30.7%) of Salmonella isolates were resistant to five antibiotics, seven isolates were resistant to seven antibiotics, two isolates were resistant to seven antibiotics were used in the study. Only one isolate of Salmonella was resistant to eight antibiotics (Table 3).

Table 3: Multi-drug resistance pattern of isolates

No. of Antibiotic	Resistant pattern and number of Isolate	Total number of resistant isolates (%)
3	TE, S, CAZ (2)	3(11.5)
	TE, S, F(1)	
4	TE, S, CTX, CAZ (2)	6(23%)
	TE, S, AZT, CAZ (1)	
	TE, S, C, CAZ (1)	
	TE, S, AMP, CAZ (2)	
5	TE, S, CTX, AMP, CAZ (5)	8(30.7)
	TE, S, CTX, F, CAZ (3)	
6	TE, S, CTX, F, AMP, CAZ (2)	7(27%)
	TE, S, CTX, F, AZT, CAZ (3)	
	TE, S, CTX, C, CIP, CAZ (2)	
7	TE, S, CTX, F, C, AMP, CAZ (1)	2(7.7)
	TE, S, CTX, F, CIP, AMP, CAZ (1)	

4 Discussion, Conclusion And Recommendations

The overall prevalence of salmonella was 27/280 (9.6%) in different types of samples collected from fish production lakes found in Bishoftu town the prevalence of salmonella in current study was agreed with the prevalence of Salmonella out of the 112 fish sampled was 9.8% (11/112) which reported by Esonu et al., (4) in his study on the prevalence of Salmonella organisms in fresh and smoke-dried fish within parts of Kaduna Metropolis, Kaduna State, Nigeria, however, it was higher than the previous studies reported by Agmas et al., (1)) in his study on Microbiological quality assessment of fish origin food along the production chain in upper Blue Nile watershed, Ethiopia with the prevalence of Salmonella 27 (6.82%) However, the prevalence in the current study was lower than that reported by Fikre et al., (5), who determine the prevalence of Salmonella with 45 (14.5%) from Fish Harvested from Haramaya Lake, Eastern Ethiopia. When the current study was compared with the study of Agmas et al. (1) , which reveals the overall prevalence of Salmonella in the study area with 36.4 %. There was a decrease in the prevalence of Salmonella in our study area.

In the current study, the Salmonella spp. Was isolated from skin, gill, muscle and intestinal content with prevalence of 5 (7.1%), 7(10%), 12(17.1%) and 3(4.3%) respectively as reviewed Porto et al., (12), which stated that the Salmonella spp. has been isolated not only from the viscera but also from the gills and skin of the fish, contributing to an increased risk of cross-contamination during handling, processing, storage and commercialization due to failures in hygienic-sanitary care or using equipment, surfaces and utensils that are inadequately sanitized during the production chain. High prevalence of Salmonella in intestinal content in the current study was because Salmonella species are thought to be part of the normal microbiota of an animal's gut or gallbladder; these animals may also play a role in the pathogen's indirect or direct transmission to humans (8).

Among the three lakes selected for the current study, there was a significant difference (P-value < 0.05) (Table 1). This may be due to the Salmonella serovars are widely present and can enter water bodies through wild animals, domestic stock, poor sanitation and inappropriate disposal of human and animal waste(3). According to the current study, there was found statistically significant difference was found between the lakes from which the samples were collected (P-value =0.042) (Table 1). With preva1lence of 3(7.5%) from kilole, 9(10.3%) from hora and, 5(25%) from cheleleka lakes. Thus, the Environmental factors, such as water quality, play a significant role in the incidence of Salmonella in fish and pose a great risk for those consuming fish caught in contaminated waters without sanitary control (16). from our observation, we found that there was hygienic difference among the three study All Salmonella isolates (100%) were resistant to Tetracycline (TE) and Streptomycin (S). Similarly, 59.2% of the isolates were resistant to Cefotaxime (CTX). The current findings revealed that most isolates (53.8%) exhibited intermediate resistance to Ciprofloxacin (CIP), whereas an equal proportion (53.8%) were susceptible to Azithromycin (AZT) (Table 3).

This result was relatively agreed with that reported by Fikre et al., (5) in which all of the Salmonella isolated were resistant to Ampicillin (100%), Tetracycline (93.3%), followed by Erythromycin (53.3%), Chloramphenicol (46.7%). However, the

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susceptibility profile of Gentamycin was 100% followed by 17.8% of, Chloramphenicol and 4.4% Erythromycin. Whereas, Erythromycin, Chloramphenicol, Tetracycline, Amoxicillin and Ampicillin were intermediate susceptible by 42.2%, 35.6%, 6.7%, 6.7%, and 2.2% respectively

All 26 Salmonella isolates subjected to antibiotic susceptibility all isolates were resistant to three (3) or more antimicrobials. This pattern of antibiotic resistance was agreed with the report of (Mumbo et al., (9). This multi-antibiotic resistance may be due to antimicrobial resistance genes (ARGs) that were developed in various classes of medically and veterinary important antimicrobials. Clinically relevant ARGs included carbapenems (blaIMI-2, blaNDM-1) and extended spectrum β-lactamases (15) Several factors influence the risk of microbiological contamination in aquaculture products, mainly location, cultivated species, breeding practices, processing and cultural habits. Some of these potential microbiological hazards may be associated with poor hygiene patterns and sewage and livestock drainage. Leaching, for example, carries environmental pollutants into river waters and; consequently, fish, causing deleterious effects in this system microbiological contamination in aquaculture (16).

Accordingly, the current study the overall prevalence of salmonella was 27/280 (9.6%) in different types of samples collected from fish production lakes found in Bishoftu town with the statistical difference among the lakes of the study. Tetracycline, ceftazidime, and streptomycin showed resistance to all isolates selected for this purpose, and the rest also showed a high prevalence of resistance. Multidrug resistance is also common, as previously recorded by other authors in different livestock sectors. Therefore, A high degree of cleanliness in lakes and surrounding environment and preventing of people to dispose waste, drainage and flood entry to lakes is critical for Fish contamination with salmonella, Food safety training should be given for community involved in fishers and consuming of fish and fish products. Although standard tests were used to assess the frequency and antibiotic resistance of salmonella, additional molecular characterization of the isolates and genetic resistance should be investigated. Further study on the should be conducted both on the prevalence and antibacterial tests.

Conflict Of The Interest

Hence all individuals who contributed the study was included the authorship; therefore there is no one who may complain the publication of these papers.

Abbreviations

AAU	Addis Ababa University
AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
AS	Antibiotic Susceptible Test
BPW	Buffered Peptone Water

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