

## Occurrence, Associated Risk Factors and Drug Resistance Profiles of Salmonella Isolated among Fish Value Chain, North West Ethiopia

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### Abstract:

Salmonella became a challenge to the global food system, from production to consumption. In this study cross-sectional study- conducted from October 2018 to June 2019 with the purpose of estimating the occurrence of Salmonella and to assess drug resistant profile of Salmonella isolated from fish value chain of North West Ethiopia. Multistage sampling technique was applied for selecting study districts, kebeles and landing sites. Systematic random sampling was used to sample individual fish. The muscle of fish was tested using routine biochemical tests. Data of potential risk factors were collected using structured questionnaire through face-to-face interview. Data were entered and analyzed using STATA version 12. The overall prevalence of Salmonella in the study area was 36.43%. Twenty five percent of Salmonella isolates showed resistance at least for one drug. Ciprofloxacin (CIP-5µg) was found the highest (9.8%) followed by Ceftazidime (CAZ-30µg), 4.88% which salmonella developed resistant from the other drugs. Factors independently predict to salmonella occurrence were presence of contamination 1.1 (AOR= 1.06; 95%CI=1.04, 1.4), fish that was noticed after landing 2 (AOR= 2.2; 95%CI=0.01, 5.41) and poor handling practice 2 (AOR=1.8; 95%CI=0.001, 3.32). In conclusion the biochemical finding and the questionnaire survey indicated that Salmonella is widely distributed in fish harvesting in Lake Tana. hence appropriate control measures including awareness creation, code of practice, quality control measures and regular surveillance on landing sites, processing areas, processors, processing materials, retailer markets and the public in general should be implemented to mitigate the problem.

**Keywords:** Associated risk factors, Drug resistance, Fish, Lake Tana, Salmonella, enumeration

## Introduction

More than 70% of the planet is covered with water and aquatic foods and providing an essential component of the global food and contributing to improve nutrition, health and well-being of humans (Tacon and Metian, 2013). Fish and shellfish are the most nutritive and highly desirable food. It provides a good quantity of animal protein in the diet with high protein and omega three fatty acid, vitamins (D and B2) and different minerals(iron, zinc, phosphorus) (Bujjamma, 2015). Fishes accounts for approximately 17% of the global animal protein intake (FAO, 2014).

In developing countries, despite the low consumption of fish by weight, it contributes 180 kilocalories per capital per day, in a few countries with a developed fish preference (Lokuruka, 2009). Fish food, in addition to being a healthy food with nutritional value can act as a source of food borne pathogens on the other hand(Lin et al.,2017). For instance, fishes skin surface, intestine and gills, carries high microbial load ( $1.72 \pm 0.68 \times 10^8$  to  $7.00 \pm 3.39 \times 10^8$ ) (Mhangoetal., 2010).Therefore, fish and fishery products have been recognized as a major carrier of food borne pathogens (Nayarit-Balteser et al., 2016).

Food borne pathogens remain a public health threat globally and Salmonella is considered as one of the primary bacterial food borne pathogens to humans (Lin et al., 2017). Aquatic environments are the major reservoirs of Salmonella especially in tropical regions (Amare and Endalew, 2018). Salmonella have strong association with animals and as such foods of animal origin must be considered potentially contaminated in a fresh, unprocessed condition (Soltan et al., 2010).

Salmonella is one of the leading causes of human gastroenteritis. About 1.3 billion annual cases of human gastroenteritis are resulting from the ingestion of contaminated food products such as shell fish and fish (Pouokam, 2017).The non-typhoid Salmonella enterica is a common cause of a number of different disease syndromes including gastroenteritis, bacteremia, and typhoid fever, with the most common being gastroenteritis, which is often characterized by abdominal pain, nausea, vomiting, diarrhea, and headache in humans (Legesse et al., 2015).

Salmonella has been isolated from fish and other seafood (Elhadi, 2014). It may be transferred to the seafood due to the poor hygienic conditions during

transportation and marketing (Sarter, 2007).Prevalence of Salmonella in sea foods, have been studied in different regions of the world and health risks were evaluated. Distribution of Salmonella in seafood on a regional basis indicated to be highest in central Pacific and Africa; 12% and lowest in Europe/Russia and North America; 1.6%(Al-iedaniet al., 2016). In country basis prevalence of salmonella on fish food in Khartoum, Sudan 9.2%(Ali and Hussein, 2010), fish and crustaceans in Coimbatore, India 14.7% (Lakshmanaperumalsamy, 2014),seafood in Greece 11.2% , cooked shellfish in UK 5.2% has been reported.

In spite of constant surveillance and intensive efforts, food-poisoning outbreaks due to salmonellosis are increasing in western countries and fishery products account for significant portion of the outbreaks reported (Gandra, 2014). In developing country like Ethiopia, there is no such continuous monitoring system and the number of exact cases is not known (Delia, 2015). In recent years, the increasing popularity of unprocessed foods containing raw fish potentially increases the risk of salmonellosis. In Ethiopia, in addition to contamination during food preparation and under cooked fish may a cause for human Salmonellosis. There is scant study on occurrence and antimicrobial susceptibility profile of Salmonella in fish origin foods in Ethiopia.

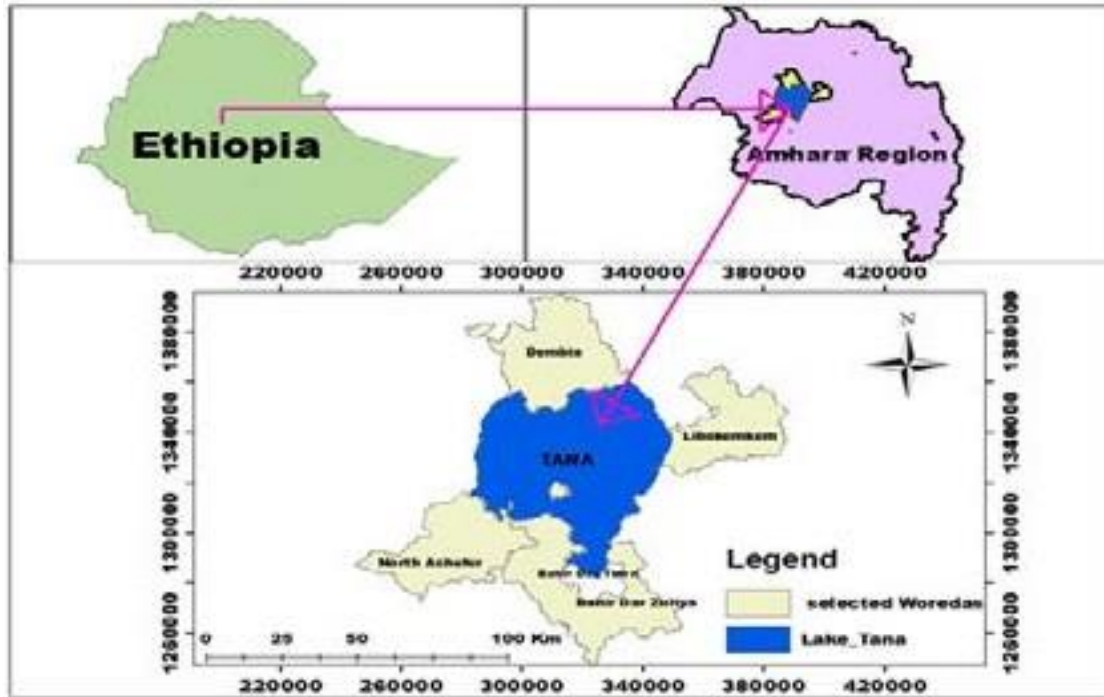
Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance and the breadth of resistance in single organisms are unprecedented and mounting (Bujjamma, 2015). Nevertheless, the resistance problem was perceived by some, most notably those in the industrialized world, as a curiosity of little health concern confined to gastrointestinal organisms in distant countries (Pouokam, 2017).

Previous studies on Salmonella of fish foods and drug resistance pattern were not adequately addressed. However drug resistance is mobile, the genes for resistance traits can be transferred among bacteria of different taxonomic and ecological groups by means of transduction, conjugation, and transformation (America Food and Drug Administration, 2001). These genes are generally directed against a single family or type of antibiotic, although multiple genes, each bearing a single drug resistance trait, can accumulate in the same organism. The resistance mechanisms are varied, like the antibiotics themselves (Ali., 2017).

There is no study conducted on associated risk fac-

tors and occurrence of Salmonella from fish origin foods in the study area /North West Ethiopia. Nevertheless, fish foods may be found contaminated through handlers along the value chain; the occurrence in fish food is unknown adequately. Finally, this work may serve as a source of information for further

investigations and to take corrective interventions. Up to our knowledge, a few studies have yet been conducted in Ethiopia to assess the occurrence, associated risk factors and drug resistance profiles of Salmonella isolated from fish value chain, but none has been conducted in North West Ethiopia.



**Figure 1:** Map of the study areas, 2019

### Sampling Strategy

Multistage sampling technique was applied for selecting study districts, kebeles and fish origin foods. The districts were selected purposively based on annual fish production, number of personnel or operators and their geographical locations to Lake Tana to be representative. While simple random sampling technique was used for selecting kebeles. Systematic random sampling was used to sample individual fish foods/muscle tissue. A total of 140 fish muscle tissue samples were sampled. For the purpose of identification of associated risk factors of Salmonella from fish a total of 40 operators, 10 from each district were interviewed.

### Questionnaire survey Data Collection

Face-to-face interview with semi-structured questionnaire was used to collect the data with respondent of the selected fish meat sellers, fish harvesters and fish filletors. The questionnaire consists of their experience and objective of fish harvesting, harvesting area and equipment, types of fish species, questions to monitor handling and hygiene, landing infrastructure, equipment and materials.

### Laboratory Sample collection and analysis

Over a period of 9 months (October- June 2019) of 140 samples from fish muscle were collected. The fish tissue samples were weighed, wrapped in a pre-cleaned aluminium foil and then packaged into zip lock plastic bags. Then were stored in an ice box and transported to Amhara Public Health Institute (APHI) Microbiology Laboratory within two hours on average for processing and isolation of Salmonella.

Of 140 samples among value chain (50 samples from landing site, 45 from fish retailers and 45 from hotels and restaurants) were collected. Salmonella was isolated from fish samples based on the conventional methods for the detection of Salmonella following the standard guidelines from ISO 6579 (Mooijman et al., 2019).

### Antibiotic sensitivity test

Antimicrobial susceptibility test was done based on the criteria of the Clinical and Laboratory Standards Institute (CLSI), (2018) for all isolates of Salmonella. Taking pure isolated colony, bacterial suspension was adjusted to 0.5 McFarland turbidity standards.

*E. coli* (ATCC 25922), which was susceptible to all tested drugs, was used for quality control. Antimicrobial was selected based on the groupings of antimicrobial agents with Ethiopian food and drug administration which are commonly used for treatment of Salmonella in animal and human were selected. The anti-microbial susceptibility test was

conducted for 6 antimicrobial by Kirby–Bauer disc diffusion method on Mueller-Hinton agar (MHA). The zone of inhibition was measured with caliper meter in millimeter (mm) and interpreted according to the standard of CLSI guideline and manufacturers recommendation as susceptible, intermediate or resistant Table 1; (CLSI, 2018).

**Table 1:** Clinical Laboratory Standard Institute breakpoints for Enterobacteriaceae (CLSI, 2018)

Antimicrobial	Concentration (µg/disc)	Susceptible*	Intermediate*	Resistant*
Co-trimoxazol	25	≥20	15-19	≤ 14
Ciprofloxacin	5	≥21	16-20	≤15
Ceftazidime	30	≥18	14-17	≤13
Chloramphenicol	30	≥18	13-17	≤12
Amoxclav (AMP)/	10	≥17	14-16	≤13
Gentamicin	10	≥15	13-14	≤12

#### Data Management and Analysis

Data were entered and analyzed using the statistical software, STATA 12.0. Descriptive statistics was applied to summarize the data and expressed using frequency and percentage. Bivariate logistic regression then followed by a multivariable logistic regression models were used finally to identify potentially strong predictors of the outcome variable. Confidence Interval (CI) of 95% and P value less than 0.05 used as cut of point of significance. The odds ratio was used to assess the magnitude of associations.

## Methods

### Description of Study Area

This study was conducted in North West Ethiopia along the value chain of Lake Tana fish produced for human consumption. Lake Tana is located North West of Ethiopia at latitude and longitude of 12°0'N 37°15'E respectively. Lake Tana is the head of the Blue Nile and is the largest Lake in Ethiopia (Ethiopian Meteorology Agency, 2016). The lake has three main commercially important fish groups such as: large Labeobarbus spp., African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*). About 15,000 tons of fish are landed each year at Bahir Dar; with an estimation of 15% of its sustainable amount (Largen and Spawls, 2010). There are 55 fishery enterprises with total 1217 operators 1154 males and 63 females. This lake is the major fish contributor of North West Ethiopia (Amhara Regional State Agriculture Office, 2017). The sample was taken in fish value chain of four districts (Bahir Dar Town, Dembia, North Achefer and Li-

bokemkem) around the Lake Tana (Figure 1).

Figure 1: Map of the study areas, 2019

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## Result and Discussion

### Occurrence of Salmonellosis

A total of 140 samples collected from value chain of fish samples obtained, 51 (36.43%) were found *Salmonella* positive (Table 4.1). The occurrence of *Salmonella* in this study was found in line with the previous study conducted by (Nguyen et al., 2016) in Vietnam with the occurrence of 36.6%. This result was found in contrary with the occurrence of previous study in china 12.4% (Zhang et al., 2015), 11.5% in Nigeria (Raufu et al., 2014) and 4% in Brazil (Ferreira et al., 2014). This difference might be due to low attention about contamination of fish in processing chain in this study area.

### Antibiotic Susceptibility Profile

Twenty five percent of *Salmonella* isolates (13 of 51) showed resistance to at least one antibiotic. Antimicrobial susceptibility pattern of *Salmonella* isolates from fish sample of Lake Tana is depicted below (Table: 2).

Ciprofloxacin was found the highest (9.8%) followed by Ceftazidime, 4.88% from the other drugs (Table 2). In Morocco, (Setti et al., 2009), 49% of *Salmonella* isolates (28) showed resistant to ampicillin (22 isolates), nalidixic acid (9 isolates), sulfonamide compounds (2 isolates) and tetracycline (1 isolates). Six isolates showed resistance to two antimicrobial substances (Setti et al., 2009).

In Nigeria, Raufu et al. (2014) isolated 23 *Salmonella* strains and overall antimicrobial resistance patterns indicated that most isolates were resistant to streptomycin 10 (43.5%), sulfamethoxazole 8 (34.8%) and trimethoprim 5 (21.7%), which constitutes a serious health risk for humans. Zhou et al. (2019) evaluated the antimicrobial resistance profile for *Salmonella* of fish showed resistance to tetracycline (35.9%), ampicillin (28.2%), nalidixic acid (26.2%), trimethoprim sulfamethoxazole (25.2%), chloramphenicol 4% and streptomycin (18.4%).

**Table 2:** Antimicrobial susceptibility pattern of *Salmonella* isolates from fish value chain, 2019.

Drugs with their concentration	Susceptible (%)	Intermediate (%)	Resistance (%)
Co-trimoxazol (SXT-25µg)	45.09	41.17	9.8
Ciprofloxacin (CIP-5µg)	90.19	7.84	1.9
Ceftazidime (CAZ-30µg),	78.43	14.72	4.88
Chloramphenicol (C-30µg)	74.5	22.56	2.92
Amoxclav (AMC-30 µg),	90.19	7.84	1.96
Gentamicin (CN- 10 µg)	84.31	11.76	3.92

In Brazil, Carvalho et al., (2009) isolated 103 Salmonella strains; 4% of the strains showed resistance to tetracycline, 2% to nalidixic acid and 2% to sulfamethoxazole. In a study conducted by Costa et al. (2016) cited by Fernandes et al., (2018) the antimicrobial susceptibility of 21 Salmonella strains obtained from Tilapia (*Oreochromis spp.*), both whole and in fillets, in the state of São Paulo was determined. Isolates were sensitive to Gentamicin (95%), amikacin (66%) and ciprofloxacin (66%), and resistant to florfenicol (80%) which is surprising, since its use is relatively recent in veterinary medicine in Brazil. Salmonella antibiotic-resistant strains have been isolated in fish in Brazil and worldwide, which evidences the transference of resistance genes among the aquatic microbial population, which can lead to more severe and difficult to

treat food borne infections (Olgunoğlu, 2012).

**Risk Factors Associated with Occurrence of Salmonella**

In the simple logistic regression, from variables under computed (Table 3); form of the fish presented to the consumers, harvesting area, contamination, storage of the fish, fish iced after landing, fish handling, protection of tools from contamination and availability of refrigeration during transportation were significant in bivariate logistic regression and selected as candidate for multivariable logistic regression.

In multivariable logistic regression contamination of the lake, fish handling and fish iced after landing were independent predictors of Salmonella in a fish (Table 3).

**Table 3.** Predictor Variables for the Occurrence of Salmonella in Lake Tana, 2019

Associated factor		Salmonella occurrence		COR (95% CI)	AOR (95% CI)
Variables	Variable category	No	Yes		
Contamination of lake	Yes	1	11	1	1
	No	4	24	0.02 (0.08-0.12)	1.06 (1.04- 1.4) *
Iced soon after landing	Yes	2	8	1	1
	No	3	27	0.05(0.07-0.17)	2.2 (0.01 – 5.41) *
Good Fish handling	Yes	1	5	1	1
	No	4	30	0.11(0.04-0.26)	1.8 (0.001-3.32) *
Form of the fish	Whole fish	2	8		
	Gutted	2	15	1	
	Filleted	2	11	0.03 (0.009-0.72)	
Harvesting area	Bahir Dar town	2	14	1	
	Dembiya	2	11	0.04 (0.00-0.08)	
	Northachefer	0	2		
	Libo-kemekem	1	8		
Species type received	Tilapia	1	8	1	
	Labeobarbus	3	17	0.08 (0.04-0.14)	
	Catfish	2	9		
Storage used Tools	Yes	2	9	1	
	No	4	25	0.1 (0.01-0.22)	
Hygiene protection	Yes	2	9	1	
	No	4	25	0.1 (0.01-0.22)	

The occurrence of Salmonella in a fish in contaminated environment was about 1.1(AOR= 1.06; 95%CI=1.04, 1.4) times more likely compared to the environment which is kept non-contaminated. In addition to contamination, fish iced after landing was one of the predictors of occurrence of Salmonella in a fish. Salmonella in a fish among not iced fishes was 2(AOR= 2.2; 95%CI= 0.01, 5.41) times more likely than those iced. In addition to this Salmonella in a fish among poor handling practice was about 2(AOR=1.8; 95%CI=0.001, 3.32) times more likely than those which were in good handling (Table 3). From the computed variables contamination, absence of icing immediately and poor hygiene were found predictor variables for the occurrence of Salmonella.

The complex concept of fish quality consists of safety, nutritional value, availability, integrity, freshness, eating quality, product size and type (Abbas et al., 2008). The most serious problem related to fish product safety is the contamination with microbial pathogens. Fish products are highly sensitive to spoilage because of their high-water content, neutral pH, and high amount of amino acids and naturally present autolytic enzymes (Jeyasekaran et al., 2006). In addition to contamination, fish iced af-

ter landing was one of the predictors of occurrence of salmonella in a fish. Salmonella in a fish among not iced fish was 2(AOR= 2.2; 95%CI= 0.01, 5.41) times more likely than those iced. Mol and Tosun (2011) investigated the quality of fish purchased from retail markets in Istanbul among poor handling practice was about 2(AOR=1.8; 95%CI=0.001, 3.32) times more likely than those which were in good handling.

#### Microbial load and its public health risk

The enumeration of Salmonella in the samples was determined using the three-tube most probable number (MPN) method. For the MPN method, 25 g of homogenized samples were mixed with 225 ml of BPW (Huankai). Then, 10 ml of this mixture was added to three empty tubes and transferring in triplicate 1 ml of the mixture into three tubes containing 9 ml of BPW followed by making 10-fold dilution. Salmonella detection of each tube was the same with qualitative method. The MPN value was determined on the basis of the number of positive tube(s) in each of the three sets using the MPN table.

Table. microbial load of Salmonella in retail fish processing markets around lake Tana bahir dar Ethiopia.

**Table 3.** Predictor Variables for the Occurrence of Salmonella in Lake Tana, 2019

Type of fish spp. and sample		Samples tested no.	No.(%) samples positive for salmonella	No. of samples Salmonella(MPN/g)			
Fish spp.	Sample type			0.3-1	01-Oct	10-110	>110
tilapia	muscle	50	21(15%)	11	3	1	3
Labeobarbus	muscle	45	15(10.7%)	0	0	2	7
Cat fish	muscle	45	15(10.7%)	0	0	3	5
Total		140	51(36.4%)	11	3	6	15

### Conclusions

Salmonella was found from fish muscle tissue samples; this indicates hygiene protocols should be applied for harvesting and processing of fish from farm to mouth to prevent the acquirer of Salmonella by human beings. Isolated Salmonella was showed resistance at least for one drug. Regular surveillance of fishes sold for consumption to know the level and types of antimicrobial resistance is important. Among the associated risk factors considered contamination, low habit of icing soon after landing and poor handling practice were statistically significant independent predictors of occurrence of Salmonella. Fish harvester societies and handlers should take the training about harvesting and processing of fish to prevent contamination of fish. Processing plants should be built in each landing sites with their facilities. Code of practice should be installed for fish harvesters, processors and sellers to ensure safe fish products.

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