Ethiop. J. Agric. Sci. 32(1) 79-88 (2022)

Occurrence, Associated Risk Factors and Drug Resistance Profiles of *Salmonella* Isolated from Fish Value Chain, Northwest Ethiopia

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ሳልሞኔላ በዓለም ላይ ከሚተላለፉ ዋና ዋና የምግብ ወለድ በሽታ አምጪ ተህዋሲያን አንዱ ሲሆን የዓሳ ምግቦች ደግሞ ለተዋሃሲው መተላለፊያ ዋንኛው ነው። ይህ ጥናት የተካሄደው በሰሜን ምዕራብ ኢትዮጵያ ካለው የዓሳ ዕሴት ሰንሰለት ያለውን የሳልሞኔላ ክስተት፣ መድኃኒትን የመቋቋም ችሎታና እና ተዛማጅ አደጋዎችን ለመገመት ነው። የጥናት ወረዳዎችን፣ ቀበሌዎችን እና የማረፊያ ቦታዎችን ለመምረጥ ባለብዙ ደረጃ ናሙና ዘዴ ተተግብሯል። የዓሳ በሳልሞኔላ መብል በተመረጡ ሚዲያዎች በመጠቀም በማሳደግና በመለየት ጥናት ተደርጓል፣ በመቀጠል አንቲባዮሱም ሳልሞኔላ ፖሊቫን-ኦን በመጠቀም የተለመደው የባዮ-ኬሚካል ምርመራዎች እና የሴሮሎጂ ማረጋገጫ ተሰርቷል። በበሽታው አጋላጭ ሊሆኑ በሚችሉ የአደጋ ምክንያቶች ላይ መረጃ የተሰበሰበው በተዋቀረ መጠይቅ በመጠቀም አምራቾችን ፊት ለፊት ቃለ-መጠይቅ በማድረግ ነው። በጥናቱ አካባቢ የሳልሞኔላ አጠቃላይ ስርጭት 36.43በመቶ ነበር። ከተለዩት የሳልሞኔላ ናሙናዎች መካከል 25 በመቶው ቢያንስ በአንድ መድሃኒት የመቋቋም ችሎታ አሳይቷል። Ciprofloxacin (CIP-5μg) ከፍተኛው የሳልሞኔላ መድሃኒት መቋቋም (9.8በመቶ) ሲሆን ሲፍታዚዲሜ (CAZ-30μg) በተከታይ 5.88በመቶ ሆኖ ተገኝቷል። ሁለት የሳልሞኔላ ናሙናዎች (3.92በመቶ) ለሶስት አንቲባዮቲኮች የመቋቋም ችሎታ አሳይተዋል። የሳልሞኔላ መከሰትን በተናጥል የሚገመቱ ምክንያቶች ብክለት 1.06 (95በመቶ CI: 1.04፣ 5.4) ፣ በረዶ ቤት ያልተቀመጠ ዓሳ 2.2 (95በመቶ CI: 1.09 ፣ 11.41) እና ደካማ አያያዝ 1.8 (95በመቶ CI: 1.02 ፣ 13.32) ናቸው። በአጠቃላይ የጥናታችን ውጤት ሳልሞኔላ ለዓሳ የምግብ መበከል እና የህዝብ ጤና አደጋ ሊጥል ይችላል። ስለዚህ ችግሩን ለማቃለል የግንዛቤ ማስጨበጫ እና በአጠቃላይ አዲስ ተገቢ የጥራት ቁጥጥር ርምጃዎች መተግበር አለባቸው። በሰሜን-ምዕራብ ኢትዮጵያ የዓሳ ፍጆታን በመጠቀም የሳልሞኔሎሲስ አደጋን ለመቀነስ በማረፊያ ጣቢያዎች፣ በዓሳ ቸርቻሪዎች፡ በሆቴሎች እና በምግብ ቤቶች ውስጥ ብክለትን ለማስወገድ በዓሳ ዕሴት ሰንሰለት መካከል ንፅህናን ማሻሻል ወሳኝ ነው።

Abstract

Salmonella *is one of the major foodborne pathogens worldwide that fish food as one of the vehicles for its transmission. The study was conducted to estimate the occurrence, drug-resistant profile, and associated risk factors of Salmonella isolated from the fish value chain in Northwest Ethiopia. A multistage sampling technique was applied to select study districts, kebeles, and landing sites. Salmonella contamination of fish muscle was tested using selective media, followed by conventional biochemical tests and serological confirmation, using Antiserum Salmonella Polyvalent-O. Salmonella enumeration was accomplished using a traditional three-tube Most Probable Number (MPN) approach. Data on potential risk factors were collected using a structured questionnaire through a face-to-face interview. Data were entered and analyzed using STATA version 12. The overall prevalence of Salmonella in the study area was 36.4 %. Twenty-five percent of Salmonella isolates showed resistance to at least one drug. Ciprofloxacin (CIP-5μg) was found to be the highest Salmonella drug resistance (9.8%) followed by Ceftazidime (CAZ-30μg) 5.9%. Two isolates (3.9%) of salmonella were resistant to three antibiotics. Factors that independently predict the occurrence of Salmonella were the presence of contamination 1.06 (95 % CI: 1.04, 5.4), non-iced fish after* *landing 2.2 (95 % CI: 1.09, 11.41), and poor handling practice 1.8 (95 % CI: 1.02, 13.32). In conclusion, our study results highlighted Salmonella as a potential fish origin food contaminant and a public health risk. Therefore, appropriate control measures, including awareness-raising and the creation of a new code of quality in general, should be implemented to mitigate the problem. Improving hygiene along the fish value chains to avoid cross-contamination at landing sites, fish retailers, hotels and restaurants is crucial to reduce the risk of salmonellosis through fish consumption in North-Western Ethiopia.*

Keywords: Drug resistance; fish; risk-factors; Salmonella

Introduction

Fish and shellfish are the most valuable part of the human diet (Venugopal and Gopakumar, 2017). It offers a noble amount of essential amino acids in the diet, omega-3 fatty acids, vitamins (D and B_2), and minerals such as iron, zinc, phosphorus (Bujjamma, 2015). According to FAO (2018), fish contributes around 17% of the global animal essential amino acid intake.

Fish food, indeed although it is being solid nourishment with wholesome esteem, can act as a vehicle of food-borne pathogens on the other hand. For instance, the fish's outer body, gastrointestinal and gills parts, carry a critical amount of microbial quantity ($1.72 \pm 0.68 \times 10^8$ to $7.00 \pm 3.39 \times 10^8$) (Lin *et al*., 2017). Hence, fish and fishery products have known as the main hauler of food-borne pathogens (Nayarit-Ballesteros, 2016; Siciliano, 2019). Food-borne pathogens stay an open wellbeing danger of the globe and *Salmonella* is considered as one of the essential bacterial foodborne pathogens to people (Lin *et al.*, 2017). *Salmonella* occurs in aquatic environments especially in tropical regions because of sewage effluents; agricultural run-off and direct fecal contamination from natural fauna. Aquatic environments, fishery equipment and handling practices are known to influence the bacterial loads in the harvested fish. Thus, fish and fishery products have been known as a major transporter of *Salmonella* especially in fresh and unprocessed conditions (Seel *et al*., 2016, WHO, 2018a).

Salmonella is one of the leading causes of human gastroenteritis. Contaminated shellfish and fish foods contribute approximately 1.3 million yearly cases of human gastroenteritis (Kurtz *et al*, 2017). The non-typhoid *Salmonella enterica* is a common cause of several 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 (WHO, 2018).

Salmonella has been isolated from fish and other seafood (WHO, 2018b). Nutritional constituents and water content available in seafood have become useful growth ingredients for the proliferation of *Salmonella*. *Salmonella*, at room temperature highly proliferate and the pathogenicity of the organism remained high and active in fish foods (Kumar *et al*., 2015). It may be transferred to the seafood due to the poor hygienic conditions during transportation and marketing (Getu *et al*, 2015). The prevalence of *Salmonella* in seafood has been studied in different regions of the world, and health risks were evaluated. The distribution of *Salmonella* in seafood on a regional basis is indicated to be highest in the central Pacific and Africa (12%) and lowest in Europe/Russia and North America (1.6%) (Kumar *et al*., 2003). In-country basis, the prevalence of *Salmonella* on fish food in Khartoum, Sudan 9.2% (Ali and Hussein, 2010), seafood in Greece 11.2%, and cooked shellfish in UK 5.2% has been reported (European Food Safety Authority, 2019).

Despite steady observation and in-depth efforts, fish food born salmonellosis is expanding in developed countries (Daniel *et al*., 2018). In developing countries such as Ethiopia, there is no such ceaseless observing framework and a precise number of cases isn't documented. In recent years, the increasing popularity of unprocessed foods containing raw fish potentially increases the risk of salmonellosis (Gezai, 2019). In Ethiopia, in addition to contamination through handlers along the value chain and undercooked fish, may also be a cause of human salmonellosis. There is no research on the burden and drug resistance profile of *Salmonella* in fish-root nourishments in Ethiopia.

Human activities are distorting antimicrobials use and creating risks of resistance. The number of resistant organisms, the strain of resistance, and the degree of resistance in each organism are unknown unless studied for a specific organism (Bujjamma, 2015). *Salmonella* is one of the organisms that develop resistance have a hazard for people of the glove. Infections with *Salmonella* that are resistant to antimicrobials may result in increased human morbidity, increased human mortality, reduced effectiveness of related antibiotics used in humans, increased human care costs, increased potential for carriage and dissemination, facilitated the emergence of resistance in human pathogens. (EFSA and EU CDC, 2019). The antibiotic resistance of one microbe can be shared with other microbes through transduction, conjugation, and transformation (Divek *et al*., 2018). One organism can have multiple drug resistance genes, which lead to different resistance traits. Resistance mechanisms are varied, like the antibiotics themselves (Ali., 2017).

Antibiotic resistance *Salmonella* monitoring allows the identification of emerging or specific patterns of resistance. Finally, suggest research priorities and propose administrative and educational measures that can help to stop this development of antimicrobial resistance (Gove and Hancock, 2019). Nevertheless; there is no previous study on the occurrence of *Salmonella* in fish foods and drug resistance patterns in Ethiopia. Thus, this study could serve as a source of information for further investigations and corrective interventions.

Materials and Methods

Description of Study Area

This study was conducted in Northwest Ethiopia along the value chain of Lake Tana fish production and 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. The commercially important fish groups in Lake Tana include large *Labeobarbus* spp., African catfish (*Clarias gariepinus*), and Nile tilapia (*Oreochromis niloticus*). There are 55 fishery enterprises with a total of 1217 operators (1154 males and 63 females). This lake is the major fish contributor of North West Ethiopia (Amhara Regional State Agriculture and Livestock Development Bureau, 2017). The samples were taken along the fish value chain of four districts (Bahir Dar Zuriya, Dembia, North Achefer, and Libokemkem) around Lake Tana (Figure 1).

Sampling Strategy

A multistage sampling technique was applied for selected study districts, 'kebeles'. The districts were selected purposively based on annual fish production, number of personnel or operators involved, and their geographic proximity to Lake Tana and assumed to be representative of the lake's value chain. Whereas simple random sampling technique was used for selecting 'kebeles' and systematic random sampling was used to sample individual fish foods/muscle tissue. A total of 140 fish muscle tissue samples were collected. For identification of associated risk factors of *Salmonella* from fish, a total of 40 operators, 10 from each district were interviewed.

Questionnaire Survey

Direct interview with a semi-structured questionnaire was used to collect the data with the respondent of the selected fish meat sellers, fish harvesters, and fish filleters. 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, commonly used equipment, and materials.

Laboratory Sample Collection and Analysis

Over a period of 9 months, 140 fish muscle samples were collected along the fish value chain. The fish tissue samples were weighed, wrapped in a pre-cleaned Aluminum foil, and then packaged into zip-lock plastic bags. Then were put away in an icebox and transported to Amhara Public health Institute (APHI) Microbiology Laboratory within two hours on common for processing and isolation of *Salmonella*. Of 140 samples among value chains (50 samples from landing sites, 45 from fish retailers, and 45 from hotels and restaurants) were collected. *Salmonella* was isolated from fish samples based on the conventional biochemical methods following the standard guidelines and finally process by serological confirmation.

A 25-g portion of fish muscle was added into 225 ml buffer peptone water in a sterile plastic bag and then homogenized using a stomacher. *Salmonella* isolation followed the ISO 6579-1:2017 technique (ISO-6579, 2002) on the enrichment and isolation media. The combined sample that homogenized one was incubated for 16–20h at 37°C on enrichment media. Thus 1ml of aliquot inoculated on Muller Kauffmann Tetrathionate (TT) broth and a Modified Semisolid Rappaport Vassiliadis (MSRV) agar plate was inoculated around 50 μl. Both media were incubated for 16–20h in 37°C. This selection phase was repeated, by using one loop (approximately 10μl) of TT and MSRV, to inoculate Xylose Lysine Tergitol 4 (XLT4) and Ramback agar plate selective media. The appearance of brown, gray, or black per plate were taken for further biochemical confirmation of Salmonella and another one to two colonies to inoculate Nutrient Agar (NA) to grow *Salmonella* for serological confirmation, using Antiserum *Salmonella* Polyvalent-O.

Salmonella concentration was enumerated using a traditional 3-tube MPN (Most Probable Number) approach (Pavic *et al*., 2010). The concentrations of 25g of homogenized solute were buffered 225 ml of buffer peptone water. Then 10 ml of this mixture was added into 3 empty tubes. Another 1ml of homogenized solute and 9 ml of buffered peptone water mixture into 3 tubes. Finally, 0.1ml solute and 9.99ml buffered peptone water dilution into 3 tubes then incubate at 37^0c for 24 hrs. *Salmonella* detection of each tube was the same as the qualitative method. The value was cross-read based on the number of positive tubes in each of the three sets using the MPN standard table (Pavic *et al*., 2010).

Antibiotic Sensitivity Test

Antimicrobial susceptibility testing was done based on the criteria of the Clinical and Laboratory Standards Institute (CLSI) (Humphries *et al.,* 2018) for all isolates of *Salmonella.* Taking the pure isolated colony, the bacterial suspension was adjusted to 0.5McFarland turbidity standards. According to Mahon and Lehman, (2019) the recommended control organism for susceptibility testing of gram-negative microorganism drugs was *E. coli* (ATCC 25922). Since *E. coli* (ATCC 25922), which was susceptible to all tested drugs, was used as a reference control. Anti-microbial was chosen according to Ethiopian food and drug administration (EFDA), which are commonly used for the treatment of *Salmonella* in animals and humans were selected. The anti-microbial susceptibility test was carried out for six antimicrobials using the disk diffusion method on Mueller-Hinton agar (MHA). Inhibition zone was measured with caliper meter and cross read with CLSI guideline standard and the manufacturer's recommendation as susceptible, intermediate, or resistant in Table 1; (CLSI, 2018). *Salmonella* isolates that fall under the resistance inhibition zone for three or more classes of anti-*Salmonella* drugs were considered multiple drug-resistant (MDR) (Sweeney *et al*., 2018).

Data Management and Analysis

Raw data were entered, cleaned, and processed using the statistical software, STATA 12.0. Descriptive statistics was applied to summarize the data and expressed using frequency and percentages. To identify the potential strong predictors of the outcome variable, bivariate logistic regression followed by a multiple logistic regression model was employed. 95% Confidence Interval (CI) and P value less than 0.05 were used as a cut of point of significance. The odds ratio was used to assess the magnitude of associations.

Results and Discussion

Occurrence of Salmonellosis

Out of a total of 140 samples collected from fish value chain samples, 51 (36.4%) were found to be *Salmonella* positive. The occurrence of *Salmonella* in this study was found to be consistent with the previous study conducted by Elhadi (2014) in Saudi Arabia, with an occurrence of 31-60 % in freshwater fish samples. This result was found to be higher than with the occurrence of the previous study in China from the shanghai area of fish farms (12.4 %) (Zhang, 2015), in Nigeria from selected fish farms and fish collected in the market (11.5 %) (Raufu *et al*., 2014) and in Brazil from the fish market (4 %) (Ferreira *et al*., 2014). The reasons for these prevalence variations might be due to water quality and environmental distinctiveness (rainfall, temperature, sewage effluents; agricultural run-off, and direct fecal contamination from natural fauna) (Percival and Williams, 2014; Fernandes *et al*., 2018). In addition, food handlers' characteristics such as poor handling and processing practices might contribute to such differences along the value chain. This high prevalence in our study finding might be due to a lack of awareness in fish product handling and poor post-harvest management practices from fishermen to end users along the value chain.

Salmonella Concentration and Its Public Health Risk

The estimated microbial concentration of *Salmonella* in fish muscle varied from zero to > 110 MPN/g. *Salmonella* concentration in fish muscle was of low levels compared with that of many studies on *Salmonella* concentration in meat products (Techathuvanan *et al*., 2010, Wang and Mustapha, 2010).

There is too much variability to suggest a definitive cutoff point below which *Salmonella* would cause illness (Jennifer *et al*., 2014). The likelihood of infection is dose-dependent. While there may be a level of exposure below which illness is unlikely, reducing exposure to high levels of *Salmonella* will undoubtedly reduce the risk of *Salmonella* illnesses, thus improving public health (Jennifer *et al*., 2014, Dang-Xuana *et al*., 2018). Fish products containing these higher levels of *Salmonella* pathogen are identified to show a high level of contamination in Northwest Ethiopia. Thus, fish-origin foods have a public health risk of salmonella. Qualitative and quantitative (enumerative) measures of *Salmonella* can support activities that will reduce the actual levels of *Salmonella* in fish-origin foods. In turn, this will allow the development and evaluation of mitigation to reduce both levels and prevalence and thus, reduce the public health burden of salmonellosis (Dang-Xuana *et al*., 2018).

Antibiotic Susceptibility Profile

Twenty-five percent of *Salmonella* isolates (13 out of 51) showed resistance to at least one antibiotic. Ciprofloxacin was found to be the highest at 9.8 %, followed by Ceftazidime at 5.9 %. Similar to our results, a study in Brazil by Carvalho cited by Fernandes *et al*. (2018) isolated *Salmonella* strains of fish origin foods showed 4 % resistance to tetracycline, 2 % to nalidixic acid, and 2 % to sulfamethoxazole. Slightly lower levels of resistance for ciprofloxacin (2.3%), gentamicin (3.2%), ceftazidime (0.5%) were recorded by Zhang (2015) in China. On the other hand, there are also reports higher than our investigations, for instance, in Morocco, 49 % of *Salmonella* isolates showed resistance to ampicillin, nalidixic acid, sulfonamide compounds, and tetracycline (Setti *et al*., 2009). In Nigeria, Raufu *et al*. (2014) Isolated 23(100%) antimicrobial resistance *Salmonella* strains from fish food. Zhou *et al*. (2019) in China, an antimicrobial resistance profile for *Salmonella* in fish was evaluated showing resistance to tetracycline (35.9%), ampicillin (28.2%), nalidixic acid (26.2%), trimethoprimsulfamethoxazole (25.2%), chloramphenicol (4%) and streptomycin (18.4%). Another study in Saudi Arabia showed that the highest antibiotic resistance was observed for tetracycline (90.7%), followed by ampicillin (70%) and amoxicillin-clavulanic acid (45%) (Elhadi, 2014). The causes for these high antibiotic resistance variations might be attributed to variation in strain, quality of water, and consequence of natural selection and genetic mutation variation which exacerbated by human factors such as inappropriate use of antimicrobials (EFS A and EU CDC, 2019).

Multidrug resistance features of *Salmonella* were analyzed of which only 2 (3.9%) isolates were resistant for three antibiotics (Ciprofloxacin (CIP-5 μg), Gentamicin (CN-10 μg), and Ceftazidime (CAZ-30 μg). Even though a relatively low percentage of drug resistance of *Salmonella* isolates in our study area was detected, it showed the potential significant threat of the resilient isolates along the value chain of fish food. This resistance in our study area might be due to indiscriminate use of antimicrobials in human, agriculture, and veterinary medicines, contamination of the aquatic environment, or poor hygiene condition in filleting of fish food that aids the spread of resistant *Salmonella* isolates. In addition, resistance genes can be moved from other bacteria to *Salmonella* isolates using transduction, conjugation, and transformation (Divek *et al*., 2018). *Salmonella* antibiotic-resistant strains isolated in fish-origin foods may cause dissemination of resistance genes to freshwater microbiomes, which can lead to harder and more problematic to treat food-borne infections (Manyi-Loh *et al*., 2018; Fernandes *et al*., 2018).

Risk Factors Associated with Occurrence of *Salmonella*

In multivariable logistic regression analysis of contamination of the lake, fish handling and fish iced after landing were independent predictors of *Salmonella* in a fish. The occurrence of *Salmonella* in a fish in a contaminated environment was about 1.06 (95% CI: 1.04, 5.4) times extra probable compared with the environment which is kept non-contaminated. The water quality, the fishery equipment, and landing site have a straightforward effect on bacteriological fish pollution because the burden of *Salmonella* in freshwater fish is on average 3.4 - 64% (Fernandes *et al*., 2018).

In addition to contamination, putting fish in ice after landing was a predictor of *Salmonella* occurring in a fish. *Salmonella* in fish among non-iced fishes was 2.2 (95 % CI: 1.09, 11.41) times more likely than those iced. This might be because water content available in fish food, the temperature is can facilitate fully the proliferation of *Salmonella* with virulent genes (Kumar *et al*., 2015).

Besides, *Salmonella* in fish with poor handling practices was about 1.8 (95 % CI: 1.02-13.32) times more likely than those which were in good handling. Similar to our finding, a study done by Mol and Tosun (2011) in Istanbul found that poor handling was about 2 (95 % CI=1.08, 3.32) times more likely to contaminate than in good handling. Quality fish food comprises of safety, nutritious, hygienic, and psychologically acceptable (fit the customer needs, specifications (internal and external /actual production) (Serena *et al*., 2017). Out of those the most uncompromised fish food quality problem is microbial contamination (Liu, *et al*., 2018).

Conclusions

Salmonella contamination was common in fish meat products in northwest Ethiopia. This indicates hygiene protocols needed to be applied to harvesting and processing fish from harvest to mouth to prevent *Salmonella* contamination. Isolated *Salmonella* is resistant to at least one drug. Thus, regular monitoring of fish muscle sold for consumption to know the level and types of antimicrobial resistance is important. Among the associated risk factors considered: contamination, low icing habit soon after landing, and poor handling practice were statistically significant independent predictors of *Salmonella* occurrence. Therefore, fishery associations and traders should undertake training on fish harvesting, processing, and storage to prevent contamination. Processing plants should be built at each landing site with the required facilities. A new code of practice to control *Salmonella* infection and antimicrobial resistance should be installed in Ethiopia to ensure safe fish products.

Competing interests

The authors declare that they have no competing interests

Funding

The study was financed by Addis Ababa University (Ref. No. LT/PY-089/2021
) and the National Fishery and Aquatic Life Research Centre of Ethiopian Institute of Agricultural Research (Ref. No. 17.2/0409/2019).

Acknowledgments

We would like to thank Bahir Dar University for its financial, material, and ideal support. We would like to thank the fish processors in our area of study for openly providing their information.

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