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Research article

Prevalence and antibiotic susceptibility pattern of *Escherichia coli* O157:H7 isolated from harvested fish at Lake Hayq and Tekeze dam, Northern Ethiopia

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ABSTRACT

Fisheries play a significant role in food security, livelihood, and source of income in developing countries. Although fish are a healthy source of protein, they can also spread diseases caused by pathogenic micro-organisms they may harbor. Epidemiology of foodborne pathogens is not well studied in Ethiopia. To address this issue to some extent, a cross-sectional study with a simple random sampling approach was conducted from October 2017 to May 2018 with the objectives of to isolate and estimate the prevalence of *Escherichia coli* O157: H7 in fish, and to evaluate the antimicrobial susceptibility pattern of the isolates in selected Lakes of Northern Ethiopia. All the microbial identification and isolation procedures were conducted based on ISO 6887-3:2017 recommendations. Antimicrobial susceptibility test was also performed following the standard procedure of Kirby-Bauer disk diffusion protocol. From the total 410 fish samples examined, six (1.46%) of them were found contaminated with Shiga toxin-producing *Escherichia coli* O157: H7 strain. The organism was isolated from landing sites (5/293) and local retail markets (1/75). Besides, *Escherichia coli* O157: H7 was isolated from filleted fish (5/214) and whole fish (1/125); however, it was not isolated from samples of ready to eat fish and working environments of restaurants. The antibiotic susceptibility test revealed that the isolates were resistant to Ampicillin and Streptomycin disks. However, Ciprofloxacin, Gentamicin and Nalidixic acid were found effective in inhibiting the growth of all of the isolates. Since pathogenic *Escherichia coli* strain was detected from fish, raw and undercooked fish consumption in Ethiopia may result in contracting infections. The occurrence of such pathogenic organisms in fish indicates the need for intervention by stakeholders. Supports like freezers, generators, the establishment of fish processing plants and on job training about proper fish handling practices may play a tremendous role in decreasing the level of contamination of fish in Ethiopia.

1. Introduction

Fisheries play a significant role in food security, livelihood, a source of income and social development in developing countries (Hossain et al., 2015). New technological advances and increased demands for fish as a source of animal protein are the main reasons for the industry's growth. Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world (Garcia-Rodríguez and De La Cruz-Aguero, 2011). World per capita fish supply has outpaced population growth in the past five decades and reached a new record high of 20 kg in 2014 due to its vigorous growth. In the last two decades, dramatic growth in aquaculture production has boosted the average consumption of fish and fishery products at the global level (FAO, 2016).

Despite being a good source of high-quality animal protein and income for the poor, fish can be a source of foodborne infections and intoxications if it is not harvested and processed under hygienic conditions. Fish can be a vehicle for several bacterial disease transmissions (Elsaidy

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et al., 2015; Zhao et al., 2018). Fish can be contaminated with pathogens through direct contact with water as well as during processing after harvest in the environment. *Enterobacteriaceae* are widely distributed in water bodies and can be detected in fish skin, gills and intestines (Ribeiro et al., 2015). The unhygienic conditions of the landing sites, storage, and domestic retail markets are causing a tremendous public health risk. The fecal contamination of natural water bodies has emerged as a challenge in developing countries. The fish harvested from such areas often contain human pathogenic microorganisms. *Escherichia coli* has been traditionally recognized as an indicator organism of fecal contamination of water and fish (Albuquerque, 2013).

Foodborne pathogens are the leading causes of foodborne human illness and death in the world (Agüeria et al., 2018). The reason for the increased risk can be attributed to many reasons; changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are few of them (Bevilacqua et al., 2017; Lopez-Campos et al., 2012). *Escherichia coli* O157 is one of the virulent strains under the species *Escherichia coli.* It is the leading cause of hemorrhagic colitis, hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in man. These illnesses may lead to death due to improper absorption of nutrients and the destruction of specific tissues in the target organs (Cobbaut et al., 2009; Earley and Leonard, 2006).

Epidemiology of foodborne pathogens, especially that of pathogenic *Escherichia coli,* is not well studied in Ethiopia. However, recently there is an increasing trend of reporting the occurrence level of the organism in beef and dairy products (Abebe et al., 2014; Desta et al., 2016; Hailesellassie et al., 2013; Hiko et al., 2008; Tassew et al., 2010; Taye, 2013). This may be because beef and dairy products are considered the risk for *E. coli* due to close contact with cattle manure. However, studies showed that the organism had been isolated from mutton, chicken, fish, and vegetables. *Escherichia coli* O157: H7 have been reported from fish in India and USA. A study conducted in the USA showed that *E. coli* O157: H7 genes had been detected in a gastrointestinal sample from fish with a prevalence of 6.95% (Ribeiro et al., 2015). In India, O157 genes were identified from fish samples taken from the street market (Sanath Kumar et al., 2001).

Fishes and fish products can be contaminated by Enterobacterial species that can cause a severe foodborne outbreak (Ribeiro et al., 2015). The risk can be worse in developing countries like Ethiopia due to many reasons which include but not limited to; lack of awareness of fishermen about food-borne infections or the consequences of non-hygienic handling of fish, extensive cattle production around water bodies and landing sites, suitable weather condition for, extensive cattle production around water bodies and landing sites, suitable weather condition for the flourishing of microorganism in feces, widespread open defecation by rural population, high flooding that drain contaminants to Lakes and landing sites, and unavailability of fish processing plants can be few of them. All those listed reasons indicate that consumption of raw and undercooked fish and fish products in Ethiopia should not be practiced. As it is well known contaminated water (contaminated with ruminant feces) is one of the most important source of *E. coli* O157:H7, therefor any study dealing with the detection of *E. coli* O157:H7 from lake water samples in the country primarily in study areas (Tekeze & Lake Hayq).

Even though fish pose a significant risk of foodborne pathogens, to the best of our knowledge, there is no single study conducted in Ethiopia reporting *Escherichia coli* O157:H7 associated with fish. In addition to problems related to poor handling practices of fish and fish products, post-harvest loss and causes are not yet studied in the study areas. If fishermen and policymakers are being able to make informed decisions in an attempt to reduce contamination and losses, the significance of these problems need to be scientifically studied, and recommendations should be generated. Bearing this idea in mind, the study was designed with the objectives of assessing the level of contamination of fish with Shiga toxin-producing *Escherichia coli* O157: H7 and determining *in vitro* susceptibility pattern of the isolates to commercially available antibiotics.

2. Material and methods

2.1. Study areas

The study was conducted in the Amhara region in Northern Ethiopia within the surrounding of Tekeze human-made reservoir and Lake Hayq. The reservoir is built on the Tekeze river which is a tributary of the Nile. The Dam has an average area and depth of 160.4km^2 and 58m, respectively (Teame et al., 2016). It has a tremendous resource regarding fish production that reaches an average of 5,065 tons of fish annually which created invaluable job opportunities for the surrounding community. Lake Hayq is a freshwater lake located in South Wollo zone of Amhara region, Northern Ethiopia. It is one of the Highland Lakes of Ethiopia, located at an altitude of 2,030 m above sea level. It receives water from many small seasonal streams and one perennial river named Ankerka. The catchment area of the lake is 65 km^2. The lakes had a maximum depth of 88.2m and 81.44m recorded in 1941 and 2013, respectively (Seid, 2016). This lake is well known for its fishery resource for the community and nearby towns. It has the potential of an average of 500 tons of fish production per year (Tesfaye and Wolff, 2014). The two study Lakes are illustrated in Figure 1.

2.2. Study design, sampling method, and sample size determination

A cross-sectional study design with a simple random sampling approach was implemented from October 2017 to May 2018. Fish harvested from the two Lakes were sampled in different locations (landing site, local retail market, and restaurants). In the study areas, fish is sold in a jerry can (*casa*) and sac containers. Before reaching restaurants, the fish stay for more than 6h in landing sites. In this location, containers (casa and sacs) were randomly selected in a simple random sampling method. On average, 20–30 casa and sacs of fish were sampled in each visit randomly from the total of 75–100 casa and sacs available in average. From a selected jerry can or sac container, few swabs from different fish were taken and pooled together into a single transport media as a single sample, which represents one sample of the total sample size. Besides, for fish tissue samples, a container was randomly selected as well. Fish were randomly selected and approximately 20g of the muscle was cut and added to stomacher bags. In local markets, samples were collected with a simple random sampling approach, as well. All restaurants were included in the study sites of Hayq city because of the absence of enough restaurants that serve fish. From each restaurant, a single working environment sample. However, from these restaurants, fish samples and working environment samples were also taken in a simple random sampling approach. Workers and processed fish were randomly selected, whereas, from working knives and containers, swabs were pooled together as a single sample.

The prevalence of *E. coli* O157: H7 in fish, the sample size was determined by (Thrusfield, 2007) as follows;

$$n = \frac{1.962^{*}pexp\ (1 - pexp)}{d^2}$$

where *n,* is the required total sample size, p*exp* is the expected prevalence of the organism in fish and d, stands for the desired absolute precision of 5%. Because there was no prior information regarding the prevalence being estimated, a more conservative expectation of 50% prevalence of *Escherichia coli* O157: H7 was considered. Hence, the sample size was estimated to be a minimum of 384 fish as well as other expected sources of contamination samples from the landing sites and restaurants, and we sampled 410 fish and working environment samples from the two Lakes. 250 samples were taken from lake Hayq, while the 160 samples were from Tekeze dam. This sample size imbalance was due to the absence of local retail markets and restaurants around Tekeze dam. Samples from local retail markets and restaurants were taken only from Lake Hayq and nearby Hayq city.

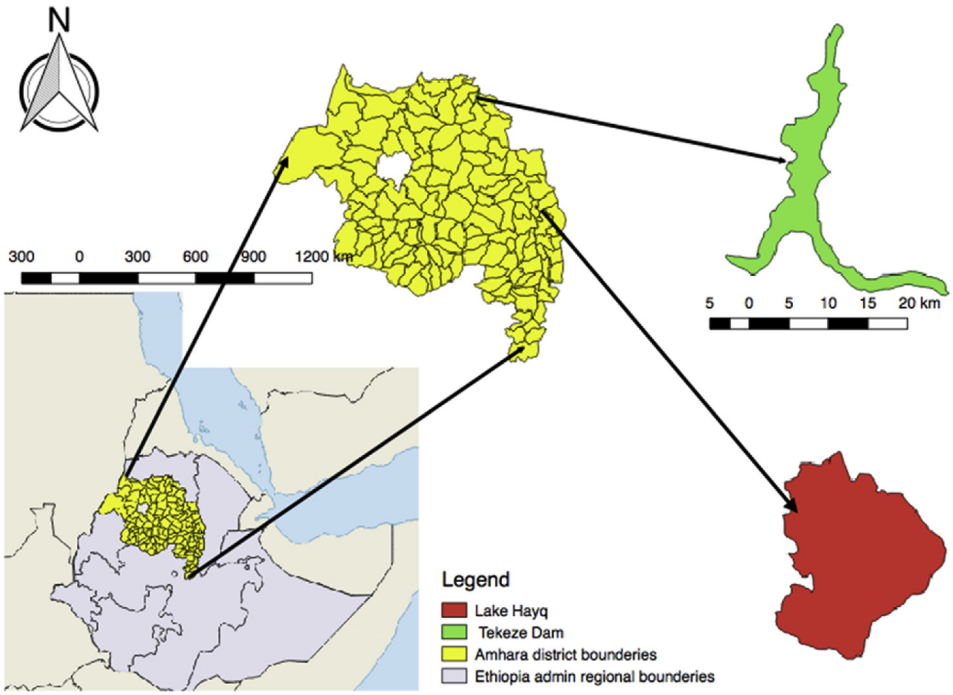

Figure 1. A map that shows study sites.

2.3. Source of fish and sample collection procedure

The source of the fish used in the study were collected from fishers operating in the two study lakes.

Filleted fish swab, filleted fish muscle (tissue), whole fish (skin) swab, knife and cutting board swab, ready to eat fish from restaurants, workers hand swab, and container swab were collected by following recommended procedures (ISO6887-3:2017, 2017). Approximately 20g of fish was cut and inserted into a stomacher bag that contained 200ml of transport media (buffered peptone water). Besides, swabs from the whole fish by swabbing the almost all the surface of the fish, a hand of the worker, container and, a knife was collected in a universal bottle that contains 10ml buffered peptone water as a transport media. Samples collected from the respective sites were transported to the nearest laboratory in the shortest possible time and processed upon arrival.

2.4. Sample preparation and identification procedure of Escherichia coli O157:H7

All the isolation and identification procedures of the organism were performed based on ISO 6887-3:2017 recommendation for microbiological analysis of fish samples (ISO6887-3:2017, 2017). Samples collected from study sites were transferred appropriately to the nearby animal health laboratories (Sekota Dryland Agricultural Research Center for samples taken from Tekeze dam, and Kombolcha Regional Animal Health Laboratory for samples taken from Lake Hayq) with a transport media. After reaching the laboratory, samples were incubated at 41.5 °C for 6h to increase the recovery rate of stressed cells. Muscle samples of fish placed in a plastic bag were homogenized using a homogenizer. From each incubated suspension (swabs in 10ml of BPW, and homogenized muscle samples) approximately, 200μl of the sample were streaked onto Sorbitol MacConkey agar plates and incubated at 37 °C for a maximum of 24h. Following incubation, sorbitol negative (colorless) colonies were identified by their color and further streaked onto sorbitol MacConkey agar plates again to get a clear colorless typical *E. coli* O157 isolates. From the pure culture, isolates were sub-cultured onto nutrient agar for further preservation and transportation of isolates with glycerol stocks.

The final serological confirmation of positive samples was not carried out in the above laboratories. For that matter, isolates were prepared for transportation to Addis Ababa University-college of veterinary medicine and agriculture (AAU-CVMA) laboratory as follows; isolates from the nutrient media were sub-cultured onto Trypton soya broth (Oxoid, Ltd., Basingstoke, UK) for 24 h at 37 °C. One milliliter of the Trypton soya broth (TSB) bacterial suspension was mixed with an equal volume of sterilized 50% glycerol in sterilized Cryovial tubes. These bacterial-glycerol stocks were stored at -80 °C deep freezer and transported with icebox packed with ice to AAU-CVMA lab. Samples were stored in a deep freezer and when Latex kit is secured, bacterial stocks were revived by culturing onto TSB and further subculturing onto nutrient agar. From nutrient, agar colonies were further subjected to biochemical tests. Indole test and dextrose/lactose fermentation test by culturing onto TSB and streaking onto Klinger iron agar (KIA) media respectively, were carried out to screen positive samples. Based on these tests, red ring formation by indole test, and yellow slant and butt formation for the sugar fermentation test was presumed to be positive for *Escherichia coli* O157: H7. Positive cultures were further subjected to a serological test using a latex kit for the confirmation of *E. coli* O157: H7 strain. The bacterial colony was picked and subjected to a slide agglutination test using an *E. coli* O157 latex kit (Oxoid Ltd., Hampshire, UK). A drop of test latex and sterile saline water was dispensed into the reaction card separately. Up to five presumptive *E. coli* O157: H7 colonies were picked by lightly touching the center of the colony with a sterile inoculating needle. The picked colonies were thoroughly emulsified with the saline on latex card and then finally with the test latex reagent. The formation of agglutination within 1 min was regarded as positive.

2.5. Antibiotic susceptibility test

All *E. coli* O157 isolates were subjected to susceptibility testing against eight antibiotics. Gentamycin, Kanamycin, Chloramphenicol, Ciprofloxacin, Streptomycin, Tetracycline, Nalidixic acid, and Ampicillin disks were used to assess the susceptibility pattern of the isolates. The method used for the susceptibility test was the Kirby-Bauer disk diffusion on Muller-Hinton agar plates prepared according to the manufacturer's recommendation. The isolated bacterial colonies from pure fresh culture

Table 1. Susceptibility decision criteria for the *Enterobacteriaceae* (CLSI, 2015).

Antibiotic disk	Disc concentration	Zone of inhibition to the nearest mm		
		Susceptible	Intermediate	Resistant
Ampicillin	10 μgm	≥17	14–16	≤13
CAF	30 μgm	≥18	13–17	≤12
Ciprofloxacin	5 μgm	≥21	16–20	≤15
Gentamycin	10 μgm	≥15	13–14	≤12
Kanamycin	30 μgm	≥18	13–17	≤13
Nalidixic acid	30 μgm	≥19	14–18	≤13
Streptomycin	10 μgm	≥15	12–14	≤11
Tetracycline	30 μgm	≥15	12–14	≤11

CAF, chloramphenicol.

were transferred to a test tube of 5 ml Trypton soya broth (TSB) and incubated at 37 °C for 6 h. The turbidity of the culture broth was adjusted using a sterile saline solution to obtain turbidity comparable to 0.5 McFarland turbidity standard. A sterile cotton swab was immersed in the suspension and swabbed uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed and gently pressed using sterile forceps and incubated at 37 °C for 24 h. The diameter of the inhibition zone formed around each disk was measured using a digital caliper. The results were interpreted according to clinical laboratory standard protocols (CLSI, 2015) depicted in the following Table 1.

2.6. Data management and analysis

All data generated in the field and laboratory were entered, coded, and filtered in Microsoft Excel® version 2016 software. From the excel sheet, data were further exported and analyzed using Stata 14 (StataCorp. Stata, Statistical Software: Release 14. College Station, TX) for statistical handling purposes. Descriptive statistics (frequency tables and graphs) were used to visualize the findings, while logistic regression and Fisher's exact test were used to make statistical inferences about the findings. A p-value ≤ 0.05 was considered as significant.

3. Results

The overall prevalence of *Escherichia coli* O157: H7 in fish was found to be 1.46% (6/410). Samples analyzed were filleted fish swab, filleted fish muscle (tissue), whole fish swab, working knife and cutting board swab, ready to eat fish from restaurants, workers hand swab, and container swab). The occurrence of the organism was numerically higher in Lake Hayq than Tekeze Dam. Besides, it was also higher in filleted fish than whole fish swabs. However, these differences were not found statistically significant. The organism was not isolated from ready to eat fish sampled from restaurants, knife and cutting board sab, workers hand, and container swabs. The prevalence of organisms was numerically higher in samples taken from landing sites than local retail markets and restaurants. The prevalence of the organism in different sample types, sampling location, and study Lakes is presented in Table 2.

Because there were no enough positive observations in some variables, the data were not suitable for chi-square analysis. Due to this issue, Fisher's exact test analysis was performed. However, Fisher's exact statistics do not show the direction of the association between the occurrence of the organism and risk factors included. To overcome this problem, we used logistic regression analysis. However, due to a few positive observations, some of the variable levels were omitted during analysis. A univariable logistic regression analysis was performed and the crude odds ratio with P-value and 95% confidence interval was extracted and displayed (Table 2).

Antibiotic susceptibility test was performed against antibiotic disks of gentamycin, kanamycin, CAF, ciprofloxacin, streptomycin, tetracycline, nalidixic acid, and ampicillin. Antibiotics were selected based on their usage level and availability in the market. Based on the susceptibility test result, Ampicillin and Streptomycin performed poorly against the isolates. On the other hand, Ciprofloxacin and Nalidixic acid were found to be effective in preventing the growth of most of the isolates. Tetracycline, Gentamycin, and kanamycin also performed reasonably well.

The maximum number of resistances recorded was only resistant to two drugs in five of the isolates. This indicates there were no multi-drug resistant isolates. The overall resistance and resistance index pattern were calculated and depicted in Table 3.

Table 2. Prevalence of *E. coli* O157 in different sample types, Lakes and sampling sites.

Variables	Total sampled	Positives(n)	p-value	Crude OR	95%CI
Sample type					
Filleted fish swab	125	3		Reference	
Filleted fish muscle (tissue)	89	2	0.942	0.93	0.15, 5.7
Whole fish (skin) swab	125	1	0.3	0.33	0.03, 3.2
Knife and cutting board swab	10	0	-	*	-
Ready to eat fish	13	0	-	*	-
Workers hand swab	24	0	-	*	-
Container swab	24	0	-	*	-
Study Lake					
Hayq	250	4		Reference	
Tekeze	160	2	0.77	1.28	0.23, 7.09
Sampling Sites					
Landing site	293	5		Reference	
Retail market	75	1	0.82	0.778	0.089, 6.76
Restaurants	42	0	-	*	-
Total	**410**	**6**			

* Omitted values because of the absence of positive observations.

4. Discussion

Escherichia coli O157: H7 is one of the most feared foodborne pathogens worldwide. It is a reportable organism in the USA and Europe. The organism has been investigated for its occurrence in different foodstuffs as well as cattle globally. In Ethiopia, there is a limitation in estimating and identifying the occurrence of the organism in foods as well as cattle and human patients. However, one can argue that recently, there is a good trend of studying the organism in different foods of animal origin. Reports in milk and meat estimated the overall prevalence of the organism to be 4.9% (Assefa, 2019). The present study reported that the organisms' occurrence in fish was 1.46% in the two study sites. This prevalence is, however, lower than reported elsewhere from fish and

Table 3. Antimicrobial resistance pattern of the isolates.

Isolate code	Antibacterial resistance profile	Sample type	Source (study Lake)	Number of resisted antibiotics	MDR index
P1	S&AMP	Whole fish (skin) swab	Hayk	2	0.25 (2/8)
P2	S&AMP	Filleted fish swab	Hayq	2	0.25 (2/8)
P3	S&AMP	Filleted fish swab	Hayk	2	0.25 (2/8)
P4	S&AMP	Filleted fish flesh	Hayq	2	0.25 (2/8)
P5	S&AMP	Filleted fish swab	Tekeze	2	0.25 (2/8)
P6	AMP	Filleted fish flesh	Tekeze	1	0.125 (1/8)

S=Streptomycin, AMP = Ampicillin MDR = multi drug resistance.

related species. A study conducted in the USA reported a prevalence of 6.95% in fish (Ribeiro et al., 2015), while in India from shrimp samples, the prevalence was found to be 5.8% (Surendraraj et al., 2010). In France, the organism was isolated from shellfish, with a prevalence of 4.16% (Gourmelon et al., 2006). This difference might be due to the level of sensitivity of tests used.

The prevalence of the organism in this particular study was somehow lower than other foods of animal origin in Ethiopia, as well. This difference can be attributed to the fact that fish is a cold blood animal in which the organism cannot flourish in it. The source for the occurrence of the organism can be poor handling practice by fishers and retailers operating in the study sites. As noted during the study period, fishermen process fish in soil, grass, and stones without any clean bedding material. This unhygienic handling practice can be one of the sources of contamination. In addition to this, fishermen do not prevent or reduce the activity of flies that wonder in the fish during processing and marketing. A study showed that *Escherichia coli* O157 occurrence was 2.7 times higher in house flies (*Musca domestica*) than manure from dairy farms (Burrus et al., 2016), in which the authors recommended the diagnosis of flies that wander in restaurants can give a better result than foods sold. In light of this, the occurrence of *Escherichia coli* O157: H7 in fish can also be attributed to the free activity of flies in the study locations.

The occurrence of *Escherichia coli* O157: H7 was numerically higher in Lake Hayq than Tekeze dam harvested fish. This difference can be due to 1) the sample size is relatively larger in Lake Hayq, 2) higher agricultural practices around Lake Hayq were noted. Farmers that live near Hayq water their cattle in the Lake, graze around the Lake where fish is gutted and filleted, they also use cattle manure to crop papaya, sugarcane, banana, onion and many more crops around the Lake. The remaining of the manure drain to the landing site where fish is filleted and arranged for sale, 3) Lake Hayq is relatively humid than Tekeze (Mulugeta et al., 2017) which makes it a favorable environment for the organism to thrive and for increased activities of flies than the hot climate Tekeze area.

The prevalence of the organism was also found numerically higher in filleted fish (swab and flesh) than whole fish samples. This may indicate that the processing environment (filleting and gutting) can be a source of contamination. A whole fish harvested from the Lakes may have not enough time to be contaminated from the environment than filleted fish. During the process of filleting, almost all parts of the body come in contact with the ground. Consequently, causing a higher contamination rate than whole fish indicating unhygienic processing may expose fish to this and other pathogenic organisms.

Regarding the sampling sites, the organism's prevalence was numerically higher at landing sites than nearby retail markets and restaurants. In Tekeze there were no local markets as well as restaurants in the nearby district (Abergele) that serve fish. Fish harvested in that Dam is directly sold in landing sites for whole sellers who store in the freezer and load it to Addis Ababa and Mekelle cities. Due to this reason, samples from restaurants and retail markets were taken only from Hayq city. Hence, the sample size from the landing site, retail market, and restaurants may not be proportional. This situation further can lead us to the conclusion that the higher occurrence of the organism in landing sites can be due to a higher number of fish samples taken from landing sites. The other conclusion can be, in landing sites, fish filleted and mishandled exposed to dust, dung, and flies. However, fishermen wash their harvest after finishing the filleting process which may lower the chance of recovery of the organism further in the value chain.

Though we did not sample ready enough to eat fish from restaurants, the occurrence level of the organism was zero out of thirteen samples examined. This is somehow a relief that the organism may not reach to end-users. However, this argument may need further detailed investigation with a representative sample size in fish serving restaurants to have a sound conclusion. In addition to restaurants, the organism was not also isolated from other working environment samples. Containers, knife, and workers' hand swabs were free from the organism. This suggests that the need for further investigation of environments of landing sites that pose a risk of contamination during processing.

As *E. coli* is an indicator organism for the presence of contamination in food samples, the existence of other pathogenic organisms in fish harvested from those areas is imminent. Future researches may focus on detecting other pathogenic bacteria and their level of concentration in fish. Besides, risk analysis of contracting infection due to *E. coli* O157 and other pathogenic organisms from fish consumption should be analyzed based on data generated from every value chain.

The antibiotic sensitivity test result showed that essential drugs in the field of veterinary and human medicine performed reasonably well. While drugs like Streptomycin and Ampicillin were poor in inhibiting the growth of the isolates. Even though some illness conditions caused by this organism may not be treated by antibiotics at all, this situation is an indication that Streptomycin and Ampicillin should not be used as a treatment option for infections caused by this organism. Recent studies showed that the organism demonstrated a high level of resistance in Ethiopia as well as elsewhere for these drugs (Abdissa et al., 2017; Bedasa et al., 2018; Sunde and Norström, 2005). It is also said that Streptomycin and Ampicillin are the two most frequently co-transferred resistance phenotypes among sulfonamide-resistant *E. coli* isolates recovered (Sunde and Norström, 2005). Chloramphenicol, one of the most frequently and widely used drug, performed reasonably well in inhibiting the growth of 4 the isolates. However, one isolate was found to be an intermediately resistant to this drug.

Even though there were no resistant isolates for at least three drugs, yet the multidrug resistance index calculated showed a relatively higher index. From the total six isolates, five of them were resistant to two drugs (Ampicillin and Streptomycin), while one isolate was resistant to ampicillin only. Hence the MDR index of the isolates was found between 0.125 and 0.25. This value is somehow higher than the accepted international standard for a multi-drug resistance index of $\leq$0.2. This can be due to widespread irrational drug use in both animals and humans in the country. Irrational drug use can result in resistant gens to develop and further transfer to susceptible organisms from the environment (Von Baum and Marre, 2005). This situation may need attention sooner than later before losing effective drugs regarding cost and efficiency. Even though it is out of the scope of this paper, it is wise that responsible bodies like drug authority and control agency (DACA) should identify actors involved in irresponsible drug use; develop and implement strict control measures to the adhesion of rational drug use at the national level.

5. Conclusion

This study is the first of its kind in Ethiopia in investigating *Escherichia coli* O157: H7 from fish. Handling practices by fishermen during filleting and gutting might be the cause of contamination. Ready to eat fish sampled from restaurants were found free of the organism indicating that it may not reach end-users provided that proper cooking is in place. Since this study has a limitation of including enough sample size from restaurants, this argument may need further investigation of fish serving restaurants with a representative sample size. Drugs like ciprofloxacin, gentamycin, tetracycline, and nalidixic acid were effective in inhibiting the growth of the isolates while streptomycin and ampicillin performed poorly. In light of this, raw and undercooked fish consumptions in Ethiopia may result in contracting infections. Since this study identified a single bacterium, other critical foodborne pathogens associated with fish should be routinely examined. Besides, future studies shall consider taking samples from the environments, the lake water as well as livestock grazing around that areas, particularly cattle as they are the main carriers of *E. coli* strains, running water that can bring cattle feces to the lake and then looking at the genetic relatedness of the isolates from these different sources shall be the focus of research.

Declarations

Author contribution statement

Fikru Regassa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Dinka Ayana, Kebede Amenu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Fufa Abunna: Conceived and designed the experiments.

Ayalew Assefa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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