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Prevalence and Serotypes diversity of *Salmonella* Species in the Nile Perch (*Lates niloticus*) of the Lake Victoria, Tanzania

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Abstract

A cross-sectional study was conducted to estimate the prevalence, serotypes, antimicrobial resistance and plasmids profiles of *Salmonella* spp. in Nile perch of Lake Victoria, Tanzania. *Salmonella* spp. and *Escherichia coli* in water and fish samples were investigated by the bacteriological methods. Antimicrobial resistance profiles of *Salmonella* spp. were determined using the minimum inhibitory concentration (MIC) method. A total of 324 samples were analysed including Nile perch, water and swabs from surfaces of facilities used for fish transport. The finding showed the prevalence of *Salmonella spp.* in fish at fishing ground were 16/60 (26.7%), landing sites and markets were 9/60 (15%) and 18/60 (30%) respectively. A significant difference ($p<0.05$) was observed between the prevalence of *Salmonella spp.* in fish from fishing ground and those at landing sites, but not with those from markets ($p>0.05$). The main serotypes recovered were *Salmonella ser.* Waycross (41: z4z23 :-) and *Salmonella enterica ssp. salamae* (42: r :-). Most *Salmonella* serotypes showed the low resistance profiles against most of antimicrobials, but few isolates were resistant to Nalidixic acid 3/64 (4.7%), ampicillin 5/64 (7.8%), azithromycin 14/64 (21.9%) and sulfamethoxazole 22/64 (34.4%). Plasmids were detected in few *Salmonella ser.* Waycross compared to none of *Salmonella ssp. salamae*. *Escherichia coli* count ranged 0.77 to 2.44 $\log_{10}$ cfu/g in fish and 0.44 to 1.71 $\log_{10}$ cfu/ml in water. Contaminated fish with different *Salmonella* serovars imply pollution of the lake attributable to waste from point and nonpoint sources that may contain antibiotic residues accounting for resistant bacteria in aquatic environment. Un-hygienic fish handling and poor conditions of markets account for the high prevalence of *Salmonella* spp. in Nile perch at markets.

Keywords: fishing ground, landing sites, local markets, antimicrobial resistance, plasmids

1. Introduction

The genus *Salmonella* is classified into two groups: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies: enterica I, salamae II, arizonae IIIa, diarizonae IIIb, houtenae IV and indica VI [Ke *et al*., 2014]. Subspecies I comprise most of pathogenic serovars especially non-typhoid responsible for foodborne disease [Puah *et al*,.2016]. Reservoirs of *Salmonella spp.* are diverse and include warm and cold-blooded animals and also from water, plants and soil was reported to harbour the bacteria [Carrasco *et al*, 2012; Ke *at al.*, 2014; Smith *et al.*, 2015]. Subspecies II-VI of *Salmonella* is usually found in poikilothermic animals and include reptiles, amphibians and also reported to be isolated in environmental samples [CFSPH 2013]. Worldwide, human infections that are caused by non-typhoid *Salmonella* are mainly associated with contaminated food and include seafood where, the contamination may occur in fishing ground (fishing area) also along the value chain [Amaglian *et al*, 2012]. Some non-typhoid serovars like *Salmonella* ser. *Weltevreden*, *Salmonella* ser. Agona, *Salmonella* ser. Newport and *Salmonella* ser. Senftenberg have been reported in water and seafood such as shrimps [Uddin *et al.*, 2015]. Non-typhoid *Salmonella* have been representing a food safety, causing salmonellosis due to the consumption of contaminated foods such as fresh produce, eggs, pork, vegetables and seafood [Campioni *et al.*, 2012]

Lake Victoria is associated with different pollutants due to anthropogenic activities most important waste from households, agricultural and hospitals containing pathogens such as *Salmonella* spp. and other bacteria of public health importance [David *et al*., 2009; Norman *et al.*, 2013; Mdegela *et al.*, 2015]. The waste may contain *Salmonella* serovars of animal and environmental sources, also antibiotic residues resulting to resistance bacteria. The resistance character of bacteria is expressed by the genes carried either in chromosomes or plasmids that can be transmitted from bacteria to human [Rychlik *et al.*, 2006; Winfiel *et al*, 2003]. The river Nile perch can be contaminated with *Salmonella* serovars in the aquatic environment being the resistant traits against different classes of antibiotic [Yang *et al.*, 2015]. Either, poor fish handling along the value chain may contribute to cross-contamination of fish with *Salmonella* spp. So far, little is known about antimicrobial resistance of *Salmonella* spp. from river Nile perch value chain of artisanal fishers in the developing countries such as Tanzania.

After fishing, the rivers' Nile perch conveyed into two processing chain/markets, the artisanal fishers mainly targeting domestic and regional markets and those who process fish for export markets mainly to European Union (EU) countries [Kirema-Mukasa, 2012]. The river Nile perch for export are processed following quality management system where in most cases fish products meet national and international microbiological safety standards, though exported products still facing challenges such as alerts and/or rejection due to *Salmonella* spp. and/or *Enterobacteriaceae* contamination as reported by EU through food notification system. The alerts indicated faecal contamination of fish and/or their products. The fish sold in local markets are handled without following quality management system as a result fish are displayed on open tables, the situation which may compromise the quality of fish [David *et al.*, 2009; Baniga *et al*, 20176]. Currently, the river Nile perch market is expanding domestically and regionally however, microbiological quality and safety of fish from these

markets is still overlooked and no information to whether they meet national and international standards [Kirema-Mukasa, 2012].

The aim of the study therefore, was to estimate the prevalence, serotypes, antimicrobial resistance and plasmids profiles of *Salmonella spp.* isolated in water and in the Nile perch from artisanal fishers of Lake Victoria, Mwanza, Tanzania.

2. Materials and Methods

2.1 Study Site

The study was carried out in Mwanza area of the Lake Victoria basin and sampling points were fishing ground, landing sites and local fish markets located in Ilemela and Nyamagana districts in Mwanza region. Other sampling points include water from rivers crossing urban area and drain into the lake. Sampling sites were selected based on value chain of the Nile perch from artisanal fishers.

2.2 Study Design and Sampling

A cross-sectional study was conducted from January to July 2017, whereby a total of 352 samples were collected and analysed for *Salmonella* spp. and *E. coli* counts. Samples include 60 Nile perch and 60 waters from six sampling points in fishing ground, 60 Nile perch and 36 waters from six selected landing sites and 60 Nile perch from six local markets. Also, 48 swabs were collected in the boats before putting fish in fishing ground and buckets used to put fish at landing sites. Size of each sample was 1-2 kg fish and water 300 ml in 500 ml glass bottle.

2.2.1 Sampling

Nile perch and water were collected in fishing areas where fishermen were fishing on particular day of the visit. Each sampling point visited, 5-15 fish and water samples were collected. Samples were collected while wearing gloves to avoid any possibility of cross-contamination during sampling. Fish samples were collected before were taken on boat, and then put into sterile plastic zip-lock bags. Both fish and water samples were preserved and transported into a cool box containing cooling elements to National Fish Quality Control Laboratory (NFQCL) based in Mwanza for microbiological analysis. In the laboratory, samples were stored refrigerated until the time of analysis which was within 24 hours following sampling.

Fish at landing sites were collected in the morning where fishermen were landing, in each boat; three to four fish were collected depending on how much were available on the day. Water was collected from onshore and offshore of the lake. Onshore water samples, in this study are defined as water collected within 100 metres from the shore of the lake and offshore were the ones collected over 100 metres. Point sources water was collected in the rivers at the course of entry in the lake and at wastewater treatment plant where; samples were taken before and after treatment. Swabs were collected per area of 100 cm^2 in facilities as recommended in food contact surface sampling guideline [NSW, Government Food Authority. 2013]. The Nile perch from local markets were purchased and taken into sterile plastic zip-lock bags and preserved in a cool box containing cooling elements.

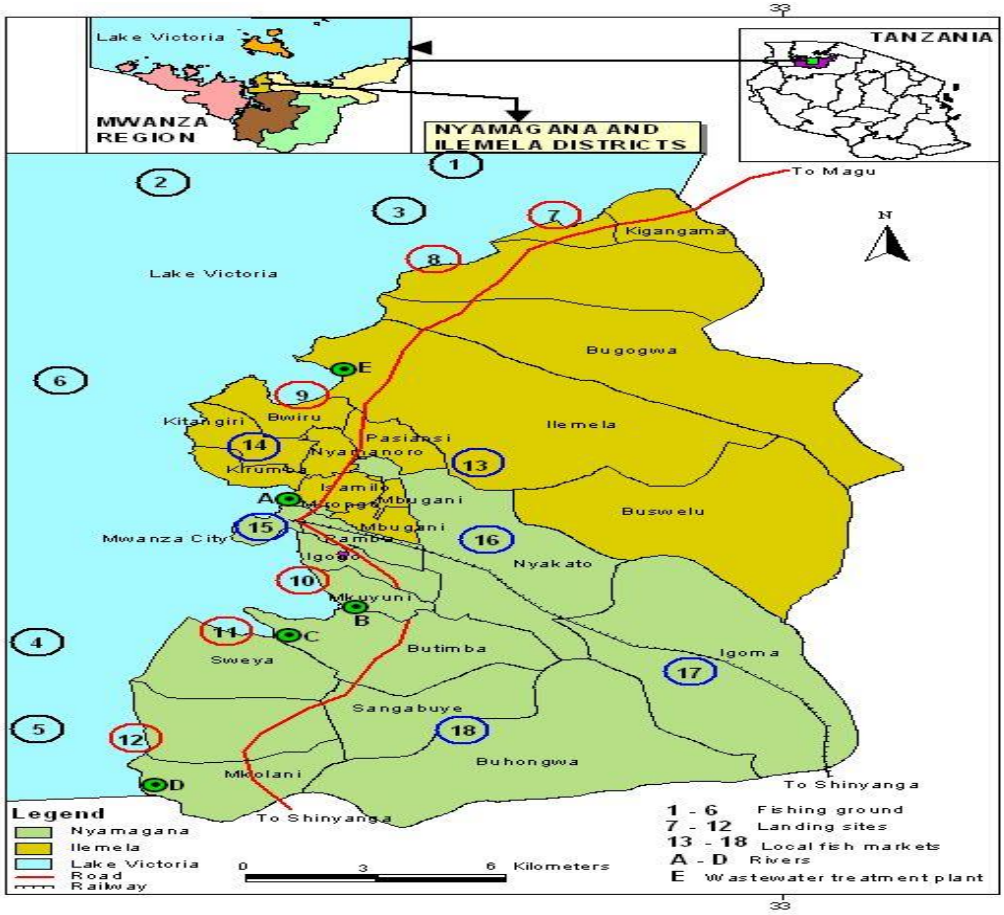

Figure 1. Sketch map showing sampling points

2.3 Sample Preparation and Microbiological Analysis

The Nile perch sample was aseptically prepared by taking four sub-samples comprise intestines, gills, flesh and surface. Fish intestines, gills and flesh were measured into sterile stomacher bag in a ratio of 1:9 between sample and sterile Buffered Peptone Water (BPW) (Oxoid Ltd, England), then homogenised for 60s using Stomacher (Seward 400, UK). Fish surface; a fish in sterile stomacher bag was placed on a tray inclined to about 60°, then 225 ml of BPW was poured and fish was macerated to obtain mucus. The inclination of fish was important to avoid washing the head part which might cross-contaminate the gills. Afterward, BPW containing fish mucus was used for analysis. After washing, fish was transferred on sterile tray, by using 70% methylated spirit, part of surface was disinfected, then sterile razor blade was used to remove skin in disinfected area, then another razor blade was used to chop flesh to 25 grams. The weight was transferred to 225 ml BPW, mixed and homogenised ready for analysis. For gills, a sterile scissor was used to remove gills cover, then using another sterile scissor, gills was chopped, weighed and BPW was added, put into 500 ml glass bottle, mixed and analysed. For intestine, a sterile razor blade was used to open fish carcass longitudinally, then with sterile scissor, lower part of intestine was cut, weighed and BPW was added, mixed and then analysed. Water sample, a 25 ml was measured and mixed into 225 ml of BPW, then analysed. Both fish and water were analysed. according to the proposed protocols.

2.3.1 Detection of *Salmonella* Species

The *Salmonella* spp. was detected according to the proposed standard bacteriological method (European Committee for Standardization, 2007). Briefly, pre-enrichment was done on BPW, and then sub-cultured for enrichment into Rappaport Vassiliadis broth (Oxoid Ltd, England) and Mueller-Kauffman Tetrathionate-novobiocin (Oxoid Ltd, England). Plating out was done onto Xylose Lysine Deoxycholate (XLD) (Oxoid Ltd, England) and Bismuth Sulphate Agar (BSA) (Sigma-Aldrich, France). Suspect *Salmonella* colonies from XLD and BSA were biochemically confirmed on Triple Sugar Iron Agar slant (Oxoid Ltd, England), agglutinated with polyvalent *Salmonella* O- and H- antisera (Rapid Lab Ltd, UK) and confirmed also by Polymerase Chain Reaction using ST11-ST15 primer. Certified Reference Material (CRMs) *Salmonella* ser. Typhimurium ATCC 13311 (Public Health England, UK) was used as positive control in parallel with samples analysed. The isolates were stored in 50% glycerol at -80ºC for further testing. Then, *Salmonella* isolates were packed and shipped to the *Salmonella* reference laboratory in Padova, Italy; Instituto Zooprofilattico Sperimentale delle Venezie for Serotyping according to the Kauffmann-White Scheme.

2.3.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined using Sensititre EUVSEC plates for *Salmonella* spp. (Trek Diagnostic System, East Grinstead, UK) based on the European Committee on Antimicrobial Susceptibility Testing [EUCAST 2015]. *Salmonella* isolates were sub-cultured onto blood agar and incubated at 37°C for 24 hours. Subsequently, a loop full of bacterial cells were resuscitated into test tube contain 5 ml sterile distilled water, bacterial turbidity was adjusted equivalent to 0.5 McFarland standard. Afterward, 10 µl were transferred into 11 ml of Mueller Hinton broth II (Oxoid Ltd, England), vortexed and 50 µl were inoculated into each well of Sensititre EUVSEC plate that contain a known concentration of antimicrobials and incubated at 37°C for 16-24 hours. Then, plates were examined using SWIN Software supplied with Sensititre EUVSEC. Antimicrobials used were ampicillin (AMP), cefotaxime (FOT), ceftazidime (TAZ), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfamethoxazole (SMX), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP).

2.3.3 Plasmids Extraction and Analysis

The plasmids of *Salmonella* serotypes were isolated using bacterial pelleted from 1 ml of overnight culture in Luria Bertani (LB) broth using phenol-chloroform-isoamyl alcohol (25:24:1) extraction method. Extracted plasmids were run in the gel electrophoresis with 0.8% w/v agarose (SRL, India), parallel with 39R and V517 markers of different size (2kb to 147kb). The plasmids DNA bands were visualised and photographed under UV trans-illuminator using quantity one gel documentation system (UVP, England).

2.3.4 *Escherichia Coli* Counts

The *Escherichia coli* were enumerated using pour plate technique on commercially available chromogenic *E. coli* agar medium, Brilliance *E. coli*/Coliform selective agar (Oxoid Ltd, England) following incubation at 37ºC for 24 hours as recommended by the manufacturer.

Purple colonies of *E. coli* on plates were counted with the aid of colony counter. Positive control was run in parallel using CRMs *E. coli* ATCC 8739 (Oxoid Limited Remel Inc, UK).

2.4 Data Analysis

Data collected were stored in Microsoft® Excel version 2010, then analysed using Stata version 14 (StataCorp LP). *Escherichia coli* counts were analysed using negative binomial regression model specified in generalised linear model with a log link function (Hilbe, 2011). All valid counts (<300 colony forming unit (cfu)) on each plate in dilutions and the weight of fish samples represented by each plate were summed and used as an offset in the analysis. Predictors were fishing ground, landing sites and sample type (intestines, gills and surface). The differences between sampling points are expressed as count ratios (incidence ratios in Stata output). The estimated prevalence of *Salmonella* spp. in the sample categories were analysed by the Chi-square (Epi Info ™, 2015). Antimicrobial resistance testing (MIC) were interpreted based on epidemiological cut-off points (ECOFFs) values as stated in (EUCAST, 2015) or Clinical and Laboratory Standards Institute (CLSI, 2014) where, all intermediates were classified together as sensitive. The statistically significant difference was considered at $p<0.05$.

3. Results

Table 1. Prevalence and *Salmonella* serotypes distributions in water and Nile perch sample types from different sampling points along the Lake Victoria, Tanzania

Location	Sample type& No. of positives per sample tested (%)	Sub-sample type	No. of Positives per sub-sample tested (%)	Sampling point (No. of positives samples)	(Sampling point) Serotype [Sub-sample &No. of positives]
Fishing grounds	Fish 16/60 (26.7)	Intestines	12/60 (20)	1 (5); 3(4); 4(7)	(1) *Salmonella* ssp. *salamae* [**S; 4, I;2**], *S.* ser. Hvittinfoss [**G;1**]; (3) *S.* ser. Waycross [I; 2, S;2] and (4) *S.* ser. Waycross [**I;7; G;2**].
		Gills	3/60 (5)		
		Flesh	0/60 (0)		
		Surface of fish	6/60 (8.3)		
	Water 5/60 (8.3)	Lake water	5/60 (8.3)	1 (4); 4(1);	(1) *Salmonella* ssp. *salamae*[**w;4**]; (4) *Salmonella* ser. Typhimurium [**w;1**]
Landing sites	Fish 9/60 (15)	Intestines	6/60 (10)	7 (1); 8(2); 9(1); 11(1); 12 (4)	(7) *Salmonella* ser. Waycross [I; 1], (8) *Salmonella* ssp. *salamae* [**I;1**] and *Salmonella* ser. Typhimurium [**I;1**]; (9) *Salmonella* ser. Waycross [I;1], (11) *Salmonella* ser. Hvittinfoss [**I;1**] and (12) *Salmonella* ser. Waycross [**I;1; G;1; S; 2**].
		Gills	1/60 (1.7)		
		Flesh	0/60 (0)		
		Surface of fish	2/60 (3.3)		
	Water 3/36(8.3)	Onshore water	3/18 (16.7)	8(1); 12 (2)	(8) *Salmonella* ser. Senftenberg [**w;1**]; (12) *Salmonella* ssp. *diarizona*[**w;1**]; *Salmonella* ser. Newport [**w;1**].
		Offshore water	0/18 (0)	N/A	N/A *Salmonella*
	Water 3/4	River outlet water	N/A	A (2); B (1); D (1)	(A) *Salmonella* ser. Singapore [**w;1**], *Salmonella* ser. Tilene [**w;1**]; (B) and (D) *Salmonella* ser. Waycross [**w;2**].
	Swabs 0/48(0)	Swabs of Boats surface	0/24 (0)	N/A	N/A

		Swabs of Buckets surface	0/24 (0)	N/A	N/A
Markets	Fish 18/60 (30)	Intestines	5/60 (8.3)	13(5); 14(2); 15(1); 16(4); 17(4); 18(2)	(13) *Salmonella* ser. Waycross [**S;1, G;1**]; *Salmonella* ssp. *salamae*[**G;1**]; *Salmonella* ser. Typhimurium [**I;1**]; *Salmonella* ser. Hvittinfoss [**S;1**]; (14) *Salmonella ser*. Singapore [**I;1**], *Salmonella* ser. Waycross [**G;1**]; (15) *Salmonella enterica* ssp.*enterica**[**S;1**]; (16) *Salmonella* ser. Newport [**S;2**], *Salmonella* ser. Waycross [**S;1, I;1**], *Salmonella* ser. Senftenberg [**S;1**]; (17) *Salmonella* ser. Waycross [**S;1**], *Salmonella* ser. Newport [**S;1**], *Salmonella* ser. Hvittinfoss [**S;1**], *Salmonella* ser. Senftenberg [**I;1**]; (18) *Salmonella* ser. Waycross [**S;2**] and *Salmonella* ser. Typhimurium [**S;1**].
		Gills	3/60 (5)		
		Flesh	0/60 (0)		
		Surface of fish	12/60 (20)		
Wastewater	Water 2/4	Water in and outlets	N/A	E (2)	(E) *Salmonella* ser. Typhimurium [1], *Salmonella* ser. Senftenberg [1]

Legend: S= fish surface; **G**= fish gills; **I**= fish intestines and **w**= water sample. *Serovar with only one flagellar antigen.

Table 2. Antimicrobial resistance and plasmids profiles of different *Salmonella serotypes* isolated from water and Nile perch of Lake Victoria, Tanzania

Serotypes	**Plasmid's "kb" (Serotypes)**	**Resistance at Epidemiological cut off points (ECOFFs)**
Salmonella ser. Waycross	63, 7.2, 5.1, 3.0 (1)	SMX, COL; GEN (1)
Salmonella ser. Waycross	3.0, 2.7, 2 (3)	Se (3)
Salmonella ser. Waycross	63, 2 (2)	AZI; NAL; SMX (2)
Salmonella ser. Waycross	63 (1)	SMX (1)
Salmonella ser. Waycross	N/A (23)	AMP; NAL; SMX (1), AZI (2), SMX (1), AMP; AZI (2), AMP (1), Se (15)
Salmonella enterica ssp. *salamae*	N/A (12)	AZI; SMX (1), SMX (1), Se (9)
Salmonella ser. Typhimurium	100, 3.0, 2.7 (1)	COL (1)
Salmonella ser. Typhimurium	36 (4)	AZI; SMX (3), Se (1)
Salmonella ser. Hvittinfoss	N/A (4)	SMX (4)
Salmonella ser. Senftenberg	7.2, 4.4, 2.7 (3)	AZI; SMX (2), TET (1)
Salmonella ser. Senftenberg	N/A (1)	Se (1)
Salmonella ser. Newport	N/A (4)	SMX (3), Se (1)
Salmonella spp.	N/A (2)	Se (2)
Salmonella enterica. ssp. *enterica**	N/A (1)	Se (1)
Salmonella enterica ssp. *diarizona*	100, 3.0, 2.7, 2 (1)	AZI; SMX (1)
Salmonella. ser. Tilene	N/A (1)	AMP; AZI; SMX (1)

Legend: AMP = Ampicillin; AZI = Azithromycin; CHL = Chloramphenicol; CIP = Ciprofloxacin;COL = Colistin; FOT = Cefotaxime; GEN = Gentamicin; NAL = Nalidixic acid; MERO = Meropenem; SMX = Sulfamethoxazole; TAZ = Ceftazidime; TET = Tetracycline; TGC = Tigecycline and TMP = Trimethoprim. Se = Sensitive.

* Serovar with only one flagellar antigen.

4. Discussion

The Nile perch at markets had the highest prevalence while those at landing sites had the lowest compared to the one at fishing ground. Fish intestines had higher prevalence of *Salmonella* spp. compared to fish surfaces and gills, except Nile perch from markets where they had higher prevalence on surface than in intestines and gills. Three sampling points out of six in fishing ground were contaminated with *Salmonella* serovars. Moreover, waters collected from rivers were also contaminated with *Salmonella* spp. However, none of *Salmonella* spp. was detected in fish flesh and swabs samples. A significant difference ($p<0.05$) was observed between the prevalence of *Salmonella* spp. in Nile perch at fishing ground and those at landing sites while not with those at markets ($p>0.05$). Also, significant difference ($p<0.05$) was observed between the prevalence of *Salmonella* spp. in Nile perch at landing sites and those sampled at markets. *Escherichia coli* counts at fishing ground; for intestines was 2.44 $\log_{10}$cfu/g, gills 1.60 $\log_{10}$cfu/g, surface 1.13 $\log_{10}$cfu/fish surface and for water was 0.35 $\log_{10}$cfu/ml. Overall *E. coli* in Nile perch at landing sites were 1.81 $\log_{10}$cfu/g for intestines, gills 1.35 $\log_{10}$cfu/g and surface 0.77 $\log_{10}$cfu/fish surface. No significant difference ($p>0.05$) was found between the *E. coli* counts in intestines, gills and surfaces of fishes at fishing ground and those counts in the same sample types at landing sites. However, most of fish and all swabs had counts below detection limit ($<1.0x10^1$ cfu/g/cm^2). Data reported for *E. coli* is only of swabs, water and Nile perch at fishing ground and landing sites, but we were unable to analyse for fish from markets because was not part of study.

Findings imply that, Lake Victoria is polluted with waste from animal and environmental sources as evidenced by the presence of both *Salmonella* serotypes in Nile perch and water. Most serovars recovered in water and fish at fishing ground were *Salmonella enterica* ssp. *salamae* and *Salmonella* ser. Waycross. *Salmonella enterica* ssp. *salamae* generally has more restricted distributions and commonly found in reptiles, amphibians and other cold-blooded animal while rarely in poultry a [Chaudry *et al.*, 2012]. This subspecies is not commonly associated with a virulence in human and animal, though occasionally causing infection to man [13Chaundry *et al.*, 2012; Ke *at al.*, 2014]. Sources of *Salmonella* ssp. *salamae* in aquatic environment could be attributable to waste from cold-blooded animal found along and perhaps in the lake and also environmental waste which are drained into the lake via rivers and water runoff. *Salmonella* ser. Waycross was not only detected in the Nile perch of fishing ground, but also in fish at landing sites, markets and water from rivers. Previous studies reported *Salmonella* ser. Waycross in poultry, human, farmed animal and monkey in USA and Greece as well isolated from beef in slaughter house in Ghana [Al-Nkhil *et al.*, 1999; Haddock *et al.*, 1991; Halatsi et al., 2006; Stevens *at al.*, 2008]. *Salmonella* ser. Hvittinfoss (16: b: e, n,x) also is rare serotype found in Nile perch from Lake Victoria, previously reported in environmental and vegetables in Thailand [Ananchaipattan *et al.*, 2014]. The presence of *Salmonella* ser. Waycross and other serovars in aquatic environment is due to the tendency of reservoirs to shed these serovars in environment where can stay alive over a period of time and when it rains are carried with water to the lake [Amaglian *et al.*, 2012]. The existence of most non-typhoid *Salmonella* serovars in environment for longer than *E. coli* and other commonly non-typhoid (*Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis) is due to the lack of special host

adaptations and ability to colonise a wide range of organisms which ensures their passage to aquatic environments, hence invade the aquatic organisms including Nile perch [Waldner *et al.*, 2012; Winfiel *et al.*, 2003]. To our knowledge, it is the first study to document these serovars in freshwater Nile perch sampled in open water.

The study noted that, boats were not the source of *Salmonella* serovars in fish at fishing ground, because none of *Salmonella* serovar was detected in the swabs. The contaminated fish with *Salmonella* spp. can be the source of pathogen to human if hygienic handling is not implemented efficiently. [Awuor *et al.*, 2011; Winfiel *et al.*, 2003].

The low prevalence of *Salmonella* spp. in the Nile perch at landing sites is due to the possible dying phase of these bacteria which were present in fish at the time of catch. Additionally, since Nile perch sampled at landing sites were not the same catch as those at fishing ground therefore, *Salmonella* spp. in fish at landings could be from contaminated water in fishing ground where fish were caught. Also, *Salmonella* spp. in fish at landing sites could be due to possible cross-contamination with contaminated water at landing sites or poor fish handling in boat after fishing since were handled using bare hands. Serovars detected in Nile perch intestines and gills at landing sites were *Salmonella* ser. Waycross, *Salmonella* ssp. *salamae*, *Salmonella* ser. Hvittinfoss and *Salmonella* ser. Typhimurium (4: i:1,2) previously reported in birds, poultry, animal and environmental samples [Al-Nakhli *et al.*, 1999; Stevens *et al.*, 2008]. The serovars in Nile perch at landing sites are associated with polluted fishing ground where fish were caught as evidenced by the presence of the same serotypes in water and/or Nile perch sampled from fishing ground. Moreover, serovars detected in onshore water at landing sites were *Salmonella* ser. Senftenberg (19: g, [s], t:-), *S.* ser. Newport (6,8: e, h:1,2) and *Salmonella enterica* ssp. *diarizona* (61: l, v: z35). The reservoirs of *Salmonella* ssp. *diarizona* are reptiles, amphibians and farmed animals and are capable of causing infection to man as reported. [Bonke *et al.*, 2012] *Salmonella enterica* ssp. *diarizona* was reported in goats, sheep environment in Switzerland and Great Britain, also from pet snakes in Germany [Schroter *et al.*, 2004; Bonke *et al.*, 2012]. The presence of ssp. *diarizona* and other serovars reported in onshore water are associated with animal such as goats and sheep normally graze along the lake, also might be the number of birds around the shore. However, buckets which were used by fish vendors to put fish at landing site were not the source of *Salmonella* spp. in Nile perch because, none of *Salmonella* spp. was detected in swabs and *E. coli* were below detection limit. The result implies *Salmonella* serovars and *E. coli* found in Nile perch were not cross-contaminated with buckets, but rather from polluted water in fishing ground. Since *E. coli* is an indicator of pathogens in a recent faecal pollution especially of warm - blooded animal, then the reported serovars in this study showed no correlation between *E. coli* and *Salmonella* serovars detected in fish and water.

Poor fish handling could be the source of *Salmonella* spp. in fish at markets. It was observed that, most of fish vendors were using sand from the shore of lake to spread on fish, believing that it reduces spoilage, without considering as potential source of *Salmonella* spp. This is reflected by having more *Salmonella* isolates on Nile perch surfaces than in intestines and gills. Serovars which were detected on fish surfaces at markets were *Salmonella* ser. Waycross, *Salmonella* ser. Singapore (6,7: k: e, n, x), *Salmonella* ser. Newport, *Salmonella*

ser. Typhimurium, *Salmonella* ser. Hvittinfoss, *Salmonella enterica* ssp. *enterica* (16: a:-) and *Salmonella* ser. Senftenberg having varied sources include poultry, animal and environment [Al-Nakhli *et al.*, 1999].

The presence of *Salmonella* serovars on fish surfaces at market showed a phenomenon of cross-contamination of fish associated with animal, poultry and environmental waste around the markets. Since fish were displayed on open tables where flies and insects were present, the presence and coupled with the poor waste disposal nearby to the markets, the flies and other insects probably acted as vehicles for transmission of *Salmonella* spp. to displayed fish. Also, the tendency of some vendors to remove fish maws before selling could be another source of *Salmonella* spp. in fish at markets due to cross-contamination. This could be indicated by *Salmonella* ser. Typhimurium and *Salmonella* ser. Waycross found in gills and intestines, though could be possible that the fish were caught in contaminated areas, as evidenced by *S.* ser. Typhimurium detected in water sample from fishing ground. Detected *Salmonella* spp. in Nile perch at markets in this study is similar to a recent study that reported *Salmonella* spp. in sardines' fish species sold in local markets in Mwanza [Baniga *et al.*, 2017]. It is an indication of persistence poor conditions of local markets linked with faecal contamination in fish sold in retails markets as also reported by others [David *et al.*, 2009; Begum *et al.*, 2010; Budiat *et al.*, 2013]. Since seafood especially Nile perch are eviscerated to remove intestines, cleaned and often cooked before consumed, then *Salmonella* spp. is not considered as potential health risk, though good hygiene should not be neglected during fish processing and handling along the value chain to minimise the risk of cross-contamination [Amaglian *et al.*, 2012].

Table 2 shows *Salmonella* serotypes isolated in water and Nile perch having very low resistance profiles against antibiotic tested. The result demonstrated that few of *Salmonella* serotypes showed resistance to Nalidixic acid 3/64 (4.7%), ampicillin 5/64 (7.8%), azithromycin 14/64 (21.9%) and sulfamethoxazole 22/64 (34.4%) however, all isolates were susceptible to most of antibiotic having MIC values equivalent to ECOFFs and CLSI as recommended by EUCAST guideline. Moreover, Table 2 shows the plasmid profiles of *Salmonella* serovars detected in this study where; *Salmonella* ser. Senftenberg, *Salmonella* ser. Typhimurium and *Salmonella* ssp. *diarizona* had plasmids compared to few *Salmonella* ser. Waycross, however, none of *Salmonella enterica* ssp. *salamae* had plasmids. The antimicrobial resistance profiles in this study are in agreement with other studies in United States, China, Burkina Faso, Malaysia, and Tanzania reported *Salmonella* spp. from different fish species which showed resistance to one or more of these antibiotics [Folster *et al.*, 2015; Uddin *et al.*, 2015; Traore *et al.*, 2015; Hao *et al.*, 2015; Yang *et al.*, 2015]. The existence of *Salmonella* serotypes with multidrug resistance is associated to overuse and/or improper use and disposal of antibiotic resulting to exposure in different environments including aquatic, which in turn contaminate seafood in its natural environment. Natural existence of resistance effects in bacteria against different classes of antibiotic could be the case for *Salmonella* spp. finding on antimicrobial resistance reported in this study. Contaminated fish with multidrug resistant *Salmonella* spp. can act as intermediate for transferring resistance genes to human as had reported in other studies [Amaglian *et al.*, 2012; Ke *et al.*, 2014; Abakpa *et al.*, 2015].

Resistance genes are carried in chromosomes and/or plasmids of bacteria and are transmitted between and among bacteria then to human through infection. Since most *Salmonella* serotypes reported had no plasmids, it suggests that resistance genes could be located in chromosomes but not in plasmids. The finding noted that, there was no relationship between resistance of *Salmonella* serotypes in different antibiotic and possession of plasmids in isolate. Follow up study is required such as whole genome sequencing of *Salmonella* serotypes obtained in this study to determine locations and type of resistance genes of the isolates.

5. Conclusion

The present study has demonstrated that, the Nile perch along its value chain of the Lake Victoria basin are contaminated mainly with uncommon environmental serovars of non-typhoid *Salmonella.* The predominant serotypes reported were *Salmonella* ser. Waycross and *Salmonella enterica* ssp. *salamae* which are rare serovars among *Salmonella* spp. The isolates have low resistance patterns in different classes of antibiotic. Poor hygienic fish handling and conditions of local markets are the key factors contributing significantly to *Salmonella* spp. contamination in fish sold in local markets around Mwanza city.

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