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Charaterisation of *Salmonella* Isolated from Nile Tilapia (*Oreochromis niloticus*) along Lake Victoria Beaches in Western Kenya

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***Abstract*—**Foodborne *Salmonella* infections have become a major problem world wide. Salmonellosis transmitted from fish are quite common. Established quality control measures exist for export oriented fish, none exists for fish consumed locally. This study aimed at characterization of *Salmonella* isolated from Nile tilapia . The study was carried out in selected beaches along L. Victoria in Western Kenya between March and June 2007. One hundred and twenty fish specimens were collected. *Salmonella* isolates were confirmed using serotyping, biochemical testing in addition to *malic acid dehydrogenase (mdh)* and *fliC* gene sequencing. Twenty *Salmonella* isolates were confirmed by *mdh* gene sequencing. Nine (9) were *S. enterica* serotype *typhimurium*, four (4) were *S. enterica* Serotype, *enteritidis* and seven (7) were *S. enterica* serotype *typhi.* Nile tilapia have a role in transmission of Salmonellosis in the study area, poor sanitation was a major cause of pollution at the beach inshore waters.

Keywords*—fliC, mdh,* Salmonellosis, Serotype

I. Introduction

SALMONELLA causes a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia. Gastroenteritis has the greatest adverse effect on children's growth and development [1]. In normal host, bacteremia accompanies gastroenteritis about 5% of the time, whereas in Acquired Immune Deficiency Syndrome (AIDS), the incidence of bacteremia is much higher [2]. The majority of 1.3 billion annual cases of *Salmonella* – caused human gastroenteritis result from ingestion of contaminated food products such as undercooked beef, pork, eggs, milk, shell fish and fish [3],[4],[5]. *Salmonella* infections can also be contracted following consumption of fresh fruits or vegetables contaminated by fertilizer [6]. Birds and flies are important vectors for rapid widespread dissemination of *Salmonella* in the environment [7]. Compared to *E.coli*, *Salmonella* withstands a wider variety of stresses associated with environmental fluctuations and may persist in water environment for sometime. *Salmonella* can be disseminated as a result of water currents, underground springs and rain run off carrying contaminated material [8],[9]. Like *E.coli, Salmonella* is constantly released into environment from infected human, farm animals, pets and wildlife [10].

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Pathogenic and potentially pathogenic bacteria associated with fish and shell fish include *Mycobacteria, Streptococcus iniae, vibrio vulnificus, vibro spp, Aeromonads, Salmonella spp, Shigella* and the others [11],[12],[13]. Human infections by these fish pathogen are usually through contact with infected fish while handling them, water or other constituents of fish life environment [14]. The initial microflora on the surface of fish is directly related to the water environment while the flora in the gastrointestinal tract corresponds to the type of food and condition of fish [15]. Most of the animal protein consumed by the local population in western Kenya comes from L. Victoria [16]. Mitigation of environmental problems in L. Victoria indicates four immediate microbiological pollution sources, namely municipal untreated sewage, run off, storm water, animal waste and maritime transport wastes. Municipal untreated sewage, run off and storm water are the most important immediate microbiological pollutants [17]. The low standard of health in the winam gulf region is caused by a general lack of awareness of good hygiene practices, direct contamination of beach waters through bathing and washing and uncontrolled waste disposal around the shoreline [17].Other sectors like wildlife, agriculture, forestry, urban and rural settlements have been implicated to contribute to microbiological pollution of the lakes [17] These activities increase eutrophication process thus creating a vast conducive environment for the survival of microbes which eventually infect fish. Analysis of fish tissue slurry indicated that fish harvested from landing beaches along Winam gulf are infested with *Enterobacteriaceae* namely; *Salmonella, Shigella* and *E. coli* [41].

Veterinary authorities from Spain and Italy detected unacceptable level of bacteriological contamination in fish from all the three East African counties [16]. In 1998, European Union (EU) banned fish exports from East Africa and Mozambique due to cholera outbreak. While established quality control measures exist for export oriented fish according to the European Union Directive on Hygiene (91/493/EEC), that deals with handling and processing of fish from the point of capture to its eventual arrival on the market. None exists for the fish consumed locally and this poses a great danger to the health of local consumers [18].

Prevalence of *Salmonella enterica* serotype *typhimurium* was found to be 23.3% and 60% among immunocompromised and immunocompetent children in western Kenya [19]. Septicemic Salmonellosis due to *Salmonella enterica* serotype *typhimurium* is documented to occur seven times more frequently than typhoid infections, with mortality rate of 18% in malaria endemic areas [20]. Clinic based surveillance by CDC/KEMRI for diarrhoeal diseases (May 1997 – April 1998) in Asembo along Winam gulf found 14% of the total isolates to be *Salmonella* [21]. [22],[23], also found 12% of nontyphoidal diarrhoea in the same region. In Kenya on average 6.6% of pediatric inpatient present with bacteremia. NTS has been second in importance only to invasive pneumococcal disease. However, there is scant information on likely reservoirs and sources of infection [24]. Given the prevalence of water and foodborne disease; Salmonellosis in western Kenya, it was important that all possible infection routes of the pathogens be investigated and prevention measures recommended. This study aimed to characterize *Salmonella* isolated from Nile tilapia (*Oreochromis niloticus*) along L. Victoria beaches in western Kenya as a purported source of Salmonellosis.

II. MATERIALS AND METHODS

A. StudySite

Lake Victoria is the second largest fresh water lake in the world, with an area of 69,000 sq km [16]. The lake is shallow with a maximum depth of 84 m, and a mean depth of 40 m. It has a catchment area of 193,000 Km^2 shared between Kenya, Tanzania and Uganda. Kenya owns 6%, Uganda 45% and Tanzania 49% of the total area. The basin supports over 30 million people. The major portion of Kenyan water of L. Victoria is a narrow gulf known to various authors by several names; the Victoria Nyanza, Kavirondo gulf, Nyanza gulf and Winam gulf. Winam gulf lies south of the Equator between $0^0$6 S – 0^0 32'S and $34^0$13' - 34^0 52'E at an altitude of 1134 m above sea level and covers an area of 1,920 sq. km (approximately 6% of whole lake) between 6 km and 30 km [16]. It has a catchment area of 3,600 km^2 drained by five major rivers (Nzoia, Kuja, Nyando, Yala, Sondu) through which it contributes approximately 30% of total riperrine inflow into L. Victoria [25]. It receives a mean annual rainfall of about 1,153 mm and experiences a mean annual temperature of 22^0C and a mean annual potential evaporation of 1968 mm. Winam gulf experiences long rains from March to May with peak in April and short rains in August and October. Sample collection was done in four selected beaches, Uhanya to the northern end of the lake, Luanda Kotieno; an open inshore beach, Homabay to the southern end of the lake and Dunga beach in Kisumu city; a closed shore in industrialized area (Fig 1).

Fig. 1 Map showing the Winam Gulf (Kenyan part) and the selected fishing beaches. (Adapted from knowledge and experiences gained from managing Lake Victoria ecosystem LVEMP 2005, Mallya, G. Wambede, J.Kusewa, M.(Eds)

B. Study Sample

The study involved the harvesting of wild Nile tilapia fish from selected beaches in Lake Victoria Kenya waters. Nile tilapia (*Oreochromis niloticus*) is the most sought fish species locally and the third commercially important and fish in lake Victoria. The study was done during long rainy season (March – June 2007). Ten table size Nile tilapia fish was harvested by fishermen using various fishing methods as described hereafter. Dunga beach by use of angling rods and beach seine, Luanda Kotieno by beach seine, angling rods and gill nets, Homa bay by use of beach seine, gill nets and trawling, Uhanya by beach seine, gill nets an angling rods. The fish were placed in sterile plastic bags and transported to Zoology laboratory, Maseno University, 6 hours after collection in a cool box at 8^0C. In the laboratory, the fish sample were processed according to Newaj, Fyazul *et al.,* [26] protocol for microbiological analysis with minor modifications.

C. Phenotypic Isolation and Identification of Salmonella species in Nile Tilapia Fish Samples

Working surface was cleaned using 10% jik solution and 70% ethyl alcohol before and after inoculation work. Fish samples were externally disinfected by dipping them in ethyl alcohol for 2 minutes followed by three washings with sterile distilled water.

Whole fish excluding bones was macerated using a sterile mortar and pestle, immersed in phosphate buffered saline (PBS) of pH 7.5 to achieve, 10% w/v suspension of fish. Five milliliters of each fish tissue was used to inoculate selenite F medium (Himedia laboratory pvt Ltd Mumbai India), incubated at 37^0C overnight (18-24h) for maximum recovery of the *Salmonella* pathogen. Isolates in selenite F medium

were subcultured onto both MacConkey agar (Oxoid No. 3 CM 115 Basingstoke, England) and Deoxycholate citrate Agar (DCA) (Himedia laboratories pvt Mumbai India), by streaking technique and incubated at 37^0 for 18 – 24 hrs. Colonies from DCA were transferred to Nutrient broth (CDH JO 0003-Himedia laboratories pvt Mumbai India) and preserved at 4^0C for later use in genetic analysis.

Biochemical tests (IMViC – Indole, Methyl red, Voges, proskaurs, Citrate), was performed to identify bacteria genus based on their biochemical activities in different culture media. Isolates from Triple Sugar iron (TSI) were subcultured in methyl red medium and incubated at 35^0C for 48± 2h. To obtain agglutination test for 'O' antigen of *Salmonella*, circular areas were marked off on surface of a glass slide using a crayon pencil marker heavily marked, circular areas containing cell suspension inhibits running onto each other or off the slide. A drop of cells suspended in normal physiological saline (0.9% ml NaCl) was placed in each circle and a drop of antisera was added to each. Agglutination will only occur when there is reaction between antibodies in the antisera and their homologous antigens on cell wall of bacterium. The 'H' antigen test was done in a test tube following same procedure as in 'O' antigen. The serovars were grouped according to their 'O' antigen reaction according to Popoff *et al*., [27] and Kauffman *et al.,* [28] and Brenner *et al.,* [29].

D.Genotypic Isolation and Analysis of Salmonella

Pure isolates of *Salmonella* obtained from a series of subcultures in selective medium DCA and stored in nutrient broth (CDH JO 0003 -Himedia laboratories pvt Mumbai India) were picked from solid agar plates using heat sterile chromium inoculation wire loop and reconstituted in 200ul of 0.9% NaCl solution ready for DNA extraction. Pure isolates of 1.5 ml were spinned at 15,000 x g for 2 minutes in microcentrifuge and supernatant removed. The pellet was resuspended in 56ul TE buffer 30ul of 20 mg/ul proteinase K, vortexed and incubated for 1h at 37^0C. 100 ul of 5 M NaCl was added and vortexed. 80ul of cetyltrimethyl ammonium bromide (CTAB/NaCl) was then added to the mixture, vortexed and then incubated at 65^0C for 10 min. An equal volume of chloroform/isoamyl alcohol and spinned at 15,000 x g for 5 min.

An aqueous phase was transferred to a fresh tube and DNA extracted by adding phenol/chloroform/isoamyl alcohol and spinned at 15,000 x g for 5 min. 0.6 volume isopropanol was used to precipitate DNA and DNA precipitate washed with 70% ethanol, centrifuged and supernatant remove. The pellet was dried for 24 hrs and re – suspended in 100ul TE buffer ready for PCR.

For the presence of *malic acid dehydrogenase (mdh)* and *fliC* gene and *sef A* gene amplifications were performed in a final volume of 50*ul* containing 5 *ul* of both primers (Table 1, 2 and 3) as well as 8*ul* of dNTP, 5*ul* of 10 x buffer, 3*ul* Mg Cl_2, 0.2. *ul Taq* polymerase, 5*ul* DNA containing sample and 23.8*ul* water to make up the volume.

The cycling conditions were as follows 94^0C for 5 minutes, followed by 94^0C for 25 sec, 54^0C for 45 sec then 72^0C for 1 min for 30 cycles and post elongation 72^0 C for 7 minutes. The amplicons were then loaded onto a casted 2% agarose gel alongside 50 bp DNA marker, a negative control and resolved at a constant voltage of 100 for 25 min prior to UV visualization.

III. RESULTS

Out of 120 fish specimens collected, 63 were positive for various bacteria isolates from fish tissue slurry, *Shigella spp* (39.6%) was the most isolated *Enterobacteriaceae* followed by *Salmonella* (31.7%), *E.coli* (25.3%), *proteus* (1.58%) and *E. aerogenes* (1.58%). A total of twenty *Salmonella spp* were isolated, 9(14.3%) were *Salmonella enterica* serotype *typhimurium*, 4(6.3%) *S. enterica* serotype *enteritidis*, 7(11.1%) *S. enterica* serotype *typhi*. Dunga beach had the highest *Salmonella spp.* 8(40%) isolated as compared to Uhanya 6(30%), Homabay 4(20%) and Luanda Kotieno 2(10%) (Table 1).

TABLE I
DISTRIBUTION OF SALMONELLA ISOLATES WITHIN SELECTED BEACHES ALONG WINAM GULF

***Salmonella* serotype**	**Beaches**			
	Dunga	**Uhanya**	**Homa bay**	**Luanda Kotieno**
S. typhimurium N=9	4 (44.4)	3 (33.3%)	1 (11.1%)	1 (11.1)
S. enteritidis N=4	1 (25%)	1 (25%)	2 (50%)	0 (0.0%)
S. typhi N=7	13 (42.8)	2 (28.5%)	1 (14.29)	1 (14.2%)
Total	8 (100%)	6 (100%)	4 (100%)	2 (100%)

A. Genotypic characterization of Salmonella

Phenotypic identification was confirmed by amplification of *Salmonella* housekeeping gene *malic acid dehydrogenase* (*mdh*) that gave a band of 261bp (Fig 2). For identification of *S. enterica* serotype (*typhimurium*, primer combination of *fli*15 and *Tym* were used to amplify *fliC* gene and a band of 559bp was obtained for 9 samples (Fig 3). *S.enteritidis* identification was genotypically confirmed by 312bp gene amplification of *sef A.* (Fig 4).

IV. DISCUSSION

Lake Victoria basin is one of the areas where environment has continued to deteriorate [40]. Microbiological pollution through wildlife, agricultural activities, forestry, agrobased industries, rural and urban settlement as well as surface runoff and storm water in the Kenyan catchment side of the lake, have negative effects on water quality of rivers draining into

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lake Victoria [30]. These activities have contributed to the rising population of fish microbes within Winam gulf.

Primer pair:

mdh – F, 5′- TGCCAACGGAAGTTGAAGTG - 3′
mdh – R, 5′ – CGCATTCCACCACGCCCTTC - 3′
M Ø 3 4 5 6 7 8 9 10 11 12 13

Fig. 2 PCR gel showing *Malic cid dehydrogenase* gene products for *Salmonella*. Isolates from Dunga (Lane 3 ,4 ,5 & 6), Uhanya (Lane 7 & 8), Luanda Kotieno (Lane 10) and Homa Bay (Lanes 11 ,12 & 13). Molecular DNA maker 50bp (lane 1), Ø; negative control (lane)

Primers	**Oligonucleotide sequence**
Fli 15 -5′ F	- CGGTGTTGCCAGGTTGGTAAT - 3′
Tym - 5′ R	- ACTCTTGCTGGCGGTGCGACTT- 3′

M Ø 3 4 5 6 7 8 9 10 11 12 13 14

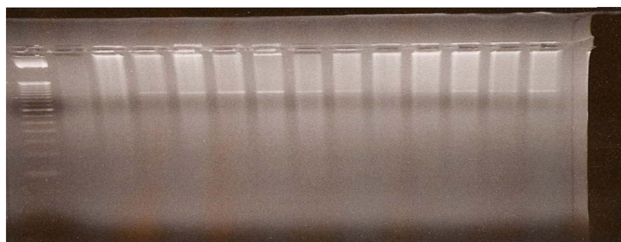

Fig. 3 PCR gel showing *fliC* gene products for *Salmonella*. Isolates from Dunga (Lane 4 ,5 ,6 & 7), Uhanya (Lane 8 & 9), Luanda Kotieno (Lane 12) and Homa Bay (Lane 13 & 14). Molecular DNA maker 50bp (lane 1), Ø; negative control (lane 2)

Primers	Oligonucleotides sequence
Sef 167 5′ F	- AGGTTCAGGCAGCGGTTACT - 3′
Sef 478 5′ R	- GGGACATTTAGCGTTTCTTG - 3′

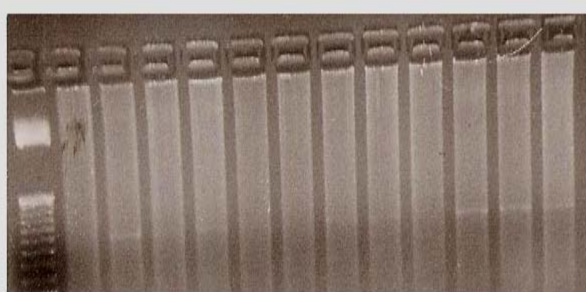

Fig. 4 PCR gel showing sefA gene products for *Salmonella*. Isolates from Homa Bay (Lane 3) Dunga (Lane 11 & 12), Uhanya (Lane 13), and. Molecular DNA maker 50bp (lane 1), Ø; negative control (lane 2)

In the present study, analysis of fish tissue slurry indicated that harvested Nile tilapia were infested by *Salmonella.* Twenty *Salmonella spp* were isolated. Dunga beach had the highest *Salmonella* distribution (40%) of the total isolates. The beach area is densely populated with a closed shoreline, this have led to poor water circulation in the shore line. The effects of urbanization have serious implications on L. Victoria along Dunga beach in Kisumu city. Kisumu city is littered with pools of sewage spills from broken sewage pipes and tanks. This forms part of the surface run off after heavy rainfall that ends up into the Lake [31]. This accounted for the high *Salmonella* distribution in Dunga. These results concur with findings of Onyango *et al.,* [41], who found *Salmonella, Shigella* and *E. coli* as some of the serious pathogens associated with fish from Dunga fish landing beach. [32], also identified fish landing environment to be a major source of fish quality problems in L. Victoria. Homabay beach had 20% of total *Salmonella* isolates. Presence of *Salmonella* in fish at Homabay beach was due to pollution as a result of untreated sewage effluents disposed directly into the lake due to dilapidated sewage treatment system. These results are typical of what was found in previous studies by [33], [34], [35],[36] who isolated *Salmonella, Shigella*, and *E. coli* from fish harvested from waters subjected to human sewage pollution.

Distribution of *Salmonella* isolates at Uhanya beach was 30%. Lack of portable water supply in addition to high latrine density in overcrowded nuclear settlements were the causes of high *Salmonella* distribution. The beach area has a closed shoreline hence poor water circulation leading to high levels of *Salmonella* counts. Luanda Kotieno had the lowest distribution of *Salmonella* (10%) in fish collected. This was attributed to the lake area being an open inshore with adequate water circulation. Dilution effect at the shoreline led to low levels of *Salmonella.*

The results of this study indicated presence of *Salmonella* in fish harvested from lake Victoria, an indication of contamination of the lake waters by the pathogen. These results are consistent with the National Agricultural Research Organization of Uganda (NARO) [37] Report which indicated the presence of certain species of *Enterobacteriaceae* like *E. coli, Salmonella* species at some fish landing sites along the shores of L. Victoria [38]. Previous studies by NARO (1997) also established that faecul or thermo tolerant coliforms and therefore prevalence of *Salmonella* species were higher at the inshore waters where beaches are located than offshore water, especially beaches which are densely populated with inadequate sanitary facilities.

The most commonly isolated *Salmonella* serotype was *typhimurium*, followed by t*yphi* and the least was *enteritidis*. There being probability that the main source of *Salmonella* contamination in the marine environment is of human or animal origin, the different population structures of *S. enterica* serotype *typhimurium, S. enterica* serotype *enteritidis* may be attributed, to the different rates of growth and survival of these serovars in the aquatic environment. *Salmonella* serovar *typhimurium* was the clinically important serovar mostly identified in this study which attests to its capacity of

adaptation and survival in the environment as has been suggested by other authors [39],[10]. The presence of *Salmonella* in Nile tilapia was as a result of contamination of fish during off loading but not during loading offshore. For absolute safety, elimination of initial contamination of fish by *Salmonella* from the source should be ensured. Landing and marketing of fish from beaches with essential sanitary facilities can reduce the risk of cross contamination. Additional safety measures should include training in personal hygiene, sanitation and ensuring water quality.

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REFERENCES

[1] R.E. Black,K.H. Brown,S. Becker " Effects of diarrhoea associated with specific enteropathogens on the growth of children in rural Bangladesh."vol 73,pp 799-805,1984.
[2] L.M.D. Gooze " Bacterial Infections associated with HIV", HIV Insight KnowledgeBase Chapter. Stanford Univ. Sch. of Medicine,1998.
[3] J. Pang, Z.A. Bhutta, B. B. Finlay ,M. Altwegg , "Typhoid, fever and other Salmonellosis: a continuity challenge'Trends microbial vol 3, pp 253-255, 1995.
[4] T.M. Gomez ,Y. Motrjemi ,S. Miyagawa ,F.K. Kaferstein, K. Stohr , "Foodborne Salmonellosis" World Health Stat. Q.;vol 50, pp81-9,1997.
[5] H.Esaki , A. Morioka , K. Ishihara, " Antimicrobial susceptibility of Salmonella isolated from cattle, swine, and poultry (2001 – 2002): Report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program", Antimicrob. Chemother vol 53, pp 266-70,2004.
[6] R.V. Tauxe , "Emerging food borne diseases: an evolving public health challenge. The fish exporting and fishmeal industries of Lake Victoria - structure, strategies and socio-economic impacts in Kenya "Annual Report, Emerge. Infect. Dis. vol 3 , pp 425-434,1997.
[7] R.H. Davies, C. Wray, " Seasonal variations in the isolation of Salmonella typhimurium, Salmonella enteritidis, Bacillus aureus and Clostridium perfringens from environmental samples", J. vet. Med. Ser.vol 43, pp.119-127,1996.
[8] W. Chao, R. Ding, R. Chen, "Survival of pathogenic bacteria in environmental microcosms", Chinese J. Microbial Immun. Vol. 20, pp.339-348,1987.
[9] M.H. Abdel-Monem, A.A. Dowidar, "Recoveries of Salmonella from soil in Eastern region of Saudi Arabia Kingdon', J. Egypt. Public Health Assoc. vol, 65, pp.61- 75, 1990.
[10] J. Baudart , K. Lemarchand , A. Brisabois , P. Lebaron, " Diversity of Salmonella strains isolated from the acquatic environment as determined by serotyping and amplification of ribosomal DNA spacer regions", Appl. Environ. Microbiol.; vol. 66, NO. 4, pp.1544 –1552, 2000.
[11] E.K. Lipp , J.B. Rose, " The role of sea food in foodborne diseases in the United States of America," Rev. Sci. Tech. OIE, vol. 16, pp.620-640,1997.
[12] A. Zlotkin ,A. Eldar , C. Ghifino , H. Bercovier , " Identification of Lactococcus garvieae by PCR,". J. Clin. Microbiol.,vol. 36 pp. 983-985,1989.
[13] P. Bhaftopadhyay , " Fish – catching and handling ," In: Robinson R.K. (ed.): Encyclopedia of Food Microbiology, Academic Press, London, vol.2, pp. 1547,2000.
[14] P.N. Acha, B. Szyfres, "Zoonoses and communicable diseases common to man and animals," Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication ,vol.1, No. 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA,; ISBN 92 75 31580 9. pp..384,2003.
[15] J. Liston "Microbiology in fishery science", In Connell, JJ. (ed). Advances in Fish Science andTechnology; Jubilee Conference of Torry Research Oxford: Fishing News Books Ltd, Farnham, UK, 1980.
[16] R.O. Abila, E.G. Jansen, "From Local to Global markets: The fish exporting and fishing meal industries of Lake Victoria– Structure, Strategies and Socio-economic Impacts in Kenya," IUCN Eastern Africa programme. Socio-economics of Lake Victoria fisheries: Report No.2, The World Conservation Union, Nairobi, 1997.
[17] S.Kayambo, E.J.Sven , " Lake Victoria. Experience and lessons learnt. A case Study for Preliminary Risk Assessment Report.; pp. 431 – 446, 2006.
[18] V.O. Ogwan'g, M. Muchiri, P. Thakor , " Investigation of bacteriological quality of smoked fish," In: Knowledge and experiences gained from managing The Lake Victoria Ecosystem. Mallya G,Wambede, J, Kusewa, M. (eds). Published by LVEMP Regional Secretariat, Dar es Salaam.; pp. 552-569,2005.
[19] D. Onyango, F. Machoni, R. Kakai, E.N. Waindi , " Multidrug resistance of Salmonella enteric serovars Typhi and Typhimurium isolated from clinical samples at two rural hospitals in Western Kenya," J. Infect Developing Countries; vol.2, no.2, pp. 106-111, 2008a.
[20] A. Nesbitt, B.M. Nazir, "Salmonella septiceamia in Kenyan children", J. Tropic. Paediatrics. Vol. 35 pp. 35-39, 1989.
[21] H.S. Roger, L. Kumar, P. Philips-Heward , J.G. Wells , P. Adrock, J. Brooks, et al. "Antimicrobial resistant bacterial diarrhoea in rural Western Kenya" J. infect. Dis. Vol. 183: 1701-4, 2001.
[22] J.T. Brooks , L.S. Roger ,L. Kumar, J.G. Wells , A. Penelope, Philip-Howard et al. " Epidemiology of periodic Bloody Diarrhoea in Rural Western Kenya ," Am. J. Trop. Med. Hyg.vol. 68, no. 6 pp. 671-677, 2003.
[23] T.B. Brooks , J.B. Ochieng , L. Kumar, G. Okoth ,R.L. Shapiro, J.G. Wells , et al., " Surveillance for Bacterial Diarrhoea and Antimicrobial Resistance in Rural Western Kenya",2006.
[24] S. Kariuki, Revathi G, Muyodi JM, Munyalo A, Kagendo D, Murungi L. et al. (2004): Increasing prevalence of multidrug resistant non-typhodal Salmonella, Kenya. Intnatl. J. Antimicrob. Agents;vol. 25, pp.38-43, 2004.
[25] P.G. Njiru , " An overview of the present status of the water quality in L.Victoria, Kenya," A limnological perspective: In: Knowledge and experiences gained from managing The Lake Victoria Ecosystem. Mallya G. Wambede, J. Kusewa, M. (eds). Published by LVEMP Regional Secretariat, Dar es Salaam; pp. 679-690, 2005.
[26] A. Newaj-Fyzul, A. Abiodun, A. Adesiyun, Mutani A, "Prevalence and antimicrobial resistance of Salmonella sp isolated from apparently healthy ornamental fish and pond water in Trinidad," J.of food. Agric. and Environ; Vol 4, no. 1, pp.27-29,2005.
[27] M.Y. Popoff, Le Minor, "Antigenic formula of the Salmonella serovars," 7th edition World Health Organization, collaborating center for reference and research on Salmonella; Pasteur institute, Paris, France, 1997.
[28] F. Kauffman, "The bacteriology of Enterobacteriacae," Munksgaard, Copenhagen,Denmark, 1997.
[29] F.W. Brenner, "Modified Kauffmann-White Scheme," CDC. Atlanta,Georgia, 1998.
[30] R.C.M. Crul, "Monographs of African great lakes; Limnology and Hydrology of L. Victoria Part" 1UNESCO/HIV-IV project comprehensive & comparative study of great lakes. A project of the fourth phase of the International Hydrological Programme of the Division of water sciences of UNESCO.(UNESCO/IHP-IV Project M-5-1), 1995.
[31] B.A. Ogindo, "Bacterial pollution indicators and their physico-chemical environment in the inshore waters of L.Victoria, Kisumu City in Relation to Enteric Diseases," A PhD thesis Maseno University, 2005.
[32] G.F. Nanyaro, J. Makene , " Report on studies of Fish handling in water, landing beaches, at markets and during transportation in Mwanza gulf," Fisheries management component. Lake Victoria Fisheries Organization; vol. 1: 19-30, 1989.
[33] J.M. Shewan, "The Microbiology of sea water fish ," In Borgstrum, G. (ed) Fish as food; vol. 1, pp. 487-9, 1961.
[34] G.J. Banwart, "Basic Food Microbiology," Westport Connecticut: The AVI publishing company Inc.A bridged edition, 1981.

[35] J.H.J. Huis in't veld, "Microbial and Biochemical spoilage of foods," An overview. Internatl. J. Food Microbiol.vol. 33 pp. 1-18, 1996.
[36] H.H. Huss, "Assurance of seafood quality," Food and organization Fisheries Technical Paper 334. Rome: FAO.1994.
[37] NARO – National Agricultural Research Organization Uganda : " A survey of sanitary and hygienic conditions of the industrial fish processing sector, from landing sites through plant to exit routes (airport)," NAROSEC report, 1997.
[38] J. Atyang , " A manual for Training Staff in Uganda Fish Processing Plants on Hygiene and Sanitation and application of HACCP Principles," J. of UNU – Fisheries Training Programme vol. 1 pp.43-57,1999.
[39] E. Dupray, A. Derrien , " Influence of the previous stay of Escherichia coli and Salmonella spp. in waste water on their survival in seawater," Water Res. Vol. 29, pp.1005-1011,1995
[40] A.F Rwabinge "Investigation of bacteriological ecological sanitation in the lake zone: Awareness Creation and Action" In: Knowledge and experiences gained from managing The Lake Victoria Ecosystem. Mallya G,Wambede, J, Kusewa, M. (eds). Published by LVEMP Regional Secretariat, Dar es Salaam; pp. 624-634, 2005.
[41] D. Onyango, S. Wandili, R. Kakai, E.N. Waindi, Isolation of Salmonella and Shigella from fish harvested along winam Gulf of lake Victoria, Kenya, J. infect. Dis. In Developing Countries,Vol. 2, pp.106-111, 2008b.

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