



**INVESTIGATING THE LEVEL OF CONTAMINATION OF FRESH NILE PERCH
(*Lates niloticus*) BY *ESCHERICHIA COLI* AND *SALMONELLA* FROM LALLE
LANDING SITE, ARAPAI MARKET AND SOROTI MAIN MARKET.**

By

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**RESEARCH DISERTATION SUBMITTED TO THE FACULTY OF AGRICULTURE
AND ANIMAL SCIENCES IN PARTIAL FULFILLMENT OF AN AWARD OF A
BACHELOR'S DEGREE IN ANIMAL PRODUCTION AND MANAGEMENT OF
BUSITEMA UNIVERSITY.**

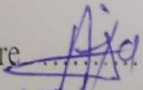
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DECLARATION

I hereby declare that, this work is truly my original work and it has never been submitted in any institution for any academic award.

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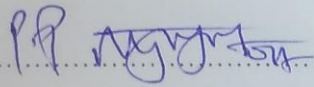
This research proposal/thesis is submitted by the approval of

APPROVAL

This research was written under my supervision and guidance it will be submitted to the department of Animal Production and Management for examination with my approval as the supervisor.

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DEDICATION

I dedicate this dissertation to my friends and family members more especially to my beloved mother Ms. Binengo Cecilia, Sister Akumu Grace, my brother Dr Kissa Charles, and my academic supervisor and mentor Dr. Matovu Henry.

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I would like to take this opportunity to thank my academic supervisor for the guidance. My beloved brother Dr Kissa Charles and sister Akumu Grace for great financial support they rendered to me and my sincere appreciation and thanks goes to all the management staff, lecturers, and stakeholders of Busitema University Arapai campus

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LISTS OF ABBREVIATIONS

Dr	Doctor
E.coli	Escherichia coli
ie.....	That is to say
XLD.....	Xylose Lysine Deoxycholate
⁰ C	degree celcius
cfu/g.....	coliform forming units per gram
ml	Mill in litres
FDA	Uganda Food and Drug Authority
WHO	World Health Organisation
df.....	Degree of freedom
f.....	f values
sig	level of significance (p- values)
UNBS	Uganda National Bureau of standards

ABSTRACT

Fish and fish products are a food with a high nutritional value, however fish may contain *Escherichia coli* and salmonella which cause foodborne infection outbreaks in humans. The aim of the study was to identify and compare *Escherichia coli* and salmonella in skin, muscles and intestine for fresh Nile fish samples collected at Arapai market, Lalle landing site and Soroti main market. Fifteen fish samples were collected, 5 samples each from different traders and fishermen. MacConkey agar was used for isolation of *Escherichia coli*, Xylose Lysine Deoxycholate media (XLD) for isolation of salmonella, *Escherichia coli* was tested using indole ring test.

The total coliform microbial load count from the Nile perch fish samples was higher than the standard mean log (2.00-6.00 cfu/g) required by UNBS hence this microbial count causes food poisoning hence fish were not recommended for human consumption. P-value ($P \leq 0.05$) indicates that there was a significant difference in total coliform count on hence implying that the different areas of study had different pathogens of food poisoning which would cause intoxication of fish. The mean log for microbial E-coli count was higher than the standard required by UNBS hence this microbial count causes food poisoning hence fish were not recommended for human consumption. The P value ($P \geq 0.05$) for the mean log for the microbial load of E-coli indicates that there was no significant difference of E-coli for the study areas. The mean log for salmonella was within the range of the standard mean log hence this microbial count does not cause food poisoning hence fish were recommended for human consumption. The P-value ($P \geq 0.05$) for the mean log of Salmonella shows that there was no significant difference in total microbial load of Salmonella hence implying that the different areas of study had statistically similar levels of microbial count of Salmonella which caused fish intoxication in these respective areas of study.

It was concluded that total coliform microbial and E-coli count were higher than the standard recommended by UNBS however the count for salmonella was within the standard range hence the fish is recommended for human consumption. Therefore more research should be done on *Bacillus cereus* count in Arapai market, Soroti and Lalle landing site.

CHAPTER ONE

1.0 Introduction

1.1 Background

According to Bondad-Reantaso *et al.*(2012),fish is the most important source of protein for human nutrition and a vital part of the diet for people everywhere. Fish is a highly nutritious and protein-rich food source that makes about 60% of the world's protein (Emikpe *et al.*, 2011). However, improper handling, processing, and storage methods cause bacterial contamination, which poses serious health risks to the public with regard to fish. Fish deterioration begins as soon as it is harvested and landed, post-harvest losses can result (Obar *et al.*, 2015).

According to Allocati *et al.*(2013),*Escherichia coli* is the primary bacterium causing food intoxication worldwide, food-borne intoxication infection rates estimate that 7.69% (600 million) of the world's population is intoxicated by food, 7.8 billion are afflicted with food-borne diseases, and 7.5% (420,000) of deaths worldwide are attributable to food-borne illness (Kirk *et al.*, 2015). In Africa *Escherichia coli* and salmonella causes major challenges to the health of the people leading to illness (Akhtar *et al.*, 2014).About 1.3 million Ugandans are diagnosed with food-borne illnesses each year, accounting for 14% of all infection (Afolabi *et al.*, 2021).

Among other pathogens invading fish and fish products, salmonella and *Escherichia coli* have emerged as the most common food poisoning germs (Herikstad *et al.*, 2002). *Escherichia coli* and salmonella species are carried and transported by fish and fish products as a result of unsanitary handling and processing, faecal contamination of the water where fish are harvested and inadequate sanitation practices (Bibi *et al.*, 2015). Fish are major carriers of salmonella that cause pathogenic diseases, the microbes are acquired by the fish from their contaminated living environment and from handling procedures. Both industrialized and developing nations experience a worldwide burden from salmonella gastroenteritis (Majowicz *et al.*, 2010).

References

- Abbas, K. A., Saleh, A. M., Mohamed, A., & Lasekan, O. (2009). The relationship between water activity and fish spoilage during cold storage: A review. *Journal of Food, Agriculture and Environment*, 7(3–4), 86–90.
- Afolabi, I. B., Aremu, A. B., Abdullahi, L. A., Mansir, B. A., Ilori, O., & Nwanna, K. U. (2021). *Predicting Relationship between Food-Borne Disease Information-Adequacy and Food Handling Practices among Food - Handlers in Selected Restaurants in Ggaba Kampala , Makindye Division Uganda*. 6(8), 837–844.
- Akhtar, S., Sarker, M. R., & Hossain, A. (2014). Microbiological food safety: A dilemma of developing societies. *Critical Reviews in Microbiology*, 40(4), 348–359.
<https://doi.org/10.3109/1040841X.2012.742036>
- Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: An overview. *International Journal of Environmental Research and Public Health*, 10(12), 6235–6254. <https://doi.org/10.3390/ijerph10126235>
- Amagliani, G., Brandi, G., & Schiavano, G. F. (2012). Incidence and role of Salmonella in seafood safety. *Food Research International*, 45(2), 780–788.
<https://doi.org/10.1016/j.foodres.2011.06.022>
- Amuna, N. N. (2014). Food safety management practices in the traditional fish processing sector in Ghana and the microbiological safety of selected processed fish products from Ghana. *University of Greenwich*, 1(11), 1–285.
- Angelica María Abdallah Ruiz. (2018). *Growth and survival of Salmonellaspp., microbial indicators and the sensory and color properties of catfish fillet subjected to slush-ice chilling*. 0(September), 160–164.
- Bibi, F., Qaisrani, S. N., Ahmad, A. N., Akhtar, M., Khan, B. N., & Ali, Z. (2015). Occurrence of salmonella in freshwater fishes: A review. *Journal of Animal and Plant Sciences*, 25(3), 303–310.
- Bondad-Reantaso, M. G., Subasinghe, R. P., Josupeit, H., Cai, J., & Zhou, X. (2012). The role of crustacean fisheries and aquaculture in global food security: Past, present and

future. *Journal of Invertebrate Pathology*, 110(2), 158–165.

<https://doi.org/10.1016/j.jip.2012.03.010>

Costa, R. A. (2013). *Escherichia coli* in seafood : A brief overview. *Advances in Bioscience and Biotechnology*, 2013(March), 450–454.

Da Silva, M. L., Rogério Matté, G., Germano, P. M. L., & Matté, M. H. (2010). Occurrence of pathogenic microorganisms in fish sold in São Paulo, Brazil. *Journal of Food Safety*, 30(1), 94–110. <https://doi.org/10.1111/j.1745-4565.2009.00192.x>

David, O. M., Wandili, S., Kakai, R., & Waindi, E. N. (2009). Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *Journal of Infection in Developing Countries*, 3(2), 99–104. <https://doi.org/10.3855/jidc.56>

Department, F. and E. H. (2014). Microbiological guidelines for food. *Centre for Food Safety*, 2014(August), 1–38.

Deribe, G., Abdela, E., Dagne, A., & Tsedey, A. (2020). *Journal of fisheriessciences.com*. 14(6), 1–5.

Determining Sample Size Degree Of Variability. (n.d.).

Emikpe, B. O., Adebisi, T., & Adedeji, O. B. (2011). Bacteria load on the skin and stomach of *Clarias Gariepinus* and *Oreochromis Niloticus* from Ibadan , South West Nigeria : Public health implications. *Journal of Microbiology and Biotechnology Research*, 1(1), 52–59.

Ertas Onmaz, N., Abay, S., Karadal, F., Hizlisoy, H., Telli, N., & Al, S. (2015). Occurrence and antimicrobial resistance of *Staphylococcus aureus* and *Salmonella* spp. in retail fish samples in Turkey. *Marine Pollution Bulletin*, 90(1–2), 242–246. <https://doi.org/10.1016/j.marpolbul.2014.10.046>

Franz, E., & Van Bruggen, A. H. C. (2008). Ecology of *E. coli* O157:H7 and *Salmonella* enterica in the primary vegetable production chain. *Critical Reviews in Microbiology*, 34(3–4), 143–161. <https://doi.org/10.1080/10408410802357432>

Gallo, M., Ferrara, L., Calogero, A., Montesano, D., & Naviglio, D. (2020). Relationships

- between food and diseases: What to know to ensure food safety. *Food Research International*, 137, 109414. <https://doi.org/10.1016/j.foodres.2020.109414>
- Getu, A., & Misganaw, K. (2015). Post-harvesting and Major Related Problems of Fish Production. *Fisheries and Aquaculture Journal*, 06(04). <https://doi.org/10.4172/2150-3508.1000154>
- Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences*, 7(7), 846–864. <https://doi.org/10.3844/ajassp.2010.859.877>
- Henson, S., & Caswell, J. (1999). Food safety regulation: An overview of contemporary issues. *Food Policy*, 24(6), 589–603. [https://doi.org/10.1016/S0306-9192\(99\)00072-X](https://doi.org/10.1016/S0306-9192(99)00072-X)
- Herikstad, H., Motarjemi, Y., & Tauxe, R. V. (2002). Salmonella surveillance: A global survey of public health serotyping. *Epidemiology and Infection*, 129(1), 1–8. <https://doi.org/10.1017/S0950268802006842>
- Javadian, S. R., Shahosseini, S. R., & Ariaii, P. (2017). The Effects of Liposomal Encapsulated Thyme Extract on the Quality of Fish Mince and Escherichia coli O157:H7 Inhibition During Refrigerated Storage. *Journal of Aquatic Food Product Technology*, 26(1), 115–123. <https://doi.org/10.1080/10498850.2015.1101629>
- Jessie, B. C. (2019). *LSU Scholarly Repository Prevalence and Control of Salmonella spp . and Sanitary Indicator Microorganisms in Wild Caught and Farm Raised Catfish (Ictalurus Punctatus) PREVALENCE AND CONTROL OF SALMONELLA SPP . AND SANITARY INDICATOR MICROORGANISMS IN WI. October.*
- Johari, F., Zapri, N., Academia, S. M.-J. of, & 2022, undefined. (2022). Prevalence of escherichia coli and salmonella in fish and blood clam (anadara granosa) from wet markets and hypermarkets in Kuala Pilah. *Ir.Uitm.Edu.My*, 10(2), 97–107.
- Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., Döpfer, D., Fazil, A., Fischer-Walker, C. L., Hald, T., Hall, A. J., Keddy, K. H., Lake, R. J., Lanata, C. F., Torgerson, P. R., Havelaar, A. H., & Angulo, F. J. (2015). World Health Organization Estimates of the Global and Regional Disease Burden of 22

- Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. *PLoS Medicine*, 12(12), 1–21. <https://doi.org/10.1371/journal.pmed.1001921>
- Lougovois, V. P., & Kyrana, V. R. (2014). Freshness Quality and Spoilage of Chill-Stored Fish. In *Food Policy, Control and Research* (Issue January 2005).
- Mahmoud, B. (2015). *A DANGEROUS* (Issue January 2012).
- Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., Jones, T. F., Fazil, A., & Hoekstra, R. M. (2010). The global burden of nontyphoidal salmonella gastroenteritis. *Clinical Infectious Diseases*, 50(6), 882–889. <https://doi.org/10.1086/650733>
- Margaret, M., & Edgar, T. S. (2015). *Microbiological Safety of Small Pelagic Fishery : Case of L. Albert , Uganda*. 4(7), 19–23.
- Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*, 33(2), 181–192.
- Matjila, S. A. (2015). *The microbial quality of locally harvested snoek (Thyrsites atun) as influenced by the current supply chain management*. March, 5–10.
- Mhango, M., Mpuchane, S. ., & Mpuchane, B. . (2010). Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. *African Journal of Food, Agriculture, Nutrition and Development*, 10(10). <https://doi.org/10.4314/ajfand.v10i10.62898>
- Microbiological Quality of Traditionally Smoked Fish from Lake Victoria Crescent, Uganda. (2021). *Food Science and Quality Management*. <https://doi.org/10.7176/fsqm/104-05>
- Mitiku, B. A., Mitiku, M. A., Ayalew, G. G., Alemu, H. Y., Geremew, U. M., & Wubayehu, M. T. (2023). Microbiological quality assessment of fish origin food along the production chain in upper Blue Nile watershed, Ethiopia. *Food Science and Nutrition*, 11(2), 1096–1103. <https://doi.org/10.1002/fsn3.3147>
- Noveir, M. R., & Halkman, A. K. (2000). A Study on Selective Broths and Agar Media for the Isolation of Escherichia coli 0157:H7 Serotype. *Turkish Journal of Veterinary and*

Animal Sciences, 24(5), 459–464.

Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V., & Bērziņš, A. (2016). Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, 66(1), 1–15. <https://doi.org/10.1007/s13213-015-1102-5>

Obar, J. A., Shitandi, A. A., Mahungu, S. M., & Lameck, A. O. (2015). The Abundance of Lactic Acid Bacteria in the Gastrointestinal Tract of Lake Victoria Nile Perch. *Issn*, 42, 2224–6088.

Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 19(2), 311–331. <https://doi.org/10.1111/1541-4337.12526>

Padhye, N. V., & Doyle, M. P. (1992). Escherichia coli 0157:H7: Epidemiology, pathogenesis, and methods for detection in food. *Journal of Food Protection*, 55(7), 555–565. <https://doi.org/10.4315/0362-028X-55.7.555>

Songe, M. M., Hang'ombe, B. M., Knight-Jones, T. J. D., & Grace, D. (2017). Antimicrobial resistant enteropathogenic escherichia coli and salmonella spp. In houseflies infesting fish in food markets in Zambia. *International Journal of Environmental Research and Public Health*, 14(1). <https://doi.org/10.3390/ijerph14010021>

Taylor, W. I., & Schelhart, D. (1971). Isolation of Shigellae. 8. Comparison of xylose lysine deoxycholate agar, hektoen enteric agar, Salmonella-Shigella agar, and eosin methylene blue agar with stool specimens. *Applied Microbiology*, 21(1), 32–37. <https://doi.org/10.1128/aem.21.1.32-37.1971>

Tzouros, N. E., & Arvanitoyannis, I. S. (2000). Implementation of Hazard Analysis Critical Control Point (HACCP) system to the fish/seafood industry: A review. *Food Reviews International*, 16(3), 273–325. <https://doi.org/10.1081/FRI-100100290>

UNBS. (2020). *Safety of foodstuffs — Requirements*.

Valdimarsson, G., Cormier, R., & Ababouch, L. (2004). Fish safety and quality from the

perspective of globalization. *Journal of Aquatic Food Product Technology*, 13(3), 103–116. https://doi.org/10.1300/J030v13n03_10