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Microbial Assessment of Dried Fish Sold in Streets and Supermarkets in Harare Central Business District: Zimbabwe

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Abstract

The study focused on the microbiological quality of dried fish sold in the streets and supermarkets in Harare Central Business District. The analysis was based on determining the microbiological load of dried fish samples. Samples were systematically collected from three different streets and three different supermarkets each week. The study period was from January 2020 to April 2020.The samples were analyzed for Salmonella spp., Staphylococcus aureus, Escherichia coli, Aspergillus spp and Penicillium spp. The methods used were pour plate, streaking and microscopy. Results obtained from this study showed that street sold fish microbial levels were within unacceptable levels food safety standards. Supermarket sold dried fish microbial levels were also within the unacceptable levels. In comparing the levels of indicator microorganisms between streets and supermarkets, the results showed that the contamination of level was similar for all the microorganisms except Salmonella. Purchasing dried fish in supermarkets does not guarantee food safety. It was concluded that street and supermarket sold dried fish pose high food safety concerns and can be a potential source of foodborne illnesses.

Keywords: *Food borne illnesses, food contamination, Microorganisms, Microbial load.*

Introduction

Fish is a limbless cold-blooded vertebrate animal with gills and fins living wholly in water [1]. It is highly perishable. Fish is prone to contamination at various stages of handling and processing. Contamination is a very important aspect, as this is the mode that most unwanted microorganisms may be transmitted to seafood. Poor handling and storage facilities allow rapid post-harvest deterioration, thereby limiting availability of fish as stated [2]. There is substantial evidence that sea foods are high on a list of foods associated with outbreaks of food-borne diseases [3]. Bacteria are present on the surface slime, skin, gills and intestine of fish.

Fish harvested from eutrophic and warm waters will present higher bacterial numbers than fish from clean and cold waters, but potential human pathogens may be present in both cases [4].

The fungi associated with dried fish, the most prevalent fungi were *Aspergillus* and *Penicillium* species [2]. There is a general perception that street-vended foods are unsafe [5]. Food borne illness of microbial origin is a major international health problem associated with food safety in developing countries [6]. Potential health risks are associated with contamination of fish by *Escherichia coli*, *Salmonella typhi*, *Pseudomonas spp*, *Staphylococcus aureus* and *Proteus spp* during preparation, post cooking and handling.

Fish is sold in different varieties, namely haddock, hake, mackerel, cod, herrings, bream, trout and kapenta amongst others. In a study carried out in the rural and urban areas of Zimbabwe [7], mackerel, bream and dried

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kapenta are the most consumed fish species. Lake Chivero is hypereutrophic due to high levels of pollution, with major sources of pollutants being sewage and industrial waste. Bream caught by local fishermen in small reservoirs is usually sold fresh, smoked or dried to people in the nearby area. For the majority of urban residents who are low on incomes lower value fish such as mackerel, bream and kapenta are on greater demand [7]. Different water bodies have different physical, chemical, and biological characteristics which influence the plankton, diversity of prey in water bodies, and the substances to which the fish are exposed, henceforth offering different environments and nutrient sources [8]. Fish is a healthy food, rich in quality animal protein, polyunsaturated fatty acids especially the (ω)-3 eicosapentaenoic acid and docosahexaenoic acid, vitamins (A, D, E, K), water-soluble vitamins B complex, vitamin C and micronutrients [9].

Harare is known to harbour cases of water-borne disease outbreaks like typhoid. An estimated number of 30000 people suffer from food-borne diseases annually [10]. Due to faltering economic development as a result of various factors, street food vending has become increasingly important in the economies of many African countries. Street foods are a source of inexpensive, nutritious meals. There is a general perception that street-vended foods are unsafe, mainly because of the environment under which they are prepared and consumed, which exposes the food to numerous potential contaminants. Some studies, however, have shown that food prepared on the street can be safe, thereby providing alternative outlets for consumers [11, 12].

Statement of Problem

According to the 2018 health statistics on cholera outbreak in Zimbabwe, there has been a rapid increase in the number of suspected cases reported per day. A total of 3621 cumulative suspected cases, including 71 confirmed cases, and 32 deaths were reported. This gives a case fatality ratio of 0.8%, out of which 98% (3564 cases) were reported from the densely populated capital Harare [13]. This is suspected to have been caused by poor sanitation and a lot of illegal vending activities, due to the high rate of unemployment. People prefer purchasing dried fish because it is cheap, convenient and there is no need for refrigeration [7]. However, street-sold fish are considered among the contributors to foodborne diseases. Microorganisms such as *Escherichia coli* can be pathogenic causing illnesses.

It is in this regard that there was a need to identify the pathogenic microbes present in street sold dried fish. The safety of eating dried fish from informal market was not known thereby possibly posing risks for human health despite the fish being preserved using hurdle technology. It was against this background that the researcher undertook this study.

Research Objectives

1. Determine the microbial load for selected microorganisms related to food borne illnesses for both street and supermarket vended dried fish.
2. Compare the microbial load for the selected microorganisms from the supermarket and streets dried fish.
3. Compare the microbial load of these to world health standards for the microbial load on fish.

Literature Review

Microorganisms Associated with Fish

The initial microflora of fish can contain human pathogenic bacteria, posing a concern for seafood borne illnesses [4, 12]. Unwanted microorganisms may access fish processing environments through raw material, personnel or mobile equipment, through leakage and openings in buildings or through pests and some pathogens may even become established in the processing plant and form niches where they can survive for long periods of time, Fish consists of on average 70% water, in fatty fish it has a

percentage of about 65% and in lean fish about 80% [14, 15]. Hence, with such high levels of internal moisture, bacteria can grow rapidly.

Foodborne bacterial pathogens commonly detected in street vended foods in developing countries include *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella spp* [16]. Street foods are often prepared by hand, which may lead to an increased incidence of contamination with the potential foodborne pathogens. *Escherichia coli* is a classic example of enteric bacteria causing gastroenteritis. *Escherichia coli* including other coliforms and bacteria as *Staphylococcus spp* and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish [17]. Contamination of food of fish origin with pathogenic *Escherichia coli* probably occurs during handling of fish and during the production process [18]. Inadequate processing of raw products or cross contamination of raw materials and prepared foods and poor time and temperature control is a contributing factor for multiplication of *Escherichia coli* [19]. Presence of *Escherichia coli* indicates potential for there to have been contamination of faecal origin from poor hygienic practices or there has been inadequate processing.

Fish appear to be passive carriers of *Salmonella*, demonstrate no clinical disease, and can excrete *Salmonella spp*. Contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella spp*. *Salmonella* is likely caused by inadequate processing of raw products, cross contamination or contaminated raw materials and poor time and temperature control [17, 19]. Enterotoxins produced by *Staphylococcus aureus* are another serious cause of gastroenteritis after consumption of fish. *Staphylococcus aureus* is likely caused by inadequate temperature control and poor hygienic practices [19].

Dried fish rely on a combination of salt and heat treatment for their safety [20]. If the product has insufficient salt, or fails to achieve a required temperature, *Clostridium botulinum* can grow. Some fungi are thought to enhance the acceptability of the product while others indicate spoilage, but the main concern is the presence of toxigenic fungi and their toxic metabolites [21]. Dried fish are dominated by *Aspergillus* species that include several important mycotoxin producers during a study of fungal spoilage of dried fish from Indonesia [21]. Less specialised xerophiles (*Eurotium* and *Aspergillus* species) cause spoilage in these products if they are not dried quickly to a safe moisture level, or if they are stored in humid environments where water may be reabsorbed [21]. *Penicillium* is a diverse genus which causes destructive rots in the food industry, where they produce a wide range of mycotoxins [22].

Practices used during food preparation such as handling, cleaning, sorting and grading, packaging, storing and wrapping in low grade plastics are some of the critical factors that increase the risk of inadequate food safety [23]. Foodborne bacterial pathogens commonly detected in street vended foods in developing countries include *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella spp*. The consumption of street foods potentially increases the risk of food-borne diseases caused by a wide variety of pathogens which include *Escherichia coli, Salmonella typhi, Pseudomonas spp., Staphylococcus aureus* [24].

They can contribute to foodborne illnesses as a result of presence of chemical hazards, heavy metals and process contaminants they may contain. Henceforth the quality of street vended food is of great food safety concern, as consumers are continuously exposed to the risk of getting ill [25]. *Salmonella spp* and *Staphylococcus aureus* are the most common foodborne pathogens responsible for food poisoning and food-related infections [26]. Food-borne illnesses are a widespread problem globally.

The microbiological quality of informally vended foods in Harare sadza and stews had few

contaminated samples by *Bacillus cereus* and *Salmonella spp* [12]. The application of hygienic practices during the preparation and sale of street food could reduce microbial risk associated with street vended food [26]. Street food plays an important socio-economic role, it provides a regular source of income for millions of low or unskilled people in developing countries [7].

Microbiological foodborne diseases immensely influence the economies of developing countries in a negative way, most of the outbreaks have been closely linked to a number of factors including use of microbiologically poor-quality raw materials in food preparation, improper handling of prepared foods and unsound vendor hygiene practices among others [26]. In many instances, street-food vendors are uneducated and untrained in basic food hygiene and pre-requisite systems. In South Africa, the researchers stated that the overall hygiene of pre-prepared street food is poor [28, 29]. A study in Santa Fe de Bogota, Colombia revealed that over 30% of a group of food handlers examined were carriers of pathogenic microorganisms including *Salmonella typhi*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Shigella* [30].

Sources of Fish Contamination

Many pathogenic bacteria are naturally present in the aquatic environment [3]. Other microorganisms are of the animal or human sources, particularly *Salmonella*, *Shigella*, and *Escherichia coli*. Thus, there is always a possibility that these microorganisms may be passed on to the raw material during production and processing [3]. During the processing of dried fish, other ingredients such as salt and spices could be potential sources of contamination. Contamination of fish products through processing equipment is a source of contamination [14]. Unclean, insufficient or inadequate cleaned processing equipment has been identified as a source of bacterial contamination in processed fish. Containers, pumps or tanks used for holding or transporting unprocessed raw materials, have occasionally been used for processed products without any cleaning and disinfection [31].

Water, is a vehicle for transmission of many agents of diseases and it continues to cause significant outbreaks of disease in developed and developing countries worldwide [32]. Potable water should be used throughout the production process for cleaning equipment and washing fish [33]. Transfer of microorganisms by food handlers is a major source of contamination. Poor hygiene and or absence of hand washing has been identified as the causative mode of transmission [14].

Standards Regarding to the Safety of Dried Fish

There is no universal agreement on which indicator microorganism(s) is most useful, henceforth different indicators and different indicator levels identified as standards are used in different states, countries, and regions [34]. Microbiological criteria for fish products include quantification of the counts of *Escherichia coli* is performed during the production. At the finished product stage, the measure monitored is the quantification of the count of *Staphylococcus aureus* and detection of bacteria of *Salmonella* genus, as their presence indicates recontamination of a finished product.

Food Standards Australia New Zealand stated that [20], Microbiological assessment is defined based on the detection or level of microorganism found under the following: Satisfactory**,** results will be within expected microbiological levels and present. There is no food safety concern, and no action is required. Marginal is when results are within expected microbiological levels but are at the upper range. Some action may be required to ensure food handling controls continue to be effective. Unsatisfactory, this is when the results are outside expected microbiological levels and indicate poor food handling practices.

Further actions are required to re-establish effective food handling controls.

Microbiological guidelines are used by regulatory agencies to check that food for sale is safe and suitable and the food handling controls and hygienic practices of a food business are adequate. Guideline criteria indicate whether the microbiological status of a food product is within the normal or acceptable range and in this way are alert mechanisms to signal conformance with food safety controls. Potentially hazardous is when the results exceed expected microbiological levels to a level that presents an immediate food safety concern. Further action is required to prevent affected product still available from being distributed or sold to determine the likely source or cause of the problem and ensure corrective actions are implemented [1] (Table1.0).

Table 1.0. Interpretation of Levels of Microorganisms in Accordance to (FSANZ, 2016)

Indicator	**Result (cfu/ml)**	**Interpretation**
Escherichia coli	$>10^2$	Unsatisfactory
	$3- < 10^{-2}$	Marginal
	< 3	Satisfactory
Salmonella	$>10^4$	Unsatisfactory
	$10^2 - 10^4$	Marginal
	$< 10^2$	Satisfactory
Staphylococcus aureus	$>10^4$	Potentially hazardous
	$10^3 - \leq 10^4$	Unsatisfactory
	$10^2 < 10^3$	Marginal
	$< 10^{-2}$	Satisfactory
Yeasts and moulds	5×10^6	Acceptable
	3×10^7	Unacceptable

Materials and Methods

Sample Collection

Research data is any information that has been collected, observed, generated or created to validate original research findings. The data was collected at six different sites in Harare CBD [35]. Three sites were for street sold dried fish while three other sites were for supermarket sold dried fish. Three sites were used for both supermarket and street sold dried fish to improve accuracy of the research. Dried fish were bought from supermarkets and street vendors in Harare CBD. Dried fish were aseptically transferred into sterile stomacher bags and labelled with identities from location of collection. Dried fish samples were transported to the laboratory for testing.

Study Area

The study took place in Harare. Sampling was done in Harare CBD and supermarkets; experiments were done at the University of Zimbabwe. The study took place in six different sites in Harare. Sampling of the dried fish and experiments for the microbial analysis were carried out from January to April 2020. The one hundred and eighty samples were aseptically transferred into sterile stomacher bags.

Preparation of Agar Mediums and Broths

Different types of agar mediums and medium broths were prepared. The solutions were put in universal bottles. Lids were put on universal bottles and closed. The universal bottles were wrapped with aluminium foil just in case the contents were likely to explode. The autoclave was used to sterilise the medium. Sterilisation in an autoclave was done under pressurised steam at a temperature of 121˚C for 15 minutes. The universal bottles and pipettes in aluminium foil were removed from the autoclave and left to cool.

Preparation of Petri Dishes

The petri dishes were labelled using sellotape. The media was poured into petri dishes. The different types of prepared agar solution were poured into petri dishes such that 12-15 ml of agar medium was put. After pouring the agar medium, all petri dishes were kept in room temperature so that agar medium solidified. The petri dishes were inoculated upside down. The samples were spread on the medium surface rapidly in a smooth back and forth motion. Swirling the cell suspension was done before removing aliquot for plating. A metal loop was flamed with a Bunsen burner before obtaining inoculum for the plate. The spread plate method was a technique used.

Experimental Design

A total of 180 samples of dried fish were systematically randomly purchased from six different sites in Harare. Eighteen samples were collected at each location. The samples were analysed according to the Official Methods of Analysis of AOAC International.

Determination of Escherichia coli

Ten grams of each dried fish sample was weighed into a stomacher bag and homogenised. Addition of 90ml of 0.1% of Trypticase Soy Broth (TSB) into the stomacher bag with the homogenised sample followed. The sample and diluent were mixed together. Serial decimal dilutions were made. *Escherichia coli* was enumerated using the most probable number method. Pure colonies were sub cultured in peptone water, incubated at 37°C for 24 hours. After incubation, the broth will be streaked on to MacConkey agar and incubated for 24 hours at 37ºC.

Determination of Salmonella

Ten grams of each dried fish sample was weighed into a stomacher bag and homogenised. Addition of 90ml of 0.1% of Buffered Peptone Water (BPW) broth into the stomacher bag with the homogenised sample followed. The sample and diluent were mixed together. Serial decimal dilutions were made. Brilliant Green Agar (BGA) was poured into each petri dish. The inoculated plates were incubated at 37°C for 24 hours under aerobic conditions.

Determination of Staphylococcus Aureus

Ten grams of each dried fish sample was weighed into a stomacher bag and homogenised. Addition of 90ml of 0.1% of Buffered Peptone Water (BPW) into the stomacher bag with the homogenised sample followed. The sample and diluent were mixed together. Serial decimal dilutions were. Mannitol Salt Agar (MSA) was poured into each petri dish. The inoculated plates were incubated at 37°C for 24 hours.

Determination of Aspergillus and Penicillium

Ten grams of each dried fish sample was weighed into a stomacher bag and homogenised. Dried fish were examined under a magnifying lens for fungal growth. Pieces of mycelia or spores were picked from the surface using a sterile needle and transferred to inoculating media, Potato Dextrose Agar (PDA) and Malt Extract Agar, respectively. The dried fish were also press-plated in the same media. The petri dishes were incubated at 25˚C for 7 days in a dark place. The visible growths were identified. This procedure was adopted and adapted from [2].

Results and Discussion

Below are results of the microbial levels in fish from both the streets and the supermarket.

Samples from Hellena Street had the highest levels of *Salmonella*. All samples were contaminated with *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Aspergillus* [24] who stated that in street foods potentially increase risk of food-borne diseases. *Aspergillus* was detected in very low levels; this might have been caused by insufficient drying of the dried fish to safe moisture levels [21]. *Aspergillus* is most likely to be caused by not drying fish to a safe moisture level [21].

Hellena Street samples had highest levels microbes probably because the fish were not wrapped in any packaging material exposing the fish to various microorganisms as compared to other sites, though the packaging material was of inferior quality. *Penicillium* was not detected at Tol Street, this might have been caused by effective preservation methods [15] who said that when sufficient water is removed from fish, microorganisms can no longer grow. High levels of *Salmonella* this might have been caused by inadequate processing of raw fish [20] who stated that *Salmonella* is likely caused by inadequate processing.

Results on Pathogenic Microorganisms obtained from Street Sold Dried Fish

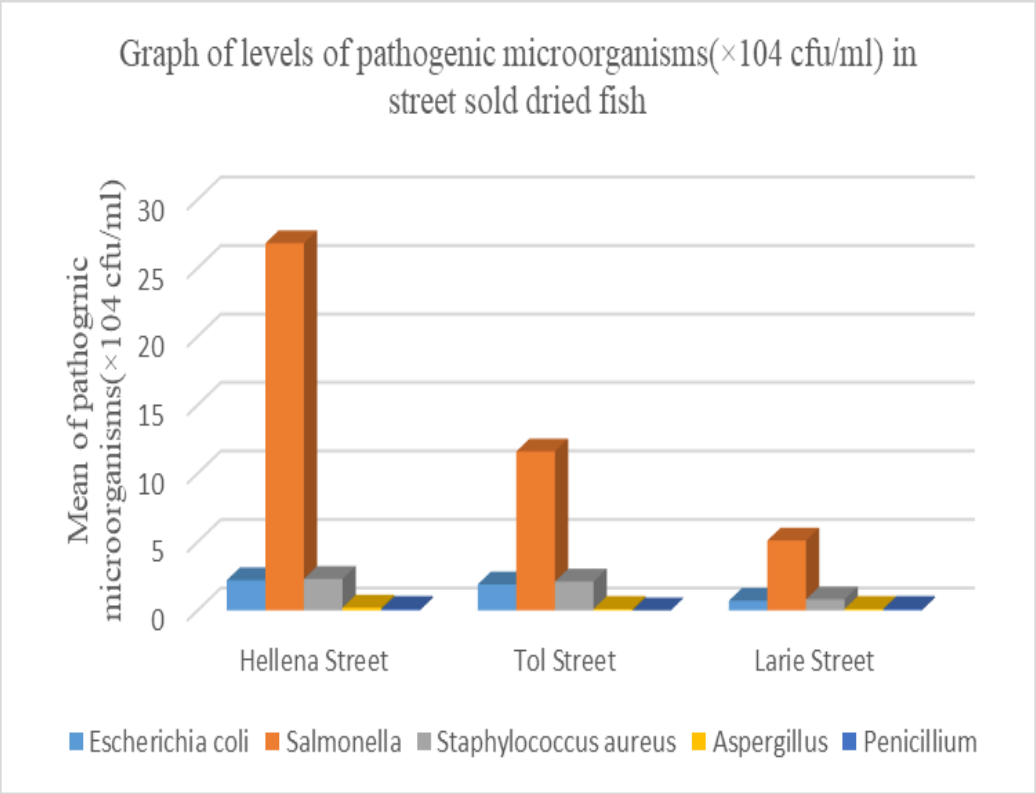

Figure 1. Mean of Pathogenic Microbes in Street Sold Dried Fish Collected from Different Streets

Results on Pathogenic Microorganisms Levels in Supermarket Sold Dried Fish

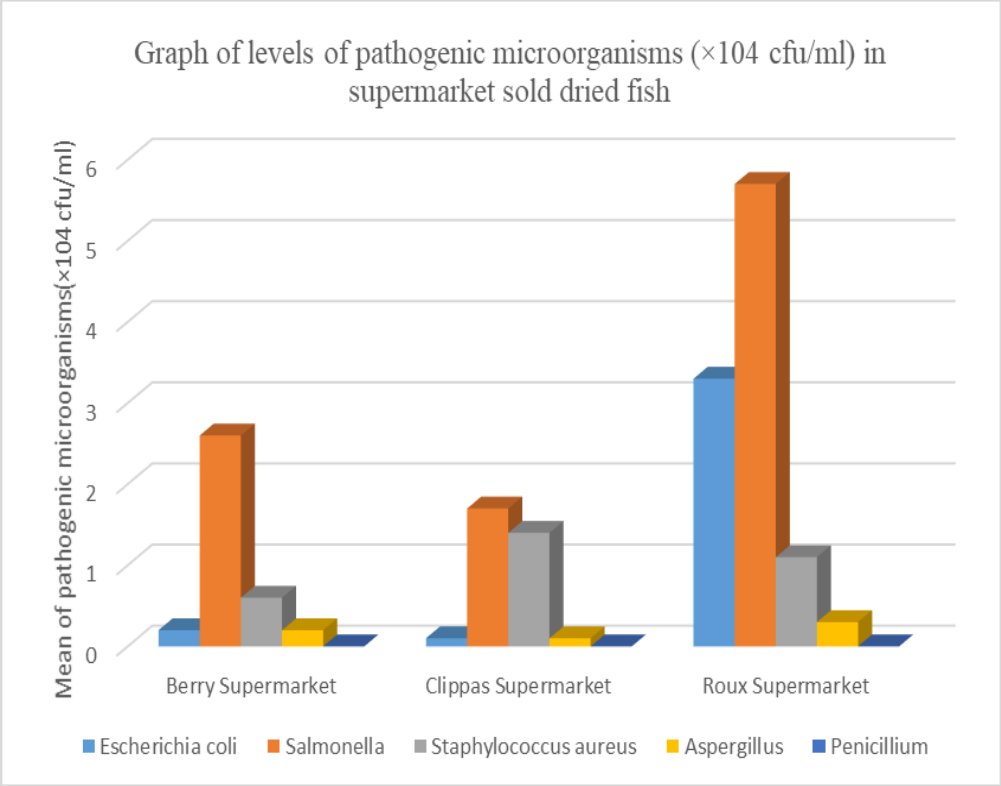

Figure 2. Mean of Pathogenic Microbes in Supermarkets Sold Dried Fish Collected from Different Supermarkets

Results indicate that samples from Roux Supermarket had the highest levels of *Salmonella* and *Escherichia coli*. Presence of *Salmonella* might have been caused by poor time and temperature control [20] which states that *Salmonella* is likely caused by poor time and temperature control. Presence of *Staphylococcus aureus* might have been caused by poor hygienic practices during handling and processing of fish [20] who stated that *Staphylococcus aureus* is likely caused by poor hygienic practices during handling and preparation. *Aspergillus* was relatively low in supermarket sold dried fish. Detection of *Aspergillus* might have been due to the fact that the fish might have been stored in a humid environment, as suggested [21] (Table 1.2).

The supermarket sold dried fish samples were not contaminated with *Penicillium* (Table 1.1). This indicates effective preservation of the fish as stated by [15] who cited that when sufficient water is removed from fish, microorganisms can no longer grow. Low levels of fungi might have been attributed to the good quality packaging material [23] who cited that wrapping in low grade plastics increases the risk of inadequate food safety, in the supermarkets food safety risk was reduced owing to choose of packaging material.

The *E. coli* and *Salmonella* levels in both the streets and supermarkets were in the unsatisfactory range [20], This is when the results are outside expected microbiological levels and indicate poor food handling practices [20]. Hellena Street had the highest levels of *Escherichia coli*. Higher values greater than 100 cfu/ ml were recorded in all the streets, rendering the dried fish unsafe. Presence of *Escherichia coli* on fish is a clear evidence of contamination from terrigenous source [33]. *Salmonella* levels were high on the different streets. Presence of *Salmonella* indicate poor food handling practices [20]. hence there are food safety concerns and is required.

Staphylococcus aureus levels for all six sites were in the potentially hazardous range [20], which is when the results exceed expected microbiological levels to a level that presents an immediate food safety concern. Presence of *Staphylococcus aureus* might have been due to contamination during processing [3], who said that *Staphylococcus aureus* is caused by contamination or recontamination of seafood during processing. *Aspergillus* and *Penicillium* were well within acceptable ranges. The main concern is the presence of toxigenic fungi and their toxic metabolites [21]. but in this case the levels were low.

Results of the comparison levels of pathogenic street and supermarket sold dried fish were established using two tailed t-tests. $P > 0.05$ at 95% confidence level therefore we can say that there were no significance differences between the street sold and supermarket sold dried fish with regards to the level of pathogenic microorganisms.

There were no significant differences in the levels of pathogenic microbes between supermarket and street sold dried fish for *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus* and *Penicillium*. This might have been due to the fact that most supermarkets do not carry microbial tests on incoming food products, they usually carry acceptance tests by use of sensory evaluation. This might have been also due to the fact that, in most instances, street vendors are uneducated and untrained in basic food hygiene and pre-requisite systems [26]. Some of the street vendors stock their dried fish in drainages to avoid harassment by city authorities displaying a relatively small number of dried fish on the street pavements, thereby posing health risks to consumers.

There was a significant difference in the levels of *Salmonella* between supermarket and street sold dried fish. This might have been caused by recontamination of dried fish [36] detection of *Salmonella* indicates recontamination of a finished product. Supermarkets usually receive products from processing companies and these companies carry out quality control tests as opposed to street sold dried fish where recontamination is likely to occur. In the processing of dried fish, poor time and temperature control might have caused *Salmonella* proliferation [20] due to lack of proper processing equipment like thermocouples and automated temperature recording devices.

Safety of the Street and Supermarket Sold Dried Fish Results according to (FSANZ, 2016) Guidelines

Table 1.1. Interpretation of Levels of Microorganisms in Street and Supermarket Sold Dried Fish

Indicator microorganism	**Street sold dried fish mean ($\times 10^4$cfu/ml)**	**Supermarket sold dried fish mean ($\times 10^4$cfu/ml)**	**Interpretation**
Escherichia coli	1.60	1.20	Unsatisfactory
Salmonella	14.5	3.33	Unsatisfactory
Staphylococcus aureus	1.73	1.03	Potentially hazardous
Aspergillus spp	0.13	0.20	Satisfactory
Penicillium spp	0.07	0	Satisfactory

T-tests Results on the Comparison between Street and Supermarket Sold Dried Fish at p > 0.05 at 95% Confidence Level

Table 1.2. T-tests Results on the Comparison between Street and Supermarket Sold Dried Fish

Indicator microorganism	T calculated	Hypothesis results
Escherichia coli	0.35	Accept H_0
		Reject H_1
Salmonella	17.05	Accept H_1
		Reject H_0
Staphylococcus aureus	1.36	Accept H_0
		Reject H_1
Aspergillus	1.19	Accept H_0
		Reject H_1
Penicillium	0.71	Accept H_0
		Reject H_1

T tabulated value = 2.776

Practical Analysis during the Research

Plate 1. The culture Media and Pipettes in Autoclave (sterilisation)

Plate 2. Start of incubation process

Plate 3. Results of the incubation process

Plate 4. Sample of Dried Fish

Conclusion

From the findings, street and supermarket sold dried fish microbial levels were within the unacceptable standards. Detection of these microorganisms is of great food safety concern and poses health risks to the consumers. Presence of these microorganisms might have been caused by lack of knowledge of basic food hygiene by food handlers. Poor time and temperature controls could have caused a proliferation of microorganisms. Use of unclean water during preparation and processing of the fish might have contributed to the presence and proliferation of microbes. In comparing the levels of indicator microorganisms between streets and supermarkets, the results showed that they were no significant differences except *Salmonella*. Recontamination of dried fish as well as inadequate processing might have caused the difference. Purchasing dried fish in supermarkets does not guarantee food safety. Packaging material, storage conditions, time-temperature combinations during processing, salt concentration, method of salt addition to the fish play a pivotal role in the final dried fish. In conclusion, street and supermarket sold dried fish poses high food safety concerns, they can be a health risk to consumers and can be a potential source of foodborne illnesses.

Conflict of Interest Statement

The authors Joe Phaeton Mukaro, Fenton Ruparanganda and Primrose Katsande declare that there are no conflicts of interest.

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