MICROBIAL RISK ASSESSMENT OF FISH ALONG ITS VALUE CHAINS IN KADUNA AND ZARIA METROPOLISES, KADUNA STATE, NIGERIA

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BY

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE
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ZARIA, NIGERIA

APRIL, 2018

DECLARATION

I declare that the work in this thesis entitled "**Microbial risk assessment of fish along its value chains in Kaduna and Zaria metropolises, Kaduna State, Nigeria**" has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Hafsat Ali GREMA _____ _____

Signature **Date**

CERTIFICATION

This thesis entitled "**Microbial Risk Assessment of Fish Along Its Value Chains in Kaduna and Zaria Metropolises, Kaduna State, Nigeria**" by Hafsat Ali GREMA meets the regulations governing the award of the degree of Doctor of Philosophy (Veterinary Public Health and Preventive Medicine) of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I dedicated this work to my mum and Dad; Hajiya Yagana Abba Mustapha and Mallam Ali Grema, my husband; Dr. Abubakar Suleiman, my two daughters; Zainab and Yasmeen

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ABSTRACT

Global demand and consumption of fish and fish products have increased tremendously over the last two decades. In Nigeria, a combination of population growth, rising prices of other animal sourced proteins and the growing awareness of the health implications of red meat significantly increased the demand and consumption of fish. Majority of foodborne outbreaks in developed and developing countries are largely related to microbiological hazards notably *Staphylococcus aureus* and *Salmonella* species. As such, microbial risk assessment of fish along the value chains of Kaduna and Zaria metropolises is important in providing food safety information suitable for public health policy development. To achieve these goals, this thesis examined the microbial burden of fish and fish products traded and processed for consumers and determined the risk factors influencing fish contamination levels. Results of this research identified discrete fish value chains for imported frozen fish distribution and that of locally farmed fish production channels which consisted of key producers, fish traders and fish processors. Almost all the stakeholders in both fish value chains had good food safety knowledge and attitude but poor food safety practices. Fish consumers interviewed in the study had good knowledge but poor attitude particularly their belief that fish safety can be judged by fish smell and appearance (96.5%). Fish consumers in this study had less concern for microbial hazards than chemical hazards (84.2%). The risk factors that influenced fish quality along the entire value chains were re- and cross-contaminations due to lack of personal hygiene such as washing hands before and after handling fish (94.5%), lack of storage and temperature control regulations (62.2%), lack of food safety training (97.3%), poor infrastructural development (100%) and poor environmental sanitation (100%). Results from this study indicated high percentages of unsafe fish handled from fish farms (93.3%), fish markets (73.4%) and fish processing units (64.9%) due to high aerobic plate

counts. Similarly, fish samples collected from fish farms (37.8%), fish markets (68.7%) and fish processing units (8.1%) were categorized as unsafe for human consumption due to high coliform counts. An isolation rate of 15.1% for *S. aureus* and 11.6% for *Salmonella* species were detected from all the fish samples collected in the value chains. *Salmonella* isolates were resistant to erythromycin, tetracycline and azithromycin but highly sensitive to chloramphenicol, gentamicin, ciprofloxacin and enrofloxacin. Similarly, *S. aureus* isolated from this study were resistant to cefoxitin, oxacillin, tetracycline and cephazolin. Simulated food borne outbreaks due to consumption of uncooked fish contaminated with *S. aureus* enterotoxin was 513 cases cases for ready-to-eat fish. Total predicted food borne cases for consumption of *Salmonella* contaminated processed fish was 111 cases per annum. The study has succeeded in mapping out the two common fish value chains in Kaduna and Zaria Metropolises. Most stakeholders had good food safety knowledge and attitude but poor practices of fish handling. The study confirmed a prevalence rate of 15.1% and 11.6% for *S. aureus* and *Salmonella* species, respectively. *S. aureus* isolated in this study were resistant to cefoxitin, oxacillin, cephazolin, erythromycin and tetracycline while *Salmonella* isolates were resistant to erythromycin, azithromycin and cephazolin. Most of the fish products produced and marketed along the fish value chains of Kaduna and Zaria metropolises were classified as unsafe for human consumption. Hence, it is paramount for food safety authorities to enforce policies with direct objectives on improving consumer health and protection such as continuous vendor education and fish processing outlets routine inspection. Policies with direct microbial risk reduction strategies that will minimize microbial cross and re-contamination from “farm to fork” are necessary. The government should provide basic infrastructure such as supply of potable water

and electricity, waste disposal services, good drainage, and toilet facilities to help facilitate translation of knowledge into practices.

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LIST OF ABBREVIATIONS

AIFP	Aquaculture and Inland fishery project
AST	Antimicrobial Susceptibility Testing
CAC	Codex Alimentarius Commission
CA-MRSA	Community Association Methicillin Resistant *Staphylococcus aureus*
CDC	Centers for Disease Control and Prevention
CFS	Center for Food Safety
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
DFID	Department For International Development
DNA	Dioxyribonucleic Acid
EC-ASEAN	European Comission-Association of South East Asian Nations
EFSA	European Food Safety Agency
EU	European Union
FAO	Food and Agricultural Organization
FBD	Food Borne Diseases
FDA	Food and Drug Adminstration
FDF	Federal Department of Fisheries
FSA	Food Safety Attitude
FSANZ	Food Standards Australia New Zealand
FSK	Food Safety Knowledge
FSP	Food Safety Practice
FST	Food Safety Training
FVC	Fish Value Chain
HACCP	Hazard Analysis and Critical Control Points
HA-MRSA	Hospital Associated Methicillin Resistant *Staphylococcus aureus*
HCl	Hydrochloric acid
ICLARM-GTZ	International Center For Living Aquatic Resource Management- Gesellschaft für Technische Zusammenarbeit
ICMSF	International Commission on the Microbiological Specifications for Foods
ILRI	International livestock Research Institute
ILSI	International Life Science Institute
IWGCSCCF	International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC)
KAP	Knowledge, Attitude and Practices
LA-MRSA	Livestock Associated Methicillin Resistant *Staphylococcus aureus*
LGAs	Local Government Areas
MAR	Multiple Antibiotic Resistance Index
MARD	Ministry of Agriculture and Rural Development
MHA	Mueller Hilton Agar
MIC (s)	Minimum Inhibitory Concentration (s)
MLST	Multi locus Sequence Typing
MRA	Microbial Risk Assessment
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSF	Medecins Sans frontiers

MT	Metric tones
NaCl	Sodium Chloride
NAFDAC	National Agency for Food and Drug Administration and Control
^{o}C	Degrees centigrade
PBP2a	Penicillin Binding protein 2a
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
RA	Risk Assessment
RF	Raw fish
RTE	Ready-To-Eat
SCC*mec*	Staphylococcal Cassette Chromosome *mec*
SSA	*Salmonella*-Shigella Agar
TAPC	Total Aerobic Plate Count
TCC	Total Coliform Count
TSB	Tryptose Soy Broth
USA	United States of America
$USDA_1$	United States Development Agency
$USDA_2$	United States Department of Agriculture
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Global demand and consumption of fish and fish products have increased tremendously over the last two decades (World Bank, 2013; FAO, 2014). According to FAO (2014), the global production of fish has gradually increased from 13, 074, 679 metric tonnes (MT) in 1990 to 66,633,253 MT in 2012. In addition, consumer preference for fish shifted from packaged ready-to-eat fish to fresh fish (World Bank, 2013). In Nigeria, a combination of population growth, rising prices of other animal sourced foods such as beef and poultry and growing awareness of the health implications of red meat significantly increased the demand for consumption of fish (Alagoa *et al*., 2011; WHO, 2016). Cultured fish, particularly Catfish and Tilapia, which are the dominant fish species farmed in Nigeria, have the potential to bridge the demand-supply gap of healthy animal protein, especially now that there is decline in both marine and fresh water catches (Veliu *et al*., 2009). These species are favourably cultivated in earthen ponds and tanks where animal dung is used to stimulate natural food supply (Plumb, 1999). However, the quantity of these locally produced species is not sufficient to meet the national demand. As such, imported frozen fish contribute a large proportion of fish consumed in the country. In this regard, Nigeria has been ranked as the highest importer of frozen fish in Africa; importing 740,000 tons of frozen fish annually to meet the demand of 2.66 million tons of fish (Jatto *et al*., 2013).

Poor hygienic practices during production, handling, marketing and processing of fish can pose serious threats to the safety of fish for human consumption. Food safety is a significant aspect of food value chain (WHO, 2000; Painter *et al*., 2013), but in Nigeria, lack of adequate regulation of food safety issues by public health authorities coupled with limited information on the

incidence and sources of food borne outbreaks have made public health interventions difficult (Dairo *et al*., 2010). Animal sourced foods such as fish, meat and milk have been incriminated as vehicles for transmission of food borne microbial pathogens (Center for Food Safety, 2015). In the United States of America (USA) where effective systems of disease surveillance are well documented, episodes of food borne illnesses associated with microbial contamination have been estimated to range from 20-58% (Hall *et al*., 2005; Painter *et al*., 2013).

Public health and environmental studies have implicated bacteria as major cause of food borne illnesses among other pathogens (USDA, 2008; Humphrey *et al*., 2007). The most commonly recognized bacterial pathogens associated with fish borne illnesses are *Listeria monocytogenes*, *Clostridium* spp., *Salmonella* spp., *Campylobacter* spp. and *Staphylococcus aureus* (*S. aureus*) (Center for Food Safety, 2015). These organisms are often found in fish feed, water environment and at the fish processing factories (Svanevik *et al*., 2015). *Staphylococcus aureus* and *Salmonella* species are more often associated with food borne diseases due to cross-contamination along production line (Amagliani *et al*., 2012). The major clinical signs of food borne diseases include fatigue, abdominal pain, vomition, diarrhoea and fever. Contamination by *S. aureus* in fish industry is mainly through handling of fish by fish handlers, especially those with skin infection or nasal colonization (Kluytmans and Wertheim, 2005).

The ability of these pathogens to develop resistance to a wide spectrum of antimicrobials has limited the alternatives for managing related infections. Presence of methicillin resistant *S. aureus* (MRSA) in livestock and several foods of animal origin have raised concerns about possible transmission to humans (Wendlandt *et al*., 2013). In Nigeria, studies on food value chains of animal sourced foods such as beef and fish have documented the presence of MRSA (Ndahi *et al*., 2013; Grema *et al*., 2015a). These findings demonstrate potential risk to exposure

of MRSA amongst fish handlers and fish consumers. The emergence and spread of pathogens harbouring antimicrobial resistance genes, poses major risk for human and animal health (Sundsfjord *et al*., 2004; Guzman-Blanco *et al*., 2009). Polymerase chain reaction is a suitable technique for detection and amplification of genes for identification of *S. aureus* and *Salmonella* species (Sundsfjord *et al*., 2004).

Microbial risk assessment (MRA) is a valuable tool for disease surveillance and control (Kaplinsky and Morris, 2000; FAO, 2012). It is a scientifically based decision making tool which involves hazard identification, hazard characterization, exposure assessment and risk characterization. Microbial risk assessment involves both qualitative and quantitative risk estimation of a hazard and its determinants along food value chains (FAO/WHO, 1997). Value chains are the linked groups of people and processes by which specific commodity is supplied to the final consumer (FAO, 2012). Understanding the value chain mapping is important in assessing the risk of disease spread within production and distribution systems as well as stakeholder attitudes and practices that influence the safety or otherwise of final product for human consumption (Luu, 2015). Hence, the present study aims to assess microbial food safety risk of fish with focus on *Staphylococcus aureus* and *Salmonella* spp. and the possible risk factors associated with the spread of these pathogens along the fish value chains in Kaduna and Zaria metropolises.

1.2 Statement of Research Problem

Outbreaks of food borne illnesses associated with the consumption of ready-to-eat foods have been increasing globally (Painter *et al*., 2013). Ready-to-eat fish products have been recalled by the Food and Drug Administration (FDA) in the USA as a result of both *Salmonella* and *S. aureus* food borne outbreaks (Painter *et al*., 2013). Food borne illness has been reported in the

USA due to ingestion of food contaminated with *S. aureus* toxins (CDC, 2013). The increase in outbreak related to microbial contamination may be due to the changes in dietary habits of consumers who prefer processed food for ease of cooking, a trend that is associated with increase in the per capita consumption of fish (Githaiga, 2012). According to FAO (2007), yearly consumption of street processed food including fish have increased, whereby about 2 billion people eat street vended food daily.

Although governments globally are trying to improve the safety of food supplied to the general public, occurrence of food borne diseases is still a significant public health concern in both developed and developing countries (WHO, 2015). Estimation of the global incidence of food borne diseases is difficult, but reports from WHO (2015) revealed that in 2005 alone, 1.8 million people died from food borne illnesses attributed to microbial contamination of food. Food borne illnesses caused by *S. aureus* intoxication has led to 458 outbreaks, 6795 illnesses, 333 hospitalizations and 3 deaths in the USA from 1998-2008, while *Salmonella* caused 1, 341, 873 outbreaks with 15, 398 illnesses, 553 deaths and US$2.4 billion in economic losses (CDC, 2013). In developing countries where food vending is common, chances of bacterial food contamination and subsequent reports of food borne illnesses, the incidences could have been high except due to lack of disease surveillance, documentation and appropriate diagnostic techniques (Ifenkwe, 2012; Mensah *et al*., 2012). In Nigeria, there are reports of food borne outbreaks traced to microbial contamination of food which causes relatively high morbidity and mortalities (Ojeniyi and Montefiore, 1986; Oni *et al*., 2009; Fatiregun *et al*., 2010).

Lack of proper knowledge and guidance on safety of processed fish products, street vended fish and the hygiene/sanitary status of food handlers involved in preparation of fish for public consumption can be attributed to most outbreaks (FAO/WHO, 2005). Nurudeen *et al*. (2014)

reported that street vended foods in Nigeria were contaminated with microorganisms associated with food borne illnesses as well as poor environmental and preparatory hygienic practices. Varying rates of *S. aureus*, *Salmonella* spp., Coliforms, *Bacillus* spp. and *Clostridium* spp. isolation have been reported in food, food handlers, and processing units in Kenya and United States of America (Nyenje and Njip, 2013; Wendlandt *et al*., 2013). On the one hand, slaughterhouses and food processing units have been reported to be contaminated with *S. aureus*; and on the other hand contaminated foods subsequently serve as sources of infection to the food handlers or personnel (Borch *et al*., 1996; EFSA, 2009).

In addition, integrated fish farming and farm practices such as prior fertilization of pond using untreated animal manure can pose serious food safety risk through colonizing fish with pathogens potential of causing foodborne diseases (Plumb, 1999). With the report of high antimicrobial use in fish farms (WHO, 2001) and occurrence of *S. aureus* and *Salmonella* spp. in faeces of poultry, pigs and cattle, there is high chance of microbial resistance and contamination as well as colonization of fish handlers during handling/processing of fish (Lee, 2003).

1.3 Justification of the Study

Fish is one of the cheapest sources of animal protein in Nigeria and its production has the potential to make substantial contributions towards addressing the food and nutrition security challenges of the country (Amiengheme, 2005). In order to realize this potential, however, it is essential to understand the public health threats associated with the development and expansion of the fish value chains in Nigeria. Currently, ongoing research on animal sourced food value chains seemed to have focused mainly on high turnover livestock sectors, particular poultry and dairy industry, with little attention being paid to the fish sector. As such, one of the justifications

for this research project was to generate information that would guide public health policy development for the Nigerian fish industry.

With tremendous increase in consumption of fish, there have been only a limited number of studies conducted which focused on hygiene and safety of both domestic fish and frozen fish value chains. The results of the studies therefore addressed the shortfalls in information regarding the food safety and hygiene measures of stakeholders in fish value chains in Zaria and Kaduna metropolises.

The study was designed to provide useful potential risks associated with consumption of fish, it's processing and handling practices through evaluation of the microbial burden. The level of microbial contaminants along fish value chains will provide baseline information for policy development on the hazard analysis and critical control points (HACCP). Cultural, biochemical and molecular evaluation will emphasize the presence of these organisms and biochemical characteristics such as the burden, pathogenic strains, and resistance profile. Antimicrobial resistance profile of circulating strains of *S. aureus* and *Salmonella* spp. could help clinicians in selecting the efficient antibiotics in cases of infections. The basic phenotypic characteristics of these agents to antibiotics will help in development of meaningful strategy for disease surveillance and control programme.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of the study was to assess the microbial food safety risk of fish along the entire fish value chains in Kaduna and Zaria metropolises.

1.4.2 Objectives of the study

The objectives of the study were to:

1. Describe and document the operations involved in fish value chains of Kaduna and Zaria metropolises using semi-structured questionnaire.
2. Identify and profile the risk factors associated with bacterial contamination along the fish value chains in Kaduna and Zaria metropolises using questionnaire and checklist.
3. Determine the bacteriological quality of fish at different stages of the value chains using total aerobic and coliform counts.
4. Isolate and identify *Staphylococcus aureus* and *Salmonella* species from fish value chains in Kaduna and Zaria Metropolises using conventional and molecular methods.
5. Determine *in vitro* antibiotic susceptibilities of *Staphylococcus aureus* and *Salmonella* isolates using disc diffusion techniques and minimum inhibitory concentrations.
6. Assess the food safety knowledge, attitude and practices of fish handlers along the fish value chains using semi-structured questionnaire.
7. Assess the behaviour and fish consumption pattern of consumers towards fish safety in Kaduna and Zaria Metropolises using semi-structured questionnaire.
8. Simulate total food borne diseases in the population of interest attributable to consumption of fish contaminated with *Staphylococuus aureus* and *Salmonella* species using Risk Ranger Analyses tool.

1.5 Research Questions

1. How do the fish value chains in Kaduna and Zaria metropolises operate?
2. What are the risk factors associated with microbial contamination of fish along the fish value chain?
3. What are the microbial loads of fish consumed in Kaduna and Zaria metropolises?
4. Are fish handled by operators of fish value chains contaminated with *S. aureus* and *Salmonella* spp.?
5. What are the antimicrobial profiles of *S. aureus* and *Salmonella* species isolated from fish value chains of Kaduna and Zaria metropolises
6. Do the fish operators in fish value chains of Kaduna and Zaria metropolises as well as the fish counsumers have good fish safety KAP?
7. Do the fish consumers' residents in Kaduna and Zaria metropolises have good behavior towards fish safety?
8. What is the estimate of the microbial risks along the fish value chains of Kaduna and Zaria metropolises?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish Demand and Supply in Nigeria

Fish demand from capture fisheries and aquaculture is presently rising and the record still remains higher than other animal sourced proteins in terms of significance to food security (FAO, 2014). Fish provides an average of about 15 percent of total animal protein supplies globally (FAO, 2014). Currently, the production of fish in Nigeria is only 780,000 metric tones (MT) which is supplemented through importation of 750,000 MT of fish worth USD 600 billion (Oota, 2012) to meet the projected demand of at least 2.66 million MT of fish per annum (Belton and Thilsted, 2014). There is a considerable gap in the demand-supply of at least 1 million metric tons of fish which need to be closed not by importation but domestic production. In Nigeria, the contribution of capture fisheries and aquaculture to national demand is not sufficient for the general population (Grema *et al*., 2011).

Despite a higher average fish supply in recent years, the annual rate of growth has not been adequate to meet domestic demand. Fish supply in Nigeria is from three major sources including Capture fisheries production, aquaculture production and fish importation. Nigeria consists of diverse natural and created water resources such as lakes, streams, rivers and reservoirs in various sizes and lengths categorized into marine, brackish and inland waters (Kudi *et al*., 2008). Main water bodies with high production of fish include Niger-Benue water and Chad basin system (Ita, 1993) which produces around 512, 000 million MT annually on a 12.5 million hectare of land (Ekpo and Essien-Ibok, 2013). Due to recent decline from capture fisheries, there are policies for domestic culture of the most common fish species such as tilapias, clariid and the carps (Vanden Bossche and Bernacsek, 1990) because of their resistance to tropical

environmental condition and low technological requirement. Despite the potential of aquaculture production, national demand is still being supplemented with importation from Europe, Asia and America. About 56% of fish supplied in Nigeria come from importation (Gomna, 2006) ranking the country as the highest importer in Africa in 2004 (Akinrotimini *et al*., 2011).

2.2 The Fish Value Chain in Nigeria

The major components of fish value chain actors in Nigeria involve five principal components namely: input suppliers, producers, marketers, processers and consumers (Alagoa *et al*., 2011). It consists of the input components such as the feed mills and hatcheries, the production component which involves primary producers and the marketing component which consists of the distributors, wholesalers and the retailers. The final points are the processors such as the restaurants and vendors. Each stage of the value chain consists of various actors who maintain the functions and linkages between those enterprises (Alagoa *et al*., 2011). The pre-production component of the fish value chain including the feed mill and hatcheries are still in their premodial stage of development.

2.3 Fishborne Diseases

Global incidence of food borne illnesses may be difficult to estimate, although it has been reported that in the year 2000 alone, 2.1 million people died from diarrhoeal diseases (WHO, 2016). Most of the outbreaks are associated with the consumption of unsafe food leading to acute and life threatening diseases ranging from diarrhoeal diseases to toxaemia (WHO, 2016). Painter *et al*. (2013) attempted to estimate food borne illnesses in data collected from several organizations and thus, concluded an estimate of 9,638,301 illnesses, 57,462 hospitalizations, and 1,451 deaths annually caused by known microbial pathogens. Most of the outbreaks

particularly in developing countries are related to consumption of contaminated food particularly those of animal origin (FAO, 2005). In Nigeria, confirmed cases of food borne illnesses directly related to consumption of food are not frequently reported due to lack of diagnostic investigation and proper disease surveillance and documentation (Bichi and Dawaki, 2010; Dairo *et al*., 2010; Adeyemi, 2013). However, there are several reports of high rates of bacterial isolation in raw and ready-to-eat fish sold within major cities for consumption (Ekundayo *et al*., 2014; Jimoh *et al*., 2014; Grema *et al*., 2015a; Grema *et al*., 2015b). The most frequent bacteria isolated in contaminated fish products include *S. aureus*, *Salmonella* spp., *E. coli, Clostridium* spp. *and Listeria monocytogenes* (Oni *et al*., 2009). Studies have been conducted in different parts of the country and *Campylobacter*, *Salmonella*, *Staphylococcus aureus* and *Escherichia coli* were isolated from livestock farms, abattoirs/processing plants and catering establishments (Ifenkwe, 2012). Most of the abattoirs and slaughter slabs are not suitable for appropriate management, hygienic and sanitary handling and processing of safe food products (Nwanta *et al*., 2008; Lawan *et al*., 2013). As such, contact surfaces of these facilities are mostly contaminated with pathogenic microorganisms that may pose risk of food borne illnesses (Zailani *et al*., 2016). Among the most dominant bacterial pathogens are *S. aureus* and *Salmonella* which are the leading aetiological agents in bacterial food borne diseases (D'Aoust, 1991). *Salmonella* has been isolated from non protein sources such as vegetables and the environment (Abakpa *et al*., 2011). A 4% prevalence of *Salmonella* isolation has been reported from raw and fermented milk samples collected from Kaduna State (Tamba *et al*., 2016). Similarly, *Salmonella* has also been isolated from retail raw beef and corresponding processed products from Kaduna state with a characteristic multidrug resistance at a prevalence rate of 2.3% (Tafida *et al*., 2013). In addition, *S. aureus* has been detected in food products such as milk, beef, poultry and fish intended for

human consumption at varying prevalence ranging from 20.3 to 30.9% (Kwaga and Adesiyun, 1984; Suleiman *et al*., 2013; Ndahi *et al*., 2013).

2.4 *Staphylococcus aureus*

Staphylococcus aureus is a pathogen of significance in fish industry due to its intrinsic virulence and ability to cause diverse disease conditions (Lowy, 1998; Waldvogel, 2000). Being a normal flora associated to skin, skin glands and mucous membrane of warm blooded animals (Aklilu, 2010), contamination of fish and contact surfaces from colonized handlers can pose risk to consumers. This organism can persistently or intermittently colonize the skin and mucous membranes of upper respiratory, gastrointestinal, and lower urogenital tracts of humans and animals, particularly the anterior nares as the ecological niches. A total of approximately 37% of the general population are carriers of *S. aureus* (Wertheim *et al*., 2004). Two cases of diseases related to fish borne illnesses can be elucidated in the fish industry either from contact with fish via consumption or by handling. On one hand, the fish handlers themselves are at risk of getting colonized from *S. aureus* through contact with external surfaces of fish contaminated with *S. aureus* and potentially cause invasive diseases ranging from skin infections to life-threatening illnesses such as pneumonia, endocarditis, bacteraemia or septicaemia (Chambers and DeLeo, 2009). On the other hand, fish handlers who serve as consistent or intermittent colonizers can serve as sources of fish or surface contamination particularly in poor hygienic and sanitary conditions with onward risk of food borne transmission to consumers. Furthermore, *S. aureus* has been reported in many animal species, both domesticated and wild animals including fish, capable of causing various skin and systemic infections (Wardyn *et al*., 2012; Loncaric *et al*., 2014).

2.4.1 General characteristics of *Staphylococcus aureus*

Staphylococci are Gram-positive spherical bacteria that occur in clusters at a size of 1 micrometer in diameter (Sass, 2008). *Staphylococcus aureus* is facultative anaerobic coccus which occurs either singly, in pairs and irregular clusters. The organism is non motile, non-spore forming, catalase and coagulase positive. It can grow at a temperature range of 15 to 45°C and at NaCl concentrations as high as 15% (Willey *et al.,* 2008). However, the optimum growth temperature of *S. aureus* is 30-37°C which correlates with body temperature of the human host. It is a facultative anaerobe that grows by fermentation yielding principally lactic acid (Willey *et al.,* 2008). It forms a fairly large yellow colony on rich medium and often haemolytic on blood agar (Hahn *et al.,* 2004). The yellow pigment confers some protection from the antimicrobial effect of sunlight and produces toxins that contribute to its pathogenicity (Tortora *et al.,* 2010). It grows and survives in nasal secretions, on the skin and in foods containing high osmotic pressure or low moisture which normally inhibits the growth of other organisms (Tortora *et al.,* 2010).

2.4.2 Epidemiology and transmission

Staphylococcus aureus is widely distributed in nature and is carried by 25-33% of normal individuals in their anterior nares and skin. It can colonize and infect both healthy, immunologically compromised individuals and hospitalized patients with decreased host defenses. It has a high propensity to colonize abnormal skin surfaces and open wounds, where it may cause active infection. Although the primary habitat of this microorganism is the mucosa of the nasopharynx and the skin of humans and animals (Casey *et al.,* 2007); it has, however, been found in water, dust, air and food (Hamann, 1986). The presence of *S. aureus* in food is often

attributed to inadequate hygiene during handling by food handlers involved in production of food (Björkroth *et al.,* 2000). It can be acquired by humans through alternative pathways such as person-to-person transmission, environmental exposure, and direct exposure to animals or through consumption of contaminated food. Bacterial infections are commonly acquired by fishermen and fish handlers through occupational hazards from fish or the aquatic environment. Transmission from fish to humans could be through injury from cleaning aquarium with bare hands (Alinovi *et al.,* 1993), exposure to fish tank water (Kern *et al.,* 1989), handling tropical fish ponds (Guarda *et al.,* 1992), contact with rare tropical fish (Bhatty *et al.,* 2000), injuries from fish spikes (Said *et al.,* 1998), fish bite (Seiberras *et al.,* 2000), contact with fish living in the wild (Darie *et al.,* 1993), contact with a fresh- or salt-water environment (Hayman, 1991; Jernigan and Farr, 2000) and consumption of contaminated fish (Rhee and Woo, 2010; Hammad *et al*., 2012).

Similarly, some *S. aureus* strains can cause food poisoning by production of enterotoxins during multiplication on foods. These enterotoxins have been divided into several serological types depending on severity of the clinical condition (Omoe *et al*., 2005; Chiang *et al*., 2008). A study on analytical modelling of *S. aureus* strains with various virulence genes showed the role of food animals in pathogen transmission (Guardabassi *et al.,* 2004).

2.4.3 Virulence factors of *Staphylococcus aureus*

Staphylococcus aureus causes various lesions ranging from superficial lesions, systemic infections to toxemic syndromes depending on the virulence factors present (Lipsky and Stoutenburgh, 2005). In pyogenic infections, the pathogenesis results from the combined action of a variety of factors (Gosden *et al.,* 1997). Infection is initiated when a breach of the skin or mucosal barrier which allows staphylococci access to tissues or bloodstream. Whether an

infection is contained or spreads depends on a complex interplay between *S. aureus* virulence determinants and host defense mechanisms (Lipsky and Stoutenburgh, 2005).

Staphylococcus aureus adheres to cells and mucosa through extra cellular matrix components and the microbial surface component recognizing adhesive matrix molecules (MSCRAMM) and the secreted expanded repertoire adhesive molecules. These MSCRAMMs bind molecules like collagen, fibronectin and fibrinogen to the host-tissue component (Foster and Höök, 1998). These molecules act in systemic infections such as endovascular infections, endocarditis and bone or joint infections. Coagulase is an extracellular protein, belonging to the secreted expanded repertoire adhesive molecules class, which binds to prothrombin to form a complex called staphylothrombin. Coagulase is produced by almost all clinical isolates of *S. aureus;* thus it is a traditional marker for identifying *S. aureus* in clinical infections (Foster, 1996). The invasion of host tissues by staphylococci involves the production of a large number of extracellular proteins which includes α-, β- and y-Toxins (Foster and Höök, 1998). The α-toxin is expressed as a monomer that binds to the eukaryotic cell membranes, the subunits oligomerize to form heptameric rings with a central pore through which cellular contents leaks. This toxin initiate the destruction of cells produced from innate and acquired immunity, interferes with the metabolism of arachidonic acid, exocytosis and induces dysfunctions in contractility, leading to bacterial spread and alterations of the hemostasis (Bhakdi and Tranum-Jensen, 1991).

The β-toxin or sphingomyelinase C is a haemolysin, which targets lipid-rich membranes and causes lysis of erythrocytes and mononuclear cells that induce strong inflammatory response. The majority of human isolates of *S. aureus* do not express ß-toxin, but it is frequently found among strains responsible for bovine mastitis (Marshall *et al.*, 2000). There are two major

biologically and serologically distinct *S. aureus* exfoliative toxin isoforms, namely exfoliative toxin A and exfoliative toxin B primarily responsible for the skin manifestations of staphylococcal scalded skin syndrome and bullous impetigo (Amagai *et al.*, 2002). Protein A is a multifunctional virulence factor produced by almost all clinical isolates of *S. aureus*. It inhibits opsonophagocytosis via binding of immunoglobulin Fc fragment and the promotion of B cell activation "superantigen". Protein A plays a pro-inflammatory role, via the activation of the tumor necrosis factor receptor (TNFR1) present on the respiratory epithelium (Turnidge *et al*., 2008). The V8 protease is an extracellular serine protease which possesses many structural similarities with exfoliative toxin. It cleaves the peptide bonds, inactivating the action of antibodies both *in vitro* and *in vivo* as well as to protect against antimicrobial peptides such as neutrophil defensin proteins and bactericidal platelet proteins which cause tissue protein destruction during bacterial invasion (Postier *et al.*, 2004). Leukocidins are toxins that consist of two proteins acting synergistically to promote eukaryotic cell lysis. They include γ-haemolysin, Panton Valentine Leukocidin (PVL), leukocidins (LukD/E and LukM/F). The γ haemolysin is produced by almost all (≥ 99%) *S. aureus* strains (von Eff *et al*., 2004). The Panton-Valentine leukocidin is a pore forming toxin which damages human neutrophils and has been recovered from less than 2% of *S. aureus* strains. It has been epidemiologically linked to skin and soft tissue infections and sometimes in deep seated infections such as necrotizing pneumonia and osteomyelitis in immunocompetent patients (Turnidge *et al*., 2008).

Staphylococcus aureus can produce a large diversity of exoproteins belonging to the family of superantigens which stimulate polyclonal T-cell proliferation. The T-cells known as antigen-presenting cells are activated by *S. aureus* enterotoxins leading to the release of large amounts of various cytokines/lymphokines which are deleterious for the host (Dinges *et al.*, 2000). Toxins

are characterized with superantigenic, pyrogenic and enteropathogenic properties which explain their implication in both staphylococcal toxic shock symdrome (TSS) and food borne illnesses. Enterotoxin-producing *S. aureus* plays an important role as causative organism of food intoxications in many countries. Symptoms like vomiting, abdominal pain and diarrhoea usually occur approximately 2–6 hours after the consumption of food containing the enterotoxins (Tranter and Brehm, 1990). These enterotoxins are thermostable and resistant to proteolytic enzymes of the human intestinal tract with a molecular weight of approximately 27–31 kDa (Balaban and Rasooly, 2000). Food containing the enterotoxin can cause food borne illnesses with no viable bacteria isolated from the suspected foodstuff. In addition, food borne illnesses from such *S. aureus* strains are facilitated by the expression of virulence factors such as immune-modulatory factors and exoenzymes during growth in contaminated food (Bien *et al*., 2011) as well as enterotoxigenic genes such as staphylococcal enterotoxin a, staphylococcal enterotoxin b, staphylococcal enterotoxin c, staphylococcal enterotoxin d, staphylococcal enterotoxin e, staphylococcal enterotoxin f and staphylococcal enterotoxin j (*sea, seb, sec, sed, see, sej*) (Balaban and Rasooly, 2000). Consumption of food containing 10^5 cfu/g of *S. aureus* may result in clinical syndrome characterized by nausea, vomiting, retching, abdominal cramping and prostration (Khetarpaul, 2006). Similarly, the enterotoxins have also been implicated in a number of autoimmune disorders such as rheumatic arthritis, psoriasis, and atopic dermatitis (McCormick and Yarwood, 2001).

2.4.4 Treatment of staphylococcal infections

For patients showing signs of staphylococcal food borne disease, treatment is usually symptomatic. The mild form of the condition is normally for short period of time and clinical symptoms disappear with time. In severe cases of staphyloccal food intoxication with prolonged

period of diarrhoea and vomition, intravenous administration of fluid becomes necessary. Antiemetic and antidiarrhoeal drugs are given to stop fluid loss. Antibiotics are not to be administered in cases of food borne illnesses due to toxins are not affected by antibiotics (Murray, 2005).

Surface infections such as staphylococcal scalded skin syndrome (SSSS) and erythroderma caused by colonization or infection with strains of *S. aureus* that produce epidermolytic toxin can be treated by application of effective antimicrobial agents which limits spread of the organism and treat the infection. However, *S. aureus* were reported to have developed resistance to the most commonly used antibiotics (Ayliffe, 1997). Antibiotics such as trimethoprim-sulphamethoxazole, clindamycin and doxycycline are reported to be effective in the treatment of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) infection (Ernst, 2012). Newer drugs such as oritavancin, tedizolid and dalbavancin have a promising impact on the treatment of MRSA. Other existing agents such as fosfomycin and fusidic acid are under investigation for potential use in the treatment of MRSA infection (Burke and Warren, 2014).

Comparative analysis of different *S. aureus* genomes revealed that many strains have independently acquired genes from members of their surrounding microflora that confer antibiotic resistance and/or encode virulence factors (Ochman *et al.,* 2000; Lindsay and Holden, 2006; de Lencastre *et al.,* 2007). Horizontal gene transfer (HGT) among bacteria and their mobile genetic elements (MGEs) is the primary mode for the spread of antibiotic resistance and virulence factors in clinically important pathogens (Ochman *et al.,* 2000; Diep *et al.,* 2006; Zanevald *et al.,* 2008). Bacteriophages, plasmids, transposons and uptake of naked DNA have been shown to be involved in the movement of DNA between different bacteria (Boyd *et al.,* 2001; Lindsay and Holden, 2006). Horizontal gene transfer (HGT) events have played a

prominent role in the rapid acquisition of antibiotic resistance in *S. aureus*. The development of resistance by *S. aureus* to penicillin is through acquisition of plasmid encoding a penicillin hydrolyzing enzyme known as penicillinase (Deurenberg and Stobberingh, 2008) which is mediated by *blaZ*, the gene that encodes Beta-lactamase (Lowy, 2003). Two adjacent regulatory genes, anti-repressor *blaR1* and the repressor *bla1* that control *blaZ* (Kernodle, 2000), are the pathways responsible for Beta- lactamase synthesis. Beta-lactamase is predominantly an extracellular enzyme synthesized when staphylococci are exposed to B-lactam antibiotics upon which it hydrolyzes the ring rendering it inactive (Lowy, 2003). Currently, 90% of *S. aureus* strains are resistant to penicillin (Freeman-Cook and Freeman-Cook, 2006) and commonly used antimicrobial agents, including the aminoglycosides, macrolides, tetracyclines and fluoroquinolones (Lee, 2003). Methicillin was the antibiotic introduced to treat infections due to *S. aureus*. A few years later, strains of *S. aureus* appeared to be resistant to the agents and are collectively termed methicillin resistant *S. aureus* (MRSA) (Guzman-Blanco *et al*., 2009).

2.5 Methicillin Resistance

The ability of *S. aureus* to adapt to pressure of antimicrobials resulted in development of resistance to multiple antibiotics used in both human and animal populations. The widespread reisitance to β-lactam antibiotics including penicillin and methicillin resulted to a term Methicillin resistant *S. aureus* (MRSA) (Wielders *et al*., 2002). These MRSA strains acquire *mec*A gene which codes for an alternative penicillin binding protein (PBP2' or PBP2a). Similarly, a novel *mec*A homologue, termed *mec*C, which encodes for PBP2a has also been identified in human and animal MRSA (Porrero *et al*., 2014). Although, there are differences in the biochemistry of the two genes, *mec*C gene also confers resistance to methicillin and most antibiotics. Hence, both genes should be correctly identified in samples for effective surveillance

(Paterson *et al*., 2014).The modified surface proteins have low binding affinity to ß-Lactam antibiotics and thereby reduces their bactericidal effect. The gene is chromosomally inserted as part of the mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Depending on the type of SCC*mec*, the added DNA can also carry antibiotic resistance genes on integrated plasmids, leading to multidrug resistance (IWGCSCCF, 2009).

2.5.1 Methicillin resistant *Staphylococcus aureus* reservoir and host specificity

Methicillin resistant *Staphylocuccus aureus* (MRSA) clones have originated in at least three separate settings according to population, infection site and route of transmission which includes the human hospitals (Hospital-Associated MRSA), human carrier individuals (Community-Associated MRSA) (Millar *et al*., 2007) and livestock animals (Livestock-Associated MRSA) (Stefani *et al*., 2012). However, reservoir distribution patterns and strategies for dealing with MRSA in each of the major setting are different. Successful epidemiological investigation of the relationship between the organisms in various settings could be achieved using typing schemes (Weller, 2014). The typing techniques are used in tracking sources, pathways of spreading infections and studying the population genetics. MRSA typing is essential for the establishment of national and international surveillance networks. Multi-locus sequence typing (MLST) and Staphylococcal cassette chromosome *mec* (SCC*mec*) have been reported to be useful for studying clonal evolution to define the clonal type of MRSA strains. International nomenclatures for these clonal types such as European MRSA (EMRSA-15), clonal complexes (CC38) were referred in interpretation of MLST and SCCmec typing (Cookson *et al*., 2007a).

Epidemiological typing of MRSA strains resulted in the recognition of different lineages that are zoonotic, humanosis and/or host specific. Seventeen epidemic strains of human MRSA have been described in the United Kingdom (Aucken *et al*., 2002) but the most dominant are EMRSA-

15 and EMRSA-16 and responsible for human MRSA infections in Europe and America (Holden *et al*., 2004). In Africa, epidemiological data on the predominant clones responsible for most epidemics is poorly documented. According to Breurec *et al*. (2011), the most predominant clones of African origin are sequence type 88-IV (ST88-IV), sequence type 5-IV (ST5-IV) and sequence type 239-III (ST239-III) which are CA-MRSA. Other lineages of human origin include clonal complex 1 (CC1), clonal complex 5 (CC5), clonal complex 8 (CC8), clonal complex 22 (CC22), clonal complex 30 (CC30) and clonal complex 45 (CC45), however MRSA lineage predominant in pigs and other food animals is CC398 (Feßler *et al*., 2012). Interspecies transmission of this strain clonal complex 398 or sequence type 398 (CC398 or ST398) is a potential hazard and can be facilitated by frequent contact, environmental contamination and individual's immunity (Declercq *et al*., 2008). The livestock-acquired methicillin resistant *Staphylococcus aureus* (LA-MRSA) has been isolated in livestock animals such as the pigs and veal calves (EFSA, 2009). The reservoirs are animal nostrils and other moist sites, as well as contaminated animal housing and surrounding environments. There is evidence of MRSA CC398 spread to humans and other animal species contributing significantly to increases in MRSA detected in human hospitals (EFSA, 2009).

While MRSA clone in companion animals are mainly associated with classical human strains, a distinct MRSA clone has been reported in livestock that is non-typeable with pulsed- field gel electrophoresis (PFGE) using the standard restriction endonuclease SmaI (Ekkelenkamp *et al*., 2006). The most predominant strain is the Clonal complex 398 which is the largest number of single-locus variants in the group (Ito *et al*., 2004). The LA-MRSA strains mainly carry SCC*mec* types IVa, V and a variant of type V, coding for resistance against tetracycline and often antibiotics like macrolides, lincosamides aminoglycosides, trimethoprim and fluoroquinolones.

The common absence of Panton-Valentine leukocidin (*pvl*) and various other virulence factors differentiates LA-MRSA from hospital-acquired methicillin resistant *Staphyloccus aureus* (HA-MRSA) and CA-MRSA strains (Argudin *et al*., 2011).

2.5.2 Methicillin resistant *Staphylococcus aureus* in fish value chains

MRSA is usually present in the animal population particularly on skin and mucous membranes of both domestic and wild animals. The spread of this organism into the food chain occurs during slaughter and processing of such animals in abattoirs and processing units with high chances of it being delivered to the food processing units. Various investigations have been conducted to evaluate to what extent MRSA enters the slaughterhouses via colonized animals (Gilbert *et al*., 2012). For instance, samples have been taken from pigs at slaughterhouse lairages or after stunning. Results indicated that slaughter and meat processing plays an essential role in the transmission of MRSA from animal to the meat products (Vossenkuhl, 2015). The high entry of MRSA into slaughterhouses via colonized animals raises the question how and to what extent MRSA is able to spread along the food value chain (Wendlandt *et al*., 2013). Although, there are no studies yet to determine the level of MRSA along fish production line, currently some studies analyzed the prevalence of MRSA in different stages of animal production chain (O'Brien *et al*., 2012; Wendlandt *et al*., 2013, Vossenkuhl, 2015). Findings suggest influence of single processing step on microbiological contamination and decontamination of carcass as regards the burden of *S. aureus*. The MRSA on surface of animal carcasses can be transmitted during meat processing via human hands, cutting tools and any surfaces with direct meat contact (Vossenkuhl, 2015). The increase in staff and manual handling during processing additionally facilitates the entry of human MRSA strains into the food production units (Vossenkuhl, 2015).

2.5.3 Molecular characterization of methicillin resistant *Staphylococcus aureus*

Different DNA typing methods notably staphylococcal chromosomal cassette *mec* (SCC*mec*), *spa* typing and MLST are used for typing MRSA strains (Cookson *et al*., 2007b). In several studies, eleven (11) different SCC*mec* types have been described; the class of *mec* gene complex and type of *ccr* gene complex carrying a set of recombinase genes responsible for integration and excision of the cassette characterize the different types of SCC*mec* elements (IWGCSCCF, 2009). *S. aureus* strains with SCC*mec* I-X which harbour *mec*A, SCCmec XI and a divergent *mecA* homologue (*mecA*LGA251) have been characterized (Garcia-Alvarez *et al*., 2011). Molecular characterization of *S. aureus* has gained more and more importance in food industry (Pimbley and Patel, 1998). PCR as a basic molecular technique has been recommended for the identification of *S. aureus* (Brakstad *et al*., 1992) and staphylococcal enterotoxin genes (Martineau *et al*., 1998). *S. aureus* genes such as thermo stable nuclease gene (*nuc* gene) which enable heat resistance to as much as 100^{o}C for at least 1 h are well characterized (Brakstad *et al*., 1992), cloned and sequenced (Kovacevic *et al*., 1985) for optimum assessment of staphylococcal hazards present in food which may compromise safety of human consumption.

2.6 The Genus *Salmonella*

The genus *Salmonella* consists of two species. The first is *S. enterica* which is divided into six subspecies; *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*; and the second is *S. bongori* (formerly called *S. enterica* subsp. *bongori*) (WHO, 2003). *Salmonella enterica* subspecies I is mainly isolated from warm-blooded animals and accounts for more than 99% of clinical isolates; whereas the remaining subspecies and *S. bongori* are mostly isolated from cold-blooded animals.

The most commonly used classification of *Salmonella* is Kauffmann-White scheme which is according to three major antigenic determinants. These include flagellar (H) antigens, somatic (O) antigens and virulence (Vi) capsular (K) antigens adopted by the International Association of Microbiologists in 1934. The agglutination of antibodies specific for the various O antigens is classified into 6 serogroups: A, B, C1, C2, D and E. For example, S. Paratyphi A, B, C and S. Typhi usually express the O antigens of the sero-groups A, B, C1 and D, respectively. Almost 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* subspecies *enterica* and sometimes cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* does occur (Pui *et al.*, 2011).

In addition, further classification of serotypes can be done based on the antigenicity of the flagellar (H) antigens which are highly specific for *Salmonella* (Scherer and Miller, 2001). The O antigens represent the lipopolysaccharide (LPS) of the outer bacterial membrane which are heat stable, resistant to alcohol and dilute acids (Yousef and Carlstrom, 2003). The H antigens are heat-labile proteins associated with the peritrichous flagella and can be expressed in one of two phases. The phase 1 H antigens are specific and associated with the immunological identity of the particular serovars whereas phase 2 antigens are non-specific antigens containing different antigenic subunit proteins which can be shared by many serovars. K antigens which are heat sensitive carbohydrates are produced by *Salmonella* serovars that express a surface-bound polysaccharide capsular antigen (Hu and Kopecko, 2003; Yousef and Carlstrom, 2003).

2.6.1 Epidemiology and pathophysiology of *Salmonella*

Salmonella belongs to the family *Enterobacteriaceae* which make up large genus of gram-negative bacilli and constitute the genus of more than 2500 serotypes that are highly adapted for

growth in both humans and animals causing a wide spectrum of diseases (WHO, 2003). The growth of S. Typhi and S. Paratyphi is restricted to human hosts and causes enteric, otherwise known as typhoid fever. The remainder of *Salmonella* serotypes, referred to as non-typhoidal *Salmonella*, can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects (WHO, 2003) and are pathogenic to humans where they mostly cause gastroenteritis, localized infections and/or bacteremia (Fuaci and Jameson, 2005). Members of the genus *Salmonella* are ubiquitous pathogens found in humans, livestock, wild animals, reptiles, birds and insects (Getenet, 2008) and can favourably multiply under various environmental conditions outside the living hosts (Pui *et al*., 2011).

*Salmonella*e are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 µm in size (Getenet, 2008). They do not require sodium chloride (NaCl) for growth, but can grow in the presence of 0.4 to 4% NaCl. Most *Salmonella* serotypes grow at temperature range of 5 to 47°C with optimum temperature of 35 to 37°C and extreme temperature of as low as 2 to 4°C or as high as 54°C . They are sensitive to heat and killed at temperature of 70°C or above. *Salmonella* grow in a pH range of 4 to 9 or 6.5 and 7.5 optimally. *Salmonella* require high moisture activity between 0.99 and 0.94 but yet can survive at moisture activity less than 0.2 such as in dried foods (de Freitas *et al*., 2010a). Complete inhibition of growth occurs at temperatures less than 7°C and pH less than 3.8 or moisture of less than 0.94 (Pui *et al*., 2011).

Like other members of the family *Enterobacteriaceae*, they produce acid on glucose fermentation; reduce nitrates to nitrite, but do not produce cytochrome oxidase. In addition, all *Salmonella*e strains are motile by means of peritrichous flagella except S. Gallinarum and S. Pullorum which are non-motile, and all but S. Typhi produce gas (H_2S) on sugar fermentation

(Fuaci and Jameson, 2005; Getenet, 2008). *Salmonella* are non- capsulated except S. Typhi, S. Sendai, S. Paratyphi A, B, C and some strain of S. Dublin (Getenet, 2008; Gal-Mor *et al*., 2014).

2.6.2 Virulence factors of *Salmonella* species

The onset of disease as well as severity of *Salmonella* infection is determined by the characteristics of the host and the bacterium. For the bacterium, the major cause of disease severity is determined by its virulence factors. *Salmonella* has three important virulence factors which are essential in pathogenicity (Van Asten and van Dijk, 2005). The *Salmonella* pathogenicity islands (SPIs) which are virulence genes clustered in regions of the chromosome called *Salmonella* pathogenicity islands (McClelland *et al*., 2001). The severity of *Salmonella* virulence, such as cell invasion, intracellular survival and the production of Vi antigens capsule are all encoded within the SPIs (van der Velden *et al*., 2000). There are several (10) SPIs which have been identified on the *Salmonella* chromosome. However, SPI-1 and SPI-2 within the islands are central for pathogenesis of *Salmonella* infections (Hansen-Wester and Hensel, 2001). The SPI-1 encodes genes necessary for invasion of intestinal epithelial cells and induction of intestinal secretory and inflammatory response for *S. enterica* (Galyov *et al*., 1997) while SPI-2 encodes genes essential for intracellular replication for systemic infection beyond the intestinal epithelium (Hensel, 2006).

Similarly, type III secretion systems are systems responsible for the production of enzymes known as Type III secretion system (TTSS). These secretory systems are specialized virulence factors that have evolved through indirect translocation of bacterial virulence proteins into the host cell cytoplasm. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope (Galan, 1998). The presence of two SPIs (SPI-1 and SPI-2) each encoding a TTSS, have been described for *Salmonella* species and

may reflect the flexibility of this highly successful pathogen in causing different forms of diseases (Fierer and Guiney, 2001). Finally, several regulatory proteins, toxins, plasmids and Vi antigens control the synthesis of multiple proteins at the level of gene transcription which are essential for *Salmonella* pathogenesis and necessary for characterization in *Salmonella* surveillance (Behlau and Miller, 1993).

2.6.3 Reservior and host specificity of *Salmonella* species

Epidemiologic classification of *Salmonella* has been conducted on the basis of host preferences. The first group of host-restricted serotypes that infect only humans such as S. Typhi was classified. A second group of host-adapted serotypes which include S. Pullorum in avian are associated with one host species, though they could cause disease in other hosts. All other remaining serotypes were classified into the third group. Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans (Boyen *et al*., 2008). These *Salmonella* serotypes are referred to as non-typhoidal *Salmonella* and cause diseases in various animals including mammals, reptiles, birds, and insects by colonizing the gastrointestinal tracts (de Freitas *et al*., 2010a). Several serotypes of these groups of *Salmonella* are pathogenic to humans causing gastroenteritis, localized infections and/or bacteremia when consumed in food or water (Fuaci and Jameson, 2005).

Salmonella species are isolated in several cases of food borne illnesses both in developed and developing countries (EC-ASEAN, 2005). Incidence of *Salmonella*'s contamination of fish and shellfish can originate from contamination occurring in natural aquatic environment or during processing (Amgliani *et al*., 2012). Therefore, the analysis of risk of food product from "farm to fork" is considered useful in evaluating the microbiological quality of fresh and ready-to-eat fish and hygiene standards during fish handling and processing.

The epidemiology of salmonellosis is difficult to determine because of several (≥2,500) distinct serotypes with different reservoirs and diverse geographic incidences (WHO, 2005). Changes in food consumption pattern, animal production systems and distribution trends have led to an increasing frequency of outbreaks associated with raw and processed animal products (Rounds *et al*., 2010). According to the WHO Global *Salmonella*-Surveillance, during 2000-2002, *S*. Enteritidis was the most common serotype reported from humans globally and accounted for about 65% of all isolates. This was followed by *S*. Typhimurium (12%) and *S*. Newport (4%) (CDC, 2013). Among the non-human *Salmonella* isolates, *S*. Typhimurium was the most commonly reported serotype within a three year period, accounting for (17%) of isolates in 2002 followed by *S*. Heidelberg (11%) and *S*. Enteritidis (9%). This made *S*. Enteritidis, *S*. Typhimurium and *S*. Typhi ranked as the three most common human serotypes causing food borne illnesses in all regions of the world (Herikstad *et al*., 2002; Galanis *et al*., 2006). *Salmonella* Agona, *S*. Infantis, *S*. Montevideo, *S*. Saintpaul, *S*. Hadar, *S*. Mbandaka, *S*. Newport, *S*. Thompson, *S*. Heidelberg and *S*. Virchow were also prevalent in human infections but not common causes of food borne illnesses. Non-typhoidal salmonellosis is acquired from multiple animal reservoirs (Fuaci and Jameson, 2005) which increase the chance of infection in humans. In Africa in 2002, *S*. Enteritidis and *S*. Typhimurium were each reported from approximately one fourth of isolates from humans and they were associated with food borne illnesses (Galanis *et al*., 2006).

The predominant sources of contamination in the environment of food value chains are the faecal wastes from infected animals and humans (Ponce *et al*., 2008). Members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and intestinal tracts of animals (Anjum *et al*., 2011). Individuals with poor personal and environmental hygiene can become

infected following contact with contaminated food, water or environment. Although environmental contamination from food processing units and untreated water are the important risk factors of food contamination, consumption of contaminated food of animal origin are the usual routes for human infections (Gracey *et al*., 1999; Anjum *et al*., 2011).

Foods of animal origin, particularly meat, poultry, unpasteurized milk and eggs are primarily considered as the most common sources of human salmonellosis (Tauxe, 1991; Nielsen *et al*., 1995; Wray and Davies, 2000; Acha and Szyfres, 2001; White *et al*., 2001). Generally, there are reports where livestock and their products contributed to as much as 96% of the total *Salmonella* infections in humans (Dahal, 2007). Most of the animal sourced food products such as beef, mutton and poultry become contaminated during slaughter and processing of gut contents of healthy animals shedding the organism. Similarly, all foods that are produced or processed in a contaminated environment may carry *Salmonella*e and thus cause outbreaks or sporadic infections (D'Aoust, 1991).

2.6.4 Pathogenesis of human salmonellosis

The most significant entry point into human body is the oral route via consumption of contaminated food or water (Mastroeni, 2006). The degree of severity of an outbreak depends on the immune status of the host. High risk groups such as infants, young children, older adults and immune-compromised individuals are at higher risk of salmonellosis (D'Aoust and Maurer, 2007). The infective dose that may cause outbreak varies ranging from 10^1 to 10^{11} cells depending on the food vehicle for transmission (D'Aoust and Maurer, 2007). The infective dose may invariably be lower than reported when the transmission vehicle is liquid food with an anti-acid property (Darwin and Miller, 1999). As the acidic nature of the gastric stomach is

unfavourable for the growth of *Salmonella*, large number of colony forming units (CFU) consumed in food may combat acidic environment and the natural microflora in the intestine to cause infection (Darwin and Miller, 1999). Several host barriers are present along the gastrointestinal tract of human such as the lymphoid tissue, intestinal epithelial cells and the antimicrobial peptides released into the gut (Baumler *et al*., 2000). Once *Salmonella* was able to breach host defense mechanisms, it migrates to the Peyer's patches within the intestinal mucosa of the ileum (Baumler *et al*., 2000) and colonize microfold (M) cells within 30 min post infection (Hu and Kopecko, 2003).

Salmonella invasion of the intestinal epithelium causes destruction of the pilli on the intestinal epithelium, thus affecting cytoskeletal rearrangement of intestinal structural membrane to cause inflammatory reaction and fluid loss (Gray and Fedorka-Cray, 2002). Systemic infection develops when the host defence fails and organisms continuously multiplied in Peyer's patches and subsequently invade host system (Baumler *et al*., 2000).

2.6.5 Route of infection and clinical signs

Clinical signs of *Salmonella* infection are divided into four syndromes, namely gastroenteritis, intestinal or extra-intestinal bacteraemia, enteric fever, and an asymptomatic carrier state (Gray and Fedorka-Cray, 2002). Based on the typical signs of salmonellosis, it has been divided into nontyphoidal salmonellosis (gastroenteritis) and typhoidal (enteric fever) (Ziprin and Hume, 2001). *Salmonella* Typhimurium, S. Enteritidis, and S. Newport are principally responsible for human and non-human gastroenteritis while S. Typhi and Paratyphi serotypes are associated with human enteric fever. Similarly, S. Choleraesuis is associated with bacteraemia in pigs. The general symptom of food borne illnesses is gastroenteritis. The incubation period is from 6 to 48

hours post ingestion of contaminated food or water (Ziprin and Hume, 2001; Molbak, 2005) depending on the CFU of *Salmonella* ingested (Molbak, 2005) and the immune status of the host (Ziprin and Hume, 2001). Initial sign exhibited by individuals infected with salmonellosis is diarrhoea which usually resolves within a week. Subsequently, symptoms of abdominal cramps, nausea, vomiting, headache, fever, chills, myalgia, and joints pain follows (Ziprin and Hume, 2001). Death is uncommon but may occur in patients infected with highly pathogenic serotypes of *Salmonella* (Ziprin and Hume, 2001).

Salmonella bacteremia syndrome is characterized by the presence of bacteria in the blood following gastroenteritis (Rubin and Weinstein, 1977). The major culprit in *Salmonella* bacteremia is S. Choleraesuis; a pathogen of swine bacteraemia. Mortality from bacteraemia is very high, more than twice the incidence of typhoid fever (Rubin and Weinstein, 1977; Jean *et al*., 2006). Secondary complications signs such as osteomyelitis, inflamed pancreas, and abscesses of the skin, tumors, and many other complications may occur (Rubin and Weinstein, 1977).

Enteric fever is also a systemic disease with symptoms such as fever and abdominal discomfort. The enteric fever caused by *S.* Typhi is known as typhoid fever. The incubation period spans from three days and one month while incubation period for paratyphoid fever is one to ten days (Molbak, 2005). Major clinical signs include headache, bradycardia, constipation, diarrhoea, muscle soreness, malaise, chills, and increased body temperature (Ziprin and Hume, 2001; Molbak, 2005).

Chronic carrier individuals, also known as asymptomatic carriers, may shed *Salmonella* in the faeces or urine for over a year (Molbak *et al*., 2006). These types of individuals could be of

significance to public health officials, particularly if they are employed as food handlers and health workers (Ziprin and Hume, 2001). With poor personal hygiene, there is a high risk of carriers to contaminate food, environment and serve as source of infection to the general public (Molbak *et al.*, 2006).

2.6.6 Treatment of human salmonellosis

The main approach to the treatment of human gastro-enteric salmonellosis is by symptomatic therapy. Each symptom is treated on the basis of clinical signs exhibited by the patient; a process known as supportive therapy. These include administration of fluids and electrolytes to reverse dehydration. In less severe cases of salmonellosis, it is advised not to give antimicrobial therapy especially when the individual is not dehydrated. This is because most illnesses are self limiting in high immune-competent individuals (Hanes, 2003).

Nonetheless, if the individual is showing signs of systemic infection like bacteraemia, administration of antimicrobial agents is necessary (Hu and Kopecko, 2003). Selection of antimicrobial agents should be based on susceptibility test and ability of the antibiotic to penetrate the phagocytic cells and destroy the bacteria at the point of replication to avoid chronic carrier state. Effective antibiotics such as quinolones and ciprofloxacin have gained favourable response in patient therapy (Ziprin and Hume, 2001). For chronic asymptomatic carriers, antimicrobial therapy using ampicillin, amoxicillin, and fluoroquinolones provided success, though very limited (Hu and Kopecko, 2003). Best line of action in this situation is cholecystectomy while focal abscess is best treated by aseptically draining the pus consistently (Gray and Fedorka-Cray, 2002).

Presently, the major problem faced by clinicians in the treatment of bacterial infections is resistance of bacterial strains to previously effective antibiotics. This is due to overuse of antibiotics, both in humans and animals. Animal agriculture is targeted as the major culprit in the development of antibiotic resistance in humans leading to two major food safety issues. Firstly, transfer of resistance genes from bacterial strains of animal origin to infectious agents in human. Secondly, residue of antibiotics in tissue of animals slaughtered for human consumption provide minute doses of the antibiotics that may confer resistance to human bacterial strains. Several studies have shown the resistance of numerous *Salmonella* serotypes to commonly used antibiotics (Threlfall *et al*., 1996; Esaki *et al*., 2004; Dechet *et al*., 2006). The most common antibiotics constituting multiple drug resistant characteristics to *Salmonella* include ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (Threlfall *et al*., 1996). To reduce burden of antibiotic resistance of *Salmonella* spp. in humans, it is necessary to discourage the improper use of antibiotics in food and companion animals (Dechet *et al*., 2006).

2.7 Risk and Risk factor Analyses of Fishborne Diseases

Risk analysis is a framework used to estimate risk of food products for evaluation of its safety within the food industry (Bassette *et al*., 2012). This model is comprised of three main components; risk assessment, risk management and risk communication. Risk assessment (RA) is a scientifically evidence-based characterization of the risk to which exposure of an individual may lead to adverse health effects. It consists of four components, namely: hazard identification, hazard characterization, and exposure assessment and risk characterization. Risk characterisation is conducted using qualitative and/or quantitative method; both involve description of information in order to estimate magnitude of risk and impacts of risk factors on FBD onset (Fazil, 2005).

Food safety measures expand along entire production chains including practices related to food production, preparation, processing, labeling, hygiene, food additives and pesticide residues, as well as policies on food biotechnology (FAO/WHO, 2006). The main obligation of food safety practices by food handlers in all food industries are to deliver safe food by maintaining high standards of personal hygiene, cleaning contact surfaces and correct storage of food at safe temperatures for specific time (CAC, 1997). Proper personal hygiene means requirement of the food handler to cover the body using clean clothing and clean hands, nails, neck, hair, and clothings which necessitates effort to become a personal activity (Guzewich and Ross, 1999). The significance of proper cleaning of all contact surfaces before and after fish handling has been recognized as an important tool in reducing the risks of contamination (Sagoo *et al*., 2003). Containers, tables, and equipment contaminated with microorganisms (Kassa *et al*., 2001; Grema *et al*., 2015a). Time-temperature regulation in fish industry is an essential factor contributing to fish-borne diseases (Ahmed, 1991; FDA, 2011). Although regulation of temperature for a certain period of time does not prevent fish and fish products from becoming contaminated; it usually prevents further multiplication of microorganisms and/or toxins production to the point of safe consumption. There are food safety guidelines which stated that chilled fish must be maintained between 0^{o}C and 4^{o}C (FDA, 2011) or at extremes of 90-100^{o}C (FDA, 2011; FDA, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Area

The study was conducted in two major cities of Kaduna State: Kaduna and Zaria metropolises. Kaduna Metropolis is the capital city of Kaduna State which consists of Kaduna North, Kaduna South and some parts of Chikun and Igabi Local Government Areas (LGAs). Kaduna metropolis has an estimated human population of 1,570,331 according to 2006 National population commission Census. Zaria metropolis consists of Zaria and Sabon-Gari LGAs and has an estimated human population of 698, 163 (Jonathan *et al*., 2011; National population commission Census, 2006). These two cities attract the largest proportion of middle income households, being the centres for Government establishments, higher education institutions and hospitality industry as well as trade opportunities. This implies comparatively higher rate of consumption of animal sourced proteins. As such, these towns were presumed to have relatively larger population of fish consumers. In addition, the towns have relatively more fish farms, fish marketing outlets and well organized fish value chains which will enable field based research (FAO, 2007; Kudi *et al*., 2008).

3.2 Sampling Strategies

A cross sectional study was employed and primary sources (fish farms/distributors), traders, processors and consumers were selected based on their willingness to participate in the study after due consultation. In order to ensure participation, discussions were held prior to fieldwork, with leaders in respective segments of fish value chains (i.e. primary sources, traders, processors) to introduce the researcher, to explain the objectives and benefits of the study and to seek for cooperation and mobilization of their colleagues.

3.2.1 Inclusion criteria

1. Fish farmers, fish traders and fish processors within the geographical location of Kaduna and Zaria metropolises
2. Fish farmers rearing fish within an intensive system of culture
3. Fish farmers rearing any kind of fish species within the geographical locations of the study
4. Fish traders handling all kinds of fish product forms for consumption in the study area
5. Fish processors that prepare and process locall farmed and imported fish products in the tudy area

3.2.2 Exclusion criteria

1. Fish farmers, fish traders and fish processors located outside the geoghraphical locations of the study area
2. Fish farmers practicing the extensive system of culture
3. Aquaculturist farming other aquatic species excluding fish species
4. Fish traders selling the imported canned fish products
5. Fish processors that used imported canned fish products for conusmption

3.2.3 Sample size estimation

3.2.3.1 Sample size estimation for fish specimens

The sample size for the study was calculated using Open Epi Info™ 7.1.5.2 software version (CDC, Atlanta, USA, 2015) with the following input parameters:

Sample size = unknown

Expected prevalence = prevalence rates of 5.0% and 6.7% respectively for *Salmonella* and *S. aureus* were used (Luu, 2015).

Confidence limit = 95%

Precision = 5%

Power of the study = 80%

Design effect = 1.0

Sample size of 118 and 89 respectively for *Salmonella* and *S. aureus* were calculated. Since 118 was bigger than 89, 118 was used as the minimum required sample size for the study. However, 20% of 118 (≈ 24) was added to the minimum required sample size in order to accommodate potential losses or spoilage of specimen prior to analysis. Therefore, a total sample size of 142 was calculated. However, 146 fish samples were collected for the study. A total of 45 fish farmers, 64 fish traders and 37 fish processors were interviewed and fish samples purchased from each of them using disproportionate sampling method.

3.2.3.2 Sample size estimation for fish consumer survey

The number of fish consumers to be sampled was estimated using the formula provided by Cheung (2005) below:

$n_h = (Z^2)\ (r)\ (1\text{-}r)\ (f)\ (k)/\ (p)\ (ñ)\ (e^2)$

Where:

n_h = number of fish consumers — K = Non-response rate (5)

Z = level of confidence (1.96) — p = Proportion of target population

r = estimate of indicator to be measured — ñ = Average household size (6)

f = design effects (default value=2.0) — e^2 = Margin of error (0.1r)

Substitution of recommended values for developing countries resulted in the formula

$n_h = (84.5)\ (1\text{-}r)/\ (r)\ (p)$

Where

r = 84% (0.84) (Gomna and Rana, 2007)

p = 37.1% (0.371) (Census, 2006)

Hence, $n_h = (84.5)\ (1\text{-}0.84)/\ (0.84)\ (0.371)$

Therefore, fish consumer sample size = 43.4 fish consumers, thus, 57 fish consumers were interviewed in the study to complement invalid questionnaire due to incorrect filling of information, and lack of questionnaire return.

3.3 Fish Value Chain Mapping

Descriptive mapping of the two fish value chains in Kaduna and Zaria metropolises was conducted from primary sources (fish farms/main distributors), traders (wholesalers/retailers) and processors (street vendors and restaurants) using face-to-face semi-structured questionnaire interview of the operators. Participatory observation technique was used for collecting information on the activities of the operators by checklisting observed activity.

3.4 Questionnaire Design, Validation and Administration

The questionnaire was designed based on information obtained from literature conducted for identification of factors influencing food handler's knowledge, attitude and practices (KAP) towards implementing food safety practices and the assessment of risk factors to contextualize local practices (Omemu and Aderoju, 2008; Ansari-Lari *et al*., 2010; Eltholth *et al*., 2015). To measure food safety KAP, the questionnaire formats were adopted from several published questionnaires (Omemu and Aderoju, 2008; Ansari-lari *et al*., 2010; Eltholth *et al*., 2015) and

rearranged to suit target objectives of this research. Pilot study was conducted to assess clarity of questions, instructions, information layout and time allocation.

Four sets of questionnaires were developed to target each segment of the fish value chains. Each questionnaire consisted of three major parts. The first part of the questionnaire consisted demographic questions on age, gender, education level, type of business, years of experience and food safety related information. The second part was designed to explore the activities regarding operational characteristics of the value chain. Thirdly, KAP of fish handlers were recorded on roles of microbes in disease transmission, risk of fish contamination and fish borne illnesses, temperature and fish storage methods and personal hygiene practices.

Questions for knowledge were provided with true, false or I don't know answers. Each response was scored in each section and classified as poor food safety knowledge when respondent did not answer 8 questions correctly or good knowledge when the respondent answered more than 8 questions correctly out of 16 questions. Responses to 12 questions on attitudes were made using a likert-scale of one to three, from 1 = disagree, 2= agree and 3 = no idea. Questions on food safety and hygiene practices were targeted for response of likert scale ranging from one to three, with 1 = never, 2 =sometimes and 3 = always and recorded and scored accordingly.

3.5 Participatory Observation

Observation of infrastructure, product flow and food safety and hygiene practices, fish storage temperature and time control, environmental sanitation, sources of contamination and recontamination within the value chain were examined and check listed in the form (Appendix 15). Each list in the subdivision was coded and reclassified as good or poor and present or absent as the case may be (Eltholth *et al.*, 2015).

3.6 Specimen Collection and Analyses

3.6.1 Collection of raw and ready-to-eat fish

About one kilogram (kg) of medium-sized fish and approximately half kg of ready-to-eat fishes were purchased from the fish value chain. Fish samples were placed in a sterilized polythene bags by the fish handlers and transported to the Bacterial Zoonoses Laboratory, Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. The procedures for microbial assessment of fish were conducted according to the United States Department of Agriculture, Food Safety Laboratory Unit manual (2015). Using sterile scalpel blade or knife, dorsal muscle and skin of each fish was cut and pooled into a single 10g of sample and placed into sterile bag, to which 90 ml of 0.1% peptone water was added and homogenized in a stomacher for 2 minutes.

3.6.2 Bacterial counts

The homogenized mixture was used for both total aerobic and coliform counts (Cheesebrough, 1993). Serial dilution using 100 fold dilution method was used and a volume of 0.1ml from final dilution was spread on nutrient agar for total aerobic count and MacConkey agar for total coliform counts respectively and incubated at 37°C for 24h. The colonies were counted for total aerobic and coliform counts with results expressed as cfu/g and TCC/g, respectively.

3.6.3 Phenotypic identification of *Staphylococcus aureus* and *Salmonella* species

3.6.3.1 Cultural and biochemical tests for Staphylococcus aureus

Using pipette, 1 ml from homogenized sample was inoculated into Tryptic soy broth (TSB) supplemented with 6.5% NaCl and incubated at 37°C for 24h. Loop full of overnight culture was streaked onto Baird Parker agar supplemented with egg yolk tellurite emulsion (Merk, Darmstadt, Germany) and incubated at 37^{o}C for 24h. The growth of the suspected *S. aureus* on Baird Parker agar as black shiny colonies with clear halo was identified as *S. aureus*. The colony with this characteristic was subjected to phenotypic tests as presented in Appendix 1. Test such as Gram staining was conducted using clean grease free slide which was smeared, air dried and heat fixed. Gram positive bacteria were viewed as blue/purple colouration of grape-like colonies while Gram negative bacteria were viewed as red/pink colouration for *S. aureus* and *Salmonella* spp, respectively. Procedure for identification and confirmation of *S. aureus* (coagulase reactions +) was conducted using the following conventional biochemical methods: Catalase, coagulase, sugar fermentation (mannitol, sucrose, maltose, lactose), haemolysis on 5% sheep blood agar and DNase activity as described by Kateete *et al*. (2010).

For coagulase and catalase tests, isolates were purified using Baird Parker agar, then inoculated onto tryptone soya broth and incubated at room temperature for 24 h. For tube coagulase, 0.5 ml of overnight culture was dispensed into sterile test tube. A drop of rabbit plasma was added and incubated at 37^{o}C for 4h. Results were monitored hourly for presence of clumping which was indicative of a positive test. In cases with no reaction in 4 h, incubation period was extended to 24h at room temperature. Catalase test was conducted by dropping 3% hydrogen peroxide

solution onto grease free slide. A loop of colony of 24 h culture was placed on the slide and mixed with an application stick, and then observed for immediate bubbling.

DNase test and sugar fermentation tests were conducted using DNase agar and prepared according to the manufacturer's instructions. A loopful from isolate was inoculated onto DNase agar and incubated for 24 h at 37°C. One-two drops of 1 N of HCl acid was flooded onto the colonies and observed for clear zones around bacterial colonies which were indicative of DNase positive colonies. Sugar fermentation tests were conducted by collecting suspected *S. aureus* colonies from pure cultures and inoculated into each of the prepared sugars, then incubated at 37^{o}C for 24-48 h. The results were observed for presence of pink or red colour which is indicative of sugar fermentation. The sugars used for *S. aureus* sugar fermentation test include Sucrose, mannitol, xylose, inositol, lactose and glucose, arabinose, galactose, fructose, maltose and raffinose while *Salmonella* species were subjected to sucrose, mannitol, xylose, inositol, lactose and glucose, arabinose, galactose, fructose, maltose, raffinose, ducitol, inositol, rhamnose and sorbitol.

3.6.3.2 Cultural and biochemical isolation of Salmonella species

Using another pipette, 1ml from homogenized sample was inoculated onto 9 ml Rappaport-Vassiliadis (RV) broth and incubated at 37°C for 24 h. Each tube was vortexed and a loopful streaked on *Salmonella*-Shigella agar plates using sterile wire loop. The plates were incubated at 37°C for 24 h. Colourless colonies with or without black centres were identified as suspected *Salmonella* isolates.

Presumptive isolates of *Salmonella* spp. from above plates were subjected to Gram staining as stated above. Characteristic colonies were picked with sterile loop and inoculated onto nutrient agar slant for incubation at 37^{o}C for 24h and then stored at 4°C for further identification. For further biochemical identification, suspected colonies of *Salmonella* were picked for inoculation of triple sugar iron, urease agar, Simmons citrate test and for sulphide indole motility (SIM) and methyl red /Voges-Proskauer tests. Similarly, suspected colonies of *Salmonella* were subjected to sugar (Sucrose, mannitol, xylose, inositol, lactose and glucose) fermentation tests.

3.6.3.3 Microbact™ STAPH 12S (MB1561A)

Suspected *S. aureus* colonies were subjected to biochemical test using microbact 12S according to manufacturer's procedure. Two colonies from pure culture were emulsified in 3ml suspending medium. Test strip was placed on holding tray while suspension containing the organisms was added in 4 drops into each well. Two drops of mineral oil (MB1093A) was added to well 7 prescribed as a black circle; the lid was replaced and the strips incubated at 37ºC for 24 h. After 24 h, tray was removed from incubator and 2 drops of fast blue reagent (MB1588A) was added to well 12 prescribed as green circle.

3.6.3.4 Microbact™ GNB 12E (MB1082)

Typical *Salmonella* colonies were subjected to biochemical tests using Microbact 12E test kit (Oxoid, England) according to the manufacturer's procedure. Plastic strips holding 12 tests in each of the strip were inoculated with saline suspensions of overnight cultures according to manufacturer's directions and incubated for 24 h at 37°C. Indole reagent, Vogues-Proskauer I and II and tryotophan deaminase reagents added to well 8, 10 qnd 12 respectively; and colour

reactions were read and compared with the chart. The octal code value was analyzed using the manufacturer's software and positive results were confirmed as *Salmonella* and presented as %.

3.6.3.5 Agglutination test for Salmonella species

Serological test of flagellar (H) and somatic (O) antigens were conducted using the agglutination card test method. A single isolated and identified colony was inoculated into 0.1% peptone water and incubated at 37°C for 24 h. Forty (40) µl of the overnight culture was placed on agglutination card and a drop of *Salmonella* antisera was placed and mixed with a sterile application stick. The presence of agglutination in the test mixture was indicative of positive *Salmonella* spp. while absence of agglutination was considered negative of *Salmonella* spp.

3.6.4 Antimicrobial susceptibility testing (AST)

The susceptibilities of *S. aureus* to 12 commonly used antibiotics were tested using disc diffusion method according to CLSI (2011). Antibiotics included Azithromycin (AZM) 15 µg, Tetracycline (TE) 30 µg, Oxacillin (OX) 1 µg, Cephazolin (KZ) 30 µg, Cefuroxime 30 µg, Vancomycin 30 µg, Chloramphenicol 30 µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (E) 15 µg, Cefoxitin (FOX) 30 µg, Gentamicin (CN) 30 µg and amoxicillin-clavulanic acid 30 µg (Oxoid, UK).

Similarly, *Salmonella* isolates were tested against a panel of 10 commonly used antibiotics for susceptibility namely; Gentamicin (CN) 10 µg, Tetracycline (TE) 30 µg, Cefazolin (KZ) 30 µg, Cefuroxime (CRO) (30µg), Azithromycin (AZM) 15µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (E) 15 µg, Chloramphenicol (CHL) 30 µg, Imipenem (IPM) 10 µg, Trimethoprim-

Sulfamethoxazole (SXT) 25µg, Enrofloxacin (ENR) 5µg, Amoxicillin/clavulanic acid (AMC) 30 µg (Oxoid, UK).

A total of 18 *Salmonella* isolates confirmed using conventional and Microbact 12E tests were subcultured onto SSA for AST. However, one *Salmonella* isolate did not grow on SSA medium during subculture; hence 17 *Salmonella* isolates were subjected to AST to determine their susceptibilities to 10 antimicrobials. Colonies from overnight culture plates were suspended directly into normal saline solution and vortexed to achieve a suspension of 0.5McFarland turbidity (10^8 CFU/mL). Sterile swab was dipped into the suspension and pressed against the tube wall to avoid excess inoculum. The cotton swab stick was swabbed over the surface of Mueller Hinton Agar (MHA) to achieve a uniform distribution of the suspension. The MHA were supplemented with 2% NaCl for oxacillin susceptibility testing. The antibiotic discs were dispensed onto the plates and incubated for 24h at 37^{o}C and zones of growth inhibition were measured and interpreted according to CLSI (2011).

3.6.5 Determination of minimum inhibitory concentration (MICs)

The MICs of oxacillin and vancomycin MICs against *S. aureus* were determined using MIC Etest strip (Biomerieux) while the MICs of amoxicillin/clavulanic acid and erythromycin were determined for *Salmonella* isolates. Using sterile wire loop, 3 or 4 pure colonies from overnight culture were taken and transferred to a tube containing normal saline. The mixture was emulsified and turbidity of inoculums was compared to that in the 0.5 McFarland standards and adjusted to match that standard. Agar plates were swabbed with adjusted inoculum within 15 minutes of preparation of inoculum by dipping sterile cotton swab into it. Swab was rotated several times against inside of the tube wall to remove excess liquid. Application was done by

streaking the swab over entire surface of the agar plate. The plate was then rotated approximately 60^0 and then repeated with motion twice. Inoculation was completed by running swab around the rim of the agar, lid was left opened for 5-10 minutes to allow any excess moisture absorbed before applying strips. Strips were placed using forceps with the 'E end' at the edge of plate with the scale placed upward and visible. Plates were incubated at 37^oC for 24h and interpreted according to CLSI (2011).

3.6.6 DNA extraction protocol for *Staphylococcus aureus* and *Salmonella* isolates

A colony of *Salmonella* and *S. aureus* each were taken from an overnight culture into a 1.5ml micro centrifuge tubes containing 600uL of cell lysis solution. The mixture was vortexed vigorously for 1 minute and incubated at 80°C for 5 minutes to lyse the cells; then cooled to room temperature. In the case of *S. aureus* isolates, the microcentrifuge tubes were transferred to a bead beating homogenizer and allowed to homogenize at full speed for 5minutes to lyse the Gram positive cells.

A 3µl of RNase solution was added into the cell lysate and the tubes were inverted for 2–5 times to mix and then incubated at 37°C for 30 minutes. The mixture was allowed to cool at room temperature. Another 200µl of protein precipitation solution was added to the RNase-treated cell lysate tubes and vortexed vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. The samples were incubated on ice for 5 minutes and centrifuged at 14,000rpm for 3 minutes. The supernatants containing the DNA were transferred into a clean 1.5ml microcentrifuge tubes containing 600µl of isopropanol and left for 5 minutes for residual liquid in the tubes to settle to avoid contaminating the DNA solution with the precipitated protein. Gently, the mixture were inverted until the thread-like strands of DNA form a visible mass and centrifuged at 14,000 rpm for 2 minutes.

The supernatants were carefully discarded and the tube contents were drained on a clean absorbent paper. Additional 600µl of 70% ethanol was added at room temperature and the tubes gently inverted several times to wash the DNA pellet and centrifuged at 14,000 rpm for 2 minutes. Careful aspiration of the ethanol was carried out and the tubes drained on the absorbent papers and allowed to air-dry for 10 minutes. About 100µl of DNA Rehydration Solution was added to the tubes to rehydrate the DNA and incubated at 65°C for 1 hour. Final genomic DNA extracts were stored at 2–8°C and quantified using Nanodrop reading.

3.6.7 Genotypic characterization of *Staphylococcus aureus*

Genomic DNA extracts of the 22 *S. aureus* isolates and 17 *Salmonella* species were sent to Inqaba Biotech laboratory, South Africa for PCR using primers presented in Table 3.1. The genotypic characterization of the *S. aureus* was carried out initially with multiplex PCR protocol to amplify the *nuc* and *mec*A genes using the method of Schubert *et al*. (2005). First, target genes were amplified using the PCR cycle conditions reported below. Master Mix of the component was prepared without the primers, and appropriate primers were added into the tube. Forward and reverse primers of 2.5 µl of 25ng each for *nuc* and *mec*A genes were added to a master mix containing 5µl of dNTP template, 2.5U of FastStart high fidelity enzyme blend and PCR grade water for adjustment to a volume of 25µl. DNA amplification was carried out with the following thermal cycling profiles: an initial activation at 95^{o}C for 4 min in single cycle followed by 30 cycles of amplification (denaturation at 95^{o}C for 1 min, annealing at 60^{o}C for 2 min, and extension at 72^{o}C for 2 min) which ended with a final extension at 72^{o}C for 7 min. Finally, 3 % agarose gel was used and fluorescence signals were detected by staining with ethidium bromide and viewed using UV light.

S. aureus isolates positive for *nuc* and *mec*A genes were then used to amplify *nuc*, *mec*A and *pvl* genes using multiplex PCR protocol. Similarly, another multiplex PCR was conducted for the 10 *S. aureus* isolates to amplify *sea, seb, sec, sed, see* and *sej* genes. Two sets of primer master mixes (Set A and set B) were prepared according to the master mixes of components from the PCR template preparation kit (Roche Applied Science, Germany). Multiplex primer set A included $MgCl_2$ concentration (2.0 µl), 1 µl of 50 pmol primers for *pvl*, 1 µl of 20 pmol each for *nuc* and *mec*A, 2 µl dNTPs, 1 µl DMSO and 0.2 µl Taq polymerase. DNA amplification was carried out in a thermocycler (Eppendorf, Germany) with the following thermal cycling profile: an initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min) and a final extension at 72°C for 7 min.

Multiplex primer set B (Table 3.1) was made of reaction mixture containing 15ng/ µl DNA templates, 1µl of primer F (6 pmol), 1 µl of primer R (6 pmol), 2.5 µl PCR buffer (Fermentas, Lithuania), 0.5 µl dNTP (6 mM, Fermantas, Lithuania), 1.5 µl $MgCl_2$ (Fermentas, Lithuania), 0.5 U Taq DNA polymerase (Fermentas, Lithuania) and distilled water was added to the final volume of 25 µl. Final master mixes from set A and B were loaded separately onto 1% agarose electrophoresis gel. A positive control DNA from *S. aureus* reference strains (ATCC™ 25923™, Rosenbach) and negative control DNA from *S. epidermidis* reference strain (ATCC™ 9491™, Rosenbach) as well as 100bp molecular size gene ladder (GeneRuler™) were added to set A. Fluorescence signals were detected by staining with ethidium bromide and visualized using UV light box.

Table 3.1: Primer sequences, gene locations, and expected amplicon sizes of *S. aureus* genes for the multiplex PCR

Target gene	Primers	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Master mix set	Reference
*mec*A	*mec*A-F *mec*A-R	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	163	A	Mehrotra *et al*. (2000)
pvl	*pvl*-F *pvl*-R	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	433	A	Mehrotra *et al*. (2000)
nuc	*nuc*-F *nuc*-R	TCAGCAAATGCATCACAAACAG CGTAAATGCACTTGCTTCAGG	255	A	Mehrotra *et al*. (2000)
sea	*sea*-F *sea*-R	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	B	Stegger *et al*. (2012)
seb	*seb*-F *seb*-R	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	B	Stegger *et al*. (2012)
sec	*sec*-F *sec*-R	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	456	B	Stegger *et al*. (2012)
sed	*sed*-F *sed*-R	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	B	Khalifa *et al*. (2015)
see	*see*-F *see*-R	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	B	Stegger *et al*. (2012)
sej	*sej*-F *sej*-R	GGT TTT CAA TGT TCT GGT GGT AAC CAA CGG TTC TTT TGA GG	306	B	Khalifa *et al*., (2015)

3.6.8 Genotypic characterization of *Salmonella* species

Monoplex PCR method was used for the amplification of *inv*A gene using DNA extracted from an overnight culture of presumptive *Salmonella* isolates cultured on *Salmonella*-Shigella agar. The DNA was subjected to the following cocktail mix using pimer presented in Table 3.2; 1× 2.5 µl PCR buffer, 1 µl $MgCl_2$, 1µl of each primer (forward and reverse) that has a concentration of 5pmol, 1 µl of DMSO, 2 µl of 2.5mM DNTPs, 0.1 µl of Taq polymerase, 3µl of 10ng/µl DNA and 13.4 µl sterile H_2O for adjustment to 25 µl. DNA amplification was carried out in a Perkin-Elmer thermocycler with the following thermal cycling profile: an initial denaturation at 94^oC for 5 mins, followed by 35 cycles consisting of denaturation temperature of 94^oC for 15 sec, annealing temperature of 65^oC for 20sec and extension at 72^oC for 30 sec. Final extension of 72^oC for 7 mins and holding temperature of 10^oC for infinity. After PCR amplification, 5µl was removed and subjected to agarose gel electrophoresis in 1.5% agarose gel with a 1 kb plus molecular size standard ladder (GeneRuler^{TM}). The gel was stained with ethidium bromide, and the amplicons visualized using a UV light box.

Table 3.2: Primer Sequences for Amplification of *inv*A gene from *Salmonella* isolates

Gene target	Gene	Primer	Sequence (5'-3')	Amplicon size	Reference
Salmonella spp.	*inv*A	F R	CGG TGG TTT TAA GCG TAC TCTT CGA ATA TGC TCC ACA AGG	796	Paião *et al*., (2013).

3.7 Semi-quantitative Risk Estimation

To estimate the microbial risk, online Risk Ranger software was used (Ross and Sumner, 2002); a tool that can be used in ranking the risk of various product/pathogen combinations by answering a series of questions in relation to food safety and risk factors contributing to food safety risk, such as susceptibility of the population, severity of the hazard, probability of exposure to the hazard and probability of food containing an infectious dose. Some of the answers to the questions relied on data presented in sections of the thesis. Questions (Q1-11) used in risk estimation in sequential order were hazard severity, susceptibility of population of interest, frequency of consumption, proportion of population consuming fish product, size of consuming population, probability of contamination of raw product per serving, pathogens prevalence and concentration data, effect of processing, potential for post-processing contamination, effectiveness of post-processing control system, and finally further cooking reheating or ready-to-eat fish product. Risk was characterized as low (Risk Ranger ratings $\leq$32), medium (Risk Ranger ratings from 32 to 48) or high (Risk Ranger ratings $\geq$48) according to Ross and Sumner (2002).

3.8 Data Analysis

Microsoft Access 2007® (Microsoft, USA) was used to store all the data generated in the study. Statistical Package for Social Science version 16 (SPSS Inc.) and Microsoft Excel were used for data analyses. Data from demographic characteristics were summarized into percentages and mean±standard deviations. Quantitative data on bacterial counts were converted to Log $_{10}$ and interpreted according to microbiological standards of fish and fisheries products reported by Huss (1993). Table 3.3 presents the microbiological standard values for raw and ready-to-eat fish according to the report by Huss, (1993) and FAO (1994). Classifications of participatory

observations were analyzed using Spearman's rank correlation to determine the relation between the risk factors and level of microbial fish contamination and likelihood of FBD in consumers

Table 3.3: Microbiological standard values for raw and ready-to-eat fish

Fish form	Microbiological test	cfu limit per gram or per cm^2			
	TAPC	N	c	m	M
Fresh raw fish		5	3	5×10^5	10^7
Frozen raw fish		5	3	10^6	10^7
Frozen processed fish		5	2	5×10^5	10^7
Dried fish		5	1	10^2	10^4
Cold roasted fish		5	3	5×10^5	10^7
	TCC			(TCC/g)	
		< 100	> 100		
Fish	*S. aureus*	5	2	10^2	10^3
Fish	*Salmonella* spps.	10	0	0	0

n= number of minimum sample units, c= maximum number of acceptable samples units with bacterial counts between m and M, m = minimum recommended counts for acceptable quality products, M= maximum recommended counts for acceptable quality products

Likewise, phenotypic characteristics, antibiotic resistance profiles, MIC and prevalences were presented as percentages. MAR index was used to analyze the risk and safety of antimicrobial agents to adopt resistance using (a/b); a = resistance pattern of each isolate to total number of antibiotics tested and b= number of isolates tested. The interpretion of Riaz *et al*. (2011) was used to determine the safe and risky levels. The range of 0.2-1 was indicative of risky phase (high risk) while value less than 0.2 was scored a safe phase.

All variables in the questionnaire were coded and analyzed. Continuous variables were summarized using mean±standard deviation while categorical variables were presented using percentages. Scores for food safety KAP were calculated by assigning correct responses. Numeric responses to questions were added to yield a range of possible scores from the lowest of 0 to highest score of 16 or 30 for attitudes. Knowledge score of ≤ 10 out of 16 points was categorized as poorly knowledgeable. Respondent with attitude score of ≤8 out of 12 points were classified as negative attitude, otherwise they were reported as positive attitude. A respondent with ≤ 15 out of a total of 30 points was considered having bad practice or otherwise good practice. Potential associations between food safety issues and other variables were made using chi square and Fishers exact tests and post hoc chi square test ($p \leq 0.05$). Correlation coefficient analysis with Pearson's ® for continuous factor such as food safety attitude while Spearman's correlation for categorical variables of all KAP questions (producers, traders and processors) were summarized and correlated ($p \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive Mapping of Fish Value Chains in Kaduna State

Two major fish value chains were identified in this study; the distribution channels of imported frozen fish and that for domestically farmed fish. The two fish value chains differed from one another by structure, channels, species handled as well as flow of fish from initial primary level to consumers. The principal actors of the imported frozen fish distribution channels comprised of the main distributors, wholesalers, retailers and fish processors (Figure 4.1) while the actors in the domestic fish value chains were the fish producers, fish traders, made up of wholesalers and retailers, and finally the fish processors (Figure 4.2). Within these chains, all principal actors were described based on demographic characteristics and food safety training, knowledge, attitude and practices in each component.

4.2 Structure of Imported Frozen Fish Distribution Channels

The core functions and flow of imported frozen fish along the distribution channel is presented in Figure 4.1. The distribution channels of imported fish in Kaduna and Zaria metropolises are relatively short and simple due to absence of processing plants. Figure 4.1 provides physical flow of frozen fish in the study area which is comprised of the main distributor (dealer), traders (wholesalers and retailers), the processors (fry shops and street vendors) and household consumers. Frozen fish flows from main distributor's cold room (depot), located each at Zaria and Kaduna metropolises to first stage wholesalers in nearby wholesale markets. Fish from this component then flows to second stage wholesalers in major locations within the cities in the study areas and finally to retailers. These retailers were located in different places characterized

by selling fish and combination of other food products in the shops (Appendix 5). Some processors and household consumers within the communities in turn purchase from either first and second stage wholesalers or the retailers. Most fish processors and households rely solely on first and second stage wholesalers and particularly mobile and stationed street vendors. Street vendors process fish in various forms mainly as roasted and/or fried forms to consumers such as individuals in market places, schools and hospitals for mobile vendors and street sides in markets or bus stations for stationed street vendors. Interconnectivity was recorded between first stage wholesaler and processors as well as stationed street vendors and first stage wholesalers. Frozen fish was the main product traded in most part of the study area. The use of refrigerator was the primary means of preservation practice while processing into fried or roasted forms were the predominant forms marketed to consumers.

4.3 Structure of Locally Farmed Fish Value Chains

The core components in a farmed fish value chain are inputs suppliers, fish producers, traders (marketing), processors and consumers. These actors were involved in different activities as indicated in Figure 4.2 which provides details of the various activities performed. Point of entry into the value chain mapping began with the primary producers, namely the fish farmers, artisanal fisher men and the local farmed fish distributors. Fish farmers in the study area farm fish and sell out all the fishes live in water without any processing to wholesalers and retailers or fish dealers as intermediary, but none of the fish farmers sold fish stock to fish processors, restaurants/hotels, and household directly. The physical flow of fish within the value chain is described in Figure 4.2. Fish flows from wholesalers to retailers, retailers to fish processors, restaurants and to household consumers. Almost all the fish farms were found to produce a single

species of farmed fish namely catfish (*Clarias gariepinus* and *Heterobronchus spp.*). At the level of the wholesalers and traders, there were no value added activities; rather the main activity was entirely distribution and preservation of raw fish products. Fish preservation was done by drying with salt (dried-salted fish) or smoking.

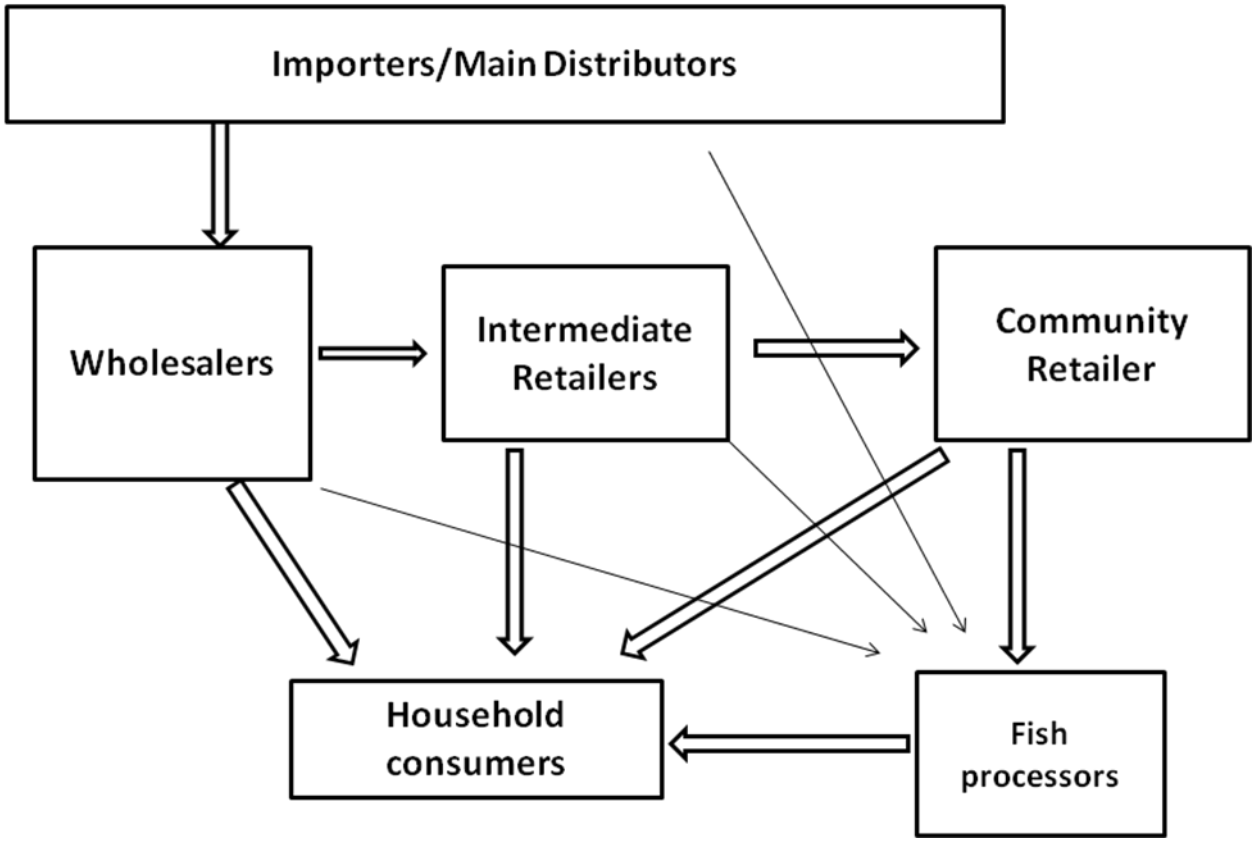


Fig. 4.1: Principal actors and patterns of flow of frozen fish along the distribution channels in Kaduna and Zaria metropolises

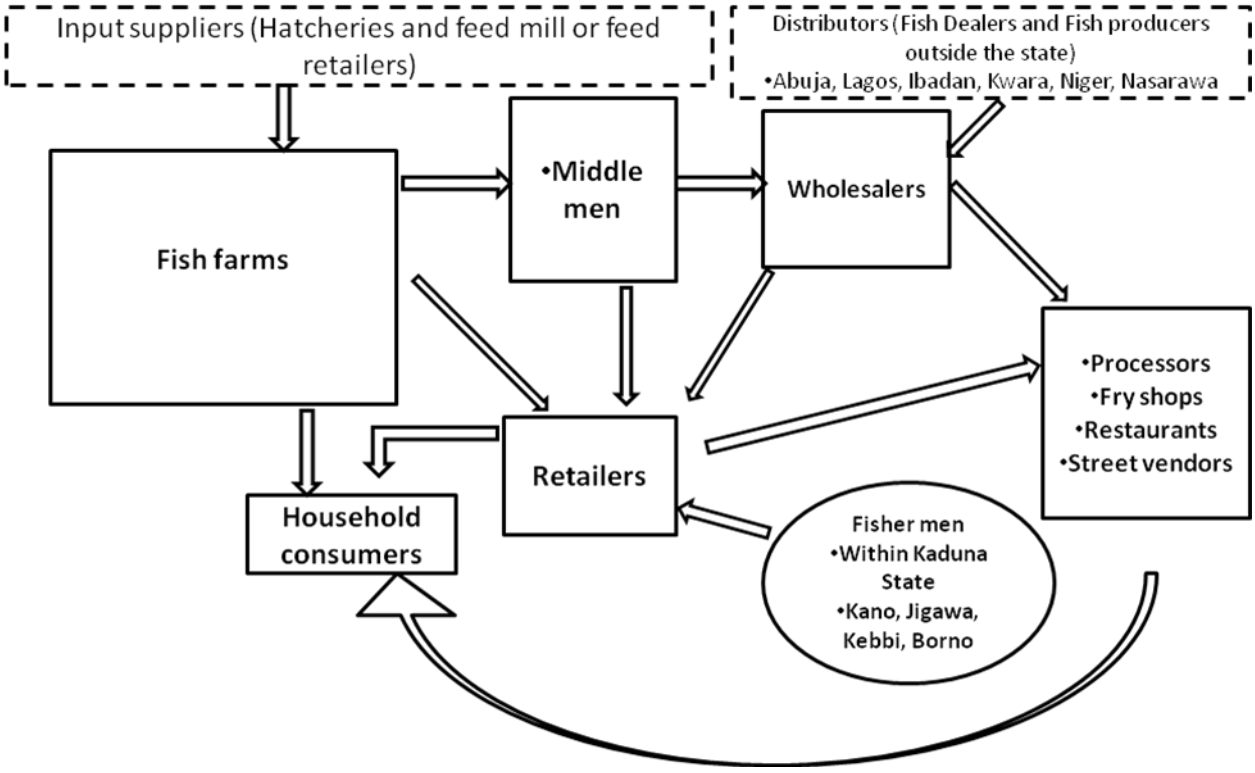


Fig. 4.2: Structure of farmed fish value chain in Kaduna and Zaria metropolises. Broken lined rectangles indicate fish value chain component not investigated in this study.

4.4 Demographic Characteristics of Fish Farmers along Fish Value Chains in Kaduna and Zaria Metropolises

A total of 45 (100%) fish farmers were interviewed in this study. Most of the respondents interviewed were owners 36 (80%) while 2 (4.4%) were managers and 5 (11.1%) employees in the farms. The highest group of fish farmers (84.4%) had less than 10 years of fish farming experience while 2 (4.4%) and 5 (11.1%) farmers had between 11-15 and 16-20 years of farming experience, respectively. Women accounted for 28.9% of fish farmers while men accounted for 71.1% of the fish farmers interviewed in the study. Six percent (6%) of the fish farmers had no formal education, 2.2% had only attained primary school education, while 31.1% and 53.3% had secondary and tertiary education, respectively. Among educated farmers, 51.1% had food safety related knowledge, 40 (88.9%) fish farmers never had formal training on food safety issues while 5 (11.1%) farmers indicated formal food safety training. Informal means of acquiring food safety information were from single source (35.6%), two sources (48.9%) and three or more sources (15.5%), respectively (Table 4.1).

4.5 Operational Characteristics of Fish Farmers along Fish Value Chains in Kaduna and Zaria Metropolises

A total of 45 farms were visited in the study area, 66.7% were in Zaria metropolis while 33.3% were located in Kaduna metropolis. Farm operational characteristics such as farm sizes were classified into three categories namely less than 1 hectare, between 1 to 2 hectares and greater than 2 hectares. A total of 22 (48.9%) fish farms had farm sizes of less than 1 hectare with 6 (13%) farms having farms bigger than 2 hectares. The most predominant culture systems practiced in the study area were earthen ponds (57.8%) and concrete ponds (33.3%) (Table 4.2). Organic manure was used for pond fertilization by 24 (53.3%) fish farmers. The primary species

cultured by 97.7% of the fish farmers was Clariid (*Clarias gariepinus* and *Heterobranchus* spp.), while only 2.3% cultured tilapia (*Oreochromis niloticus*). The main sources of water for the fish farms were boreholes 18 (40%), streams/rivers 2 (4.4%) and groundwater 24 (53.3%). Only 1 (2.2%) farmer used well water and 12 depended solely on rainwater (Table 4.2).

4.6 Demographic and Operational Characteristics of Fish Traders along Fish Value Chains in Kaduna and Zaria Metropolises

A total of 64 fish traders were interviewed in this study, of whom 42 (65.1%) were fish retailers, 20 (31.2%) wholesalers and 2 (3.1%) middle men. Fish traders interviewed in this study were managers 6 (9.4%), owners 50 (78.1%) and employees 8 (12.5%) (Table 4.3). Some (37.5%) of the fish traders interviewed sold cultured fish only, while 14 (21.8) traded imported fish only. Two traders indicated handling wild caught fish only and 22 (34.4%) handled both cultured and wild fishes. All the fish traders interviewed in this study were men (100%). Educational levels attained by the fish traders included primary (24%), secondary (40%) and tertiary education (10.9%) while 25% had no western education (Table 4.3). A total of 42 (65.5%) were not classified as knowledgeable on food safety and hygiene measures on the basis of educational background. Among those with formal western education, 12 (18.8%) of them were classified as knowledgeable on food safety and hygiene measures while 10 (15.6%) had no knowledge on food safety and hygiene measures. Eight (8) 12% of the fish traders had formal food safety and hygiene training, while 54 (84.4%) had never attended formal food safety and hygiene training (Table 4.3).

The forms of fish purchasedby the fish traders inth e study ares were mainly fresh and live fish (37.5%0, live farmed fish (37.5%) and frozen imported fish (21.8%). However, the main fish forms sold were frozen imported fish (21.8%), and combination of frozen fish, wild caught fish

and live farmed fish (78.1%). Factors that influence the preference of handling the specific fish form or a combination were availability (12.5%), cost (65.6%) and freshness/quality (21.9). Fish purchase was mainly daily (21.8%), weekly (23.4%0 and twice weekly (32.8%) purposely due to poor storage facility (53.1%), cost (18.8%) and consistency of supply (28.1%).

4.7 Demograhic and Operational Characteristics of Fish Processors along Fish Value Chains in Kaduna and Zaria Metropolises

A total of 37 fish processing outlets were visited within the study areas and majority of the fish processors interviewed were located in Zaria metropolis (73%). Among them, 73% owned the business while 27% were employees at the fish processing outlets. Six (16.2%) were operating fry shops, 5.4% operated restaurants, 29 (78.4%) were mobile and fixed street vendors. Type of fish handled by processors included farmed fishes (40.5%), imported frozen fishes (51.4%) and wild caught fishes (8.1%). Women accounted for about 51.4% of the fish processors in the study area while men accounted for 48.6%. Respondents with no formal education were 27%, those who attained primary education were 13.5%, and those with secondary school education were 48.6% whilst 10.8% had tertiary qualification (Table 4.4). By age, 32.4% of the respondents were between 21-30 years old, 35.1% were between the ages of 31-40 years and 32.4% were above 41 years of age. Formal food safety training was acquired by only one (2.7%) fish processor while 97.3% had no formal training on safety and hygienic processing of food. Traders with 6-10 years business experience were 18 (48.6%) while 10 (27%) traders had 11-20 years of business experience. A total of 7 (18.9%) and 2 (5.4%) fish traders had experience in fish trading above 21 years and below 5 years, respectively (Table 4.4).

4.8 Demographic Characteristics of Fish Consumers Interviewed from Fish Value Chains in Kaduna and Zaria Metropolises

A total of 57 respondents were interviewed in general; 59.6% of whom were located in Zaria metropolis and 40.4% from Kaduna metropolis. About fifty eight percent (57.9%) were females while 42.1% were male. The age range of respondents were 18-30 (14%), 31-40 (31.6%), 41-50 (40.4%), 51-60 (10.5%) and those above 60 years were 3.5% of respondents. Educational attainment of the respondents were primary certificate (1.8%), secondary certificates 26 (45.6%), tertiary education 26 (45.6%) and those with no western education 4 (7%). The occupation of respondents were farming 2 (3.5%), workers 22 (38.6%), retiree 1 (1.8%), students 9 (15.8%), traders 17 (29.8%) and unemployed persons 6 (10.5%). Position of respondents in the family were daughter 2 (3.5%), head 18 (31.6), son 1 (1.7%), wife 29 (50.9%) and others (daughter-in-law, cousins and housemaids) 7 (12.3%). The decision to buy fish was made mostly by the head of the family (26.3%), joint decision by both spouses (14%) or by the wives only (42%). However, 10 (17.4%) of the interviewees were reported as bachelors, students and housemaids (Table 4.5).

Table 4.1: Demographic characteristics of fish farmers in Kaduna and Zaria metropolises (n=45)

Variables	Description	No. of respondents (%)
Gender	Male	32 (71.1)
	Female	13 (28.9)
Educational level	No formal education	6 (13.3)
	Primary	1 (2.2)
	Secondary	14 (31.1)
	Tertiary	24 (53.3)
Informal food safety knowledge	Yes	23 (51.1)
	No	8 (17.8)
	Unclassified	14 (31.1)
Formal food safety Training	Yes	5 (11.1)
	No	40 (88.9)
Role of respondents	Managers	2 (4.4)
	Owners	36 (80.0)
	Workers	5 (11.1)
	Others	2 (4.4)
Years of Business experience (yrs)	<5-10	38 (84.4)
	11-15	2 (4.4)
	16-20	5 (11.1)
	>21	0

Table 4.2: Operational characteristics of fish farms visited in Kaduna and Zaria Metropolises (n=45)

Variables	Description	No. of respondents (%)
Location	Kaduna	15 (33.3)
	Zaria	30 (66.7)
Type of Production system	Concrete ponds only	15 (33.3)
	Earthen ponds only	26 (57.8)
	Tanks only	4 (8.9)
Farm sizes (ha)	Less than 1 hectare	22 (48.9)
	Between 1-2 hectares	17 (37.8)
	Greater than 2 hectares	6 (13.3)
Type of fish species cultured	Clariid	44 (97.7)
	Tilapia	1 (2.27)
Type of fertilizer used	Organic	24 (53.3)
	Inorganic	2 (4.4)
	Both	6 (13.3)
	None	13 (53.3)
Source of water supply farm	Ground water	24 (53.3)
	Well water	1 (2.2)
	Bore hole	18 (40)
	Stream/River	2 (4.4)
Product form sold	Live fish	42 (93.3)
	Dead Fresh	0
	Live and Smoked	3 (6.7)

Table 4.3: Demographic and operational characteristics of fish traders in Kaduna and Zaria metropolises (n=64)

Variables	Descriptions	No. of respondents (%)
Location	Kaduna	20 (31.1)
	Zaria	44 (68.8)
Gender	Male	64 (100)
	Female	0
Educational level	No formal education	16 (25.0)
	Primary	15 (23.4)
	Secondary	26 (40.6)
	Tertiary	7 (10.9)
Informal food safety knowledge	Food safety related knowledge	12 (18.8)
	Non-food safety related knowledge	10 (15.6)
	Not applicable	42 (65.6)
Formal Food safety Training	Yes	8 (12.5)
	No	54 (84.4)
	I can't remember	2 (3.1)
Years of business experience	≤5 Years	4 (6.3)
	6-10 years	20 (31.3)
	11-15 years	20 (31.3)
	≥16	20 (31.3)
Role of respondent in fish trade	Managers	6 (9.4)
	Owners	50 (78.1)
	Workers	8 (12.5)
Fish forms purchased	Live farmed fish	24 (37.5)
	Frozen imported fish	14 (21.8)
	Fresh wild caught fish	2 (3.1)
	Fresh and live fish	24 (37.5)
Fish forms sold	Frozen imported fish	14 (21.8)
	Fresh and frozen wild caught fish and live farmed fish	50 (78.1)
Factors for choice of fish forms	Availability	8 (12.5)
	Financial	42 (65.6)
	Freshness and quality	14 (21.9
Frequency of fish supply	Daily	14 (21.8)
	Weekly	15 (23.4)
	Twice a week	21 (32.8)
	Fortnightly	12 (18.7)
	Irregularly	2 (3.1)
Reasons for supply frequency	Storage condition	34 (53.1)
	Cost of purchase	12 (18.8)
	Consistency in supply	18 (28.1)

Table 4.4: Demographic and operational characteristics of Fish processors in Kaduna and Zaria metropolises (n=37)

Variables	Description	No. of respondents (%)
Location	Kaduna	10 (27.0)
	Zaria	27 (73.0)
Gender	Male	18 (48.6)
	Female	19 (51.4)
Educational level	No formal education	10 (27.0)
	Primary	5 (13.5)
	Secondary	18 (48.6)
	Tertiary	4 (10.8)
Informal food safety knowledge	Food safety related knowledge	11 (29.7)
	Non-food safety related knowledge	7 (18.9)
	Not applicable	19 (51.4)
Formal food safety training	Yes	1 (2.7)
	No	36 (97.3)
Age of respondents (years)	≤20	0
	21-30	12 (32.4)
	3140	13 (35.1)
	≥41	12 (32.4)
Years of business experience	≤5	7 (18.9)
	6-10	18 (48.6)
	11-15	12 (32.4)
	16-20	0
Role of respndents	Owner	27 (73.0)
	Worker	10 (27.0)
Type of fish processing outlets	Fry shops	6 (16.2)
	Restaurants	2 (5.4)
	Street vendors	29 (78.4)
Type of fish handled	Cultured fish only	15 (40.5)
	Imported fish only	19 (51.4)
	Wild caught fish only	3 (8.1)
Fish state purchased	Dead fresh fish without ice	2 (5.4)
	Frozen fish	19 (51.4)
	Live fish in water	16 (43.2)
Fish forms sold	Smoked fish	4 (10.8)
	Fried fish	14 (37.8)
	Roasted fish	11 (29.7)
	Grilled fish	8 (21.6)
Frequency of fish supply	Daily	29 (78.4)
	Twice a week	8 (21.6)

Table 4.5: Demographic characteristics of the fish consumers in Kaduna and Zaria metropolises (n=57)

Demographic characteristics		No. of respondents (%)
Location	Kaduna	23 (40.4)
	Zaria	34 (59.6)
Gender	Male	24 (42.1)
	Female	33 (57.9)
Age group	18–30	8 (14.0)
	31–40	18 (31.6)
	41–50	23 (40.4)
	51–60	6 (10.5)
	≥61	2 (3.5)
Education Level	Primary	1 (1.8)
	Secondary school	26 (45.6)
	Tertiary	26 (45.6)
	No formal education	4 (7.0)
Occupation	Farming	2 (3.5)
	Workers	22 (38.6)
	Retiree	1 (1.8)
	Student	9 (15.8)
	Trading	17 (29.8)
	Unemployed	6 (10.5)
Family position of respondent	Daughter	2 (3.5)
	Head	18 (31.6)
	Son	1 (1.7)
	Wife	29 (50.9)
	Others	7 (12.3)
Decision to purchase fish	Head	15 (26.3)
	Joint decision	8 (14.0)
	Wife	24 (42.1)
	Others	10 (17.4)

4.9 Total Aerobic Plate Counts (TAPC) Determination of Fish Samples from Fish Farmers Interviewed from Fish Value Chains of Kaduna and Zaria Metropolises

The log mean±SD of TAPC for samples obtained from fish farms, fish markets and fish processing outlets were 89.7.5±0.50 cfu/g, 19.5±8.9 cfu/g, and 11.9±9.2 cfu/g, respectively. A total of 17 (26.6%) and 47 (73.4%) fish samples from fish farmers were considered marginally acceptable and unacceptable fish samples for human consumption, respectively. Fish samples from fish traders were classified as marginally acceptable 3 (6.7%) and unacceptable fish samples unfit for human consumptions 42 (93.3%). Similarly, 8 (21.6%) and 24 (64.9%) ready-to-eat fish samples were classified as acceptable and unacceptable fish samples for human consumption (Table 4.6).

4.10 Total Coliform Counts (TCC) Testing of Fish SampleS from Fish Value Chains in Kaduna and Zaria Metropolises

The coliform counts of both raw and ready-to-eat fish samples from fish farms, fish markets and fish processing outlets are summarised in Table 4.7. The mean±SD coliform counts obtained for raw fish sampled in fish farms and fish markets were 140.1±64.5 TCC/g and 92.9±12.5 TCC/g, respectively. Raw fish samples from fish farms (37.80%) and fish markets (68.75%) as well as ready-to-eat fish from fish processing outlets (91.89%) were classified as coliform contaminated products. Fish samples from fish farms (62.2%), fish markets (31.3%) and fish processing outlets (91.9%) complied with safe microbiological TCC standard used in this survey.

Table 4.6: Total aerobic plate counts (TAPC) of fish samples from fish farms, fish traders and fish processing outlets in the study area (counts represent colony forming units per gram of sample).

S/no.	Fish value chain components	No. of fish samples	TAPC values		
			Mean ± SD (log_{10} cfu/g)	Acceptable fish samples (%)	Unacceptable fish samples (%)
1	Fish farmers	45	89.0 ± 7.5	3 (6.7)	42 (93.3)
2	Fish traders	64	19.5 ± 8.9	17 (26.6)	47 (73.4)
3	Fish processors	37	11.9 ± 9.2	8 (21.6)	24 64.9)

Table 4.7: Total coliform counts (TCC) of fish samples from fish farms, fish traders and fish processing outlets in the study area

S/no.	Fish value chain components	No. of fish samples	TCC values		
			Mean ± SD (TCC/g)	Acceptable fish samples (%)	Contaminated fish samples (%)
1	Fish farmers	45	140.1 ± 64.5	28 (62.2)	17 (37.8)
2	Fish traders	64	92.9 ± 12.5	20 (31.3)	44 (68.7)
3	Fish processors	37	38.7± 9.4	34 (91.9)	3 (8.1)

4.11 Phenotypic Characterization of *Staphylococcus aureus* Isolated from Raw and Ready-to-eat Fishes Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

Out of hundred and fourty six (146) samples tested for the isolation and characterization of *S. aureus*, 22 (15.1%) were identified as *S. aureus* based on appearance as Gram positive coccoid-cluster under the microscope and black colonial morphology on Baird-Parker and catalase and coagulase positive tests. Out of the 22 *S. aureus* isolates, 21 (94.5%) were positive for DNase test, 18 (81.8%) for PBP 2a and 19 (86.4%) for β lactamase production. All the 22 *S. aureus* isolates were interpreted as *Staphylococcus aureus* using Microbact 12A test with percentage ranging from 49.21 to 100%. Fifteen (68.2%) out of the 22 isolates were recorded with probability index of ≥ 75% (Appendix 9).

4.12 Phenotypic Characterization of *Salmonella* species Isolated from Raw and Ready-to-eat Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

One hundred and fourty six (146) fish samples were analysed and 17 (11.6%) *Salmonella* isolates were identified as Gram negative rod-shaped organisms. Out of the 17 (11.6%) *Salmonella* isolates, 16 (94.1%) showed alkaline-acid reaction, 17 (100%) were indole negative by absence of reddish ring along the surface of the substrate, and 16 (94.1%) were Urease negative. Twelve 12 (70.5%) of the *Salmonella* isolates were interpreted as *S. arizonae*, 2 (11.7%) as *Salmonella* species and 1 (5.8%) as S. Cholereaesuis using Microbact 12E test with percentages ranging from 35.38% to 98.11%. Ten (58.8%) out of the 17 *Salmonella* isolates were interpreted as various *Salmonella* spp. based on ≥75% Microbact interpretation (Appendix 12).

4.13 Isolation Rate of *Staphylococcus aureus* and *Salmonella* species on the basis of Fish product types and the Fish Value Chain Components Visited in Kaduna and Zaria metropolises

Table 4.8 presents the isolation rate and distribution of *S. aureus* and *Salmonella* species from the fish products sampled in Kaduna and Zaria metropolises. Overall detection rate of 15.1 % (22 of 146) were found to be contaminated with *S. aureus* in all the 3 fish value chain (FVC) components. Six (27.1%) *S. aureus* were recovered from raw fish collected from fish farms and fish markets while 54.5% were detected from grilled fish sampled from fish processing units. Similarly, 11.6 % *Salmonella* were detected from the all the fish samples collected from the FVC in the study area. Out of the 17 (11.6%) of the isolates recovered, 9 (52.9%) were recovered from raw fish, 2 (11.8%) from unprocessed frozen fish and 3 (17.6%) from roasted frozen fish.

Table 4.9 presents the distribution of *S. aureus* and *Salmonella* species based on the fish types sampled in this study. Six (40%) *S. aureus* isolates were detected from raw catfish while 9 (60%) from processed catfish. Eleven (84.6%) of the *Salmonella* isolates were detected from raw catfish while 2 (15.4%) and 3 (100%) were isolated from ready-to-eat catfish and imported frozen fish, respectively.

4.14 Antibiotic Susceptibility Testing of *Staphylococcus aureus* Isolated from Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

Twenty two (22) *S. aureus* isolates were tested against 13 antibiotics and a significant number (95.4%) of *S. aureus* were susceptible to gentamicin, ciprofloxacin (90.9%), cefuroxime (77.2%), chlorampenicol (72.7%), sulphadizine/trimetoprim (63.61%) and amoxicillin/clavulanic acid (63.6%). Conversely, *S. aureus* isolates were found to have reduced susceptibility to cefoxitin (18.1%), oxacillin (22.7%), vancomycin (18.1%) and erythromycin (31.8%) (Table 4.10).

4.15 Multiple Antibiotic Resistance Patterns of *Staphylococcus aureus* Isolated in Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

Table 4.11 shows the multiple resistance patterns of *S. aureus* isolates to 13 antibiotics tested in this study. Four (4) *S. aureus* isolates showed resistance to 2 antibiotics which included cefoxitin and gentimicin. Resistance to 5, 6, 7, 8 and 9 antibiotics were shown by 3 different isolates each. One *S. aureus* isolate each showed resistance to 10 different antibiotics whereas another *S. aureus* isolate showed no resistance to any of the antibiotics tested in this study. Similarly, no *S. aureus* isolate showed resistance to 11, 12 and 13 antibiotics tested in this study.

4.16 Antibiotic Susceptibility Testing of *Salmonella* species Isolated from Raw and Ready-to-eat Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

From the study, 16 (94.1%) *Salmonella* were susceptible to imipenem, 5 (88.2%) to ciprofloxacin, 14 (82.3%) to gentamicin, 14 (82.3%) to chloramphenicol, 12 (70.5%) to cefuroxime, 14 (79.5%) to enrofloxacin and 11 (64.7%) to amoxicillin/clavulanic acid. One, 2 and 3 *Salmonella* isolates were resistant to erythromycin (5.8%), azithromycin 2 (11.7%) and tetracycline 3 (17.6%), respectively (Table 4.12).

4.17 Multiple Resistance Patterns of *Salmonella* species Isolated from Raw and Ready-to-eat Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

The patterns of resistance of *Salmonella* isolates to antibiotics tested in this study are presented in Table 13. Six (35.3%) *Salmonella* isolates were resistant to 6 antibiotics tested in this study while 3 (17.6%) were resistant to all the 12 antibiotics tested. Similarly, 5 (29.4%) isolates were resistant to only 3 antibiotics, whereas 3 (5.8%) isolates were resistant to 2, 4 and 6 antibiotics tested. No isolate was found to be resistant to 1, 5 or 7 antibiotics tested concurrently in this study.

4.18 Multiple Antibiotic Resistance (MAR) Index Analysis of *Staphylococcus aureus* and *Salmonella* species Isolated Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

The MAR indices for the 22 *S. aureus* and 17 *Salmonella* species tested in this study were calculated and presented in Figure 4.3. A total of 14 *Salmonella and* 17 *S. aureus* isolates had MAR indices ≥ 0.2. Specifcally, 7 *Salmonella* isolates had MAR index of 0.3, while 3 isolates had MAR index of 0.4. Two (2), 4, 3 and 4 *S. aureus* isolates had MAR index of 0.2, 0.4, 0.6 and 0.8, respectively.

Table 4.8: Distribution of *Staphylococcus aureus* and *Salmonella* isolates based on fish value chain components and fish product forms

Fish value chain components (n)	Distribution of *S. aureus* isolates based on fish product forms (%)						Distribution of *Salmonella* species isolated based on fish product forms (%)					
	Raw	Frozen	Roasted	Grilled	Smoked	Total	Raw fresh	Frozen fresh	Roasted	Grilled	Smoked	Total
Fish farms (45)	4 (100)	0	0	0	0	4 (18.1)	7 (100)	0	0	0	0	7 (41.2)
Fish market (64)	2 (100)	0	0	0	0	2 (9.1)	2 (50)	2 (50)	0	0	0	4 (23.5)
Fish processors (37)	0	0	4 (25)	12 (75)	0	16 (72.2)	0	0	3 (50)	3 (50)	0	6 (35.3)
Total (146)	6 (27.1)	0	4 (18.2)	12 (54.5)	0	22 (15.1)	9 (52.9)	2 (11.8)	3 (17.6)	3 (17.6)	0	17 (11.6)

Table 4.9: Distribution of *Staphylococcus aureus* and *Salmonella* isolates based on fish products/types

Fish types/ Fish forms	*S. aureus* positive fish types/forms (%)				*Salmonella* positive fish types/forms (%)			
	Raw fish	Frozen fish	Read-to-eat fish	Total (%)	Raw fish	Frozen fish	Ready-to-eat fish	Total
Catfish	6 (40)	0	9 (60)	15 (68.2)	11 (84.6)	0	2 (15.4)	13 (76.5)
Tilapia	0	0	3 (100)	3 (13.6)	0	0	1 (100)	1 (5.9)
Imported frozen fish	0	0	4 (100)	4 (18.2)	0	0	3 (100)	3 (17.6)
Total	6 (27.3)	0	16 (72.7)	22(100)	11 (64.7)	0	6 (35.3)	17 (100)

Table 4.10: Susceptibility of *S. aureus* isolates from fish to thirteen antimicrobials (n=22)

Antimicrobial agents	Disc conc. (µg)	No. (%) of isolates susceptible
Amoxicillin/Clavulanic acid (AMC)	30	14 (63.6)
Azithromycin (AZM)	15	13 (59.1)
Cefoxitin (FOX)	30	4 (18.1)
Cefuroxime (CRO)	30	17 (77.2)
Cephazolin (KZ)	30	6 (27.2)
Chloramphenicol (C)	30	16 (72.7)
Ciprofloxacin (CIP)	5	20 (90.9)
Erythromycin (E)	15	7 (31.8)
Gentamicin (CN)	30	21 (95.4)
Oxacillin(OX)	1	5 (22.7)
Sulphamethoxazole/Trimethoprim (SXT)	25	14 (63.6)
Tetracycline (TE)	30	6 (27.2)
Vancomycin (VA)	30	4 (18.1)

Table 4.11: Antibiotic resistance profile of *S. aureus* isolated from fish in Kaduna and Zaria metropolis tested against 12 antibiotics (n=22)

Antibiogram	Frequency of occurrence (%)
None	1 (4.5)
FOX, CN	2 (9.1)
FOX, CRO	4 (18.1)
FOX,OX,TE,KZ,E, VA	3 (13.6)
AZM,E,FOX,KZ,TE,OX, VA	3(13.6)
AZM,E,FOX,KZ,SXT,TE,OX, VA	3(13.6)
AZM,C,E,FOX,KZ,SXT,TE,OX, VA	3(13.6)
AMC,AZM,C,E,FOX,KZ,SXT,TE,OX, VA	2(9.1)
AMC,AZM,C,CRO,E,FOX,KZ,SXT,TE,OX	1 (4.5)
	22 (100)

AZM: Azithromycin; TE: Tetracycline; OX: Oxacillin; KZ: Cephazolin; CRO: Cefuroxime; VA: Vancomycin; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; FOX: Cefoxitin; CN: Gentamicin; AMC: Amoxicillin-clavulanic acid, SXT: Sulphamethoxazole Trimethoprim

Table 4.12: Susceptibility of *Salmonella* isolates from fish to twelve antimicrobial agents (n=17)

Antimicrobial agents	Disc conc. (µg)	No. of isolates (%)
Amoxicillin/Clavulanic acid (AMC)	30	11 (64.7)
Azithromycin (AZM)	15	2 (11.7)
Cefuroxime (CRO)	30	12 (70.5)
Cephazolin (KZ)	30	0
Chloramphenicol (C)	30	14 (82.3)
Ciprofloxacin (CIP)	5	15 (88.2)
Enrofloxacin (ENR)	5	14 (82.3)
Erythromycin (E)	15	1 (5.8)
Gentamicin (CN)	30	14 (82.3)
Imipenem (IPM)	10	16 (94.1)
Sulphamethoxazole/Trimethoprim (SXT)	25	13 (76.5)
Tetracycline (TE)	30	3 (17.6)

Table 4.13: Antibiotic resistance profile of *Salmonella* species isolated from fish value chains of Kaduna and Zaria Metropolises (n=17)

Antibiogram	Frequency of occurrence (%)
AZM, E	1 (5.8)
AZM, E, TE	5 (29.4)
AZM, E, KZ,TE	1 (5.8)
AMC, AZM, E, ENR, TE, KZ	1 (5.8)
AMC, AZM, C, CRO, E, ENR,SXT,TE	6 (35.3)
AMC,AZM,C,CN,CRO,E,KZ,SXT,TE	3 (17.6)
	17 (100)

CN: Gentamicin; TE: Tetracycline; KZ: Cefazolin; CRO: Cefuroxime; AZM: Azithromycin CIP: Ciprofloxacin; E: Erythromycin; C: Chloramphenicol; IPM: Imipenem; SXT: Trimethoprim-Sulfamethoxazole; ENR: Enrofloxacin; AMC: Amoxicillin/clavulanic acid

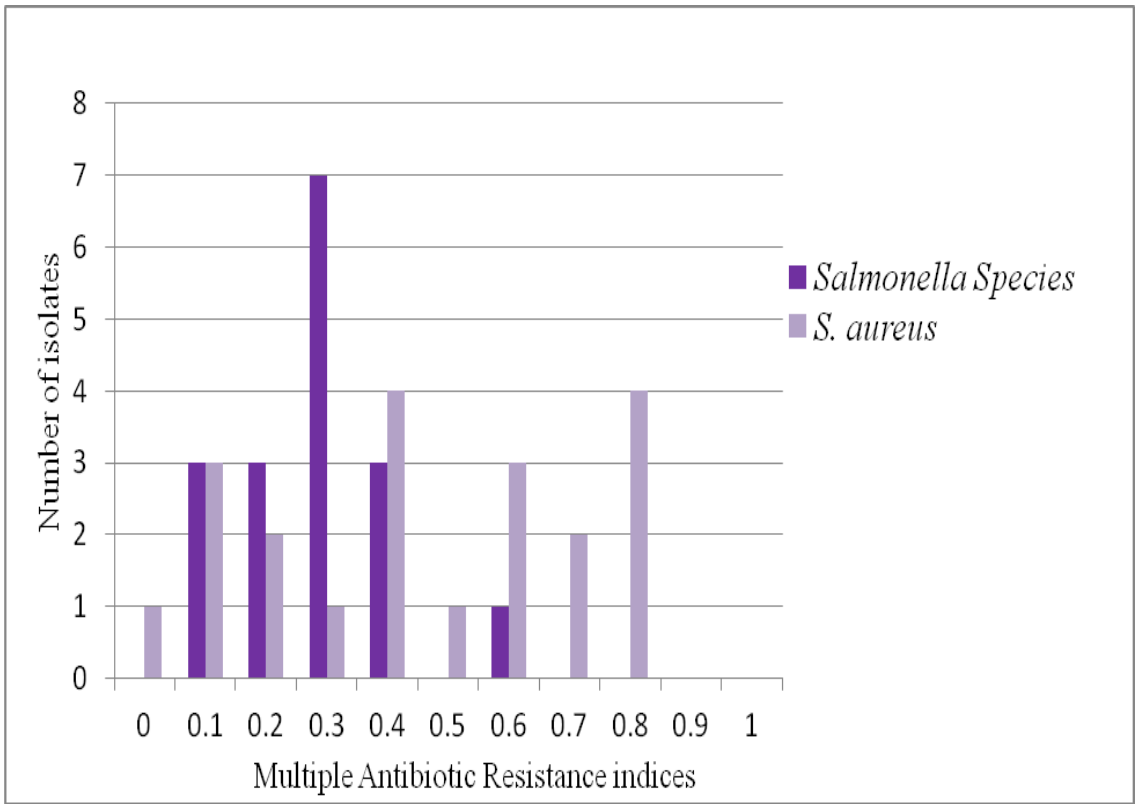


Fig. 4.3: Multiple Antibiotic resistance (MAR) indices of *Salmonella* and *S. aureus* isolates from Fish Value Chains in Kaduna and Zaria Metropolises.

4.19 Vancomycin and Oxacillin MIC for *Staphylococcus aureus* Isolated from Fish Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

The MIC values of vancomycin and oxacillin were determined for 10 *S. aureus* isolates that were resistant to these antibiotics using disc diffusion technique. Three (3) isolates had MIC values ≥ 16 µg/ml and were considered resistant to vancomycin while 9 isolates tested were resistant to oxacillin at values ≥ 4 µg/ml according to CLSI (2011) (Fig. 4.4).

4.20 Erythromycin and Amoxicillin/clavulanic acid MIC for *Salmonella* species Isolated from Fish Samples Collected from Fish Value Chains in Kaduna and Zaria Metropolises

A total of 6 *Salmonella* isolates from various components of the fish value chains which were resistant to Erythromycin and Amoxicillin/clavulanic acid by the disc diffusion tests were further subjected to MIC determination. Three (3) *Salmonella* isolates showed resistance at 256 µg/ml MIC level to erythromycin while 2 isolates showed resistance to Amoxicillin/clavulanic acid at 128 µg/ml MIC level. Using CLSI MIC break points for resistance interpretation, all the 6 *Salmonella* isolates tested for MIC of erythromycin in this study were resistant at the recommended level of ≥ 25 µg/ml while 5 isolates were resistant to Amoxicillin/clavulanic acid at recommended level of ≥ 32 µg/ml (Figure 4.5).

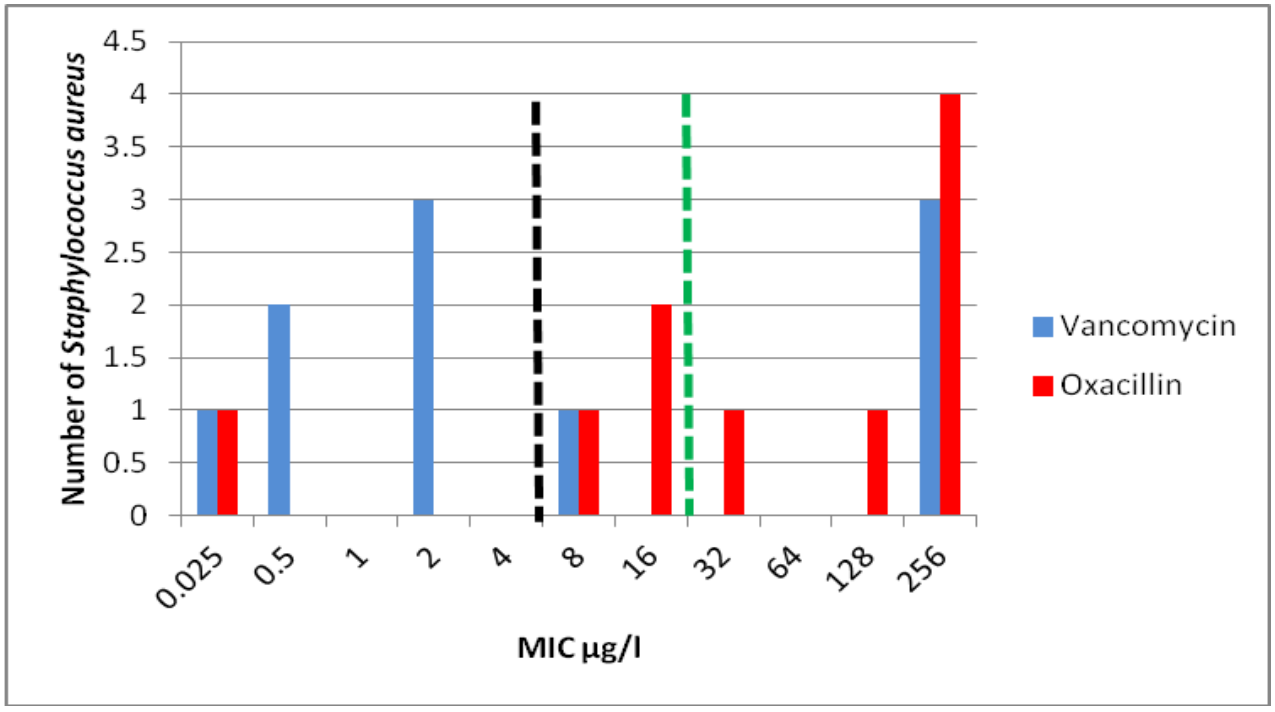


Fig. 4.4: Vancomycin and Oxacillin MIC distributions for 10 *Staphylococcus aureus* isolates collected from fish value chains in Kaduna and Zaria Metropolises, Nigeria. The thin black and green dotted lines denote MIC resistance break points for oxacillin and vancomycin respectively as recommended by Clinical Laboratory standard institute (2011). A cut off point of ≥ 4 and ≥16 µ g/ml were reported as resistant to Vancomycin and Oxacillin, respectively.

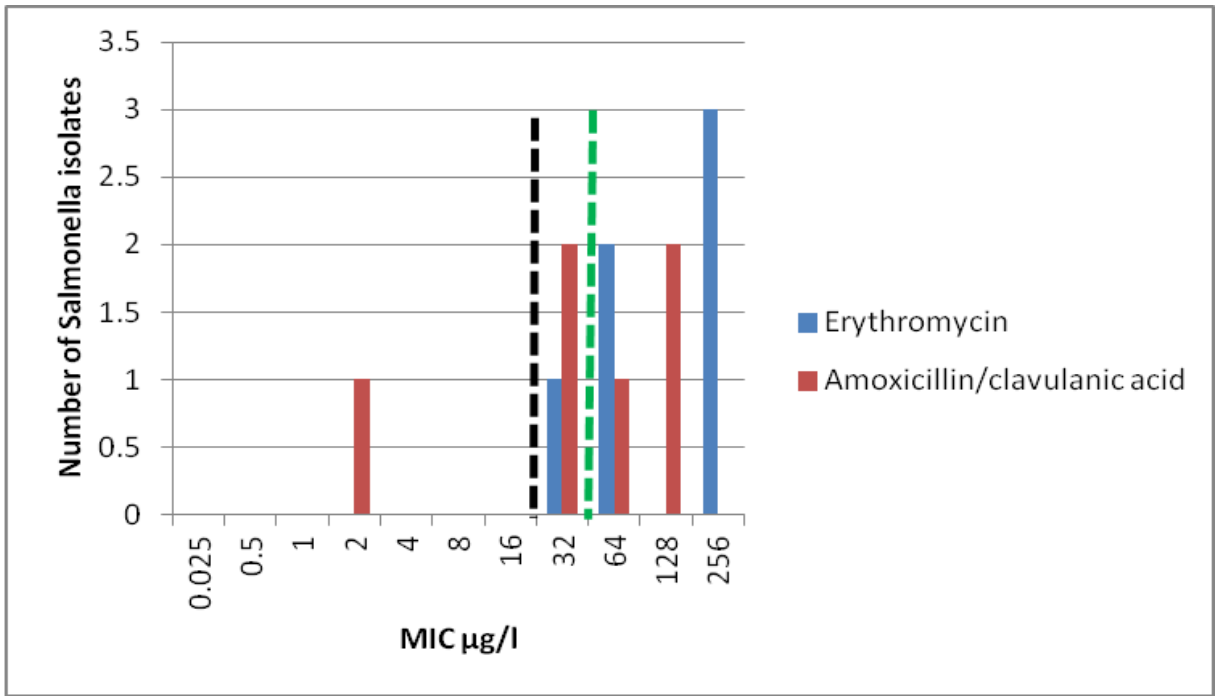


Fig. 4.5: Erythromycin and Amoxicillin/clavulanic acid MIC distributions for 6 *Salmonella* isolates collected from fish value chains in Kaduna and Zaria Metropolises, Nigeria. The black and green doted lines denote resistant MIC break points for erythromycin and Amoxicillin/clavulanic acid, respectively as reported in Clinical Laboratory standard institute (2011). A cut off point of ≥ 25 and ≥ 32 µ g/ml were reported as resistant to erythromycin and Amoxicillin/clavulanic acid, respectively.

4.21 The Amplified Genes of *Staphylococcus aureus* Isolates from Fishes Sampled from Fish Value Chains in Kaduna and Zaria Metropolises

Plate 4.1 is the agarose gel electrophoresis showing amplified *mec*A, *nuc* and *pvl* genes. All the 10 *S. aureus* isolates were confirmed by the presence of *nuc* gene (255 bp). Nine (9) of the 10 *S. aureus* isolates tested harboured *mec*A gene (163 bp), while 6 of the MRSA strains were positive for *pvl* (433 bp).

Plate 4.2 shows the detection of *sea*, *seb*, *sec*, *sed*, *see* and *sej* genes from the confirmed *S. aureus* isolates in Plate 4.1. Six (6) *S. aureus* isolates were positive for the *seb* gene (164 bp), 8 *S. aureus* isolates were positive for the *see* gene (209 bp), 7 isolates were positive for *sed* gene (278 bp) while 2 isolates were positive for *sec* gene (456 bp). Finally, 4 *S. aureus* isolates were positive for *sej* (306 bp) gene. Plate 4.3 shows the amplied *inv*A gene of *Salmonella* species isolated in this study. Seven isolates haboured the invasion gene *inv*A (796bp).

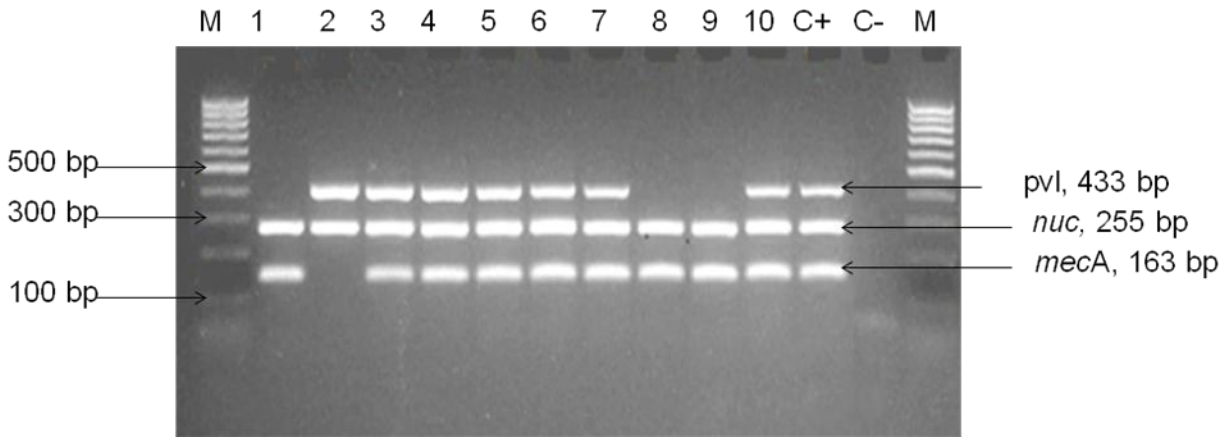


Plate 4.1: Agarose gel electrophoresis of multiplex PCR amplification for *mec*A (163 bp), *nuc* (255 bp) and *pvl* (433 bp) genes. Lane M is the molecular weight marker and lanes 1- 10 are the test *S. aureus* isolates (S2, S3, S4, S5, S10, S12, S13, S16, S18, S21), respectively. Lane C+ and C- are the positive and negative controls for *nuc* gene, *mec*A and *pvl*.

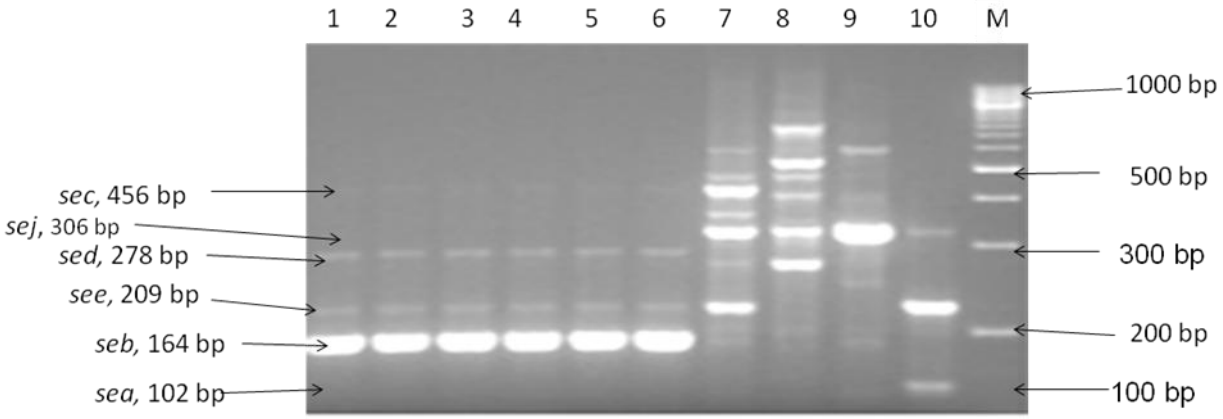


Plate 4.2: Agarose gel electrophoresis of multiplex PCR amplification of *sea* (102 bp), *seb* (164 bp), *sec* (456 bp), *sed* (278 bp), *see* (209 bp) and *sej* (306 bp) genes. Lane M is the molecular weight marker and lanes 1- 10 *S. aureus* isolates (S2, S3, S4, S5, S10, S12, S13, S16, S18 and S21, respectively).

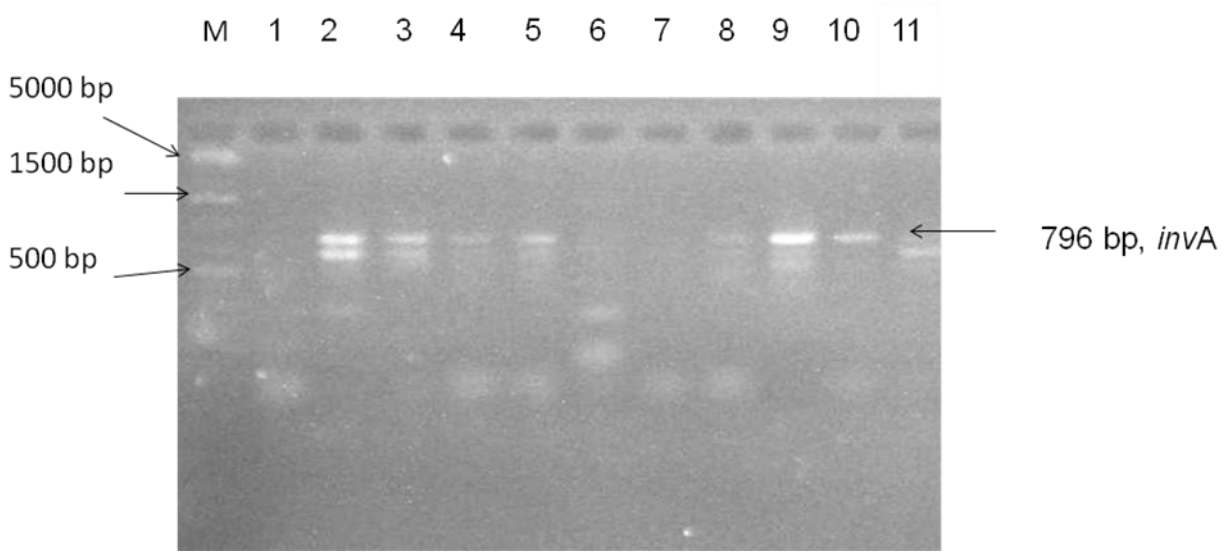


Plate 4.3: Agarose gel electrophoresis of *inv*A (796 bp) gene. Lane M is the molecular weight marker and lanes 1-11 are the test *Salmonella* isolates (Sal 2, Sal 3, Sal 4, Sal 5, Sal 6, Sal 7, Sal 9, Sal 12, Sal 13, Sal 14, Sal 18), respectively. Lane 2, 3, 4, 5, 8, 9 and 10 are positive for *inv*A (796 bp) gene.

4.22 Occurrence and Distribution of *S. aureus* and *Salmonella* species Genes Isolated from Fish Samples Collected from Fish Value Chains in Kaduna and Zaria Metropolises

The *nuc* gene was carried by 100% of *S. aureus* isolated from the fish samples collected in this study. Nine (90%) of the *S. aureus* harbouring *nuc* gene also haboured *mec*A gene while 7 (70%) harboured *pvl* genes. A detection rate for *sea* (10%), *seb* (50%), *sec* (20%), *sed* (70%), *see* (40%) and *sej* (40%) genes were found in *S. aureus* isolates in this study (Table 4.14).

Table 4.15 presents the distribution of staphylococcal enterotoxigenic genes carried by *S. aureus* isolated from fish product forms collected in the fish value chains of Kaduna and Zaria metropolises. One raw catfish collected from market carried *nuc, mec*A, *pvl* and the *sea, see* and *sej* genes while another carried *sed* and *see* genes only. Another *S. aureus* isolated from raw catfish carried the *pvl* gene in addition to *seb, sed* and *see* genes. *Staphylococcus aureus* from 2 grilled fish samples sampled from processing units carried the *mec*A and *pvl* genes with *sec, sed, see, sej* while another from grilled tilapia carried *mec*A with *sec, sed* and *sej* genes.

Table 4.14: Occurrence of *Staphylococcus aureus* genes in isolates from Fish Value Chains of Kaduna and Zaria metropolises (n=10)

Target gene	Number (%) positive for gene amplified
nuc	10 (100)
*mec*A	9 (90)
pvl	7 (70)
sea	1 (10)
seb	5 (50)
sec	2(20)
sed	7 (70)
see	8 (80)
sej	4 (40)

Table 4.15: Genotype profile of *S. aureus* enterotoxingenic genes detected in various fish samples collected from value chains of Kaduna and Zaria metropolises

Sample ID	*Source/fishtype/productform	****mec**A/*PVL*	Results for *S. aureus* enterotoxin genes amplified from isolates in this study					
			sea	*Seb*	*sec*	*sed*	*see*	*sej*
S 2	Processor/Imported fish/Roasted	+ / -	-	+	-	+	+	-
S 3	Farm/Catfish/Raw	- / +	-	+	-	+	+	-
S 4	Processor/Imported fish/Roasted	+ / +	-	-	-	+	+	-
S 5	Processor/Catfish/Grilled	+ / +	-	-	-	+	+	-
S 10	Retailer/Catfish/Raw	+ / +	-	-	-	+	+	-
S 12	Processor/Catfish/Grilled	+ / +	-	-	-	+	+	-
S 13	Processor/Catfish/Grilled	- / +	-	-	+	+	+	+
S 16	Processor/Tilapia/Grilled	+/ -	-	-	+	+	-	+
S 18	Processor/Catfish/Grilled	+/ -	-	-	-	-	-	+
S 21	Retailer/Catfish/Raw	+ / +	+	-	-	-	+	+

*Source: The component of the fish value chain where that particular fish was purchased; Fish type: The fish species in which *S. aureus* were isolated; Product form: The fish product form in which *S. aureus* were isolated.

** All isolates confirmed as *S. aureus* by detection of *nuc* gene; *mec*A gene: for methicillin resistance; *pvl*: for Panton Valentine leukocidin (*pvl*);

+/-: indicates presence/absence of a particular gene in *S. aureus* isolates.

4.23 Food Safety Knowledge, Attitude and Practices of Fish farmers along Fish Value Chains of Kaduna and Zaria Metropolises

All respondents 45 (100%) answered correctly that washing hands before work reduces the risk of fish contamination. Respondents also agreed that the use of gloves 29 (64.4%), apron 21 (46.7%), and caps 20 (44.4%) during handling fish reduces risk of fish contamination (Table 4.16). Thirty five (77.8%) of the respondents reported that they stayed away from handling fish during sickness. They also agreed that diarrhoea 28 (62.2%) and cholera 28 (62.2%) could be transmissible through consumption of contamination fish. All respondents answered correctly that the use of refrigerator is the best fish storage facility 45(100%) and transporting fish live in water reduces risk of fish spoilage 45 (100%) (Table 4.16).

Fish farmers generally had positive attitude towards use of protective gloves (71.1%) and adequate clothing 22 (48.9%) reduces risk of fish contamination, while 37.1% and 64.4% of the farmers disagreed that personal protective equipment such as caps 14 (31.1%) and face masks 9 (20%) could reduce risk of fish contamination. Fish producers did believe that food safety and hygiene training 40 (88.9%) was very important in reducing fish contamination (Table 4.17).

As presented in Table 4.18, the overall food safety practices of fish farmers was good (34/45, 75.6%), even though personal hygiene practices such as use of gloves, (100%), frequent handwashing (100%), use of apron (95.6%), use of caps (75.6%) and use of face masks (100%) were not practiced by the majority. Contrastingly, washing of contact surfaces before harvest (31.1%), during transportation (37.8%), or handwashing before (64.4%) and after (35.6%) handling raw fish were practiced. No farmer reported the practice of always feeding fish with dead animal carcasses or left over food. About 44.4% of the fish farmers always used antibiotics

in grow out ponds and keep other animal species in their farms while 73.3% of the fish farmers reported never removed dead fishes in their ponds.

4.24 Factors Associated with Food Safety Knowledge, Attitude and Practices of Fish Farmers Interviewed from Fish Value Chains in Kaduna and Zaria Metropolises

Factors that significantly influenced good food safety knowledge of fish farmers in the study area included location, gender of fish producers, years of business experience, role of respondents, food safety training and source of food safety information ($P<0.05$) (Table 4.19). In addition, level of education, gender of fish farmers, attendance of food safety training, and farm types operated significantly influenced positive fish safety attitudes of fish farmers ($P<0.05$) (Table 4.20). However, there was no statistical significance observed from the variables listed in Table 4.21 influenced fish farmers' food safety practices ($p<0.05$).

Table 4.16: Food safety knowledge statements and responses of fish farmers in the study areas (n = 45)

Statements	No. of Respondents (%)		
	True	False	I don't know
Washing hands before work reduces the risk of fish contamination	45 (100)	0	0
Using gloves during work reduces the risk of food contamination	29 (64.4)	15 (33.3)	1 (2.2)
Using apron during work reduces the risk of food contamination	21 (46.7)	21 (46.7)	3 (6.7)
Hair covering during work reduces the risk of food contamination	20 (44.4)	23 (51.1)	2 (4.4)
Proper cleaning and handling of contact surfaces reduces the risk of food contamination	41 (91.1)	4 (8.9)	0
Farming two or more fish species increases the risk of food contamination	12 (26.7)	12 (26.7)	21 (46.7)
If you have diarrhoea, it is necessary to stay away from handling fish for consumption/business	35 (77.8)	9 (20.0)	1 (2.2)
If you have skin wounds on your hands, it is necessary to stay away from handling fish	13 (28.9)	30 (66.7)	2 (4.4)
Typhoid can be transmitted by food	3 (6.7)	39 (86.7)	3 (6.7)
Dysentery can be transmitted by food	4 (8.9)	41(91.1)	0
Diarrhoea can be transmitted by food	28 (62.2)	15 (33.3)	2 (4.4)
Cholera can be transmitted by food	28 (62.2)	14 (31.1)	2 (6.7)
Abortion in pregnant women may be induced by food borne diseases	2 (4.4)	38 (84.4)	5 (11.1)
The refrigerator is the safest fish storage facility	45 (100)	0	0
Transporting fish alive in water reduces risk of fish spoilage	45 (100)	0	0

Table 4.17: Food safety attitude statements and responses of fish farmers in the study areas (n = 45)

Statements	No. of Respondents (%)		
	Agree	Disagree	No idea
Using gloves is important in reducing risk of fish contamination	32 (71.1)	11 (24.4)	2 (4.4)
Using apron is important in reducing risk of fish contamination	22 (48.9)	21 (46.9)	2 (4.4)
Using face masks is important in reducing risk of fish contamination	9 (20)	32 (37.1)	4 (8.9)
Using caps is important in reducing risk of fish contamination	14 (31.1)	29 (64.4)	2 (4.4)
Food hygiene training for farmers is important for reducing risk of fish contamination	40 (88.9)	5 (11.1)	0
Sick people should not be involved in fish handling and preparation	24 (53.3)	21 (21 (46.7)	0
Fish fed dead animal carcasses can cause cross contamination	27 (60.0)	18 (40.0)	0
Fish fed with animal manure can cause cross contamination	22 (48.9)	22 (48.9)	1 (2.2)
Fish should be transported alive to reduce contamination	41 (91.1)	3 (6.7)	1 (2.2)
Mixing fish of different sources increase risk of fish contamination	30 (66.7)	14 (31.1)	1 (2.2)

Table 4.18: Fish producer's practices towards fish-borne disease prevention (n = 45)

Statements	No. of Respondents (%)		
	Never	Sometimes	Always
Do you use gloves during work?	45 (100)	0	0
Do you wash your hands before using gloves?	45 (100)	0	0
Do you wear apron during work?	43 (95.6)	2 (4.4)	0
Do you use face mask during work?	45 (100)	0	0
Do you cover hair during work?	34 (75.6)	7 (15.6)	4 (8.9)
Do you clean contact surfaces before you harvest fish?	19 (42.2)	12 (26.7)	14 (31.1)
Do you clean contact surfaces during fish transportation?	17 (37.8)	11 (24.4)	17 (37.8)
Do you wash your hands before you handle raw fish?	7 (15.6)	29 (64.4)	22 (48.9)
Do you wash your hands after you handle raw fish?	14 (31.1)	16 (35.6)	2 (4.4)
Do you use antibiotics in grow out ponds?	20 (44.4)	5 (11.1)	20 (44.4)
Do you feed the fish with dead animal carcasses?	33 (73.3)	12 (26.7)	0
Do you feed the fish with homemade leftover food?	41 (91.1)	4 (8.9)	0
Do you remove dead fish immediately from ponds?	33 (73.3)	12 (26.7)	0
Do you keep other animal species in your fish farms	12 (26.7)	13 (28.9)	20 (44.4)

Table 4.19: Factors influencing the food safety knowledge (FSK) of fish farmers in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSK	$\varkappa^2$	P - value
Location:			10.000	0.002 (p≤0.01)
Kaduna	15	7 (46.7)		0.00
Zaria	30	2 (6.60)		0.00
Gender:			3.894	0.048 (p≤0.01)
Men	32	4 (12.5)		0.04
Women	13	5 (38.5)		0.01
Business years			6.884	0.015 (p≤0.008)
≤5-10	38	6 (15.8)		0.110
11-15	2	2 (100)		0.000
16-20	5	1 (20.0)		1.000
Role of respondent			9.757	0.021 (p≤0.006)
Manager	2	2 (100)		0.003
Owner	36	7 (19.4)		0.841
Workers	5	0 (0)		0.230
Others	2	0 (0)		0.483
FS related knowledge			2.550	0.029
FSR	23	6 (26.1)		
Non-food safety	14	3 (24.4)		
Not applicable	8	0 (0)		
Food safety training			12.656	0.000 (p≤0.012)
Yes	5	5 (100)		0.003
No	40	4 (10)		0.025
Food safety source			10.253	0.000 (p≤0.01)
One source	16	6 (10)		0.01
Two sources	23	3 (13.0)		0.04
Three or more sources	7	0 (0)		0.16

Table 4.20: Factors influencing the food safety attitude (FSA) of fish producers in Kaduna and Zaria metropolises, Nigeria

Variable	No. of respondents	Respondent with good FSA (%)	$\varkappa^2$	P – value
Location:			0.047	0.828
Kaduna	15	6 (40)		
Zaria	30	11 (36.7)		
Gender:			4.391	0.03 (p≤0.012)
Men	32	9 (28.1)		0.005
Women	13	8 (61.5)		0.035
Years of Business experience			0.569	0.927
≤5-10	38	14 (36.8)		
11-15	2	1 (50.0)		
16-20	5	2 (40.0)		
Educational attainment			13.297	0.003 (p≤0.001)
No formal education	6	0 (0)		0.034
Primary	1	0 (0)		0.423
Secondary	14	2 (14.3)		0.027
Tertiary	24	15 (62.5)		0.000
Role of respondent			0.945	0.814
Manager	2	1 (50)		
Owner	36	14 (38.9)		
Workers	5	1 (20)		
Others	2	1 (50)		
Food safety training			9.265	0.002 (p≤0.012)
Yes	5	5 (100)		0.002
No	40	12 (30.0)		0.002

Table 4.21: Factors influencing the food safety Practices (FSP) of fish farmers in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSP	χ^2	P - value
Location:			0.60	0.806
Kaduna	15	11 (73.3)		
Zaria	30	23 (76.7)		
Gender:			0.794	0.373
Men	32	23 (71.8)		
Women	13	11 (84.6)		
Farm sizes			2.670	0.410
Between 1-2 hectares	17	11 (64.7)		
Greater than 2 hectares	6	6 (100)		
Less than 1 hectare	22	17 (77.2)		
Type of production			1.048	0.915
Concrete tanks	15	11 (77.3)		
Earthen ponds	26	20 (76.9)		
Plastic tanks	1	1 (100)		
All	3	2 (66.7)		

4.25 Food safety Knowledge, Attitude and Practices of Fish Traders Interviewed from Fish Value Chains of Kaduna and Zaria Metropolises

The general food safety knowledge of fish traders was good (47/64, 73.4%). They indicated the importance of hygiene and protective clothing in reducing contamination risks such as handwashing (96.9%), use of gloves (90.6%), apron (81.3%) and cap (70.3%) were answered correctly. However, few traders were aware of the role of microbial fish contaminants in diseases such as typhoid (28.1%), dysentery (32.8%), diarrhoeal diseases (39.1%) and abortion (12.5%). They were knowledgeable on transporting live fish in water (64.1%) and storage of fish in freezer (96.9%) as being acceptable modes of reducing fish spoilage and bacterial growth by most traders (Table 4.22).

Table 4.23 shows food safety attitudes of fish traders towards prevention and control of fish borne diseases. Positive attitudes were recorded for majority of fish traders (39/64, 60.9%) who agreed that using hair cover 40 (62.5%), face masks 40 (62.5%), protective gloves 57 (89.1%) and apron 55 (85.9%) could reduce risk of fish contamination. Fish traders responded that they covered wounds (81.2%) or stayed away from fish handling when they were sick (79.7%). Similarly, most fish traders (93.8%) agreed that keeping raw and processed fish separately was important in preventing contamination from raw to processed fish. High number of fish traders (98.4%) agreed on the need for formal food safety training for reducing contamination and food borne disease burden, since 62.5% of the respondents considered fish stored at room temperature could increase chances of food borne diseases (Table 4.23).

Food safety practices of fish traders interviewed in this study showed that 50% of fish traders were found with good food safety practices (32/64); even though some traders reported not performing hygienic practices such as use of gloves (87.5%), apron (82.8%), face mask (96.9%) and caps (81.3%). Conversely, fish traders reported frequent practice of hygiene measures such

as washing contact surfaces before (8.75%) and after (81.3%) business, washing of hands before (79.7%) and after (90.6%) handling raw fish (Table 4.24).

4.26 Factors Associated with Food Safety Knowledge, Attitude and Practices of Fish Traders Interviewed from Fish Value Chain in Kaduna and Zaria Metropolises

The analyses of the relationships between the factors influencing food safety knowledge, attitudes and practices of fish traders were summarized in Table 4.25. Only informally acquired food safety knowledge was significantly associated with food safety knowledge of fish traders ($p<0.05$). Similarly, the type of fish handled by fish traders had significantly influenced the food safety attitude of fish traders ($p<0.05$) (Table 4.26). Whereas, factors such as fish form purchased by fish traders, fish form sold by fish traders, role of respondents, age of fish traders and location of fish traders significantly influenced the level of food safety practices of fish traders ($p<0.05$) (Table 4.27).

Table 4.22: Food safety knowledge statements and responses of fish traders in the study area (n=64)

Statements	No. of Respondents (%)		
	True	False	I don't know
Washing hands before work reduces the risk of fish contamination	62 (96.9)	1 (1.6)	1 (1.6)
Using gloves during work reduces the risk of food contamination	58 (90.6)	5 (7.8)	1 (1.6)
Using apron during work reduces the risk of food contamination	52 (81.3)	10 (15.6)	2 (3.1)
Hair covering during work reduces the risk of food contamination	45 (70.3)	17 (26.6)	2 (2.1)
Proper cleaning and handling of contact surfaces reduces the risk of food contamination	58 (90.6)	1 (1.6)	3 (4.7)
Eating and drinking in the work place increases the risk of food contamination	17 (26.6)	45 (70.3)	2 (3.1)
If you have diarrhoea, it is necessary to stay away from handling fish for consumption/business	54 (84.4)	6 (9.4)	4 (6.3)
If you have skin wound on hands, it is necessary to cover it when handling fish	16 (25.0)	35 (54.7)	12 (18.8)
Typhoid can be transmitted by food	18 (28.1)	35 (54,7)	10 (15.6)
Dysentery can be transmitted by food	21 (32.8)	34 (53.1)	8 (12.5)
Diarrhoea can be transmitted by food	25 (39.1)	30 (46.9)	8 (12.5)
Cholera can be transmitted by food	24 (37.5)	29 (45.3)	10 (15.6)
Abortion in pregnant women may be induced by food borne disease	8 (12.5)	41 (64.1)	14 (21.9)
The refrigerator is the safest place to store fish	62 (96.9)	2 (3.1)	0
Transporting fish alive reduces the risk of fish contamination	41 (64.1)	1 (26.6)	6 (9.4)
Mixing fish from different sources increases risk of fish contamination	25 (39.1)	34 (53.1)	5 (.8)

Table 4.23: Food safety attitude statements and responses of fish traders in the study area (n=64)

Statements	No. of Respondents (%)		
	Agree	Disagree	No idea
Using gloves is important in reducing risk of food contamination	57 (89.1)	6(9.4)	1 (1.6)
Using apron is important in reducing risk of food contamination	55 (85.9)	6 (9.4)	3 (4.7)
Using face masks is important in reducing risk of food contamination	40 (62.5)	20 (31.3)	4 (6.3)
Using caps is important in reducing risk of food contamination	40 (62.5)	21 (32.8)	3 (4.7)
Food handlers who have abrasions or cuts on hands should not touch foods without gloves	52 (81.3)	9 (14.1)	3 (4.7)
Raw and cooked foods should be stored separately to reduce risk of food contamination	60 (93.8)	3 (4.7)	1 (1.6)
Food hygiene training for workers is important for reducing risk of food contamination	63 (98.4)	1 (1.6)	0
Sick people should not be involved in food handling and preparation	51 (79.7)	11 (17.2)	1 (1.6)
Fish kept at room temperature can be contaminated	39 (60.9	17 (26.6)	6 (9.4)
Fish stored at room temperature when consumed can cause disease	40 (62.5)	17 (26.6)	5 (7.8)
Fish should be transported alive	44 (68.8)	19 (29.7)	1 (1.6)
Mixing fish from different sources increases the risk of fish contamination	29 (45.3)	30 (46.9)	5 (7.8)

Table 4.24: Fish traders practices towards fish-borne disease prevention (n=64)

Statements	No. of Respondents (%)		
	Never	Sometimes	Always
Do you use gloves during work?	56 (87.5)	7 (10.9)	1 (1.6)
Do you wash your hands before using gloves?	63 (98.4)	0	1 (1.6)
Do you wear apron during work?	53 (82.8)	9 (14.1)	2 (3.1)
Do you use face mask during work?	62 (96.9)	2 (3.1)	0
Do you cover hair during work?	52 (81.3)	9 (14.1)	3 (4.7)
Do you clean contact surfaces before you start business?	5 (7.8)	3 (4.7)	56 (87.5)
Do you clean contact surfaces after the end of the days/business?	7 (10.9)	5 (7.8)	52 (81.3)
Do you wash your hands before you handle raw fish?	4 (6.3)	9 (14.1)	51 (79.7)
Do you wash your hands after you handle raw fish?	2 (3.1)	4 (4.3)	58 (90.6)
Do you wash your hands after you finish work at the end of the day?	3 (4.7)	2 (3.1)	59 (92.2)
Do you eat or drink in your business place?	3 (4.7)	3 (4.7)	57 (89.1)
How often do you consume fish from your business?	21 (32.8)	21 (32.8)	27 (42.2)
Do you store left over fish at room temperature for sale the next day?	11 (17.2)	11 (17.2)	37 (57.8)
Do you further process left over fish for sale the next day?	34 (53.1)	34 (53.1)	17 (26.6)

Table 4.25: Factors influencing the food safety knowledge (FSK) of fish traders in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSK	χ^2	P - value
Location:			1.100	0.285
Kaduna	20	4 (20.0)		
Zaria	43	13 (30.2)		
Role of respondents			2.509	0.634
Middle men	3	0 (0)		
Retailers	40	10 (25.0)		
Wholesalers/Dealers	21	7 (33.3)		
Years of business experience			1.714	0.634
≤5	4	2 (50.0)		
6-10	20	6 (30.0)		
11-15	20	5 (25.0)		
≥16	20	4 (20.0)		
Educational level			7.422	0.060
No formal education	17	2 (11.7)		
Primary	15	3 (20.0)		
Secondary	26	8 (30.8)		
Tertiary	6	4 (66.7)		
Age of respondents			7.878	0.247
≤18	1	1 (100)		
19-25	6	1 (16.7)		
26-32	9	1 (11.1)		
33-39	14	5 (35.7)		
40-46	18	5 (27.8)		
≥47	15	3 (20.0)		
Food safety related knowledge			6.413	0.040 (P≤0.01)
Food safety knowledgeable	12	6(50.0)		0.01
No food safety knowledgeable	10	4 (40.0)		0.31
Not applicable	42	7 (16.7)		0.45

Table 4.26: Factors influencing the food safety attitude (FSA) of fish traders in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSA	χ^2	P - value
Location:			1.805	0.405
Kaduna	20	14 (70.0)		
Zaria	43	24 (55.8)		
Business years			7.076	0.070
≤5	4	2 (50.0)		
6-10	20	17 (85.0)		
11-15	20	10 (50.0)		
≥16	20	10 (50.0)		
Educational level			5.165	0.160
No formal education	17	10 (58.8)		
Primary	15	7 (46.7)		
Secondary	26	16 (61.5)		
Tertiary	6	6 (100)		
Age of respondents			4.005	0.679
≤18	1	1 (100)		
19-25	6	3 (50.0)		
26-32	9	7 (38.9)		
33-39	14	9 (64.3)		
40-46	18	11 (61.1)		
≥47	15	8 (53.3)		
Type of fish handled			8.782	0.032 (P≤0.01)
Cultured/wild fishes	26	15 (57.7)		0.68
Cultured only	24	17 (70.8)		0.01
Imported only	13	7 (53.8)		0.54
Wild fish only	1	0 (0)		0.19

Table 4.27: Factors influencing the food safety practices (FSP) of fish traders in Kaduna and Zaria metropolises, Nigeria

Variables	No of respondents	No (%) with good FSP	χ^2	P - value
Location:			11.014	0.04 (P≤0.025)
Kaduna	20	16 (80.0)		0.001
Zaria	43	16 (37.2)		0.016
Role of respondents			6.447	0.04 (P≤0.01)
Manager	6	4 (66.7)		0.36
Owners	50	21 (42.0)		0.01
Workers	8	7 (87.5)		0.02
Business years			1.714	0.895
≤5	4	1 (25.0)		
6-10	20	10 (50.0)		
11-15	20	7 (35.0)		
≥16	20	14 (70.0)		
Age of respondents			12.432	0.05 (p≤0.003)
≤18	1	0 (0)		0.317
19-25	6	1 (16.7)		0.089
26-32	9	6 (66.7)		0.271
33-39	14	5 (35.7)		0.230
40-46	18	8 (44.4)		0.548
≥47	15	12 (80.0)		0.001
Food safety training			4.296	0.231
Yes	8	6 (75.0)		
No	54	25 (46.3)		
I can’t remember	1	0 (0)		

4.27 Food safety Knowledge, Attitude and Practices of Fish Processors Interviewed from Fish Value Chains of Kaduna and Zaria Metropolises

Table 4.28 presents food safety knowledge of fish processors in the study area. Among the respondents, 64.9% of them had good knowledge of fish safety. All respondents had good knowledge on use of hand gloves (100%) and hand washing (100%) as well as proper cleaning of utensils. In addition, 35.1% and 73% did not eat in business premise and were covering wound surface were good food safety practices, respectively. They agreed that there was the likelihood of the disease/conditions such as cholera 28 (75.4%), diarrhoea related diseases 17 (45.9%) and typhoid 18 (48.6%) being transmitted through fish contamination. However, they (8.1%) did not belive abortion could occur through consumption of contaminated fish.

Most of the fish processors (70.3%) had a positive attitude to food safety and hygiene measures 26/37. Almost all participants (62.2–100%) agreed with various statements targeted at scoring food safety attitudes. Small percentages of respondents disagreed (27–40.5%) on use of protective clothings or use of storage temperature in reduction of food borne disease risks. Some (2.7–8.1%) did not have any idea on the statements for use of protective clothing and temperature control in food borne diseases (Table 4.29).

Majority (86.5%) of fish processors had good food safety practices such as use of gloves (91.9%), handwashing (94.5%), apron (45.9%) and caps (51.4%), cleaning contact surfaces before (97.3%) and after (35.1%) business, hand washing hands before (91.9%) and after (97.3%) handling raw fish and hand washing at the end of business (100%). However, majority (75.7%) ate food at work place; kept left over fish (43.2%) for resale the next day, while some did not reheat the fish (27%) before selling it (Table 4.30).

4.28 Factors Associated with Food Safety Knowledge, Attitude and Practices of Fish Processors Interviewed from Fish Value Chain in Kaduna and Zaria Metropolises

The factors that significantly influenced the food safety knowledge of fish processors are presented in Table 4.31. Factors such as age of fish processors, food safety training and level of education of fish processors' had significantly influenced the level of their food safety knowledge ($p<0.05$). Further, factors which significantly influenced FSA of respondents were years of business experience, food safety related knowledge, level of education, gender of fish processors, fish form purchased and type of business ($p<0.05$) (Table 4.32). However, none of the factors listed in Table 4.33 significantly influenced food safety practice of the fish processors.

Table 4.28: Summary of questions and responses for assessment of fish processors' food safety knowledge (n=37)

Statements	No. of Respondents (%)		
	True	False	I don't know
Washing hands before work reduces the risk of fish contamination	37 (100)	0	0
Using gloves during work reduces the risk of food contamination	3 (100)	0	0
Using apron during work reduces the risk of food contamination	23 (62.2)	13 (35.1)	1 (2.7)
Hair covering during work reduces the risk of food contamination	29 (8.2)	7 (18.9)	0
Proper cleaning of contact surfaces reduces the risk of food contamination	37 (100)	0	0
Eating and drinking in the work place increases the risk of food contamination	13 (35.1)	24 (64.9)	0
If you have diarrhoea, it is necessary to stay away from handling fish for consumption/business	27(72.9)	10 (27)	0
If you have skin wound on hands, it is necessary to cover it when handling fish	27 (72.9)	6 (16.2)	4 (10.8)
Typhoid can be transmitted by food	18 (48.6)	12 (32.4)	7 (18.9)
Dysentery can be transmitted by food	17 (45.9)	15 (40.5)	5 (13.5)
Diarrhea can be transmitted by food	34 (91.9)	2 (5.4)	1 (2.7)
Cholera can be transmitted by food	28 (75.7)	4 (10.8)	5 (13.5)
Abortion in pregnant women may be induced by food borne disease	3 (8.1)	19 (51.4)	15 (40.5)
The refrigerator is the safest fish storage facility	37 (100)	0	0

Table 4.29: Food safety attitude statements and responses of fish processors in the study area (n=37)

Statements	No. of Respondents (%)		
	Agree	Disagree	No idea
Using gloves is important in reducing risk of food contamination	37 (100)	0	0
Using apron is important in reducing risk of food contamination	2 (73.0)	8 (21.6)	2 (5.4)
Using face masks is important in reducing risk of food contamination	20 (54.1)	15 (40.5)	2 (5.4)
Using caps is important in reducing risk of food contamination	24 (64.9)	10 (27.0)	3 (8.1)
Food handlers who have abrasions or cuts on hands should not touch foods without gloves	37 (100)	0	0
Raw and cooked foods should be stored separately to reduce risk of food contamination	37 (100)	0	0
Food hygiene training for workers is important for reducing risk of food contamination	37 (100)	0	0
Sick people should not be involved in food handling and preparation	35 (94.6)	1 (2.7)	1 (2.7)
Fish kept at room temperature can be contaminated	23 (62.2)	13 (35.1)	1 (2.7)
Fish stored at room temperature when consumed can cause disease	23 (62.2)	12 (32.4)	2 (5.4)

Table 4.30: Fish processors' practices toward food-borne disease prevention (n = 37)

Statements	No. of Respondents (%)		
	Never	Sometimes	Always
Do you use gloves during work?	34 (91.9)	2 (5.4)	1 (2.7)
Do you wash your hands before using gloves?	35 (94.5)	1 (2.7)	1 (2.7)
Do you wear apron during work?	17 (45.9)	9 (24.3)	11 (29.7)
Do you use face mask during work?	37 (100)	0	0
Do you cover hair during work?	19 (51.4)	12 (32.4)	6 (16.2)
Do you clean contact surfaces before you start business?	0	1 (2.7)	36 (97.3)
Do you clean contact surfaces after the end of the days/business?	22 (59.4)	2 (5.4)	13 (35.1)
Do you wash your hands before you handle raw fish?	0	3 (8.1)	34 (91.9)
Do you wash your hands after you handle raw fish?	0	1 (2.7)	36 (97.3)
Do you wash your hands after you finish work at the end of the day?	0	0	37 (100)
Do you eat or drink in your business place?	0	9 (24.3)	28 (75.7)
How often do you consume fish from your business?	4 (10.8)	15 (40.5)	18 (48.6)
Do you store left over fish at room temperature for sale the next day?	12 (32.4)	9 (24.3)	16 (43.2)
Do you further process left over fish for sale the next day?	21 (56.8)	6 (16.2)	10 (27.0)

Table 4.31: Factors influencing the food safety knowledge (FSK) of fish processors in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSK	$\varkappa^2$	P - value
Location:			0.138	0.100
Kaduna	10	6 (60.0)		
Zaria	27	18 (66.7)		
Years of business experience			4.669	0.198
≤ 5	7	6 (85.7)		
6-10	18	13 (72.2)		
11-15	10	4 (40.0)		
16-20	2	1 (50.0)		
Educational level			12.818	0.005 (p≤0.01)
No formal education	10	2 (20.0)		0.76
Primary	5	4 (80.0)		0.42
Secondary	18	14 (77.8)		0.01
Tertiary	4	4 (100)		0.10
Age of respondents			4.738	0.044 (p≤0.05)
20-30	12	2 (16.7)		0.10
31-40	13	4 (30.7)		0.68
≥ 41	12	7 (58.3)		0.04
Food safety training			10.542	0.042 (p≤0.025)
Yes	1	1 (100)		0.00
No	36	23 (63.9)		1.32
Gender			3.301	0.059 (p≤0.02)
Men	19	15 (78.9)		0.01
Women	18	9 (50.0)		0.23

Table 4.32: Factors influencing the food safety attitude (FSA) of fish processors in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSA	$\varkappa^2$	P - value
Location:			2.533	0.110
Kaduna	10	9 (90.0)		
Zaria	27	17 (62.9)		
Role of respondents			2.553	0.011
Owners	27	17 (62.9)		
Workers	10	9 (90.0)		
Business years			10.392	0.016 ($p \leq 0.006$)
≤ 5	7	4 (57.1)		0.5481
6-10	18	17 (94.4)		0.4237
11-15	10	4 (40.0)		0.0019
16-20	2	1 (50.0)		0.0124
Educational level			11.258	0.010 ($p \leq 0.01$)
No formal education	10	4 (40.0)		0.012
Primary	5	2 (40.0)		0.109
Secondary	18	16 (88.8)		0.016
Tertiary	4	4 (100)		0.161
Age of respondents			1.222	0.543
20-30	12	9 (75.0)		
31-40	13	10 (76.9)		
≥ 41	12	7 (58.3)		
Food safety training			0.435	0.510
Yes	1	1 (100)		
No	36	25 (69.4)		
Gender			3.535	0.057 (($p \leq 0.025$)
Men	18	10 (55.6)		0.0574
Women	18	16 (88.9)		0.0574

Table 4.33: Factors influencing the food safety practices (FSP) of fish processors in Kaduna and Zaria metropolises, Nigeria

Variable	No of respondents	No (%) with good FSP	χ^2	P - value
Location:			0.145	0.704
Kaduna	10	1 (10.0)		
Zaria	27	4 (14.8)		
Role of respondents			3.187	0.740
Owners	27	2 (7.4)		
Workers	10	3 (30.0)		
Years of business experience			0.572	0.902
≤ 5	7	1 (14.3)		
6-10	18	3 (16.7)		
11-15	10	1 (10.0)		
16-20	2	0 (0)		
Educational level			3.963	0.265
No formal education	10	0 (0)		0.1336
Primary	5	0 (0)		0.3173
Secondary	18	4 (22.2)		0.0133
Tertiary	4	1 (25.0)		0.4839
Age of respondents			2.994	0.224
20-30	12	2 (16.7)		
31-40	13	3 (23.1)		
≥ 41	12	0 (0)		
Food safety training			0.161	0.689
Yes	1	0 (0)		
No	36	5 (13.9)		
Gender			0.173	0.677
Men	19	3 (15.8)		
Women	18	2 (11.1)		
Food safety related knowledge			2.892	0.236
Food safety related knowledge	11	3 (27.3)		
Non food safety related knowledge	7	1(14.3)		

4.29 Food Safety Knowledge, Attitude and Practices Scores of Fish handlers in the Fish Value Chains of Kaduna and Zaria Metropolises

Table 4.34 summarizes the results regarding food safety knowledge, attitudes and practices of all the stake holders interviewed. Mean score for knowledge was 9.06± 2.65, ranging between 2 and 15. Approximately 35% of respondents had poor knowledge about fish safety measures. Mean score for attitudes was 8.69± 2.7 and about 98% had their total attitudes score higher than 10, hence, positive attitudes towards fish safety. Mean score for practices was 9.4 ±3.18, ranging between 0 and 12 points. Ninety two percent of respondents had poor self-reported hygienic practices.

Table 4.34: Total scores for food safety knowledge, attitudes and practices of all stakeholders in fish value chains of Kaduna and Zaria metropolises (n=107)

Food safety risk components	Mean±SD	Min.	Max.
Knowledge (15 questions)	9.06±2.65	2	15
Attitude (12 questions)	8.69±2.7	2	12
Practices (12 questions)	9.4±3.18	0	12

4.30 Food safety Knowledge, Attitude and Practices of Fish Consumers Interviewed from Fish Value Chains of Kaduna and Zaria Metropolises

An overall 100% positive response was recorded for significance of hand washing in reduction of risk of fish contamination. Some (52.6%) did agree that thorough washing of fish before cooking reduce the risk of contamination and FBD. Likewise attitude of fish consumers showed that only 33.3% of the consumers agreed that germs may cause FBD and 98.2% of respondents worried more about chemical residues in fish tissues than microbial contamination. And they believed that fish safety can be judged by individual's sight and smell of fish before purchase. With this, consumers were of the opinion that there was no health effects associated with consumption of fish leftover at atmospheric temperature overnight as far as it has been processed (70.2%). Fish consumers reported ability to buy poor quality fish from retailers as far as it was cheap (47.4%) and also believed that wild fish is safer to consume than farmed fish (57.9%) (Table 4.35).

Most respondents (46.6%) preferred to buy their fish fresh from fish markets while 26.5% purchased ready-to-eat fish at the end of the day. Fish consumers in this study preferred to eat fish cooked (62%), fried (28%) and roasted forms (8%), respectively (Table 4.36). The usual time of the day respondents bought fish were early in the morning 1 (1.8%), mid day 10 (1.5%), afternoon 32 (56.1%) and evening 14 (24.6%). Fish were packaged at the point of purchase with foil paper 4 (7.0%), ordinary paper 3 (5.3%), polythene bag 43 (75.4%) and take away pack 7 (12.3%). The mean time taken to transport fish from the place of purchase to consumers' houses was 20.4±9.6 minutes with minimum of 5 and maximum of 45 minutes. The mean time fish was kept at home before cooking was 1.36±14.81 with mimumun of 0 and maximum of 60 minutes. Fish were normally stored in freezer 3 (5.3%), refrigerator 10 (17.3%) and at room temperature

41 (71.9%) before cooking. All of the respondents (100%) reported consuming left over fish. However, 15.8% ate the fish without reheating, 61.4% reheated left over fish before eating, and 22.4% reported no left overs at all while none of the respondents threw or gave away left overs. Among those respondents that reported eating left over, 6 (13.6%) reported storing left over in freezer, 10 (17.5%) in refrigerator and 28 (63.6%) at room temperature. The mean number of days left over fish were kept was 1.38±1.09 with minimum of 0 and maximum of 7 days.

4.31 Risk Factors Associated with Bacterial Fish Quality

Based on Risk Ranger scores, the following high risk factors were identified: temperature abuse, RTE fish products with extended shelf life and cross contamination of processed fish products with *S. aureus* or *Salmonella* or raw products intended to be cooked but recontaminated or cross-contaminated before consumption increases the likelihood of FBD among fish consumers (Fig. 4.6).

Table 4.35: Food safety knowledge and attitudes of fish consumers interviewed in Kaduna and Zaria metropolises (n=57)

Questions on food safety knowledge	No. of Respondents (%)		
	True	False	No idea
Washing hands before cooking reduces the risk of food borne disease	57 (100)	0	0
Washing fish thoroughly before cooking reduces the risk of food borne disease	30 (52.6)	26 (45.6)	1 (1.8)
There are chances of contaminating fish for consumption when you have diarrhea	28 (49.1)	15 (26.3)	14 (24.6
Germs can be transmitted through consumption of fish	19 (33.3)	35 (61.4)	3 (5.3)
Fish stored at room temperature can spoil or cause FBD	24 (42.1)	27 (47.4)	6 (10.5)
Questions for food safety Attitudes	Agree	Disagree	No idea
Eating germ contaminated fish can cause FBD	19 (33.3)	28 (49.1)	10 (17.5)
Unwashed hands can increase the chance of getting FBD	56 (98.2)	0	1 (1.8)
I worry more of chemicals than germs in fish***	48 (84.2)	4 (7.0)	1 (1.8)
I would eat poor quality fish if it is cheaper	27 (47.4)	30 (52.6)	0
Fish safety can be judged by sight and smell	55 (96.5)	0	2 (3.5)
There are no health effects in eating overnight left over fish	40 (70.2)	11 (19.3)	6 (10.5)
I can get sick from eating well cooked fish	12 (21.1)	45 (78.9)	0
Wild fish is better than farmed fish in terms of safety	33 (57.9)	7 (19.3)	17 (29.3)

*** 4 (7%) said both were causes of health concern to them

Table 4.36: Food Safety Practices of Fish consumers interviewed in Kaduna and Zaria metropolises

Questions	No. of Respondents (%)
What time of the day do you buy fish?	
Early morning	1 (1.8)
Midday	10 (17.5)
Afternoon	32 (56.1)
Evening	14 (24.6)
Mode of packaging at sales point	
Foil paper	4 (7.0)
Paper	3 (5.3)
Polythene bag	43 (75.4)
Others (Take away pack, plates)	7 (12.3)
Average time taken to transport fish home (min)	
Mean±SD	20.9±9.6
Minimum	5
Maximum	45
How do you store fish before cooking?	
No storage	3 (5.3)
Freezer	3 (5.3)
Refrigerator	10 (17.5)
Room temperature	41 (71.9)
Average time before cooking (min)	
Mean±SD	13.6±14.8
Minimum	0
Maximum	60
Do you consume overnight left over fish?	
Yes	57 (100)
No	0
What do you do with left over fish?	
Eat cold the next day	9 (15.8)
Eat reheated the next day	35 (61.4)
Throw away/ give away/ feed pets	0
Mostly no left over	13 (22.4)
How do you store left over processed fish?	
Frozen	6 (13.6)
Refrigerated	10 (17.5)
Room temperature	28 (63.6)
How long do you keep fish left over (days)?	
Mean±SD	1.38±1.09
Minimum	0
Maximum	7

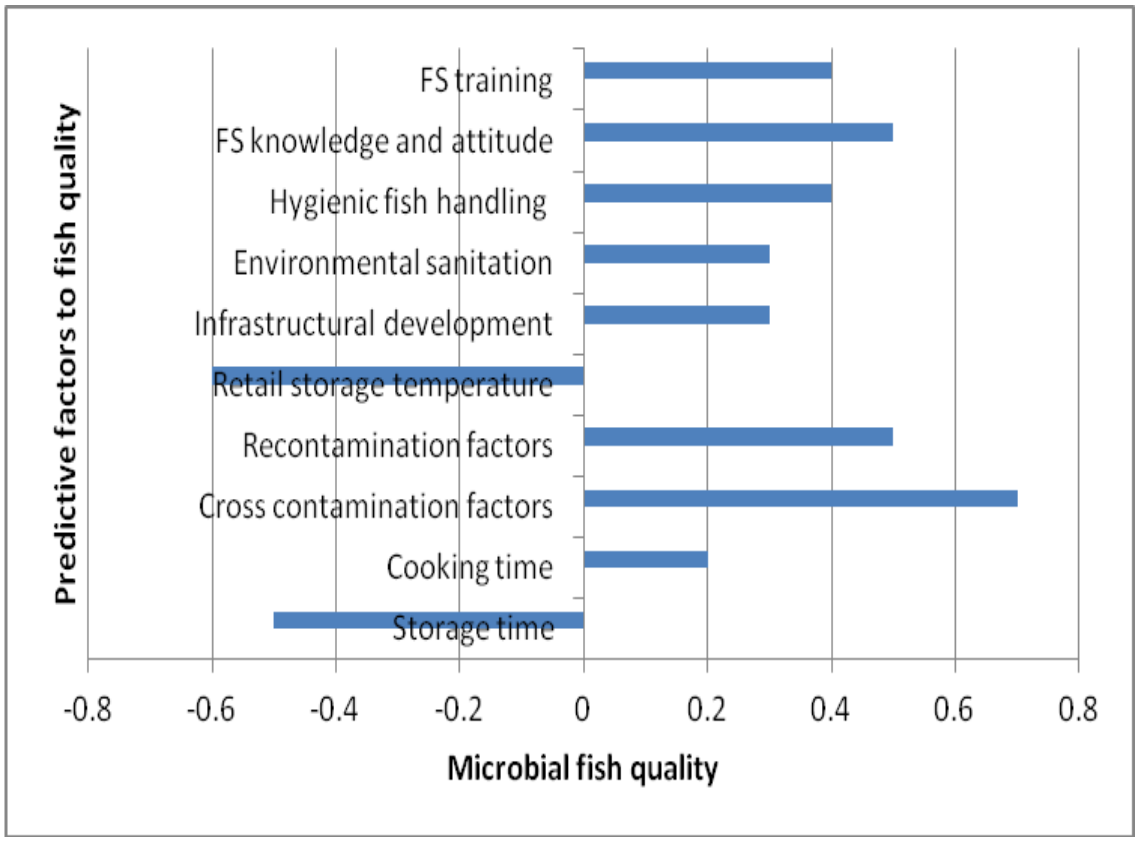


Fig. 4.6: Spearman rank correlation between predictive factors for fish contamination with *Salmonella* and *Staphylococcus aureus* and level of fish microbial quality. A positive value of the rank correlation indicates that the predictive factor is positively related with probability of safe fish, while a negative factor correlates with a decrease in microbial quality of fish.

4.32 Fishborne Disease Risk Estimation

The results of microbial risk estimation conducted using Risk Ranger tool per pathogen per fish product types are presented in Tables 4.37 and 4.38. Risk predictions were generally unsatisfactory for both raw and processed fish products. Characterization of pathogen/product combinations using Risk Ranger scores for *S. aureus* were classified as highly likely of FBD in all high and low risk groups except for low risk group consuming dried/smoked fish (≤32). Similarly, high risk for FBD (≥ 48) was recorded for all *Salmonella* contaminated fish products (raw, processed or RTE) in high risk groups. On the other hand, *Salmonella* contamination of dried/smoked fish was characterized as low risk products (≥48) for low risk groups. Total predicted illnesses for high and low risk groups were estimated in Table 4.37 and 4.38 for both pathogens. FBD due to contamination with *S. aureus* enterotoxin of uncooked fish were predicted as 156 and 513 cases annually for high and low groups with 1.00 and 0.03 probability of occurrence. Total predicted cases of FBD due to consumption of RTE fish contaminated with *S. aureus* enterotoxins in high and low risk groups were 520 and 2,220 cases annually, respectively. Consumption of dried/smoked fish contaminated with *S. aureus* enterotoxin may result in 53 and 520 FBD cases with 1.00 and 0.03 probability of occurrence in high risk and low risk groups, respectively.

Total predicted cases for consumption of *Salmonella* contaminated uncooked fish were 520 and 371 with 1.00 and 7.12×10^{-5} in high and low risk groups, respectively. *Salmonella* contaminated RTE fish consumed by high or low risk group individuals can result in 171 and 111 FBD cases annually at 0.03 and 7.12×10^{-4} probabilities of occurrence. Finally, 148 FBD cases annually at 0.28 probability of occurrence was estimated for high risk groups that consumed *Salmonella*

contaminated dried/smoked fish products whereas 4,340 cases of FBD were estimated for low risk groups at $8.2x\ 10^{-4}$.

Table 4.37: Risk estimation for high and low risk groups due to consumption of contaminated fish and fish products with *Staphylococcus aureus*

S/No.	Risk ranger questions	Raw Fish		RTE fish		Dried/Smoked fish	
		High	Low	High	Low	High	Low
Q1	Hazard Severity	Moderate	Mild	Moderate	Minor	Severe	Mild
Q2	Susceptibility of population	Extreme	General	Extreme	General	Extreme	General
Q3	Fish consumption frequency	Weekly	Monthly	Monthly	Weekly	Weekly	Monthly
Q4	Proportion of population consuming fish	75%	75%	25%	75%	25%	75%
Q5	Size of consuming population	2268494	2268494	2268494	2268494	2268494	2268494
Q6	Probability of contamination	50%	50%	50%	50%	50%	10%
Q7	Effect of processing on the hazard	Slightly 50%	Slightly 50%	No effect	No effect	No effect	Slightly 50%
Q8	Potential for recontamination of fish after processing	Major 50%	Major 50%	Major 50%	Major 50%	Major 50%	Major 50%
Q9	Effectiveness of post processing control system	Not controlled	Gross abuse	Gross abuse	Gross abuse	Gross Abuse	Gross Abuse
Q10	Increase of the hazard needed to cause illness	Slight (10 fold)	Slight (10 fold)	Slightly (10 fold increase)	Slightly (10 fold increase)	None	Slightly (10 fold increase)
Q11	Preparation of product before eating	No effect on hazard	No effect on hazard	No effect on hazard	No effect on hazard	No effect on hazard	Slightly reduces
α		1.00	0.03	1.00	0.14	1.00	0.03
β		156	513	520	2,220	520	53
RR		71	74	68	72	79	32

Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard; Q8, potential for recontamination of product after processing; Q9, effectiveness of post processing control system; Q10, increase of the hazard needed to cause illness; and Q11, preparation of product before eating.

RF =Raw fish

RTE = Ready-to-eat

RR= Risk Ranking

α =A Probability of illness per annum in population of interest.

β = Total predicted illnesses annually per consumer of interest.

Table 4.38: Risk estimation for high and low risk groups due to consumption of contaminated fish with *Salmonella* species

S/No.	Risk ranger questions	RF		RTE fish		Dried/Smoked fish	
		High	Low	High	Low	High	Low
Q1	Hazard Severity	Severe	Mild	Severe	Mild	Mild	Moderate
Q2	Susceptibility of population	Extreme	General	Extreme	General	Extreme	General
Q3	Fish consumptionfrequency	Monthly	Weekly	Monthly	Weekly	Weekly	Monthly
Q4	Proportion of population consuming fish	25%	75%	25%	75%	25%	75%
Q5	Size of consuming population	2268494	2268494	2268494	2268494	2268494	2268494
Q6	Probability of contamination of raw fish product	50%	50%	25%	50%	50%	50%
Q7	Effect of processing on the hazard	50%	50%	99%	99%	100%	50%
Q8	Potential for recontamination	50% frequency	50%	50%	50%	50%	50%
Q9	Effectiveness of post processing control system	Not controlled	Not controlled	Not controlled	Not controlled	Gross abuse	Gross abuse
Q10	Increase of the hazard needed to cause illness	None	10 fold	None	10 fold	10 fold	10 fold
Q11	Preparation of product before eating	Slightly	50%	50%	99%	99%	50%
α		1.00	$7.12x10^{-5}$	0.33	$7.12x10^{-4}$	0.28	$8.2x10^{-4}$
β		520	371	171	111	148	4390
RR		79	56	77	64	59	70

Q1, hazard severity; Q2, susceptibility of population; Q3, product consumption frequency; Q4, proportion of population consuming that product; Q5, size of consuming population; Q6, probability of contamination of raw product; Q7, effect of processing on the hazard; Q8, potential for recontamination of product after processing; Q9, effectiveness of post processing control system; Q10, increase of the hazard needed to cause illness; and Q11, preparation of product before eating.

RF =Raw fish

RTE = Ready-to-eat

RR= Risk Ranking

α =A Probability of illness per annum in population of interest.

β = Total predicted illnesses annaully per consumer of interest.

CHAPTER FIVE

5.0 DISCUSSION

The purpose of this study was to provide an in-depth understanding of the structure and activities along the fish value chains (FVCs) in Kaduna and Zaria metropolises with the aim of assesseing risks of microbial contamination as well as risk factors for possible fish contamination and the public hazards there in. Although operations involving fish production and marketing are well established in Nigeria, there are few published data describing fish value chains in the context of food safety (Alagoa *et al*., 2011). Two discrete FVCs were identified in this survey; the imported frozen fish distribution channel and the domestic fish value chains consisting of producers/distributors, traders (wholesalers and retailers), processors (street vendors, fry shops and restaurants) and fish consumers. Similar pattern of fish production and marketing activities were reported in the Southern part of Nigeria as well as other animal protein value chains such as beef value chains in Northern Nigeria (Iro, 1994; Alagoa *et al*., 2011). Structurally, the distribution channel of the imported frozen fish in this study was characterized as a monopolistic fish market where few people have major market share with homogenous products which agrees with market description by Bain (1968). This structure of market typically consists of one single seller with no competitiors or rivals in both popularity and technical power. The effect of monopolistic market structure is on market impacts such as conducts of participants, cost of product, volume and organizational operations such as implications on implementation of food safety and hygiene measures (Scott, 1995). Targeting implementation and enforcement of food safety and hygiene practices will therefore be easy in such situation where change in attitude and practices of main distributor might have greater impact on several buyers along the chain. Conversely, the market structure of farmed fish is typically oligopolistic where more than one

producer existed in the market and each contributes substantially to the existence of the fish market (Scott, 1995). There were more product differentiations as fish was supplied from various sources. As such, policies for implementation of food safety and hygiene measures may require involvement of almost all the fish suppliers such as the fish farmers, fishermen, middlemen and main fish distributors from other states. This type of market structure has more channels of product flow, less organizational and functional structure which affects success of policies for market development (Smith, 1981). In terms of product valuation, both imported and farmed fish value chains were short and simple with little processing activities such as filleting, smoking and roasting, which serves as an advantage to fish consumers as no nutrients are lost by heating or processing conditions, yet it presents a food borne disease (FBD) risk, as no temperature regulation is put in place to stop bacterial growth and multiplication (Eltholth *et al.*, 2014).

The production and marketing chains for farmed fish in this study was dominated with clariid species, mainly catfish and wild caught fish species such as carp and tilapia. Most of the clariid species farmed within the study areas were sold live in water at the farm gate. Likewise, additional fish quantities were sourced by traders in these study areas from Southern and North Central part of Nigeria to supplement shortage experienced by fish traders in the study area. The business of trading locally farmed fish and imported frozen fish was dominated by men while women dominated the processing components. This is a common trend as reported in Southern Nigeria where men are responsible for fishing, farming and trading of fish, with women being responsible for processing and preservation of fish (Gomna and Rana, 2007). Most of the fish producers in this study had tertiary education (53.3%) while traders and processors (48.6%) had secondary education. Formal food safety trainings were reported by 11.1% of the fish farmers, 12.5% of the traders and 2.7% of the processors. Thus, the poor knowledge of fish handlers

regarding safe handling and processing of fish might increase the chances of production, processing or marketing of unsafe fish to street consumers and household members. Mitchell *et al*. (2007) reported that formal education and food safety/hygiene training of food handlers are essential requirements of any food industry intending to implement intervention and control of industrial food contamination. Apart from educational level of food handlers, years of business experience is also an essential tool in the control of food contamination (Tolulope *et al*., 2014). Most fish farmers in the study areas started production within 10 years, while fish traders had 11 to 15 years marketing experience but processors had 6 to 10 years of processing experience. Hence, extended years of business experience has been found to positively influence the food safety and hygiene practices of food handlers (Tolulope *et al*., 2014).

The fish traders' products and trading evaluation in this study showed that traders handling farmed and wild fishes tend to purchase live fish in water or fresh (37.5%) while traders in the frozen fish distributing channels bought and sold fish in frozen forms. Although each component is dependent on organizational strategies, there are interdependencies within and between value chain components regarding demand-supply of fish; which is a typical feature of fish value chains (Neiland and Bènè, 2004). Most fish processors had preference for imported fish (51.4%) or cultured fish (40.5%) with few having preference for (8.1%) wild caught fish. The increased availability of imported fish may have influenced the choice of fish handlers in deteriming the market share for frozen fish. Increase in scarcity of supply of wild caught fish and the underdevelopment of aquaculture systems in Nigeria might have increased the demand-supply of imported frozen fish in the study area (Gomna, 2006). Similar reports have been presented in neighbouring states of Kastina and Kogi with increased availability and consumption of frozen fish imported from outside Nigeria (Ogbe *et al*., 2012; Dauda *et al*., 2016). Fish processors

encountered in this study were mainly fixed or mobile street vendors, restaurateurs and fry shop owners. Due to lack of infrastructural development, the entire value chain relied on daily or weekly fish supply to avoid spoilage of fish, being a perishable food that requires good temperature and storage conditions. Common forms in which fish are transported within the value chains in this study included frozen (59.5%) or live in water (35.1%); which might not be unconnected with the lack of infrastructure in most of agricultural value chains of animal sourced proteins in Northen part of Nigeria (Nwanta *et al*., 2008; Lawan *et al*., 2013). Fish handlers in this study might have preferred live fish transportation to avoid early spoilage of fish products due to microbial contamination from environments. The electricity power supply in Nigeria is still epileptic which might be the reason for daily supply. Those using generator may end up increase the unit price of fish which might affect the market (Olukunle, 2013). The production and marketing environment as well as processing equipment of fish in most Nigerian cities were found to be highly colonized with pathogenic microorganisms that may pose risk of fish contamination and spoilage (Grema *et al*., 2015 a, b; Zailani *et al*., 2016). Although fish processors considered choices such as availability (8.1%) and price (16.2%) in this study to describe frequency of purchase, freshness was an important reason for daily fish purchase. The preference of consumers for roasted (51.4%) and smoked (21.6%) fish from street vendors who display of fish products on mini tables under shade was similar to scenario observed by Luu (2015) in Vietnam emphasizing the favourable condition for contamination and multiplication of harmful pathogens.

Understanding consumer behavior in terms of purchase decision and post purchase handling practices has been reported to be a prerequisite for tackling problems of FBD caused by microbes and a necessary tool for food safety risk analysis (Rani, 2014). Consumer buying and

behavioral factors evaluated in this survey included decision and frequency of fish purchased. Many of the respondents in this study were educated (45.6%) and earned formal salary (38.6%). The observational findings in this study agrees with the concept that both social class and income level are relevant in understanding consumption pattern and safety methods because income and social class were found to be associated with values and lifestyle of consumers in relation to food safety practices (Mihić and Čulina, 2006).

This study assessed the bacterial load of fish samples collected from the various components of the fish value chains as a reflection of contamination level from handlers' practices and environment. The levels of aerobic and coliform counts are indicators for evaluating the general hygienic status of food and food industry in line with the recommendations of World Health Organization, WHO (Jackson *et al.*, 2007). A total of 73.4% and 93.3% of raw fish samples collected from fish farms and markets were considered unacceptable for human consumption due to high microbial contaminations (TAPC). High level of total aerobic counts in a fish sample is an indication of poor sanitary environment or poor personal hygiene by fish handlers (Luu, 2015). Similarly, 64.9% of ready-to-eat fish samples collected from fish processing outlets in this study were considered unacceptable for human consumption due to high aerobic plate counts; while 8 (21.6%) were microbiologically fit for human consumption. Contamination from aerobic organisms such as *S. aureus* on ready-to-eat fish samples can result from poor hygienic handling of fish (Mayrhofer *et al*., 2004; Atyah *et al*., 2010). Total coliform counts testing of fish samples from this study reavealed that fish samples from farms (37.8%), markets (68.7%) and processing outlets (8.1%) were highly contaminated with coliform organisms, thus, considered unfit for human consumption according to standard microbiological interpretation reported by FAO (1994). High coliform counts encountered in this study might be the reflection of environment

where the fishes were reared or due to factors such as faecal contamination during production or from water used in production (Raufu *et al*., 2014). The most risky of the fish samples unfit for consumption within the fish value chain in this study were the ready-to-eat fishes from the processing outlets which has direct link to consumers (Raspor, 2004; Andritsos *et al*., 2012).

Two commonly incriminated organisms mainly *S. aureus* and *Salmonella* species as causal agents of food borne diseases were investigated in this study (Painter *et al*., 2013; WHO, 2016). An overall isolation rate of 15.1% was recorded for *S. aureus* in this study and that the organism was isolated less frequently in raw fish samples (40%) and more in ready-to-eat fish samples (60%). This is a typical trend of *S. aureus* contamination, common in FVCs probably because the organism is a predominant normal flora of human and animal skin/mucous membranes; hence can be seen on ready-to-eat fish products, especially when handled without personal protective equipment (PPE) and/or in poor sanitary environments. This is in agreement with the work of Grema *et al*. (2015b) in which isolates of *S. aureus* (MRSA) were detected in animal owners and domestic animals in the fish markets as well as the water and utensils used for fish handling and processing. These MRSA have also been isolated in several studies on raw and ready-to-eat fish products as well as other animal sourced proteins along food value chains which lead to the assumption of its potential to cause food borne zoonosis (Atyah *et al*., 2006; Wendlant *et al*., 2013; Vossenkuhl, 2015). The presence of *S. aureus* on ready-to-eat fish sample was regarded as an indicator of unhygienic handling of fish during the processing and post-processing stages (Andritsos *et al*., 2012). Several studies have suggested that the presence of *S. aureus* on food substances was due to improper handling, unhygienic environmental conditions and inadequate temperature control (Snyder, 1998). The natural niche for *S. aureus* in human body is the nostril (Wertheim *et al*., 2004) and the relationship between hand and nostril in human behaviour cannot

be overemphasized. For this reason, the levels of *S. aureus* contamination of fish in this study may be attributable to lack of use of protective equipment or poor handling by personnel when processing fish. From the above findings of *S. aureus* on ready-to-eat fish samples, two possible outcomes may likely occur on post-human contact with contaminated fish products: either colonization of previously uncolonized fish handler leading to potential systemic infections or contraction of food borne diseases by consumers due to consumption of enterotoxin contaminated fish (Khetarpaul, 2006). *S. aureus* in this study harboured *pvl* genes, which is a gene for Panton-Valentine leukocidin protein responsible for destruction of human neutrophils and promotes skin and soft tissue infections resulting in necrotizing pneumonia and/or osteomyelitis especially in immunocompromised patients (Turnidge *et al*., 2008). The presence of various enterotoxigenic genes in some of the *S. aureus* isolated in this study was an indication of potential cause of staphylococcal food poisoning (SFP) since SFP is mainly caused by consumption of food containing enterotoxins produced by *S. aureus* (Khetarpaul, 2006). Staphylococcal food poisoning (SFP) is a foodborne intoxication that develops in people who ingest toxin contaminated food that has been improperly prepared or stored (Schelin *et al*., 2011). The severity of the illness depends on the amount of toxin ingested, alongside the ingested food and the general immune status of the victim. It has been shown that SFP can be caused by as little as 20–100 ng of enterotoxin (Smyth *et al*., 2004) with symptoms rapidly and abruptly manifesting such as copious vomiting, diarrhoea, abdominal pain or nausea (Asao *et al*., 2003); symptoms sometimes wears off within 24 h of manifestation (Prescott *et al*., 2005).

Similarly, a total isolation rate of 11.6% of *Salmonella* isolates was detected in this study. When considering the standard interpretation of *Salmonella* contamination in food intended for human consumption, Huss (1993) reported that as low as 1 cell recovered from 25g of fish tissue should

be considered as unsafe food for human consumption. As such, all salmples in this study in which *Salmonella* were isolated were regarded as unfit for human consumption. This is because of the recommendations by health-related organizations such as WHO and FAO that no single *Salmonella* isolate is supposed to be present on food intended for human consumption, and if found, the food should be considered unfit for human consumption (Vishwanath *et al*., 1998). The isolation rate of *Salmonella* spp. in this study was higher than that reported by Ha and Pham (2006) and Tran *et al*. (2007) where 6.6% and 18% respectively, of fish samples were contaminated. The findings of *Salmonella* on raw fish in this study might present less microbial FBD risk because the reports that *Salmonella* as a known vegetative organism could be easily destroyed by heat (Huss *et al*., 2000). However, the presence of *Salmonella* on ready-to-eat fish in this study should be considered as high risk and potential of causing infections in humans (Martinez-Urtaza *et al*., 2003); since most health-related organizations have reported that fish should be considered unfit for human consumption even when it contains a single cell of *Salmonella* (Vishwanath *et al*., 1998). The main reservoir for *Salmonella* species is the gastrointestinal tract of humans and animals or environments such as estuaries, ponds, rivers and lakes contaminated with faecal materials (Huss *et al*., 2000). Contamination of fish with *Salmonella* might be due to poor environment sanitation, display of fish at point-of -sale without good cover, resale of overnight fish with/without reheating, and the typical habits of consumers who buy cheaper fish from hawkers (Alimi, 2006). This trend of marketing and purchase behavior has been reported from several countries such as Thailand, Hong Kong, Spain, Egypt and Turkey (Herrera *et al*., 2006; Kumar *et al*., 2009; Eltholth *et al*., 2014).

In this study, factors attributable to raw fish contamination with *Salmonella* were related to terrestrial sources such as use of animal dung as organic manure in farm land located close to rivers and subsequently being washed off during rainy season leading to environmental and fish contamination (Raufu *et al*. 2014) or as organic manure in pond fertilization to stimulate growth of natural food (Plumb, 1999). These practices contribute to increased colonization of fish with human pathogens. Other factors such as poor sewage disposal, untreated waste from abattoir or domestic places and other industrial waste flushed into lakes or ponds either through run off, storm-water as well as contamination from transportation media are main predictive factors to fish contamination (Raufu *et al*. 2014). The practice of integrated farming by keeping animals such as horses, cattle, pigs, poultry and sheep/goats in farms with fish ponds or keeping companion animals such as dogs and cats in abattoirs and/or fish markets to feed on fish remnants can increase chances of fish contamination with microbes (Grema *et al*., 2015b). Results from this study were also in agreement with the findings of Makino *et al*. (1999) and Murugkar *et al*. (2003) who isolated *Salmonella* spp from humans and animal products. Fish and fish products have been incriminated as major carriers of *Salmonella* species capable of causing FBD when consumed by humans (Kamat *et al*., 2005; Upadhyay *et al*., 2010; de Freitas *et al*. 2010b). However, infection depends on likelihood of human contact with contaminated fish, dietary habits of consumers and the immune status of individuals exposed to contaminated fish (Bhaftopadhyay, 2000). Most outbreaks of FBD are associated with consumption of contaminated raw or uncooked fish products (Heinitz *et al*., 2000). Approximately, 12% of the foodborne outbreaks related to consumption of fish were reportedly caused by bacteria including *Salmonella* and *S. aureus* (Huss *et al*., 2000; Allshouse *et al*., 2004; Aberoumand, 2010).

Antibiotic resistance patterns of both *S. aureus* and *Salmonella* isolates from this study showed resistance to multiple antibiotics. *Staphylococcus aureus* showed resistance to cefoxitin (18.1%), oxacillin (22.7%), tetracycline (27.2%), cephazolin (27.2%) and erythromycin (31.8%) which conforms with the resistance patterns reported in Southern part of Nigeria (Ayepola, 2012). The pattern of resistance to tetracycline in *S. aureus* isolated in this study was in comformity to the study of Gelfand and Cleveland (2004) who reported that 90% of community-acquired strains of *S. aureus* were resistant to tetracycline. *Staphylococcus aureus* isolates were reported to be highly resistant to tetracycline, a broad spectrum antibiotic and the most prescribed antibiotic in most countries (Moodley *et al.*, 2011). However, the sensitivity patterns of isolates to gentamicin in this study was not in accordance to the findings of Wogu and Maduakor (2010) which showed all isolates as resistant to gentamicin. The susceptibilities of isolates to gentamicin, an aminoglycoside may not be unconnected to the complexity of aminoglycoside and the difficulty in its administration (Onanuga *et al*., 2005). Even though first generation cephalosporins were reported to have high activity aganst *S. aureus* infections in the study of Ameh *et al.* (2003), certain *S. aureus* isolates in this study were resistant to the tested cephalosporins. Resistance of *S. aureus* isolates in this study to β lactams is often mediated by a small plasmid encoding a penicillin hydrolyzing enzyme known as penicillinase (Deurenberg and Stobberingh, 2008). The reduced susceptibility of *S. aureus* to cefoxitin and oxacillin is a phenomenon that was considered as a standard for phenotypic characterisation of MRSA (Valesco *et al.*, 2005) and isolates resistant to cefoxitin are particularly important for detection of low level MRSA (Witte, 2008). Hence, CLSI recommended that before isolates are reported either as resistant or susceptible to oxacillin, test results should be obtained on cefoxitin; as it was the recommended method for phenotypically testing *mec*A gene of *S. aureus* (CLSI, 2010).

On the other hand, *S. aureus* isolates in this study showed high level of susceptibility to ciprofloxacin 20 (90.9%), cefuroxime 17 (77.2%), Azithromycin (72.7%), Sulphamethoxazole/trimethoprim (63.6%) and Amoxicillin/clavulanic acid (63.6%). Even though, these are over-the-counter drugs and can be purchased without prescription in Nigeria, the high susceptibilities of microbes to these agents might be because they are among the newer antibiotics, relatively expensive and thus less available for abuse (Gebretekle and Serbessa, 2016). The *S. aureus* isolates in this study showed resistance to at least two antibiotics with only one isolate not resistant to any of the antibiotics tested. According to definition of Oteo *et al*. (2005); an isolate is said to be multidrug resistant, when it is resistant to at least two or more antibiotics. Such profile of antibiotic resistance occurs rather frequently in many of the *S. aureus* isolated in this study and several other works (Voss and Doebbeling, 1995; Melter *et al.*, 1999). Multiple antibiotic resistance patterns observed in this study conforms with the findings typical characteristical of MRSA strains as often resistant to multiple antibiotics and mostly to trimethoprim-sulphonamides, gentamicin and fluoroquinolones (Dietrich *et al.*, 2004). The cause of multiple antimicrobial resistances in this study may be rooted from poor practices of cilinicians such as misdiagnosis and prescription and patients' attitude towards the use of antimicrobials including over/under dosage, reckless purchase of drugs, and lack of awareness (Malfertheiner *et al*., 1993; Usluer *et al*., 2005) as well as systematic failure in most developing countries. Lack of regulations on sales of drugs, propagation of unliscensed drug vendors, sale of counter feit and substandard drugs are some of the factors that promote antimicrobial resistance in the population (Ayukekbong *et al*., 2017). Similar trends are seen in the use of antimicrobials

for non-human purposes such as use in growth promotion, abuse for animal treatments and as prophylaxis in fish farms (Peterson *et al*., 2002; Collignon *et al*., 2005).

Most *Salmonella* isolated from this study were susceptible to chloramphenicol (82.3%), cefuroxime (70.5%), sulfamethoxazole/trimethoprim (76%), gentamicin (82.3%), ciprofloxacin (88.2%), enrofloxacin (82.3%) and amoxicillin/clavulanic acid (64.7%). Equally, the antimicrobial resistance profile revealed resistance to erythromycin (5.8%), tetracycline (17.6%) and azithromycin (11.7%). Previous studies have shown a significant number of *Salmonella* isolates from other parts of Nigeria being resistant to tetracycline and erythromycin (Ayeni *et al*., 2016; Bata *et al*., 2016). A study conducted in Cameroun revealed antimicrobial resistance patterns of *Salmonella* isolates (34.1%) to twenty-one antibiotics, which included sulfamethoxazole/trimethoprim, gentamicin, ampicillin and penicillin (Stevens *et al*., 2006). In contrast to the antimicrobial characteristics of the *Salmonella* isolates in this study, findings of SjÖlund-karlsson *et al*. (2011) showed all *Salmonella* species in that study were susceptible to erythromycin, tetracycline and azithromycin. From the work of Nhung *et al*. (2016), *Salmonella* isolates were found to be sensitive to chloramphenicol, cefuroxime, sulfamethoxazole/trimethoprim, gentamicin, ciprofloxacin, enrofloxacin and amoxicillin/clavulanic acid; however, resistance was reported for erythromycin and clindamycin. Finally, ciprofloxacin (88.2%), chloramphenicol (82.3%), imipenem (94.1%) and gentamicin (82.3%) still stand out as the effective antibiotics to *Salmonella* isolates of this study and may be the best drugs of choice for treating *Salmonella* infections in this study area. Since chloramphenicol is a banned drug for use in humans, best options for treatment of bacterial infections due to *Salmonella* in the study area are gentamicin and ciprofloxacin. Presently, azithromycin and erythromycin have been recommended for the treatment of both shigellosis and

invasive salmonellosis by the World Health Organization and the American Academy of Pediatrics (WHO, 2005) as well as management of uncomplicated enteric fever (Butler *et al*., 1999; Frenck *et al*., 2004; Parry *et al*., 2007). However, clinical breakpoints for antibiotics are necessary for detection of emerging and changing minimum inhibitory concentration (MIC) patterns of existing antibiotics to microbes; leading to difficulty in the selection of effective antimicrobial therapy by clinicians.

The MICs of erythromycin and amoxicillin/clavulanic acids were determined towards establishing their susceptibility breakpoints against *Salmonella* isolates in this study area. The MIC distributions of the antibiotic to isolates were found to be above the recommended resistance break points reported by CLSI (2011). These findings are consistent with a study which reported erythromycin and amoxicillin/clavulanic acid susceptibility among non-Typhi and typhoidal *Salmonella* isolates (Gunell *et al*., 2010; Howie *et al*., 2010). The highest MIC value observed in the present study for erythromycin was 256 µg/ml, which was similar to the findings for human *Salmonella* isolates reported by Ojo *et al*. (2004). From results of this study, ciprofloxacin, gentamicin, chloramphenicol and cefuroxime, imipenem and enrofloxacin had best *in vitro* activities against *S. aureus* and *Salmonella* spp and may serve as drugs of choice for treatment of infections caused by these organisms.

The food safety knowledge, attitude and practices of principal actors in the value chains studied were evaluated. Fish farmers in this study had poor knowledge on ways of farming safe and hygienic fishes which might explain the high contamination levels of microorganism in fishes. Several studies have indicated the importance of food safety knowledge of food producers since good knowledge of production mostly translates into positive behaviour and practices leading to

safe food production and handling (Howes *et al*., 1996). Although, Rennie (1995) speculated that formal food safety training certification of food handlers may influence practice, the acquisition of knowledge such as mere provision of information informally cannot lead to direct change in attitudes and practices as presented in this study. Fish farmers with informal knowledge of food safety acquired from family business experience or colleagues were found to have poor knowledge of basic formal food safety measures. This model of assessment of knowledge, attitude and practices of food handlers was reportedly flawed in its assumption that basic informal knowledge is the main precursor to behavioural changes such as practice (Ehiri *et al*., 1997). In the findings of this study, factors such as food safety training, sources of food safety information and gender had influence on food safety knowledge and attitude of fish farmers. Although fish farming business is a high capital intensive business that requires skilled and high income owners, most of the information on fish production was reported to be acquired from trainings, workshops and extension services on effective livestock husbandry without including safe production of fish. Information and training from these sources are mostly done specifically for promoting production but not safety; as such most of the trainings lack the skills for safe production. Majority of farmers did have knowledge on production but could not transform the knowledge into safe practice. This is a typical trend in most training that targeted animal production industries in Nigeria, which aimed at encouraging farmers to institute biosecurity measures and prevent mortality and other economic losses but not for food safety reasons (Fishsite, 2003; Delia, 2015). As such, animal disease prevention skills may be transferred successfully to farmers but problems related to food safety and hygiene practices at farm level still remains a major challenge to long term prevention and control of food borne diseases originating from animal sourced foods. Even if farmers are trained on food safety issues,

lack of legal implementation and regulations targeted towards responsible safe production and delivery of food especially in developing countries have exacerbated the problem (Ellis *et al*., 1998). Most often, food producers are not considered as essential in food production chains even in developed countries until recently when WHO emphasized the need for including producers especially animal protein producers to practice good agricultural practices (GAP), good educational practices (GEP) and good training practices (GTrP) (Raspor, 2006). In classical food chain analysis of food safety and hygiene practices, all components of the chain from "farm to fork" or "farm to table" are assessed to identify hazards, evaluate likelihood of their occurrence and identify measures for control (Raspor, 2004).

Almost all respondents agreed that practices such as washing of hands, use of PPE and proper cleaning of the utensils (91.1%), are practices that reduce fish contamination. However, respondents failed to identify diseases/clinical conditions which could be transmitted via fish such as typhoid fever. Due to the increased awareness of general public on mode of transmission of diarrhoea-related diseases such as cholera, 62.2% of fish farmers were aware of transmissible diarrhoea-related diseases contracted through consumption of contaminated food. Most of the fish producers (85%) did not know that eating contaminated fish could lead to abortion in pregnant women. The later question was considered because foods including all types of raw meat and ready-to-eat products are considered potential sources for pathogens such as *Listeria monocytogenes* which could lead to spontaneous abortion or stillbirth (Jemmi and Stephan, 2006). Likewise, among all the questions targeting perceptions of fish farmers on food safety issues, transporting fish live (91.1%) and mixing different batches of fish (66.7%) were the most correctly answered questions. These two questions were related to fish health management and biosecurity measures to maximize profit and minimize loss from fish mortality. Unlike in human

health issues, livestock production is only seen as business for sole profit; where demand is the major issue of concern to farmers with less regard to public health significance of safe meat production. From veterinary point of view, knowledge and perceptions of farmers in controlling animal health and zoonotic diseases is paramount for successful implementation of food safety and hygiene measures. The choices and decisions of farmers for safe production of meat are essential to increase environmental protection, rural sustainability as well as promoting public health (Ellis-Iversen *et al*., 2010).

The knowledge of using PPE in reducing risk of fish contamination was similarly good in fish traders in this study. However, information on risk of transmission of food borne pathogens/clinical conditions of diseases such as typhoid (28.1%), diarrhoea (39.1%), cholera (37.5%) and abortion (12.5%) were unknown by fish traders interviewed in this study. Similar trend of poor knowledge and negative attitude of food handlers in food borne diseases at meat processing units have been reported in Turkey (TokuC *et al*., 2009), Portugal (Gomes-Neves *et al*., 2007) and Iran (Ansari-Lari *et al*., 2010). Factors such as years of business experience, food safety training, level of education and food safety-related knowledge and information had no influence on food safety knowledge, attitude and practices of fish traders in this study. The trend of knowledge, attitude and practices of fish traders in this study conforms with previous report that food handlers with positive attitude towards handling of food and reduction of cross-contamination eventually result in good processing practices (Ansari-Lari *et al*., 2010). In view of this, most of the fish traders in this study had good practices influenced by factors such as location of fish traders, role of fish traders in the fish market, age of fish traders and the fish form handled by fish traders. Regarding assessment on the value of storing fish at extremes of temperature for extended period of time, most of the fish traders had good knowledge, attitude

and practices of maintaining fish at low temperature until purchased. Unfortunately, traders in this study preferred to trade fish alive due to inconsistency in power supply in most cities in Nigeria. However, the risk of food borne diseases particularly caused by *S. aureus* enterotoxin is highly likely to occur because toxins are not inactivated even in extremes of temperatures. Thus, storage of fish at low temperatures will only inactivate pathogenic/vegetative bacteria or lower their metabolic activity to inhibit multiplication but does not eliminate the bacteria (Schmidt and Rodrick, 2003). Therefore, under favourable conditions of temperature and humidity, pathogenic bacteria found in or on fish surface multiply to considerable number that may cause disease. This circumstance can be more devastating to consumers, especially when organisms contaminate ready-to-eat fish and fish products which may not be reheated. This scenario when found in fish processing unit, can lead to food borne outbreak when more individuals consume fish from the same source (Walker *et al*., 2003).

In this study, the knowledge of fish processors was good (64.9%) with positive attitude (70.3%) but poor practices (13.5%). It has been reported that even when foof processors had good knowledge and attitude towards food safety, their bad practices are considered significant in final product safety (Raspor, 2007; Delia, 2015). It is evident that most surveys where processors had good knowledge but negative attitude towards food safety could not translate the knowledge into practice (Akabanda *et al*., 2017). Further reference was made on the use of PPE such as aprons, gloves, face mask and caps in this study, and very few fish processors' self-reported use of PPE. Participatory observation of fish processors that used apron in this study showed that it was not used for protection but rather for ease of safe-keeping of money. Personal hygiene practices such as cleaning contact surfaces before and after business, washing hands before and after handling raw fish and cleaning contact surfaces at the end of the day were practices frequently

implemented by few processors in this study. However, reasons for not practicing the listed hygiene measures might be related to reasons reported by WHO in which the organization stated reasons like aesthetics and/or for development of customer confidence but not strictly for food safety (WHO, 1996). Lack of use of hand gloves when handling fish by processors in this study posed increased risk for food borne disease because the hands of food handlers were reported to be the most important vehicle for transfer of pathogens from faeces, nose, skin or environmental sites onto food (WHO, 1989). Hence, the isolation of *S. aureus* and *Salmonella* species from fish samples in this study might be due to lack of proper use of PPEs by almost all the fish handlers in the value chans as well as use of untreated animal manure for pond fertilization, respectively. Several epidemiological studies of *Salmonella, S. aureus, Campylobacter* and *Escherichia coli* have demonstrated that these organisms can survive on finger tips and surfaces of hands for long periods of time even after mild hand washing in some cases (WHO, 1989). It was evident in this study that most of the processors had good knowledge and positive attitude towards hand washing and use of other PPEs but could not practice it, which confirms the report from a survey that most street vendors never use PPE when marketing ready-to-eat meat products in most developing countries (Khairuzzaman *et al*., 2014). This may be attributed to the fact that the source of water and hand washing or toilet facilities are not readily available in most of the vending sites (Idowu and Rowland, 2006; Omemu and Aderoju, 2008). In addition, they reported that most food vendors surveyed did not have access to any toilet facility whenever they are out hawking. Absence of basic facilities for toilet and hand washing has been reported in street food studies from other developing countries like Kenya (Muinde and Kuri, 2005) and Indonesia (van-Kampen *et al*., 1998). The typical factor associated with most street food vending in urban poor communities of developing countries was unavailability of toilet and lavatory facilities which

often force vendors to use secluded areas in place of a public toilet and do not wash hands afterwards (Bryan, 1988). Conversely, a study conducted by Azanza *et al*. (2005) in Philippine University campus reported high level of knowledge and consistent practice of hand washing by street food vendors with reasons related to availability of hand washing facilities designated in specific locations within the campus.

Almost all the principal actors of the fish value chains in this study did not stay away from fish handling whenever they were sick. This agrees with the findings from a survey by Omemu and Aderoju (2008) which showed that most vendors had no knowledge on the need to stay away from handling and preparation of fish when suffering from infectious conditions such as diarrhoea or to cover topical wounds on hands before handling of fish, which is of great public health concern. Thus, high chances of fish contamination from sick fish handlers and increased poor fish quality deducted from this study. Individuals with practices of eating at business places similarly had the chances of infection with microbes responsible for typhoid, cholera and other diarrhoeal diseases. The ignorance of the risk of transmission of infectious pathogens or contracting infection from fish as well as perception of fish handlers that it is not within their responsibility to institute food control at business level may have resulted in negligence of food safety control (Omemu and Aderoju, 2008). The major concerns of most fish traders in this study were the price and volume of fish sold per day but not the freshness or cleanliness of the fish. This agrees with the work of Abdussalam and Kaferstein (1993) which reported that vendors most often consider buying low quality fish from dubious sources to maximize profit of final product by selling at lower price. This type of practice is common in most animal sourced proteins such as beef, poultry and fish; where contamination could persist through preparation and/or cooking to the level of consumption (Abdussalam and Kaferstein, 1993). In some

countries, public health codes require food handlers to undergo some form of examination or screening test before they can be employed as food vendors (Musa and Akande, 2002).

Street vended foods have also been positively implicated as vehicles of food borne diseases such as cholera and typhoid fever (Abdussalam and Kaferstein, 1993; Castillo, 1994); which makes storage time, temperature and covering of processed food essential for prevention of contamination and subsequent disease. Some fish vendors (43.6%) in this present study processed the fish and stored it several hours before sale, while only 16.2% further reheated the fish before sale. These final stages of processing are often inadequate to destroy bacteria that may be present. In addition, toxins produced from pathogens such as *S. aureus* are not inactivated by reheating of fish. Therefore, practices such as storage at ambient temperature with inadequate cooling and reheating measures, inadequate covering during display and undercooking of fish were identified as the key factors in fish handling that can contribute to FBD outbreaks (WHO, 1989). Storage of ready-to-eat foods intended for continuous serving are recommended to be protected from environmental contamination and to be kept at $\geq 60^{o}C$ for food served hot or $\leq 0^{o}C$ for food served cold while food served frozen are expected to be kept at $\leq -18^{o}C$ (Mead *et al*., 1999). Thus, any food that is not maintained within this safety temperature zones does provide favourable condition for incubation and multiplication of pathogenic bacteria particularly animal sourced proteins in raw, partially cooked or fully done forms (Roller, 1999). Animal sourced proteins such as beef, poultry, pork and fish contain high level of nutrients that favour the growth and multiplication of pathogens which increase the chances of contracting diseases (Ensminger *et al*., 1994).

Food safety knowledge and attitude of consumers evaluated in this study included personal hygiene, cooking methods, cross contamination sources, storage time and temperature as well as handling methods as recommended by Hillers *et al*. (2003), Redmond and Griffith (2003) and Unusan (2007). Fish consumers interviewed in this study had excellent knowledge of personal hygiene. However, they had less knowledge on the role of germs in the cause of food borne diseases, importance of washing of raw fish thoroughly before cooking and proper storage temperature of fish before use. This is in agreement with the report of Ilu *et al*. (2016) who state that lack of proper knowledge on hygienic handling and preparation of fish, practices that favour microbial growth at household levels would predispose families to food borne diseases. Fish consumers interviewed similarly believed that fish safety could be judged based on fish appearance and smell and were more concerned of chemical residues in fish than microbial contamination (84.2%). Reasons for fish consumers' corncerns of chemical residues in fish tissue than microbial contaminants might be due to the high level of awareness regarding chemical and heavy metal contamination of soil and water in most part of Nigeria particularly the Niger Delta region (Etchie *et al*., 2013). Even in Northern Nigeria, the crisis of lead poisoning that occurred in 2010 in villages located in Zamfara State which resulted in the death of about 400 children due to consumption of lead contaminated water had generated serious public awareness of chemical harzards in Nigeria (MFS, 2012). Cases where pollution of aquatic water has been reported, emphasis still made were more on chemical pollutants such as pesticides, agro-chemicals and industrial pollutions than microbial contamination (Obasohan, 2009). Very few of the fish consumers (21.1%) opined that an individual could get sick by eating cooked fish, but others believed that no source of disease agents could be found in cooked food. Lack of

awareness of fish consumers on the role of food intoxicants from pathogens such as *S. aureus* might be linked to poor disease awareness within the communities in the study areas.

Fish consumers purchased fish daily from fish retail oulets and street vendors in the afternoon, packaged in plastic bags or foil paper, in cases of processed fish. This scenario is similar to the pattern of food security study described by Nkiru *et al*. (2015) that most residents in Nigeria were among the lower income class who normally provide to the family on daily basis after earning on a day job. The mean time for transporting fish from point-of-purchase to respective homes was 20.9±9.6 minutes to keep fish at room temperature for average of 13.6±14.8 minutes before cooking. This type of condition can be favourable for growth, multiplication and toxin production by pathogens such as *Salmonella*, *S. aureus* and *Escherichia coli* (FDA, 2012). The packaging of food products such as fish containing high moisture contents provides favourable pH condition for microbial growth and toxin production. Although, *Salmonella* are vegetative microbes that can be killed easily at unfavourable condition, there are reports of *Salmonella* species that grow at pH level of 3.0 (Anonymous, 2001).

The risk of FBD in consumers of fish sampled from Kaduna and Zaria metropolises was estimated using Risk Ranger tool for *S. aureus* and *Salmonella* spp. in raw, ready-to-eat and dried/smoked fish traded and consumed in the study area. Accordingly, the total predicted illnesses annually within the study population due to consumption of contaminated raw fish with *S. aureus* toxin was estimated at 513 and 156 cases for low and high risk groups with probability of disease occurrence at 0.03 and 1, respectively. In light of individuals consuming ready-to-eat fish without further processing, total predicted illnesses annually was estimated at 520 and 2,220 cases for high and low risk groups with probability of disease occurrence as 1 and 0.14,

respectively. Dried/smoked fish recorded total predicted cases of FBD as 520 and 53 at probability of 1 and 0.03, respectively for high and low risk groups. Risk Ranger ranked all the three fish product forms as high risk food product for FBD. On the other hand, predicted ilnessess from contaminated fish with *Salmonella* species were estimated at 520 and 111 cases for high and low risk groups, respectively with 1 and $7.12x10^{-5}$ chances of occurrence. Although these estimates may seem low, but the cases were considered to be average to assume that cooking methods may have influenced the presence of the organisms at the time of consumption. Thus, aggregate of cases were within the range reported in United Kingdom (EFSA, 2009). Total predicted FBD for consumption of ready-to-eat fish contaminated with *Salmonella* species were 171 and 111cases for high and low risk groups and 148 cases for consumers of dried/smoked fish or fish products. It is estimated that 93.8 million cases of salmonellosis occur globally each year, with 80.3 million of these being attributed to the consumption of unspecific contaminated food products and estimated 155,000 deaths are reported annually due to *Salmonella* infection (Majowicz *et al*., 2010). Surprisingly, majority of reported food-borne outbreaks are related to low-moisture products and principally due to *Salmonella* contamination (Van Doren *et al*., 2013). This pathogen is prevalent in raw ingredients and can survive under harsh and dry conditions for lengthy periods of time (Podolak *et al*., 2010). The survival of this bacterium is not only dependent on moisture of the food product but also on factors such as matrix composition and storage temperature (Finn *et al*., 2013). Even though *Salmonella* is a vegetative microbe that may hardly survive favourably in low moisture product for a long period, there are reports of *Salmonella* being persistent in dry food stuff for several years (Finn *et al*., 2013). As, such the chances of contracting FBD by eating dried/smoked fish still exist.

Similarly, risk ranking scored *S. aureus* as highly likely to cause FBD for all low and high risk groups consuming raw, ready-to-eat and dried/smoked fish with the exception of low risk individuals consuming dried/smoked fish (≤32). For both *Salmonella* and *S. aureus*, low risk groups were characterized as unlikely to contract FBD in dried/smoked fish category because most of the fish product forms were subjected to further processing by consumers before consumption. Hence, the chances of eating fish contaminated with these organisms were presented as low risk. Preserved meat products mainly fish such as dry fish or smoked fish are considered generally safe products due to categorization of food products likely to be contaminated at an early stage of processing (e.g. animal at farm or slaughter level) but used for meals that require further processing (Argudin *et al*., 2011). As such, even when the contamination level were high at production stage, the processing steps and the cooking methods for delicacies of African origin might reduce or eliminate the organisms at final stage prior to consumption (Egwim *et al*., 2013).

Following the well-structured approach in observation of activities within the value chains, risk profiles based on data found in this study were subjected to Spearman's rank correlation. Predictive factors for risk of fish contamination with these pathogens and the likelihood of FBD in the population of fish consumers in the study area included poor knowledge and attitudes and practices of the principal actors operating in the fish value chains and fish consumers as well as the poor environmental sanitation, presence of cross or recontamination factors, high retail temperature and prolonged processed fish storage time and the cooking methods. Thus, the listed factors except high retail storage temperature and prolonged processed storage time increases risk of poor fish quality. Environment of fish production, processing, distribution and marketing of fish are significant factors contributing to safety of product. Infrastructural support highly

influences food safety and hygiene practices of workers while lack of facilities such as water sources can hinder achieving maximum hygienic working environment (Subratty *et al.*, 2004). Proper personal hygiene means requirement of food handler to cover body using clean clothing and keep hands, nails, neck, hair, and clothing clean for the purpose of prevention of fish cross and/or recontamination (Guzewich and Ross, 1999). Evidence of bacteria on surfaces of containers, working environment and equipment increases risk of fish contamination (Sagoo *et al*., 2003). Fish and fish products displayed at normal atmospheric temperature may increase chances of contamination and promotes pathogen multiplication or toxin production (Phillips, 1996).

Time-temperature regulation in fish industry is an essential factor contributing to fish-borne diseases (Ahmed, 1991; FDA, 2011). Although regulation of temperature for certain period of time does not prevent fish and fish products from becoming contaminated; it usually prevents further multiplication of microorganisms and/or toxin production to the point of unsafe level. Food safety guidelines stated that chilled or hot fish must be maintained between 0^{o} C and 4^{o} C (FDA, 2012) or at extremes of 90-100^{o} C. Finally, regulation of time and temperature along entire food value chain is an important factor in reduction of FBD incidence (Ahmed, 1991; FDA, 2012).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The fish value chains in Kaduna and Zaria metropolises are mainly made up of the distribution channels of imported frozen fish which consisted of main distributors, wholesalers, retailers and the fish processors, while the domestically produced fish chains comprised of producers, wholesalers, retailers and fish processors.
2. Overall detection rate of *Salmonella* and *S. aureus* from fish value chains studied were 11.6% and 15.1%, respectively. *Salmonella* spp were mostly recovered from fish farm samples while *S. aureus* were commonly isolated in processed fish samples.
3. A total isolation rate of *mec*A (90%), *pvl* (70%), *sea* (10%), *seb* (50%), *sec* (20%), *sed* (70%), *see* (40%), and *sej* (80%) genes were detected in this study while (63.6%) *Salmonella* isolates harbored the *inv*A gene.
4. This study revealed that the *S. aureus* isolated in this study were mostly susceptible to chlramphenicol, cefuroxime, ciprofloxacin, gentamicin, sulphamethoxazole/trimethoprim and amoxicillin/clavulanic acid but resistant to cefoxitin, oxacillin, cephazolin, erythromycin and tetracycline.
5. Similarly, *Salmonella* isolates in this study were found to be susceptible to chloramphenicol, cefuroxime, ciprofloxacin, sulphamethoxazole/trimethoprim, gentamicin, enrofloxacin and amoxicillin/clavulanic acid but resistant to erythromycin, azithromycin and cephazolin.

6. The study demonstrated poor food safety knowledge by both fish producers and fish traders, positive attitude from fish traders and food processors with good practices by fish producers.
7. Risk factors such as lack of food safety training, food safety knowledge, attitude and practices, poor fish handling, presence of factors responsible for fish cross and re-contamination and duration of cooking time were found to influence microbial contamination level of fish in the study.
8. Total predicted illnesses annualy in Kaduna and Zaria metropolises due to risk of consuming contaminated uncooked, ready-to-eat and/or dried/smoked fish with *S. aureus* toxin were estimated between 156 and 2,220 cases for both low and high risk groups, repectively. Likewise, total number of FBD cases annually due to consumption of uncooked, ready-to-eat or dried/smoked fish contaminated with *Salmonella* species spaned from 111 to 520 cases in the study area.

6.2 Recommendations

On the basis of the findings reported in this study, the following recommendations are made:

1. The fish value chains of both domestic and imported fishes should be provided with necessary infrastructure such as supply of potable water and electricity, waste disposal services, good drainage systems, and toilet facilities to help facilitate the translation of the knowledge of street food vendors into practices.
2. The provision of alternative sources of electricity and training of all cadres of personnel involved in the fish value chain on technical and hygienic skills of fish production,

marketing and processing is significant in order to improve safe and hygienic production, distributin and handling of fish along the production line.

3. Fish safety training should be incorporated in workshops targeting farming skill acquisition, disease management and biosecurity measures for all stakeholders in order to develop the value chains in terms of fish safety, quality, sustainability and security.
4. Preventive and control measures of fish contamination should be imposed at all stages of food chains including the home to avoid frequent use of antibiotics.
5. Food safety and hygiene educational programmes should be made essential for behavioural changes to occur in all aspects of fish value chains.
6. Government should implement and enforce the services of Veterinarians to include inspection of fish preparation slabs and regulations of hazards associated with animal sourced proteins.

6.3 Contributions to Knowledge

1. The study has succeeded in mapping out the two common fish value chains in Kaduna and Zaria Metropolises.
2. The results of the studies similarly characterized the demographic and operational characteristics of the stakeholders involved in production and delivery of fish from farm onto the table of consumers within the State.
3. The study has found out good food safety knowledge and attitude by the fish handlers but poor practices of fish handling.
4. The study confirmed a prevalence rate of 15.1% and 11. 6% for *S. aureus* and *Salmonella* species isolated from raw and ready-to-eat fish samples respectively.

5. Risk factors associated with contamination of fish at selling point and processing units (market and household levels) included cooking time, poor environmental sanitation, and poor use of personal protection equipment, re- and cross contamination factors.
6. *Staphylococcus aureus* were susceptible to chloramphenicol, cefuroxime, ciprofloxacin, gentamicin, sulphamethoxazole/trimethoprim and amoxicillin/clavulanic acid but resistant to cefoxitin, oxacillin, cephazolin, erythromycin and tetracycline
7. *Salmonella* isolates were found to be susceptible to chloramphenicol, cefuroxime, ciprofloxacin, sulphamethoxazole/trimethoprim, gentamicin, enrofloxacin and amoxicillin/clavulanic acid but resistant to erythromycin, azithromycin and cephazolin

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APPENDICES

Appendix 1: Summary of reagents and media used in the study

Reagents and Media
Gram-Stain Reagents
Crystal violet solution
Crystal violet 500mg
Distilled water 100ml
Gram's Iodine Solution
Iodine 1.0g
Potassium iodide 2.0g
Distilled water 300ml
Safranin solution
Safranin 1.0g
Distilled water 100ml
Media
Media and Preparation protocols
Mueller Hinton Agar (Oxoid, England)
Beef, dehydrated infusion 300.0g
Casein hydrolysate 17.5g
Starch 1.5g
Agar 17.0g
Ph 7.3 ± 0.1 at 25˚C
Suspend 38g of the powder in 1L of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121˚C for 15 min.

Mannitol Salt agar (Oxoid, England)
'Lab-Lemco' powder 1.0g
Peptone 10.0g
Mannitol 10.0g
Sodium chloride 75.0g
Phenol red 0.025g
Agar 15.0g
Ph 7.5 ± 0.2 at 25˚C
Suspend 111g of the powder in 1L of distilled water and bring to boil to dissolve completely. Sterilise by autoclaving at 121˚C for 15 min.
Blood agar base (Oxoid, England)
Approximate formula per litre
'Lab-Lemco powder 10.0g
Peptone Neutralised 10.0g
Sodium chloride 5.0g
Agar 15.0g
Ph 7.3 ± 0.2

Suspend 40g of the powder in 1L of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121˚C for 15 min. For blood agar, cool the base to 50˚C and add to 7% of defibrinated sheep blood. Mix with gentle rotation and pour into petri dishes.

Tryptone soya broth
Pancreatic Digest of Casein 17.0g
Enzymatic digest of soya bean 3.0g
Sodium Chloride 5.0g
Di-potassium hydrogen phosphate 2.5g
Suspend 30g of the powder in 1L of purified water and distribute into final containers, Autoclave at 121^0C for 15 min.

Nutrient Agar (Oxoid, England)
Peptone 5.0g
Yeast Extact 2.0g
Sodium Chloride 8.0g
Agar 15.0g
pH 7.4 ± 0.2 at 25^0C
Suspend 28g of the powder in 1L of purified water, bring to boil and sterilize by autoclaving for 15 min.

SS- Agar (Fluka)
Meat extract and Peptone 5.0g
Lactose 10g
Sodium Citrate 10.0g
Sodium thiosulfate 8.5g
Feric acid 1g
Briiliant green 0.0003g
Neutral red 0.025g
Oxbile dehydrated 10g
Agar 15.0g
pH 7.0 ± 0.2 at 37^0C
Suspend 63g of the powder in 1L of purified water, bring to boil and but do not autoclave. Cool to about 50^o C, mix and pour into petri dishes.

Baird-Parker Agar Base (Oxoid, England)
Tryptone 10g
Yeast Extact 1.0g
Lab Lemco powder 5g
Sodium pyruvate 10g
Glycine 12.0g
Lithium chloride 5g
Agar 20g
pH 7.4 ± 0.2 at 25^0C
Suspend 65g of the powder in 1L of distilled water, bring to boil to dissoive completely. Mix well and distribute into containers. Sterilize by autoclaving for 15 min. Allow to set as slope with 2.5cm butts.

MacConkey Agar (Oxoid, England)
Peptone 5.0g

Yeast Extact 2.0g
Sodium Chloride 8.0g
Agar 15.0g
pH 7.4 ± 0.2 at 25^0C
Suspend 28g of the powder in 1L of purified water, bring to boil and sterilize by autoclaving for 15 min.

Appendix 2: DNA Extraction reagents and protocol

a) **Phenol solution** (Sigma St. Louis USA)
10 mM Tris HCl pH 8.0
1 mM EDTA, pH 6.7
b) **Sodium acetate** (3M) (Sigma, St. Louis, USA)
Sodium acetate regulates the salt concentration of the DNA strands for more adhesion to each other
c) **Lysozyme** (50 mg.ml-1) (Roche Molecular Diagnostics, Germany)
Lysozyme was used for the disruption of the Gram-positive cell walls by digestion of the polymeric cell compounds which are responsible for the cell firmness.
d) **Sodium dodecyl sulphate** (SDS) solution 20% (Promega, Madison, USA)
SDS 100 g
H2O 500 g
Add SDS powder to distilled H20 and bring volume to 500 ml. Sodium dodecyl sulphate is a detergent that lysis dissolving lipid molecules and therefore causing cell disruption
e) **Proteinase K** (20 mg.ml-1) (Finnzymes, Inqaba, Pretoria)
Proteinase K is an enzyme that denatures cell proteins during cell wall disruption
f) **TE Buffer**, pH 7.4 (Promega, Madison, USA)
10 mM of Tris-HCl, pH 7.5
1 mM EDTA, pH 8.0
TE buffer is used to disolve and re-suspend DNA

g) **Sodium Tris-HCl EDTA** (STE) buffer
0.1 M of NaCl (Promega, Madison, USA)
10 mM Tris-Cl, pH 7.5 (Promega, Madison, USA)
1 mM EDTA, pH 8.0 (Promega, Madison, USA

Appendix 3: Pictures of fish farms taken to demonstrate observational survey results

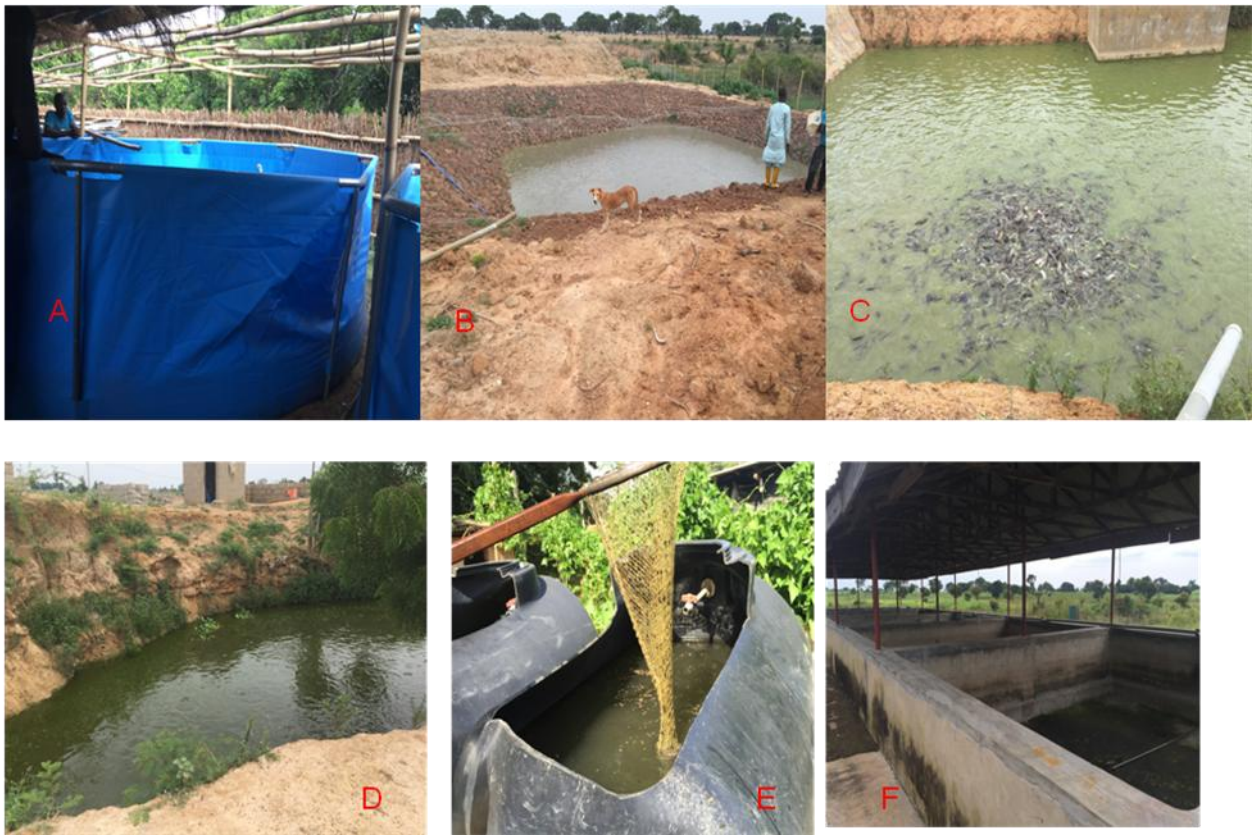


Plate I: Different types of fish culture systems practiced in fish farms visited in Kaduna and Zaria metropolises, Aand E are plastic ponds, B, C and D are earthen ponds and F is a concrete pond

Appendix 4: Pictures of fish markets taken to demonstrate observational survey results



Plate II: Different practices practices by stakeholders along the fish value chains of Kaduna and Zaria Metropolises, A shows method of fish harvesting in earthen pond, B is the process of weighing fish from farms amd transportation utensils, C shows weighing and distribution of fish in fish market visited, D is showing the weighing of fish from fish distributors transporting fish from neighbouring states, E showis display of wild caught fish in the fish markets while F shows carriage of live fish in water at the point-of-sale.

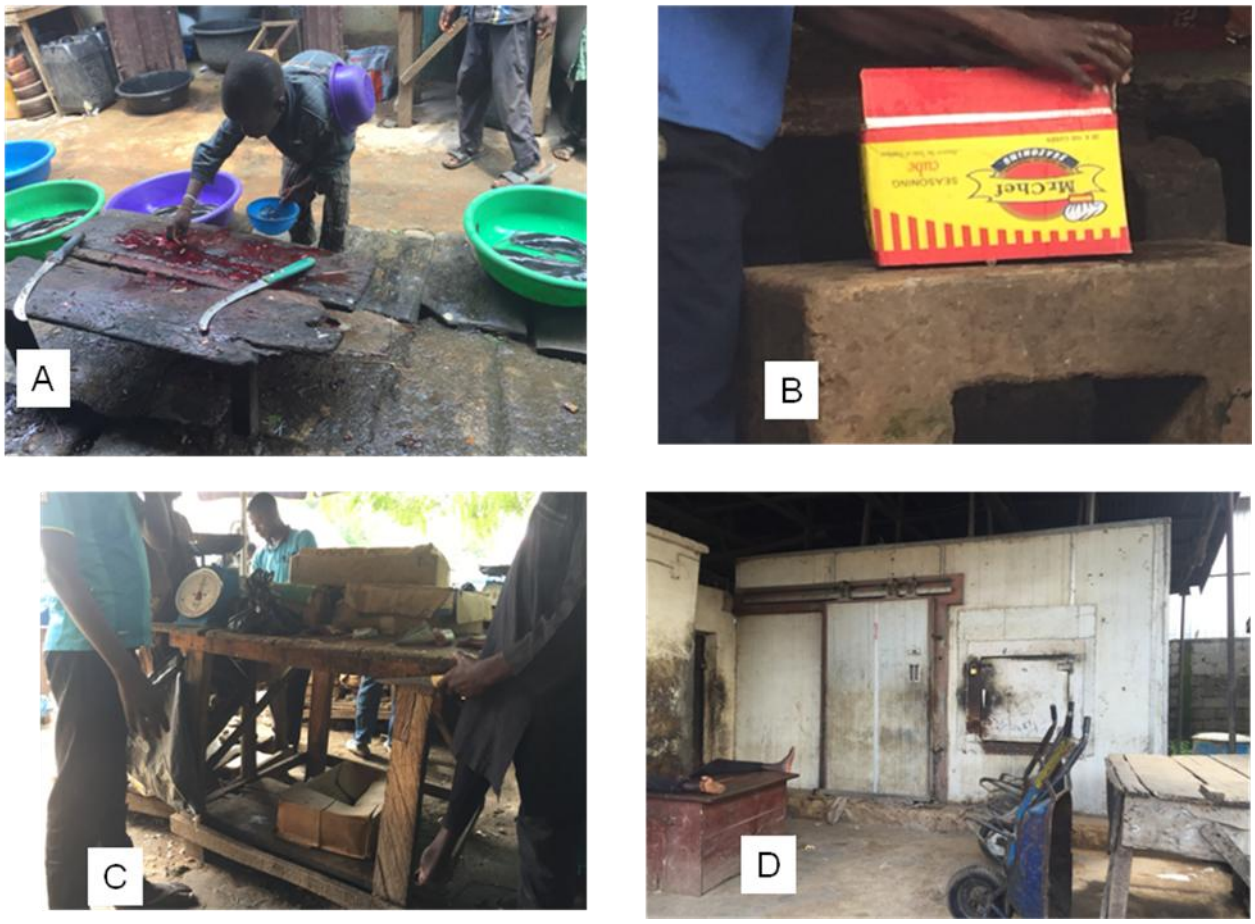


Plate III: Title A is showing the picture of improvised slaughter slab/killing slab of fish, B shows picture of packaging method for transportation of live catfish, C presents the tables and weighing scale for marketing of frozen fish, D is showing the cold room depot for storage of large scale frozen fish.

Appendix 5: Pictures of fish processing outlets taken to demonstrate observational survey results



Plate IV: Title A presents the method of roasting frozen fish, B and C presents the process of thawing and frying of frozen fish, D presents a fish processor processing wild cautgh pelagic fishes for onward frying, E is showing the processing of smoking fish for preservation/consumption while F presents the process of grilling catfish.

Appendix 6: Food safety hazards observed in participatory observation



Plate V: A is a picture of catfish seller using his teeth to label his fish stock, B is a fish seller eating food at the premises of the market, C is a boy "almajiri" scavenging on the left over visceral organs after fish was degutted for customers, D is a picture of smoked fish left open and mixed with wild animal meat for market display, E is a picture of fish left on the floor, F is a picture of a young girl urinating in a gutter beside a fish seller utensils.

Appendix 7: Total aerobic plate counts and Total coliform counts

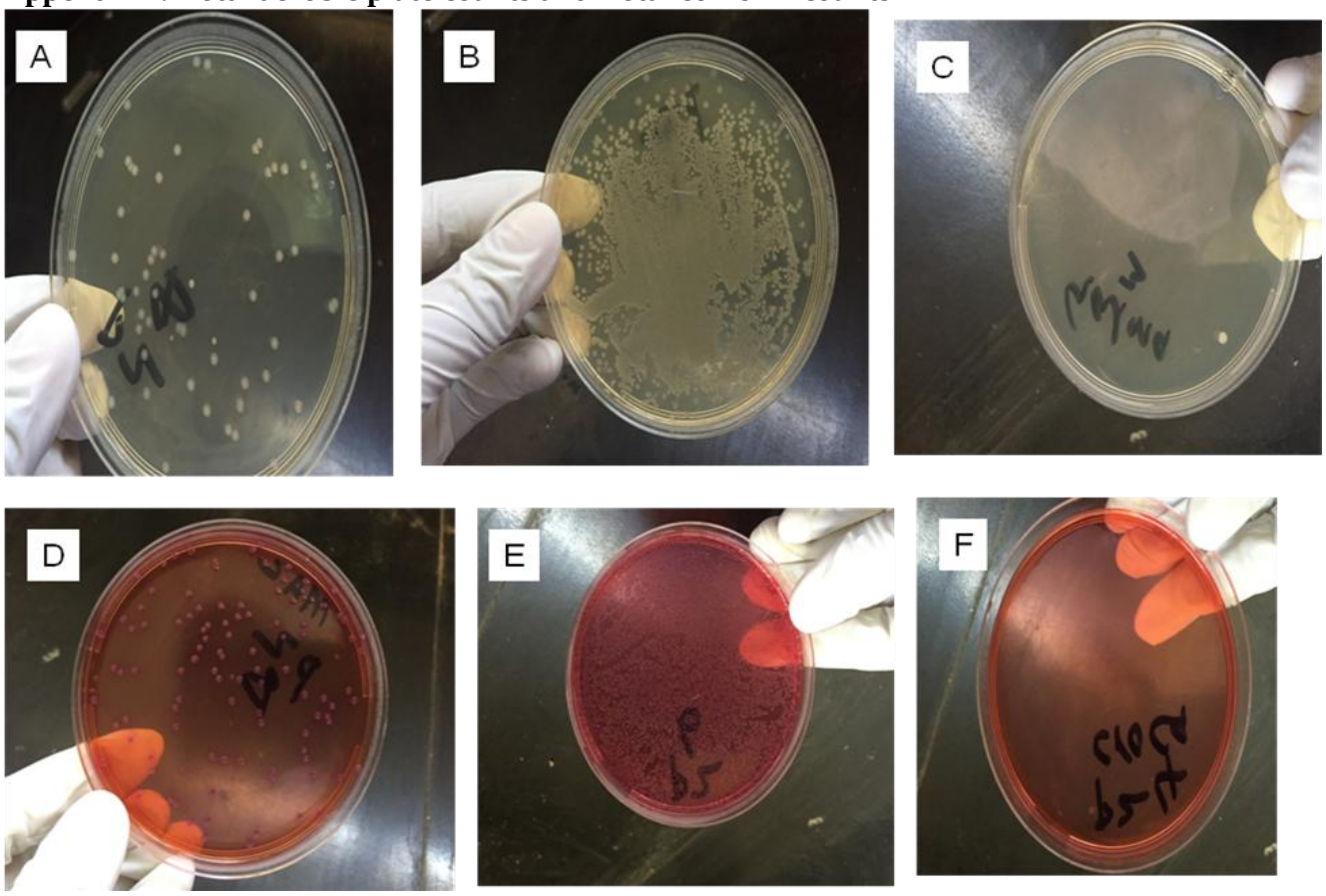


Plate VI: A is a nutrient agar plate for total aerobic plate count with a colony forming unit between 30-300, B is a nutrient agar culture plate with a colony forming unit above 300 which signifies too numerous to count (TNTC), D is a MacKoncey agar plate with colony forming unit between 30-300 for coliform counts, E is a plate with colony forming unit above 300 while F is a plate with 0 colony forming unit signifying no growth (NG).

Appendix 8: Cultural morphology of *Staphylococcus aureus* on Baird Parker medium and other biochemical tests conducted

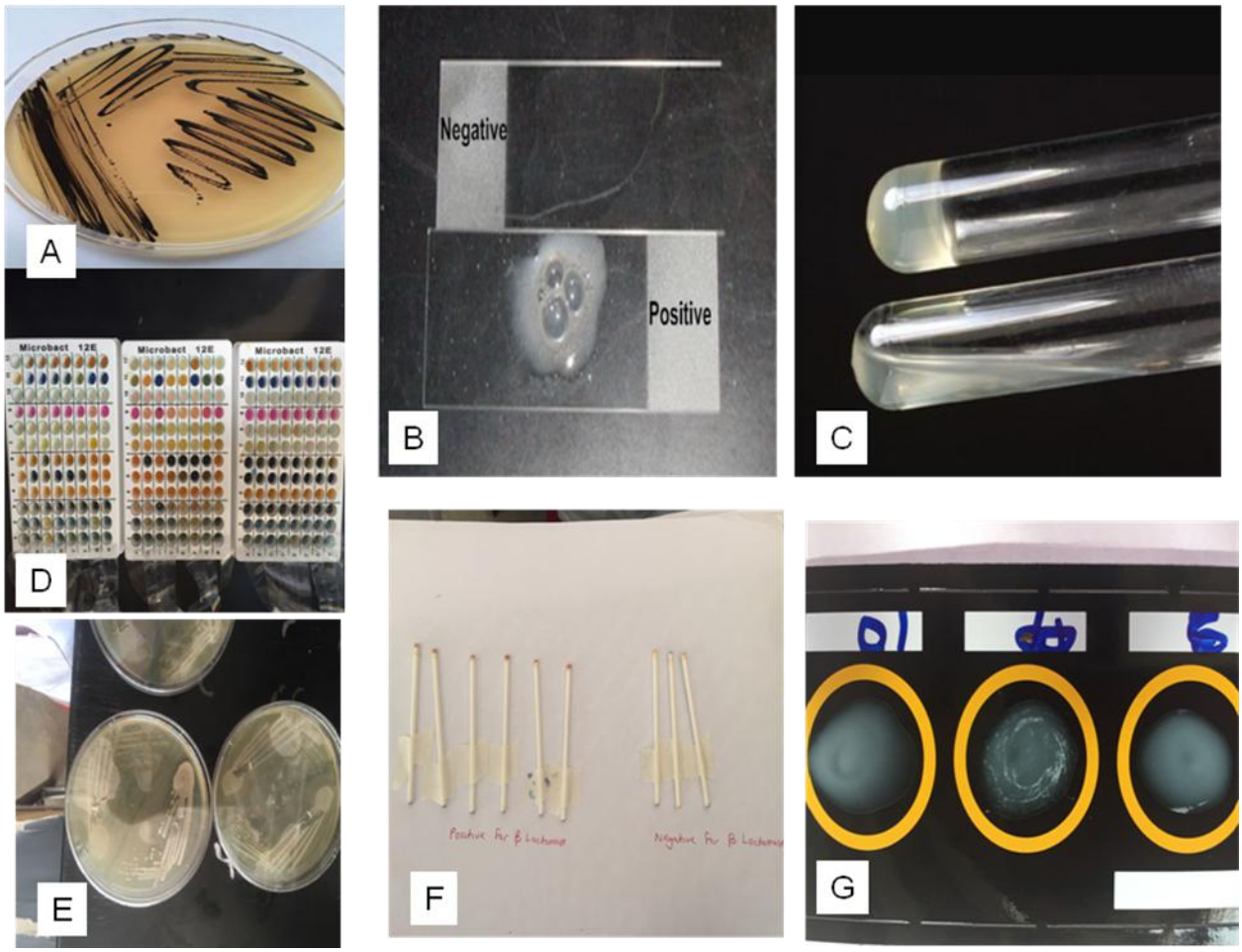


Plate VII: A is the cultural morphological of *S. aureus* on Baird Parker medium, B is a picture of positive catalase test and D is for positive coagulase test, E is a picture of positive DNase test, F is a picture of β lactamase test and G is for positive PBP 2a test.

Appendix 9: Results of Cultural and Biochemical identification of *Staphylococcus aureus* obtained from different sources of fish

ID	Color/Shape	Coa	Cat	Hem	Pig	Arab	Gal	Fruc	Lac	Glu	Mal	Man	Raf	Suc	DNase	PBP	β	Microbact	Spp %
S1	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	*S. aureus*	95.66
S2	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	*S. aureus*	99.98
S3	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	100
S4	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	95.66
S5	Black/coccoid	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	*S. aureus*	95.66
S6	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	91.47
S7	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	*S. aureus*	67.72
S8	Black/coccoid	+	+	+	+	-	+	+	+	+	-	+	-	-	+	+	+	*S. aureus*	91.47
S9	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	*S. aureus*	67.72
S10	Black/coccoid	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	*S. aureus*	99.97
S11	Black/coccoid	+	+	+	+	-	+	+	+	+	+	-	-	+	+	-	+	*S. aureus*	49.21
S12	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	*S. aureus*	99.95
S13	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	95.66
S14	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	91.47
S15	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	80.19
S16	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	99.67
S17	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	*S. aureus*	72.62
S18	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	100
S19	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	67.72
S20	Black/coccoid	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*S. aureus*	72.65
S21	Black/coccoid	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	*S. aureus*	95.66
S22	Black/coccoid	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	*S. aureus*	49.21

Key words: Coa=coagulase test, Cat=Catalase test, H=Hemolysis, P=Pigmentation, Arab=Arabinose, Gal=Galactose, Fruc=Fructose, Lac=Lactose, Mal=Maltose, Man=Mannitol, Raf=Raffinose, Suc=Sucrose, β=β Lactamase detection test, PBP=PBP 2a detection test, Spp=Microbact test speciation

Appendix 10: Antimicrobial susceptibility test of *Staphylococcus aureus* from different sources

S/No.	Sample ID	Source	Fish type	Fish form	FOX	OX	C	AZM	CRO	TE	KZ	CIP	VA	SXT	AMC	E	CN
1	S1	Retailer	Catfish	Raw	R	R	I	S	S	R	R	S	R	S	S	R	S
2	S2	Processor	Tilapia	Grilled	S	S	S	S	I	R	S	S	S	S	S	S	S
3	S3	Processor	Frozen	Roasted	R	I	S	S	R	S	I	S	S	S	S	I	S
4	S4	Farm	Catfish	Raw	R	R	R	I	R	R	R	S	R	R	R	R	S
5	S5	Processor	Frozen	Roasted	R	R	I	R	S	R	R	S	R	S	S	R	S
6	S6	Processor	Catfish	Grilled	R	R	R	R	R	R	R	I	R	R	R	R	S
7	S7	Retailer	Catfish	Raw	R	R	I	R	S	R	R	S	R	R	S	R	S
8	S8	Retailer	Tilapia	Raw	R	R	I	R	S	R	R	S	R	S	S	R	S
9	S9	Processor	Catfish	Grilled	R	R	R	R	S	R	R	I	R	R	R	R	S
10	S10	Retailer	Catfish	Raw	R	R	I	I	R	S	R	R	R	R	R	R	R
11	S11	Processor	Tilapia	Grilled	R	R	R	R	R	R	R	S	R	I	S	R	S
12	S12	Processor	Catfish	Grilled	R	S	S	S	S	S	S	S	S	S	S	S	S
13	S13	Processor	Tilapia	Grilled	R	R	R	R	I	R	R	S	R	R	S	R	S
14	S14	Retailer	Catfish	Raw	R	R	I	S	S	R	R	S	R	S	S	R	S
15	S15	Retailer	Catfish	Raw	S	I	S	S	I	S	I	S	S	S	S	I	S
16	S16	Processor	Frozen	Roasted	R	S	S	S	I	S	I	I	S	S	S	I	S
17	S17	Retailer	Tilapia	Raw	R	R	I	I	R	R	R	S	R	I	R	R	S
18	S18	Processor	Catfish	Grilled	R	R	R	R	I	R	R	S	S	R	S	R	S
19	S19	Processor	Catfish	Smoked	R	R	I	R	I	R	R	R	R	S	S	R	S
20	S20	Retailer	Catfish	Raw	R	R	R	R	I	R	R	S	R	R	R	R	S
21	S21	Retailer	Tilapia	Raw	R	R	I	R	I	S	R	S	R	S	R	R	S
22	S22	Retailer	Catfish	Raw	S	R	S	S	S	R	S	S	S	S	S	S	S

Key words: FOX=Cefoxitin, OX=Oxacillin, C=Chloramphenicol, AZM=Azithromycin, CRO=Cefuroxime, TE=Tetracycline, KZ=Cephazolin, CIP=Ciprofloxacin, VA=Vancomycin, SXT=Sulphamethoxazole/Trymethoprim, AMC=Amoxicillin/clavulanic acid, E=Erythromycin, CN=Gentamicin, R=Resistant, S=Susceptible, I=Intermediate

Appendix 11: Cultural morphology of *Salmonella* species on *Salmonella*-Shigella agar and Biochemical reactions

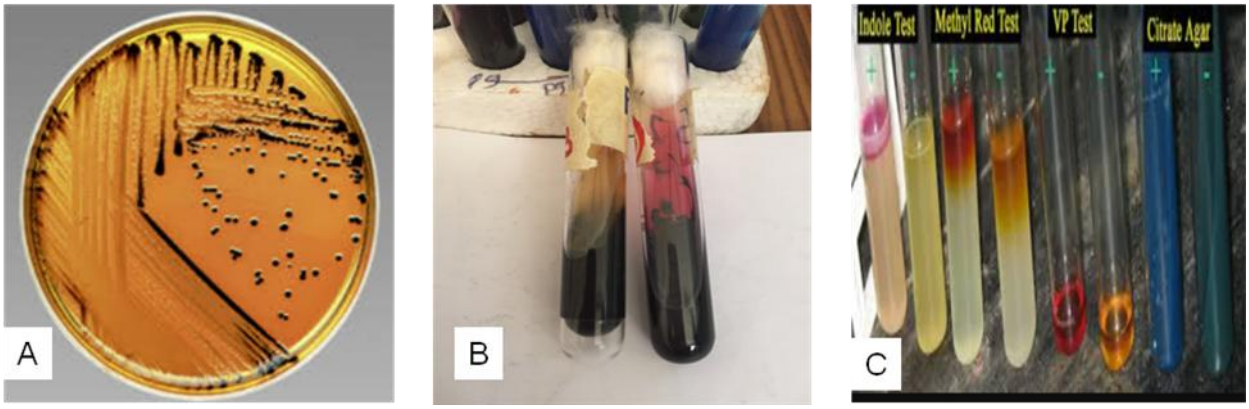


Plate VIII: A is a *Salmonella*-Shigella agar plate with cultural morphology of *Salmonella* species, B is a TSI test indicative of Alkaline/Acid + gas production signified as pinkish coloration of the slant and black coloration of the butt, C is the other biochemical test for identification of *Salmonella* species.

Appendix 12: Results of Cultural and Biochemical identification of *Salmonella* species obtained from different sources

ID	C	G	Ind	TSI	U	MR	VP	Ara	Du	Gl	In	Mal	Man	Raf	Rha	Sor	Sal	CAT	Microbact	Spp (%)
1	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	-	-	+	*S. arizonae*	47.76
2	-	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*S.cholreaesuis*	69.93
3	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	-	-	+	*S. arizonae*	98.11
4	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*Salmonella*	59.83
5	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	97.18
6	+	+	-	K/A	+	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	98.11
7	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*Salmonella*	35.38
8	-	+	-	K/A	-	+	-	-	-	+	+	+	+	-	-	-	-	+	*S. arizonae*	52.06
9	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*Salmonella*	59.83
10	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	-	*S. arizonae*	89.04
11	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	-	-	+	*S. arizonae*	47.76
12	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	-	*S. arizonae*	98.11
13	+	+	-	K/A	+	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	97.18
14	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	98.11
15	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	97.18
16	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*S. arizonae*	98.11
17	+	+	-	K/A	-	+	-	-	-	+	-	+	+	-	-	+	-	+	*Salmonella*	94.38

Key words: C=Colonial morphology, G=Gram Staining, Ind=Indole, TSI=Triple Sugar Iron Agar, U=Urease, MR=Methyl Red, VP= Voges Proskauer, Arab=Arabinose, Gal=Galactose, Du=Ducitol, In=Inositol, Mal=Maltose, Man=Mannosel, Raf=Raffinose, Rha=Rhabdose, Sor=Sorbitol, Sal=Salicin, Spp=Microbact test speciation

Appendix 13: Antimicrobial susceptibility test of *Salmonella* species obtained from different sources

S/No.	Sample ID	Product	Source	SXT	E	TE	C	CRO	CN	CIP	AZM	IPM	KZ	ENR	AMC
1	Sal 1	Raw	Market	S	R	R	S	S	S	S	R	S	R	S	S
2	Sal 2	Raw	Farm	S	R	S	I	R	S	R	R	S	R	R	R
3	Sal 3	Raw	Farm	S	R	R	I	S	S	S	R	I	R	S	S
4	Sal 4	Raw	Market	R	R	R	R	S	S	S	R	I	R	I	R
5	Sal 5	Raw	Farm	S	R	I	I	S	S	S	R	S	R	S	S
6	Sal 6	Raw	Farm	S	R	R	S	S	S	S	R	S	R	S	R
7	Sal 7	Frozen	Market	S	R	S	S	R	S	I	S	R	R	S	R
8	Sal 8	Raw	Farm	S	R	R	S	I	R	I	R	S	R	I	I
9	Sal 9	Raw	Farm	S	R	R	I	S	S	S	R	S	R	S	S
10	Sal 10	Raw	Market	R	S	R	R	R	S	S	S	S	R	S	S
11	Sal 11	Raw	Farm	S	R	R	I	S	I	S	R	S	R	R	S
12	Sal 12	Processed	Shop	S	R	R	S	S	S	S	R	S	R	S	S
13	Sal 13	Raw	Farm	S	R	R	I	S	S	S	R	I	R	S	S
14	Sal 14	Raw	Farm	I	R	R	S	R	R	I	R	S	R	I	R
15	Sal 15	Processed	Vendor	R	R	R	S	R	R	R	R	I	R	R	R
16	Sal 16	Raw	Farm	R	R	R	R	S	S	S	R	S	R	S	S
17	Sal 17	Raw	Market	S	R	R	S	S	S	S	R	S	R	S	S

Key words: IPM=Imipenem, ENR=Enrofloxacin, C=Chloramphenicol, AZM=Azithromycin, CRO=Cefuroxime, TE=Tetracycline, KZ=Cephazolin, CIP=Ciprofloxacin, VA=Vancomycin, SXT=Sulphamethoxazole/Trymethoprim, E=Erythromycin, CN=Gentamicin, R=Resistant, S=Susceptible, I=Intermediate

Appendix 14: Questionnaire for fish farmer survey, fish market and fish processing units

General Information
Name of Establishment…….Date……..
Name of respondent……
Role of respondent: Manager [ ] Worker [ ] Owner Other [ ]
Sex of respondent: Female [ ] Male [ ]
GPS: Lat……Long……Deg……Elevation……
Location of Farm……Metropolis……
Type of System: Backyard [ ] Commercial [ ] Monoculture [ ] Poly culture [ ]
Level of education: No formal education [ ] Primary [ ] Junior Secondary [ ] Senior Secondary [ ] Tertiary [ ] Arabic/Islamic [ ] Others [ ]
Area f Study: Sciences [ ] Social sciences [ ] Arts [ ] Technical [ ]
Did you attend any food safety related training? Yes [ ]No [ ] I don't remember [ ]
How do you get information on food safety measures? Business colleagues [ ] Friends/family [ ] Business association [ ] Radio [ ] TV [ ] Others [ ] (Specify)…….
How long have you been involved in this business (years)? …….
What type of fish do you handle? Catfish [ ] Tilapia [ ] Others [ ] (Specify)…….
Pond Area (s):
Nursery Pond (s):
Rearing Pond (s):
What year was the farm established? .....
Farm size < 1 hectare Between 1-2 hectares > 2 hectares
Who sell your product after harvest? Self Spouse Other family members Fish dealer
If you sell out fish, to whom do you sell your fish?
Fish retailer: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/productNone (0%) [ ]
Fish processor: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/productNone (0%) [ ]
Household consumers: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [Half (50%) of fish/product [ ] Quarter (25%) of fish/product None (0%) [ ]
Fish Dealer: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/productNone (0%) [ ]
Individual consumers: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/productNone (0%) [
Restaurant/Hotels: All (100%) of fish/product [ ] Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/productNone (0%) [ ]
Others: All (100%) of fish/product [ ]Three-quarter (75%) of fish/product [ ] Half (50%) of fish/product [ ] Quarter (25%) of fish/product None (0%) [ ]
Where are your fish sold after harvest? On-farm [ ] Fish market [ ] Others [ ] ……
In what form are they sold? Live [ ] Dead fresh [ ] Dead frozen [ ] Smoked [ ] Processed (fried, grilled, roasted etc.) [ ] Others [ ] (……)
Who determines the price of your products? Self [ ] Retailer [ ] Dealer [ ] Others …..
How are the prices determined? Prevailing market price [ ] Cost of production [ ] Arbitrary [ ]

What are the sizes of the fish at harvest (no. per Kg)? Small (3 or more fishes per Kg) [ ]
Big (A fish is ≥1Kg) [ ] Very big (1 fish is ≥2Kg) [ ]
What is price of the fish at harvest? ……
If you use antibiotics, what type of antibiotics? .....
How long do you stop applying antibiotics before harvesting? ..... days
Are there fish farms close to your farm? [_] Yes [_] No
If yes, what is the distance between your farm and the nearest farm? ..... m

Food safety knowledge

Please indicate your response to the following set of statements as true if you agree to the statement, false if you disagree or I don't know for statement (s) that you have no idea.

	Statements	**True**	**False**	**I don't know**
1	Washing hands before work reduces the risk of fish contamination			
2	Using gloves during work reduces the risk of food contamination			
3	Using apron during work reduces the risk of food contamination			
4	Hair covering during work reduces the risk of food contamination			
5	Proper cleaning and handling of contact surfaces reduces the risk of food contamination			
6	Farming to or more animal species increases the risk of food contamination			
7	If you have diarrhea, it is necessary to stay away from handling fish for consumption/business			
8	Typhoid can be transmitted by food			
9	Dysentery can be transmitted by food			
10	Diarrhea can be transmitted by food			
11	AIDS can be transmitted by food			
12	Cholera can be transmitted by food			
13	Abortion in pregnant women may be induced by food borne disease			
14	The refrigerator is the safest place to store fish			
15	Transporting fish alive in water reduces risk of fish contamination			
16	The mixing of fish from different sources increases risk of fish contamination			

Food safety Attitudes of fish handlers

Please indicate your response as agree, disagree or no idea as your level of agreement to the following set of statements.

	Statements	**Agree**	**Disagree**	**No idea**
1	Using gloves is important in reducing risk of fish contamination			
2	Using apron is important in reducing risk of fish contamination			
3	Using face masks is important in reducing risk of fish contamination			
4	Using caps is important in reducing risk of fish contamination			
5	Food hygiene training for farmers is important for reducing risk of fish contamination			

6	Sick people should not be involved in fish handling and preparation			
7	Fish fed dead animal carcasses can cause cross contamination			
8	Fish fed with animal manure can cause cross contamination			
9	Fish should be transported alive to reduce contamination			
10	Mixing fish of different sources increase risk of fish contamination			

Food hygiene practices

Please indicate your response as never, sometimes or always to reflect the frequency of performing the following practices in your business.

	Statements	**Never**	**Sometimes**	**Always**
1	Do you use gloves during work?			
2	Do you wash your hands before using gloves?			
3	Do you wear apron during work?			
4	Do you use face mask during work?			
5	Do you cover hair during work?			
6	Do you clean contact surfaces before you harvest fish?			
7	Do you clean contact surfaces during fish transportation?			
8	Do you wash your hands before you handle raw fish?			
9	Do you wash your hands after you handle raw fish?			
10	Do you use antibiotics in grow out ponds?			
11	Do you feed the fish with dead animal carcasses?			
12	Do you feed the fish with homemade leftover food?			
13	Do you remove dead fish immediately from ponds?			
14	Do you keep other animal species in your farm			
15	Do you mix fish from different sources during transportation			

Do you have any question regarding this interview?

Thank you for your time.

Appendix 15: Stakeholders Observation checklist

Facilities: Record the following for the sale location	**YES**	**NO**
a. Is there a permanent structure?		
b. Is it well ventilated and lightened?		
c. Is there pest control (Screen)?		
d. Is it a pollution prone area (close to industrial/Agricultural facility)?		
e. Is there a source of electricity?		
f. Is there a refrigerator or freezer present?		
g. Does the retailer have access to running water?		
h. Does the retailer have access to a hand-washing area with soap?		
i. Is the flooring and wall material concrete or tile?		
j. Is the area where fish are kept protected from the environment?		
k. Is the area where fish are kept clean (no obvious dirt or flies)?		
l. Are there any rotten fish or food around sale area?		
m. Is there a separate rubbish bin?		
Worker/ retailer conditions: Record the following for people selling the fish		
a. Do workers have clean designated clothing and shoes?		
b. Do workers have uncovered wounds?		
c. Do workers have any visible signs of communicable diseases?		
d. Are latrines present in the area?		
e. Are workers eating or drinking while selling fish?		
Storage conditions: ask to see where the fish are stored and record the following		
a. Are crates or storage equipment clean?		
b. Are plastic storage containers used (not baskets, etc.)?		
c. Is the fish on display?		
d. Is the displayed fish covered?		
d. Is the displayed fish in contact with other food products?		
e. Is the processed fish in refrigerator?		
Slaughter Slab Hygiene	**Yes**	**No**
a. Washing fish after evisceration		
b. Washing hands after evisceration		
c. Cleaning of slaughter slabs		
d. Cleaning of equipment		
Waste Management		
a. Presence of fish tissue left over on slaughter slabs		
b. Presence of waste bin (Covered/uncovered)		
c. Presence of drainage (Covered/uncovered)		

Appendix 16: Consumer household survey
Data entry number: _____

Interviewer name:	State
Metropolis	
LGA	

A. Household information

1. Provide the following details about the household head:

a. Sex	[__] Male	[__] Female
b. Age (years)		
c. Ethnicity		
d. Religion		
e. Level of education: (circle)	**1** = None **2** = Primary **3** = Secondary	**4** = Tertiary **5** = Vocational **6** = Other: _____
F Primary activity	1 = Crop farming 2 = Animal keeping (incl. sales) 3 = Trading in animal products (not own) 4 = Trading in ag. products ((not own produce) 5 = Formal Salaried employee	6 = Business – trade / services (non-agric.) 7 = Not working / unemployed 8 = Old/Retired 10 = Infant (<6 years) 11 = Student/ pupil 12 = Disabled 13 = Other (specify)

2. What is the position of the respondent in the household? (circle)
1 = Head **2** = Spouse **3 =** Daughter **4 =** Son **5** = Son-in-law **6** =Daughter-in-law **7** = Father of head
8 = Mother of head **9** =Mother- in-law **10** = Father-in-law **11**= Other: _____
3. Provide the number of people in the household (including the household head):

(Note: household members include all members living permanently within the compound and sharing the same meals)

	Children < 5 years	Children >5 to 18 years	Adults (>18 years)
a. Males			

b. Females			

4. How many of the following items does the household have?

Name of Asset	Number owned by HH
Domestic	
a. Cooker/ Gas Stove	
b. Refrigerator	
c. Radio	
d. Television	
e. DVD Player	
f. Mobile phone	
g. Mosquito nets	
Transport	
h. Car/Truck	
i. Motorcycle	
j. Bicycle	

5. What is the availability of the following utilities in the household? (circle all applicable)

a. Power	Electricity – constant	Electricity-intermittent	Generator	Kerosene lamps
b. Water	Piped	Well water	Borehole	Surface
c. Toilet	None	Flush	HH pit latrine	Shared pit latrine
d. Floor	Earth	Cement	Wood	Carpet tile

6. Do members of your household work in fish production or industry? Yes ☐ No ☐

7. How many months per year do you work in fish production or industry? _____mon

8. How important is this source of income for the household?

1= Only income source **2**= Major income source **3**= Same importance as other income sources **4**= Minor income source **5**= No or negligible income source

D. Purchase and handling:

13. Who usually decides whether to get fish? (circle below)

1= Head **2**= Wife **3**= Joint decision **4**= Other male **5**= Other female **6**= Other:_____

14. Of the following which do you prefer? Goat meat, chicken, rabbit, fish, Beef, others

15. On what basis do you select fish as animal protein?

[__] Price [__] Quality/ freshness [__] Size [__] Trusted retailer

What grade of fish do you purchase?

a. Grade 1 (Less than 3 fish per kg)	
b. Grade 2 (3-5 fish per kg)	
c. Grade 3 (More than 5 fish per kg)	
d. fish fillets	

e. Others (Specify)	

17. What time of day do you usually get your fish? (circle)
1= Early morning **2**= Mid-day **3**= Afternoon **4**= Evening **5**= Other:_____
From where do you get your fish?

	Always	Sometimes	Never
a. Purchase			
b. Produce on own farm			
c. From workplace (wages or purchased)			
d. Gift from workplace			
e. Gift from other people or charity			
f. Hunting/catching/fishing			

If you purchase fish, where do you purchase it from?

	Always	Sometimes	Never
a. Wholesale market			
b. Retail market (fresh fish)			
c. Supermarket			
d. Street vendor			
e. Fish fry shop (cooked fish)			
f. Restaurant			

19. How much fish is consumed in a single serving by the following people in the household?

	Number of fish
a. You	
b. Child (2-5 years)	
c. Pregnant women	

20. How are fish packaged when you get them from the source? (circle all that apply)
1= Plastic bag **2**= Sealed package **3**= No packaging **4**= Aluminium foil **5=**Other:_____
21. How long does it take to transport the fish home? _____ hours
22. How long do you usually keep the fish at home before cooking? _____hours
23. How do you usually store fish during this time (before cooking)? (circle)
1= Room temp **2**= Refrigerated/ ice **3**= Freezer **4**= Other: specify:
24. If you do not consume the fish on the same day, how long do you usually store it for? _____ days
25. How do you usually store fish during this time? (circle)
1= Room temp **2**= Refrigerated/ ice **3**= Freezer **4**= Other: specify:

26. How do you prepare the fish (include all methods that apply, ie. raw, grilled, in stew, etc.)?

Method	Cooking time (minutes)	Frequency of preparing this way (circle)
a.		Always Sometimes Rarely

b.		Always Sometimes Rarely

27. When do you wash your hands?
After using the toilet [_] before preparing food [_] before eating [_]
28. What do you use for washing your hands?
1= Tap water only **2**= Hot water only **3**= Hot water and soap **4**= Tap water and soap
5= Other:

29. Do you consume the fish with other food? Yes No
30. When you prepare fish and other foods at the same time, do you use the same knives and boards?
Yes No
31. What do you use to clean surfaces and utensils? (circle)
1= Cold water **2**= Hot water **3**= Soap **4**= Detergent **5**= Bleach **6**= Other:_____

32. What do you do with cooked fish leftovers? (circle)
1= Eat cold **2**= Eat reheated **3**= Give to animals **4**= Throw away **5**= Compost **6**= Other:_____
33. How long do you keep the fish after cooking? _____ days
34. How do you store the cooked fish? (circle) **1**= Frozen **2**= Refrigerate **3**= In kitchen **4**= Other:_____

Knowledge, attitudes and practice

35. Record whether you agree or disagree with the following statements:

Do you think…	Yes	No	I don't know
a. Eating fish is good for your health?			
b. You can get sick from eating fish?			
c. Fish is a highly nutritious food?			
d. The fish you eat may be contaminated with chemicals?			
e. Children under 1 year should eat fish?			
f. Fish safety can be judged by sight and taste?			
g. You would buy more fish if it was cheaper?			
h. You can get sick from well-cooked fish?			

C. Health information

36. Have you or any household members had any health problems in the past 2 weeks? Yes No
37. If yes, what type of health problems? (circle as appropriate)
1= Diarrhoea, vomiting, stomach pain **2**= Skin rash **3**= Fever, muscle pain or weakness **4**= Other: _
38. If you were sick, sick the disease management strategies implemented: