

BENEFITS, PUBLIC HEALTH HAZARDS AND RISKS ASSOCIATED WITH FISH CONSUMPTIONAdedeji OB¹, Okerentugba PO², Innocent-Adiele HC, Okonko IO²¹Department of Veterinary Public Health & Preventive Medicine, University of Ibadan, Ibadan, Nigeria²Department of Microbiology, University of Port Harcourt, P.M.B. 5323 Uniport Post Office, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria;

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ABSTRACT: This paper gives an overview of the benefits, public health hazards and risks associated with fish consumption. The comprehensive data regarding the environmental occurrence and levels, niches, survival, seasonality and strain diversity will be helpful for developing sea food strategies which helps to eliminate the risk of pathogens in exporting sea foods. Fish has become an increasingly important source of protein and other element necessary for the maintenance of and healthy body. Fish and seafood constitute an important food component for a large section of world population. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein. Fish and fish products constitute an important part in the international trade, more than 50 billions indicating increasingly consumer interest in commodity. The quality of our fish is of major concern to the food processors, consumers and public health authorities and provisions of safe, wholesome and acceptable fish and its product as food to consumers and control of microorganisms is essential to meet these objectives. The quality of fish degrades, due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated. However, some sea foods are processed in a modern fish industry which is technologically advanced and complicated industry in line with any other sea foods industry and with the same risk of products being contaminated with pathogenic microorganisms. Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products. Fish acts as an important food vehicle for some zoonotic pathogens such as *Salmonella* and vibrios. Contamination of fish with pathogen is a major public health concern. The presence of *Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan, Japan and Nigeria. Pathogenic and zoonotic *Vibrios* species have also been isolated from stool of diarrheic patients in recent studies, and associated with consumption of undercooked shellfish of which these microorganisms are implicated in outbreaks of food poisoning and diarrhea in humans. Some zoonotic vibrios have been detected in water samples. Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade. *V. parahaemolyticus* and *Vibrio vulnificus* among other pathogens poses a significant health threat to humans who suffer from immune disorders, liver disease, or hemochromatosis. *Vibrio* species carrying *tdh* gene in fish, oyster and diarrheic stool using PCR could be useful as basis for a preventive consumer protection policy. Recent studies have recommended further investigation for other virulent genes in pathogenic *Vibrios*. The public should be enlightened on the inherent danger that may accompany handling fresh fish or consumption of improperly cooked fish. HACCP Programs designed to prevent unsafe foods from reaching the consumer should be employed. Seafood processors must also keep in mind that HACCP does not replace GMPs or guard against fraudulent practices. Good manufacturing practices should always be observed by trade to minimise the risk of cholera and vibrio food poisoning associated with the consumption of seafood products. Hygienic qualities of fish tank water in particular the source water for keeping live seafood is also important.

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1. INTRODUCTION

Fish and seafood constitute an important food component for a large section of world population (Wafaa et al., 2011). They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein (Wafaa et al., 2011). Sea foods have traditionally being a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal

protein. Today, even more people are turning to fish as a healthy alternative to real meat (Adebayo-Tayo et al., 2012a). The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids food in fatty pelagic fish species are extremely important aspect for health conscious people particularly in affluent countries where cardiovascular disease mortality is high (Adebayo-Tayo et al., 2012a).

FAO (1994 cited by Emikpe et al., 2011) asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing world derives more than 30% of their animal protein from fish. Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe et al., 2011). Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues (WHO, 2007). However, consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics (Adebayo-Tayo et al., 2012a).

The term shellfish covers the bivalve molluscan shellfish (oysters, cockles, clams and mussels), the gastropods (periwinkles, sea snails) and the crustacean shellfish (crabs, lobsters, and shrimps). Seafood differs from other types of foods in a number of ways (Adebayo-Tayo et al., 2012a). Most seafood is still extracted from a wide population, and the fishermen are hunters with no influence on handling of their prey before it is caught (Adebayo-Tayo et al., 2012a). Thus, it is not possible to irritate the situation for slaughter animals, selecting only the most suitable specimen for slaughter and to rest and feed them well before killing (Adebayo-Tayo et al., 2012a).

When frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumer especially when the micro-organism present includes pathogenic ones (Adebayo-Tayo et al., 2012a). According to Higgins (2007), anyone who work in food safety sooner or later discover that one of the most valuable tools for prevention is simply reading about and understanding how past outbreak have occurred. Using major and frequently famous or at least newsworthy outbreaks, Phyllis (2007) illustrate critical factors come together to produce tragic and largely preventable results modern microbes often team up with old practice, short sighted decision or current consumer tend to produce an outbreak.

Bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish or shellfish (ICMSF, 1998). The microbiological flora in the intestines of sea foods such as finfish, shellfish and cephalopods is quite different being psychotrophic in nature and to some extent believes to be a reflection of general contamination in the aquatic environment. In filter feeding bivalve molluscan shellfish (oyster) and accumulation and concentration of bacteria and viruses from the environment is generally taking place (Adebayo-Tayo et al., 2012a). However, some sea foods are processed in a modern fish industry which is

technologically advanced and complicated industry in line with any other sea foods industry and with the same risk of products being contaminated with pathogenic microorganisms (Adebayo-Tayo et al., 2012a).

Several studies have demonstrated many bacteria species encountered in different fish which are potentially pathogenic under certain conditions as reported for *Pseudomonas angulluseptica* and *Streptococcus sp.* (Emikpe et al., 2011). It has been estimated that there are more than 80 million cases per annum of sea food borne illnesses on antibiotic resistance in the United States of America and that the cost of these illnesses is the order of many billions of dollars per year (Adebayo-Tayo et al., 2012a). The economic losses due to spoilage are rarely quantified but a report by the US National Research Council Committee (FND/NRC) estimated that one-fourth of the world food supply is lost through microbial activity alone (EEC, 1992). Thus, the need for control of quality of our sea foods to avoid high microbial contamination which may lead to antibiotics resistance is well documented and since the rate of seafood borne illnesses is increasing, there is also an urgent means of assuring quality of sea food (Adebayo-Tayo et al., 2012a). These studies about the human pathogenic bacterial load, particularly enterobacteria in fresh water are scanty in literature (Emikpe et al., 2012).

The consumption of fresh African Catfish (*Clarias gariepinus*) and wild Tilapia fish (*Oreochromis niloticus*) is on the increase in both rural and urban centres in Nigeria (FDF, 2007; Emikpe et al., 2011). However, there is dearth of information on the bacterial load in African catfish and Nile Tilapia sampled from ponds and natural water (Emikpe et al., 2011). Thus, the present study was designed to give an overview of the benefits and risk associated with the consumption of wild and cultured Tilapia source from different aquatic environments. The comprehensive data regarding the environmental occurrence and levels, niches, survival, seasonality and strain diversity will be helpful for developing sea food strategies which helps to eliminate the risk of pathogens in exporting sea foods. This paper therefore, gives an overview of the risks and benefits of fish consumption. The public health implications are also discussed.

2. FISHES

Fish is an important food commodity in the international trade but they deteriorate rapidly especially when storage facilities are lacking. It has been widely accepted as a good source of protein and other elements necessary for the maintenance of healthy body (Adebayo-Tayo et al., 2012c). Fishes are members of the super class Pisces, but those having a

conspicuous set of feelers surrounding the mouth are called the catfishes. The term 'Fish' encompasses all sea foods including crustaceans with a chitinous exoskeleton such as lobsters, crabs, shrimps and mollusks such as mussels cockles, clams and oysters (Adebayo-Tayo et al., 2012c). Fish are aquatic Vertebrate that is typically cold. There are more kinds of fishes than all other kinds of water and land Vertebrates put together and this various kind of fish differ so greatly in shape, colour and sizes (Adebayo-Tayo et al., 2012c).

In this review, the type and species of fish was narrowed down to the tilapia and cat fishes. Fish or catfish generally encompasses all sea foods including crustaceans with chitinous exoskeleton such as lobsters, crab, shrimps and such as muscle cockles and Oyster (Adebayo-Tayo et al., 2012c). All catfishes have either smooth or armored naked bodies with bony plate. The dorsal and pectoral fins are often edged with sharp spines that are used for defense. They can inflict severe wounds and are poisonous in some species; this feature is usually for protection from predators (Adebayo-Tayo et al., 2012c).

Tilapia is ranked as the second most widely farmed fish in the world (Adebayo-Tayo et al., 2012c). They are farmed in at least 85 countries, with most production coming from Asia and Latin America (Eknath et al., 2007; Liu et al., 2010; Adebayo-Tayo et al., 2012c). In 2007, tilapia production of China reached 1,210,000 tons, approximately up to 49% of the global yield (Li and Cai, 2008; Liu et al., 2010; Adebayo-Tayo et al., 2012c). The majority (approximately 66.7%) of tilapia production in China is sold alive in domestic market and the remaining are frozen for exportation or used for further processing (Li and Cai, 2008; Liu et al., 2010; Adebayo-Tayo et al., 2012c).

Molluscan bivalves are filter feeders and they tend to accumulate microorganisms in the surrounding waters which may also contain vibrios (FEHD, 2005). They are usually grown and harvested in shallow, near-shore estuarine waters and are therefore likely to harbour high concentrations of pathogenic organisms including pathogenic vibrios (FEHD, 2005). As they often are eaten raw or after a very mild heat treatment, they constitute a significant health risk to the consumers (FEHD, 2005).

2.1. MARKETING OF FISH IN NIGERIA

Marketing of Fish in Nigeria is mostly carried out by local fish sellers at ambient temperature, therefore knowledge of spoilage patterns of tropical fishes and their shelf life under ambient conditions is very important (Okoro et al., 2010; Adebayo-Tayo et al., 2012d). Refrigeration temperatures are also relevant because they are used by most households in Nigeria for temporary storage of fish (Okoro et al.,

2010; Adebayo-Tayo et al., 2012d). Frozen state condition is also important since most fishes consumed in Nigeria are imported and they usually come in frozen state (Okoro et al., 2010; Adebayo-Tayo et al., 2012d).

2.2. FISH QUALITY

In a study by Emikpe et al. (2011), fish samples of different sources were contaminated with total aerobic bacteria as well as enterobacteria. Fish of good quality should have bacterial count less than 10^5 per gram and what obtained from fish samples examined in the Emikpe et al. (2011) study exceeded the acceptable limit recommended by Food and Agricultural Organisation (1979 cited Emikpe et al., 2011). The study by Emikpe et al. (2011) also established the poor microbial quality of fish, both wild and cultures *C. gariepinus* and *O. niloticus* in some areas of Ibadan, southwest Nigeria (Emikpe et al., 2011). This indicates human health risks due to consumption of fish collected from pond and river from this area (Emikpe et al., 2011).

Varieties of quality attributes have been used to assess fish freshness in many cold water fish species as sea bream, sea bass, sardine and European eel (Hernandez et al., 2009; Liu et al., 2010; Adebayo-Tayo et al., 2012d). Many indices have been used for the assessment of fish quality during storage (Sallam, 2007). Such indices comprise changes in the microbial population (Hozbor et al., 2006; Sallam, 2007), chemical changes (Sallam, 2007), as well as changes in sensory attributes (Sallam, 2007; Adebayo-Tayo et al., 2012d). However, few researches were reported on quality assessment for tropical freshwater fish species (Liu et al., 2010; Adebayo-Tayo et al., 2012d).

2.2.1. Physiochemical Levels of Fishes

Fish as earlier defined are generally vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays (Adebayo-Tayo et al., 2012c). The physiochemical levels of these fishes found in Habitats that have already being polluted by industrial discharge and waste disposal may either kill these fishes or render them inedible which if not taken note of might cause a great deal of harm to man and his environment (Adebayo-Tayo et al., 2012c).

Many species of fish (catfish) normally live in fresh water lakes and rivers. The term or word "Fish" are generally defined as aquatic vertebrate that are typically cold blooded covered with scales and equipped with two sets of paired fins and several unpaired fins that use gills to obtain oxygen from humbler of skeletal called Fin-Rays, put together and thus various kinds of fish greatly in shape sizes and colour (Adebayo-Tayo et al., 2012c).

The effect of physical condition and chemical agents on the growth of microorganism in fish has

been investigated and well documented and not much of information on the spoilage of fish. Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Sil et al., 2008; Liu et al., 2010). In the recent time, modern biotechnology have introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life and also impact disease resistance to the fish (William and Michael, 2009; Okoro et al., 2010).

2.2.2. Microbiological and physiochemical changes and its correlation with quality indices of tilapia fish (*Oreochromis niloticus*)

In Nigeria, the microbiological and physiochemical analysis of Tilapia fish by Adebayo-Tayo et al. (2012c) revealed that fishes from some parts of the country contained high microbial load but are nutritionally richer. The physiochemical and Nutritional composition of the tilapia fish samples revealed that they serve as food sources of protein and mineral elements (Adebayo-Tayo et al., 2012c). In a study by Adebayo-Tayo et al. (2012c), samples from Itu had the highest moisture content than does from Uyo, these suggest that these fish samples analyzed may not be storable for a long while due to the high water activity. Crude protein was high in Uyo samples Itu which is probably due to favourable environmental conditions (Adebayo-Tayo et al., 2012c). The high level of protein source for the alleviation of Kwashiorkor arising from proteins malnutrition especially in diets meant for children. According to Adebayo-Tayo et al. (2012c), the high ash contents of the samples from Uyo compared to Itu may be due to pollution of these aquatic habitats were the fish samples were gotten by crude oil. This also suggests higher level of mineral elements in these samples as against non contaminated areas, since uptake of water, salts and mineral elements are carried out by these fish samples (Adebayo-Tayo et al., 2012c). In order to derive full nutritional potentials of these fish, they should be consumed with fat-giving foods since low energy may lead to low birth weight and increased infant mortality (Adebayo-Tayo et al., 2012c). Considering the public health implications of the poor bacteriological and mycological qualities of these fish samples, particular attentions should be paid to their safety through proper processing, storage and handling procedures as most of these organisms are from the handlers (Adebayo-Tayo et al., 2012c).

2.2.3. Microbiological and Physiological Level of Fresh Catfish (*Arius hendelotic*)

From the previous work on catfish, the microorganism isolated from the different parts of these catfish samples such as intestine, gills and skin showed slight differentiation. The skins of these

catfishes contained more of the bacterial and fungal isolates due to their constant contact with the water (Adebayo-Tayo et al., 2012d). The presence of *Aspergillus spp* reported in the study by Adebayo-Tayo et al. (2012d) brings to mind the likely presence of toxin produced by this fungal isolate. This could become the possible source Aspergillois transmission among consumer. *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium spp.* and tend to dominate spoilage in warmer climates (Doyle, 2007; Adebayo-Tayo et al., 2012d). Many aspergilli produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid (Doyle, 2007; Adebayo-Tayo et al., 2012d). Aspergilli spoil a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices (Doyle, 2007; Adebayo-Tayo et al., 2012d).

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions (Doyle, 2007; Adebayo-Tayo et al., 2012d). They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) (Doyle, 2007; Adebayo-Tayo et al., 2012d)

The physiochemical component of the catfish reveals that the fish sample serve as a good source of protein and minerals element (Adebayo-Tayo et al., 2012d). Most of the organisms isolated in the study by Adebayo-Tayo et al. (2012d) causes food poisoning such as shigellosis, *Salmonellosis* caused by *Shigella* and *Salmonellosis spp* respectively. *Bacillus* which is known to be the highest occurring bacterial isolate causes toxin-mediated disease rather than an infection. The study by Adebayo-Tayo et al. (2012d) revealed that the fish samples from Itu Market have high microbial loads of pathogenic microorganisms. The high microbial load reported in their study (Adebayo-Tayo et al., 2012d) may have been due to high level of contaminants in Itu River where these catfish were obtained. However, there should be proper processing, storage and handling measure. People should be oriented not to buy fishes that have shown signs of spoilage (Adebayo-Tayo et al., 2012d).

2.3. FISH SPOILAGE

The microbiology of fish skin and gastro intestinal tract has been subjected to many researches. Fish is one of the most highly perishable food products (Sallam, 2007; Adebayo-Tayo et al., 2012d). Fish can spoil from both outer surface and inner surfaces as fish stomach contain digested and partially

digested food which can pass into the intestine (Emikpe et al., 2011). After fish is being caught and dying the immune system collapses and bacteria are allowed to proliferate freely on the skin surface and the stomach (Emikpe et al., 2011). The walls of intestines do break down sufficiently for bacteria to move into the flesh through the muscle fibre. It has been suggested that intestinal microflora is the causative agent for food spoilage (Emikpe et al., 2011).

Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Emikpe et al., 2011). During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Sallam, 2007; Adebayo-Tayo et al., 2012d). The quality of fish degrades, due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated (Sallam, 2007; Adebayo-Tayo et al., 2012d).

Fish spoilage is a complex process in which physical, chemical and microbiological mechanisms are implicated (Hozbor et al., 2006; Adebayo-Tayo et al., 2012c). Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Sil et al., 2008; Liu et al., 2010; Adebayo-Tayo et al., 2012c). Degradation of lipids in fatty fish produces rancid odors (Doyle, 2007). In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as pseudomonads that produce siderophores that bind iron (Doyle, 2007).

Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth (Doyle, 2007). *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions (Hozbor et al., 2006; Doyle, 2007). Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria and *Photobacterium phosphoreum* (Doyle, 2007). Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by molds (Doyle, 2007). Addition of organic acids selects for lactic acid bacteria and yeasts (Doyle, 2007). Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish (Doyle, 2007).

Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures

(Doyle, 2007; Adebayo-Tayo et al., 2012d). These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen) (Doyle, 2007; Adebayo-Tayo et al., 2012d). Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures (Doyle, 2007; Adebayo-Tayo et al., 2012d). Mesophilic anaerobes (*Bacillus* spp.), growing at ambient temperatures, cause several types of spoilage of vegetables (Doyle, 2007; Adebayo-Tayo et al., 2012d). Psychrotolerant spore-formers produce gas and sickly odors in chilled meats and brine-cured hams (*Clostridium* spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.) (Doyle, 2007; Adebayo-Tayo et al., 2012d).

Pseudomonas and related genera are aerobic, Gram-negative soil bacteria, some of which can degrade a wide variety of unusual compounds (Doyle, 2007; Adebayo-Tayo et al., 2012d). They generally require a high water activity for growth (0.95 or higher) and are inhibited by pH values less than 5.4 (Doyle, 2007; Adebayo-Tayo et al., 2012d). Some species grow at refrigeration temperatures (psychrophilic) while other are adapted for growth at warmer, ambient temperatures (Doyle, 2007).

Four species of *Pseudomonas* (*P. fluorescens*, *P. fragi*, *P. lundensis*, and *P. viridiflava*), are reported to be the main food spoilage organisms (Doyle, 2007). *P. fluorescens*, *P. fragi*, and *P. lundensis* cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause formation of sulfides and trimethylamine (off-odors) and by forming biofilms (slime) on surfaces (Hozbor et al., 2006; Doyle, 2007; Adebayo-Tayo et al., 2012d). Some strains of *Pseudomonas* are adapted for growth at cold temperatures and spoil these foods in the refrigerator (Doyle, 2007). *Pseudomonas* spp. was reported as one of the dominant bacteria during the study period (Adebayo-Tayo et al., 2012d). *Pseudomonas* spp. was reported as the dominant bacteria during the ice stored period in a study by Hozbor et al. (2006).

2.3.1. FACTORS THAT AFFECT FISH SPOILAGE AND DETERIORATION OF FISH

2.3.1.1. Fish Spoilage

Spoilage is the result of a series of changes brought about in the dead fish mainly due to enzyme and bacterial action. It starts in the fish as soon as the fish dies when caught. In areas where temperature is high, fish spoil within 15-20 hours depending on the specie and the method of capture (Adedeji and Adetunji, 2004; Adedeji, 2012). Fish is extremely perishable. It spoils easily. "Spoilage" can be defined as a change in fish or fish products that renders them

less acceptable, unacceptable or unsafe for human consumption. Fish undergoing spoilage has one or more of the following signs: slime formation; discolouration; changes in texture; off-odours; off-flavours and gas production (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.1.2. Microbiological spoilage

Live fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish in varying numbers. A normal range of 102-107 cfu (colony forming units)/cm² on the skin and between 103 and 10⁹ cfu/g in the gills and intestines has been observed (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.1.3. Chemical oxidation

Chemical spoilage processes are changes taking place in the lipid fraction of the fish. Lipids are oxidised to peroxides, aldehydes, ketones and lower aliphatic acids. The hydro-peroxides are tasteless but can cause brown and yellow discolouration of the fish tissue (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.1.4. Autolytic spoilage

As fish dies, its enzymatic activity does not stop immediately but continues resulting in proteolytic changes that are responsible for early quality loss in fresh fish (Adedeji and Adetunji, 2004; Adedeji, 2012). The more these enzymes get in contact with the fish's flesh the greater the spoilage. Adenosine triphosphate (ATP) is broken down through a series of products such as adenosine diphosphate (ADP), inosine monophosphate (IMP), inosine and hypoxanthine (HX).

2.3.2. FACTORS THAT INFLUENCE THE RATE OF FISH SPOILAGE

2.3.2.1. Effects of time/temperature conditions on microbial growth

The most crucial factors determining the quality of fishery products are time and temperature tolerance. Proliferation of microorganisms requires appropriate high temperatures, while at lower temperatures close to 0°C, their activity is reduced, thereby extending the shelf life of fish products (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.2.2. Effects of hygiene on fish quality during handling

Apart from the microorganisms that fishes have at the time of capture, more is added via unhygienic practices and contaminated equipment such as storage facilities. Rough handling will result in a faster spoilage rate (Adedeji and Adetunji, 2004; Adedeji, 2012). This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. Physical mishandling in the net, such as very large catches, fishermen stepping on fish or throwing boxes, containers and other items on top of the fish,

may cause bruises and rupture of blood vessels (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.2.3. Initial bacterial load

The microflora on tropical fish often carries a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish. Basically, bacteria populations on temperate fish are predominantly psychrotrophic reflecting water temperatures of about 10°C while fish from the tropics have largely mesophilic bacteria.

2.3.2.4. Methods of capture

The fishing gear and method employed determines the time taken between capture and death (Adedeji and Adetunji, 2004; Adedeji, 2012). Fish caught in gillnets struggle much to escape, and in so doing, they are bruised by the net which increases exposure to microbial entry and subsequent deterioration (Adedeji and Adetunji, 2004; Adedeji, 2012). Fish caught by hook and line methods, on the other hand, die relatively quickly and therefore bruises and stresses are likely to be minimal (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.3.2.5. Mode of storage

In bulk-storage, the weight of the pile may crush the fish at the bottom, leading to a loss of weight (yield) as well as other physical damage (Adedeji and Adetunji, 2004; Adedeji, 2012). It has been reported that when haddock is kept in a short, deep pile of about 3 ft, the bottom fish lose 15% of their weight compared to a normal weight loss of 3-8%, which is entirely due to biochemical changes that cause a loss of water holding capacity leading to drip (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4. PRINCIPLES OF FISH PRESERVATION AND PROCESSING

In some islands, more fish is caught at times than can be consumed. Methods are used in keeping the surplus fish in good condition for later consumption (Adedeji and Adetunji, 2004; Adedeji, 2012). Again, fishermen sometimes cannot return to their villages promptly with fresh fish they have caught, and it will be of value to them to know how to preserve their catch by simple means (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.1. Fish Preservation

Preservation of fish is done to prevent spoilage. Since fish is very perishable, it is therefore, necessary to preserve fish if not consumed or disposed immediately (Adedeji and Adetunji, 2004; Adedeji, 2012). Fish preservation is the method of extending the shelf life of fish and other fishery products by applying the principles of chemistry, engineering and other branches of science in order to improve the quality of the products (Adedeji and Adetunji, 2004; Adedeji, 2012). Some of the important reasons for preserving foods are: a) To take care of the excess

produce. b) Reaches areas where the food item is not available, c) Makes transportation and storage of foods easier and d) Preserving foods at home (Adedeji and Adetunji, 2004; Adedeji, 2012).

Foods can be preserved at home by the following methods: a) Dehydration b) Lowering temperature, c) Increasing temperature and d) Using preservatives (Adedeji and Adetunji, 2004; Adedeji, 2012)

2.4.2. Proper Steps In Handling Fresh Fish

Avoid exposing the fish to sunlight. Keep them in a shaded area. Ice the fish immediately after they are caught to lower their temperature (Adedeji and Adetunji, 2004; Adedeji, 2012). Remove the gills and internal organs. Avoid soaking the fish too long in the water after death as this easily spoils the fish. Use mechanical refrigeration if there are facilities (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.3. METHODS OF FISH PRESERVATION

2.4.3.1. Salting

Salt is the preservative agent used to lengthen the shelf life of fish and fishery products (Adedeji and Adetunji, 2004; Adedeji, 2012). This is used in almost all methods of preservation except in icing, refrigeration and freezing. There many different kinds of salt, some being better than others for fish curing (Adedeji and Adetunji, 2004; Adedeji, 2012). However, in islands or in outlying places there is often no choice, and whatever is available in the way of salt has to be used, whether it is bought in a shop, prepared on the spot, or extracted from earth containing salt (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.3.2. Wet Salting

This is the cheaper, since it requires lesser amounts of salt. The principle is to keep the fish for a long time in brine. If the salt is coarse, it has to be ground or pounded first. It is then dissolved into the water by stirring with a piece of wood. To be good, the brine must float a fish (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.3.3. Smoking

Any kind of fish can be smoked. There are three main methods of smoking: (a) Smoking and roasting; (b) Hot smoking; and (c) Long smoking.

a) Smoking and Roasting: This is a simple method of preservation, for consumption either directly after curing or within twelve hours. Re-smoking and roasting can keep the product in good condition for a further twelve hours. Fresh unsalted fish is put over a wood or coconut husk fire (Adedeji and Adetunji, 2004; Adedeji, 2012).

b) Hot Smoking: The hot smoking system can be used for immediate consumption or to keep the fish for a maximum of 48 hours. Small fish can be salted first for half an hour (see wet salting). After salting

they are put on iron spits and dried in a windy place or in the sun for another half hour. It is necessary to have an oil drum to make the smoking stove (Adedeji and Adetunji, 2004; Adedeji, 2012).

c) Long Smoking: If fish must be kept in good condition for a long time, for instance, two or three months or even longer, it can be done by smoking, provided the fish is not oily. For this purpose, a small closed shed made of palm leaves or other local material can be used (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.3.4. Cooking

Fish can be kept for two or three days in the following way: Small drums (possibly oil drums) are cleaned and filled with water. Salt is added in the proportion of four parts water to one of salt (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.4. FISH PROCESSING

Processing means handling, storing, preparing, heading, eviscerating, shucking, freezing, changing into different market forms, manufacturing, preserving, packing, labeling, dockside unloading, or holding fish or fishery products (Russell, 2004). Eviscerating or heading on board a harvest vessel—not a factory trawler—with the sole intent to hold, but not process, the catch is exempt from these regulations. However, evisceration/heading carried out at an aquaculture facility before delivery to a processing plant must comply with these regulations (Russell, 2004). Methods of fish processing include a. Curing b. Icing c. Freezing d. Canning E. the use of additives or chemicals.

2.4.4.1. Fish curing: This is defined as the method of preserving fish by means of salting, drying, smoking and pickling. Fish to be cured are usually first cleaned, scaled, and eviscerated. Medium-sized fishes are split through the backbone and top of the head, with the two halves joined by the belly skin, butterfly style (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.4.4.2. Fish canning: This is a process involving heat treatment of fish in sealed containers made of tin plates, aluminium cans or glass, until the product has been fully sterilized. The canned food fish is also prevented from contamination by pathogenic organisms by storing them in a virtually airtight package. If heat treatment is properly carried out canned fish may remain in storage for several years without refrigeration (Adedeji and Adetunji, 2004; Adedeji, 2012).

3. BENEFITS OF FISH CONSUMPTION

The importance of fish to man cannot be overemphasis in the world today (Udeze et al., 2012a). The major importance of fish to human is majorly to serve as a source of protein, and they are being converted to different forms for different purpose (Udeze et al. (2012a). Fish has become an increasingly

important source of protein and other element necessary for the maintenance of and healthy body (Adebayo-Tayo et al., 2012d).

The African catfish, *Clarias gariepinus* has been reared for about 20 years in Africa with mixed success; the total farmed production of this species being only 3,978 metric tones or 7.4% of the of the total farmed fish production of 69,434mt in Africa in 1994 (Udeze et al., 2012a). *Clarias gariepinus* is a highly nutritious fish that contains high amount of vitamins, proteins, minerals and a little or no saturated fat and is low in carbohydrate (Udeze et al., 2012b). Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds (Emborg et al., 2005; Olafsdottir et al., 2005; Baixas-Nogueras et al., 2005; Dalgaard et al., 2006; Doyle, 2007; Udeze et al., 2012a).

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as good source of protein and other elements for maintenance of health body (Adebayo-Tayo et al., 2012b). The less developed Countries capture 50% of the world harvest and a large proportion of the catch are consumed internally (Adebayo-Tayo et al., 2012b). In many Asian countries over 50% of the protein intakes comes from fish while in Africa the proportion is 17.50% (Adebayo-Tayo et al., 2012b).

In Nigeria, fish constitute 40% of the animal protein intake. Fish and fish products constitute an important part in the international trade, currently worth more than US and 50 billions indicating increasingly consumer interest in commodity (Adebayo-Tayo et al., 2012b). Generally, fish are good sources of vitamins B12 and B6 (Adebayo-Tayo et al., 2012b). It is also good source of fluorine and iodine which are needed development of strong teeth and the prevention of goiter in man. However, availability of these vital nutrients depends to a large extent on the methods of storage, such as salting, roasting, drying and freezing. Iced fish of different types are of great demand by the Nigeria consumers as a relatively cheaper source of animal protein (Adebayo-Tayo et al., 2012b).

Also, seafood derived from wild fish as well as farmed fish has always been an important source of protein in the human diet (Yagoub, 2009; Adebayo-Tayo et al., 2012b). On a global scale, fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture (Håstein et al., 2006; Yagoub, 2009; Adebayo-Tayo et al., 2012b). Fishery products are important not only from a

nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Yagoub, 2009; Adebayo-Tayo et al., 2012b). Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits (Yagoub, 2009; Adebayo-Tayo et al., 2012b). In addition, they can also function as carriers of several microbial and other health hazards (Yagoub, 2009; Adebayo-Tayo et al., 2012b). Therefore maintenance of quality is of utmost importance in production and trade of fishery products. Most of current quality control techniques are time consuming and cumbersome (Yagoub, 2009; Adebayo-Tayo et al., 2012b). Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities (Adebayo-Tayo et al., 2012b). However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub, 2009; Adebayo-Tayo et al., 2012b).

4. FISH CONTAMINATION

Fish take a large number of bacteria into their gut from water sediment and food (Emikpe et al., 2011). It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (Emikpe et al., 2011). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (Emikpe et al., 2011).

Fish contamination can also be linked to raw material, personnel, processing tools such as forklifts through leakage, opening in building and pests. Some pathogens may even become established in the processing plants from niches where they can survive for a long period of time (Adebayo-Tayo et al., 2012b). The tissue of a healthy fish is normally considered sterile until bacterial invasion that leads to spoilage. According to Adams and Moses (2008), the normal bacterial load of the surface slime of fish can range from $10^2 - 10^7$ cfu/cm² and the Gills and Intestines can range up to 10^3 and 10^7 cfu/g respectively. Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). This was in agreement with earlier report by Agbu et al. (1998) in Kastina in terms of high viable counts of coliform density in the water ecosystem.

Shellfish is a food substrate for some zoonotic vibrios of which these microorganisms, cause food poisoning and diarrhea in human (Merwad et al., 2011). Shellfish make an excellent substrate for the microorganisms to live in the aquatic habitats due to loose texture of their flesh (Merwad et al., 2011).

When the aquatic system is contaminated with pathogenic *Vibrio*, these bacteria become part of shellfish microflora (Colakoglu et al., 2006). Concerning the zoonotic aspect, the hazardous pathogenic *Vibrio* causes life threatening food borne infections and poses a considerable public health threat as agents of sporadic and epidemic human infections to be represented an important microbial group in the field of food safety (Espineira et al., 2010; Merwad et al., 2011).

4.1. MICROBIAL CONTAMINATION OF FISHES

Most of the organisms found in fishes are associated with food poisoning infection and typhoid fever in humans, shigellosis food infection in humans, and the presence of *Aspergillus* reveals possible production of aflatoxins (Adebayo-Tayo et al., 2006). The total aerobic bacteria and enterobacteria load in African Catfish (*Clarias gariepinus*) and Nile Tilapia (*Oreochromis niloticus*) randomly sampled from different aquatic environments in Ibadan, Southwest Nigeria by Emikpe et al. (2012). The high microbial load of the wild catfish and wild tilapia in their study (Emikpe et al., 2012) may be due to mass pollution of the environments where the fish were caught. The presence of highly pathogenic bacterial isolates, like *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *E. coli*, *Pseudomonas* sp. and *S. aureus* in fish are organisms of public health concern. The presence of these microbes is an indication of possible contamination resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination (Adebayo-Tayo et al., 2009).

4.1.1. Contamination of fish with *Enterobacteriaceae*

Enterobacteriaceae are a large, diverse heterogeneous group of rod shaped gram negative bacilli that survive under aerobic conditions and normally inhabit the intestine of man and animals; some are motile while some others are not (Olayemi et al., 2007; Udeze et al., 2012b). The family includes many genera, some of which are part of the normal flora and incidentally cause diseases especially when given the opportunity. They are non-spore forming and some have capsules while others do not (Olayemi et al., 2007; Udeze et al., 2012b). In a study by Yagoub (2009), *Enterobacteriaceae* were isolated from gills, skin, muscles and the intestine of randomly collected fishes. Thampuran et al. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform where *Escherichia coli* was the most common contaminant and is often encountered in high numbers. This is also in accordance with what was previously reported by

Jeyasekaran et al. (2006), Yagoub (2009) and Adebayo-Tayo et al. (2012a,b) who identified *pseudomonads* as a good spoilage index.

4.1.1.1. *Salmonella*

Contamination of seafood with *Salmonella* is a major public health concern. The presence of *Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan and Japan (Ponce et al. 2008; Wafaa et al., 2011). Heinitz et al. (2000) highlighted that the incidence of *Salmonella* in seafood is highest in the central Pacific and African countries and lowest in Europe including Russia, and North America (12% versus 1.6%). During a 9-year study (1990–1998), the Food and Drug Administration noted an overall incidence of *Salmonella* in 7.2% of 11,312 samples from imported and 1.3% of 768 samples from domestic U.S. seafood (Wafaa et al., 2011). In Croatia, *Salmonella* spp. was recorded as the primary microbial pathogens responsible for the majority of food-borne illnesses (Wafaa et al., 2011).

4.1.1.2. *Escherichia coli*

Escherichia coli cause dysentery. Normal fish and human skin is a complex organ and the bacterial populations associated with it are complex in kind and number. The skin supports the growth of both aerobic and anaerobic bacteria (Adebayo-Tayo et al., 2006, 2009).

4.1.2. Contamination of fish with *Staphylococcus aureus*

Staphylococcus aureus, a mesophile have been implicated in food poisoning outbreak of some food material (Adebayo-Tayo et al., 2009). Odunfa (1988 cited by Adebayo-Tayo et al., 2006, 2009) reported that *S. aureus* levels of 10^8 ml are considered potential hazardous to consumers. The presence of *S. aureus* is an indication of contamination by food handlers and 80% of them are being harbored by man as normal micro flora (Adebayo-Tayo et al., 2009). *S. aureus* known for production of heat stable enterotoxin and potentials for multiple antibiotic resistances when they get into the living tissue makes the product of immense epidemiological danger (Adebayo-Tayo et al., 2009).

4.1.3. Contamination of fish with *Pseudomonas sp*

Pseudomonas sp on the other hand is prevalent among patients with wounds, burns, cystic fibrosis are likely to have introduced into the environment by swimmers and infected individuals who use these waters were the tilapia samples were obtained for recreational purposes (Adebayo – Tayo et al., 2006). The contamination may be as a result of human activities such as deposition of faecal matters, washing, bathing, discharge of effluents into the rivers were these fish are harvested from.

4.1.4. Contamination of fish with *Bacillus sp*

Bacillus sp causes a toxin-mediated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Adebayo-Tayo et al., 2006, 2009). The occurrences of *Bacillus sp*. in fish can be said to be as a result of prevalence of their spores in the environment (Adebayo-Tayo et al., 2009). *Bacillus* species are spore formers whose spores could survive high temperatures of processing. The organisms are present in most raw materials used in food manufacturing at concentration of $10^3/g$ or less. The infectious dose has been estimated to be $10^5/g$ (Adebayo-Tayo et al., 2009)

4.1.5. Contamination of fish with *Erysipelothrix rhusiopathiae*

Infection with *Erysipelothrix rhusiopathiae* (erysipeloid) is also known as fish handler's disease, fish handler, blubber finger, etc., in humans, since it is most commonly characterised by swollen fingers (Hastein et al., 2006). The bacterium is reported to occur on fish, and the infection is most often introduced to humans through skin wounds. Thus, the disease must be considered as occupational in humans, due to handling fish and fish products contaminated with *E. rhusiopathiae*. The disease is usually benign, but may be fatal in some cases. Fatal endocarditis has been described following the gutting of eels (Hastein et al., 2006).

4.1.6. Contamination of fish with *Klebsiella* species

Bacteria from the genus *Klebsiella* causes numerous infections in human, which are often treated with β -lactam antibiotics (Amin et al., 2009). A variety of nosocomial and community acquired (food borne) infections are caused by *K. pneumoniae*, one of the most deadly pathogens of *Enterobacteriaceae* (Amin et al., 2009, Udeze et al., 2012b). These pathogens possess β -lactamase, therefore they mediate high levels of resistance to β -lactam antibiotics and have become a global threat (Amin et al., 2009, Udeze et al., 2012b). *K. pneumoniae* is an enteric Gram-negative bacillus causing hospital-acquired infections and infections in debilitated or immuno-compromised patients accounting for up to 10% of all nosocomial bacterial infections. Mostly these infections are treated with β -lactam antibiotics, which are usually hydrolyzed by β -lactamases produced by such microorganisms resulting in failure of therapy. Because of resistance of many *Klebsiella sp.* strains to β -lactamases; alternative antibiotic therapy can make use of aminoglycosides and quinolone (Amin et al., 2009, Udeze et al., 2012b). Udeze et al. (2012) carried out a study to find out if *Klebsiella pneumoniae* bacteria isolate can survive in the fish (immunity of fish) and observation of the public health hazard that bacteria i.e. test organism and natural flora exposes the people to. This study by Udeze et al. (2012)

showed that *Klebsiella pneumoniae* may cause an infection in catfish and can act as a vector of human pathogen.

4.1.7. Contamination of fish with *Listeria* species

Listeria monocytogenes has been isolated on a regular basis from a wide variety of seafood products, including fresh, frozen, fermented, cold smoked and salted fish derived from aquaculture as well as captive fisheries. It is a problem often associated with fish and fish products from temperate climates (Hastein et al., 2006). The organism is ubiquitous in nature and regarded as a zoonotic agent, causing meningitis and abortions in sheep and septicaemia in lambs, as well as food-borne illness in humans (Hastein et al., 2006). The occurrence of *L. monocytogenes* in seafood is reported to range from 0% to 75% (Hastein et al., 2006). The Ready-to-eat food products, such as refrigerated, vacuum packed products with a long shelf life, are of particular concern for *L. monocytogenes*, especially when they are inadequately heated before consumption (Hastein et al., 2006). It has been shown that bacterial growth occurs during the fermentation process at $8^{\circ}C$ and storage at $4^{\circ}C$. The ability to grow at low temperatures, together with halotolerance, enables bacteria to reproduce in salted products (Rorvik, 2005; Hastein et al., 2006).

Listeria monocytogenes has also occasionally been found in smoked salmon and it is thought that the bacterium is introduced through water during the production process (Hastein et al., 2006). Cold smoking does not eliminate *L. monocytogenes* (Hastein et al., 2006) and, although bacterial counts are reduced by hot smoking, the bacterium is not completely eliminated from smoked products (Hastein et al., 2006). The isolation of different strains of *L. monocytogenes* from raw fish and final products indicates that contamination may take place at several stages in the production chain between harvesting and production for consumption (Hastein et al., 2006).

Outbreaks of listeriosis in humans due to contaminated seafood have been reported from many parts of the world, particularly from industrialised countries (Rorvik, 2005; Hastein et al., 2006). Outbreaks have been related to different types of food items, including products such as shrimps, vacuum packed smoked salmon and fermented fish (Hastein et al., 2006). Most cases of listeriosis in humans occur in immunocompromised people, the elderly and pregnant women, and the disease is characterised by septicaemia, intra-uterine infection and meningitis. It may also cause abortions (stillbirths) (Hastein et al., 2006). However, more recently, *Listeria* has been associated with mild gastro-intestinal symptoms (Hastein et al., 2006). Disease caused by *L. monocytogenes* is rare and a high infectious dose is

required. The zero tolerance policy established in many countries for *Listeria* in fish products may be overprotective from a public health point of view (Hastein et al., 2006).

4.1.8. Contamination of fish with Pathogenic *Vibrio* Species

Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa et al., 2011). The family Vibrionaceae is autochthonous to aquatic environments including estuarine, coastal waters and sediments worldwide, and some species are well-known pathogens of marine organisms including fish and shellfish (Merwad et al., 2011). The importance of *Vibrio* spp as a contaminant of raw or under cooked seafood has been well established (Lucan et al., 2008). Species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. hollisae* and *V. damsela* are human pathogens (Adeyeye et al., 2010). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeyeye et al., 2010). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi et al., 2004).

The presence of other species of *Vibrio* (*Vibrio parahaemolyticus*, *Vibrio fluvialis*, and *Vibrio mimicus*) has been repeatedly reported on shellfishes in previous studies by Colakoglu et al. (2006), Ali (2010) and Adebayo-Tayo et al. (2011a,b). Ristori et al. (2007) isolated *Aeromonas* spp., *Plesiomonas shigelloides*, *Vibrio cholerae* 01, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* from different organs of fishes. It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (Yagoub, 2009).

Vibrio parahaemolyticus and *Vibrio vulnificus* are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea - and brackish water of both tropical and temperate regions, sediments, and a variety of seafood especially shellfish and bivalve mollusks (Kirs et al. 2011; Wafaa et al., 2011). Although the vast majority of environmental *V. parahaemolyticus* isolates are non virulent, it is a leading cause of gastroenteritis linked to seafood consumption in the United States (U.S.) (Iwamoto et al. 2010; Wafaa et al., 2011). *Vibrio vulnificus* poses a significant health threat to humans who suffer from immune disorders, liver disease, or hemochromatosis (Wafaa et al., 2011). It enters human hosts via wound infections or consumption of raw shellfish (primarily oysters), and

infections frequently progress to septicemia and death in susceptible individuals (Wafaa et al., 2011).

Merwad et al. (2011) reported the overall prevalence of *Vibrio* spp. in water samples from Suez Canal to be 25% in their study in Egypt. Merwad et al. (2011) reported that *V. parahaemolyticus*, *V. mimicus*, *V. furnissii* and *V. hollisae* were not detected in water samples collected from the same Suez Canal. According to Merwad et al. (2011), the absence of *V. parahaemolyticus* in Suez Canal water may be associated with sample size and water clearness. This finding was supported by Zimmerman et al. (2007), who observed that *V. parahaemolyticus* levels in water are strongly correlated with turbidity during summer. On the contrary, higher reports of *V. parahaemolyticus* in water were noticed in other studies: Mahmoud et al. (2006) reported 20.8%, Japan; Masini et al. (2007) reported 6.5% in Italy; Hassanin (2007) reported 40% in Abu-Kir fishing farm, Egypt and Blanco- Abad et al. (2009) reported 5.6% in Spain.

4.1.9. Contamination of fish with other Bacteria

Aeromonas bacteria are ubiquitous in the environment and several *Aeromonas* species have been reported to cause disease in fish, as well as being potential food-borne pathogens that may cause disease in humans (Hastein et al., 2006). Food-borne pathogenic bacteria such as *Campylobacter*, *Shigella* and *Yersinia* are seldom associated with fish. Nevertheless, the fish pathogenic bacteria *Y. ruckeri* has been reported to occur in humans (Hastein et al., 2006). *Edwardsiella tarda*, which causes red disease in eels as well as enteritis in penguins, is also sporadically reported as causing gastroenteritis and septicemia in humans (Hastein et al., 2006).

4.1.10. Contamination of fish with *Aspergillus* spp.

Aspergillus spp. have also been implicated in causing mycetoma in human (Adebayo-Tayo et al., 2009). *A. flavus* is involved in allergic (aspergillosis (pulmonary aspergillo-sis) and also produces aflatoxin that is highly carcinogenic (Adebayo-Tayo et al., 2009). The presence of species of *Aspergillus* could be attributed to the prevalence of their spores in the atmosphere (Adebayo-Tayo et al., 2009). This organism was easily trapped during the post harvest processing and handling of tilapia fishes. Since most fungal spores are found in the air, the spores must have contaminated the tilapia fishes during storage, transportation and displaying of the fishes at the market. The liberated spore can easily settle on food and ceilings of room and then germinate (Adebayo-Tayo et al., 2009). Iloju and Iloh (2007) isolated and identified *A. flavus* and *A. niger* from sorrel drink.

4.1.11. Contamination of fish with *Penicillium* spp.

Penicillium spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in

these crops (Doyle, 2007). They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed foods such as jams and margarine (Doyle, 2007). A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores (Doyle, 2007).

4.2. SOURCES OF CONTAMINATION IN FISH

4.2.1. Water

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio spp.* especially crustaceans (Wafaa et al., 2011). The potential of water to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Okonko et al., 2008, 2009). Water-related diseases continue to be one of the major health problems globally. Water-related diseases continue to be one of the major health problems globally (Adebayo-Tayo et al., 2011a,b). Okonko et al. (2008, 2009) reported that both bacteria and fungi are common flora of frozen fish and fish related products during packaging. It is estimated that 80% of all illnesses are linked to use of water of poor microbiological quality (Okonko et al., 2008, 2009). One of the strategies for tackling this problem is the provision of protected sources such as boreholes, standpipes, protected wells and springs. However, such facilities are located some distances requiring transportation to homes (Taulo et al., 2008). During transportation, water gets contaminated with bacteria which grow and proliferate during storage in the homes (Okonko et al., 2008, 2009).

In Malawi, access to safe water has increased over the past 8 years through the installation of the above mentioned protected sources (Taulo et al., 2008). In Lungwena, about 82% of the households have access to portable water, with 78% of the households having access to borehole water sources. Despite this availability and promotion of the use of such facilities, water-related diseases remain the major cause of mortality and morbidity. The area experiences outbreaks of Cholerae every rainy season. The above episodes suggest that consumption of water from contaminated sources and poor environmental sanitation continue to be practiced in Lungwena (Taulo et al., 2008).

4.2.2. Hygiene

Although interventions for improving sanitation have lagged behind those for water, promising advances have been reported, especially in the development of ecologic sanitation systems (Okonko et al., 2008a). A good knowledge of the bacteriological and chemical qualities of raw water is necessary so as to guide its suitability for use. Thus,

regular physico-chemical and bacteriological analysis of water at source must be carried out to determine or check the effectiveness of treatment process (Okonko et al., 2008a).

According to Oluwafemi and Simisaye (2005 cited by Okonko et al., 2008, 2009) most of the sausage being sold as ready-to-food pose health risk to consumers, making it imperative to institute not only sanitary measures during its production and sales but for retailers selling raw or pre-processed foods to have a steady source of power supply. The presence of index of water quality and indicators of faecal contamination such as *E. coli*, *Streptococcus faecalis*, *Enterobacter aerogenes* and *Salmonella sp.* are as a result of possible contamination during sales or unhygienic handling of seafood right from the processing plants and this might have adverse effect on the health of the consumers (Okonko et al., 2008).

According to Okonko et al. (2008), the presence of *Staphylococcus aureus*, a pathogenic organism of public health concern and significance in frozen seafood products might have contaminated the processed frozen seafood products from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of ready-to-eat food and this might eventually affect the health of the consumers. It is therefore suggested that frozen seafood processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination. However, the vendors/retailers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these processed frozen seafood products.

4.2.3. Food Processors/ Handlers

Food processors/ handlers may be sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage hence, food processors/handlers may be counter productive by being responsible for public health hazard and loss of revenue (Bankole et al., 2005). Bankole et al. (2005) reported in a study carried on food handlers, that all the palms of food handlers harboured *Staphylococcus aureus* and the palms of hotel operators among the food vendors sampled were reported to have harboured the least types of microorganisms.

4.3. FACTORS DETERMINING THE MICROBIAL COMPOSITION OF FISH

World wide, all fisheries are threatened by various factor, such as pollution, which brings about the introduction of industrial waste which comprise of various inorganic and organic waste and invasive species of bacteria (Udeze et al., 2012a). In which they are majorly enteric in nature, and their target point is the colonization of the intestine of fish and other sites

in the fish (Udeze et al., 2012a). The higher density in fish may be due to their consumption of bacteria for long time through food and water (Shankar et al., 2009). The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fish are often simply their hosts (Emikpe et al., 2011).

4.3.1. Effect of water

The microbial composition of fish depends upon the microbial counts of water in which they live. However, fresh and internal organs of freshly caught healthy fish from tropical and temperate water are normally sterile because the scale and slime covering the fish serve as biological barriers to the entry of microorganisms (FEHD, 2005).

The ponds and rivers that harbour the fish may be the source of contaminants due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies (Emikpe et al., 2011). Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds (Emikpe et al., 2011). Birds can also be a significant source of bacteria. Swans; Geese and other water fowl can all elevate bacteria counts in water bodies and ponds (Doyle and Ericson, 2006).

The results of the study by FEHD (2005) implied that if *V. cholerae* is the concern when considering abstracting seawater for keeping live seafood, both the site where the water is abstracted and the *E. coli* count are important parameters to be considered. Abstraction of seawater from typhoon shelters and shoreline in urbanised areas for this purpose is therefore not recommended (FEHD, 2005).

4.3.2. Effect of temperature

Water temperature can greatly affect the vibrio levels in seafood. Vibrios can multiply rapidly between 20 and 40°C (FEHD, 2005). Growth at the optimum temperature (37°C) can be very rapid and generation times of 9 to 10 minutes have been reported (FEHD, 2005). Growth of pathogenic vibrios occurs optimally at around 37°C although the maximum and minimum growth temperatures are 43°C and 5°C respectively (FEHD, 2005). *V. parahaemolyticus* is primarily associated with coastal inshore waters rather than the open sea. It is rarely isolated from water with temperatures below 15°C (FEHD, 2005). All vibrios are sensitive to heat. In shellfish, heating to produce an internal temperature of at least 60°C for several minutes appears sufficient to eliminate the pathogenic vibrios. Chilling and

refrigeration are critical control measures to prevent growth of these microorganisms (FEHD, 2005).

4.3.3. Effect of pH and other factors

21. Vibrios are acid sensitive and grow best at pH values slightly above neutrality, i.e. 7.5 to 8.5 (FEHD, 2005). They are also sensitive to drying (FEHD, 2005). While *V. parahaemolyticus* has an absolute Na⁺ ion requirement and shows optimal growth at about 2 to 4% NaCl, freshwater inactivates this organism (FEHD, 2005).

Fish are very much susceptible to contamination with different bacteria because of their perishable protein content (Shankar et al., 2009). Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds (Dalgaard et al., 2006; Doyle, 2007; Adebayo-Tayo et al., 2012c; Udeze et al., 2012b). Degradation of lipids in fatty fish produces rancid odors (Doyle, 2007; Adebayo-Tayo et al., 2012c). In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as pseudomonads that produce siderophores that bind iron (Doyle, 2007; Adebayo-Tayo et al., 2012c; Udeze et al., 2012b).

Vibrios are abundant in the aquatic environment. Most of them require 2 to 3% NaCl or a seawater base for optimal growth (FEHD, 2005). Vibrios are associated with live seafood as they form part of the indigenous microflora of the environment at the time of seafood capture or harvest (FEHD, 2005). Healthy live fish is protected by its immune system and therefore bacteria cannot grow in its flesh. When the fish dies, the immune system no longer functions and the bacteria present are able to proliferate freely (FEHD, 2005).

4.3.4. Effect of handling, processing, transportation and storage

The contaminations of fish observed in most studies may result from rupturing fish intestine during poor processing or inadequate washing as intestinal microflora of human or animal origin are the causative agent for food spoilage (Emikpe et al., 2011). The higher density of total aerobic bacteria found in the skin and stomach of fishes might be due to quick proliferation after catching and during transportation and storage (Emikpe et al., 2011).

Preservation in low quality ice, handling with contaminated hands could also be responsible for higher density of aerobic bacteria (Emikpe et al., 2011). In addition, bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of

fish or shellfish. If subsequent handling is improper and that there is no or inadequate pathogen reduction step (e.g. cooking) afterwards, the level of bacteria in the final product may increase to such an extent that may present a health risk to consumers (FEHD, 2005).

Even though epidemiological evidence on outbreak of food borne disease is scarce, there are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipments/utensils and the raw food materials (Edema et al., 2008). Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food borne illness (Ajao and Atere, 2009). However, contamination of the fish may occur from food handlers and retailer who sell these items to the public for consumption (Adebayo-Tayo et al., 2011a,b).

5. PUBLIC HEALTH HAZARDS AND RISKS ASSOCIATED WITH FISH CONSUMPTION

Seafood is a nutritious food that constitutes one of the desirable components of a healthy diet. Nevertheless, there is health risks associated with the consumption of seafood. Fish disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish (Emikpe et al., 2011). Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other food (Emikpe et al., 2011). Fish and Shellfish not only transmit disease to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxications (Emikpe et al., 2011).

One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favourable conditions (FEHD, 2005). Consumption of raw or undercooked seafood is recognized as a health risk to consumers. Sea foods are prone to bacterial contamination, especially filter feeders such as mussels, and oysters, which concentrate these bacteria in their filtration systems and, therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water (Popovic et al. 2010; Wafaa et al., 2011).

Seafood may be a vehicle for most of known bacterial pathogens, as *Salmonella* and *Vibrio spp.* (Wafaa et al., 2011). In contrast to most other

foodborne pathogens, *Vibrio* spp. has the aquatic habitat as their natural niche. As a result, vibrios are most commonly associated with seafood as natural contaminants (FEHD, 2005). Foodborne infections with *Vibrio* spp. are common in Asia (FEHD, 2005). Regarding the public health hazard, vibrios have been implicated in food poisoning and gastroenteritis; *V. parahaemolyticus* (Fuenzalida et al., 2007); *V. fluvialis* (Ballal et al., 2010) and *V. vulnificus*, (Horseman and Surani, 2011). Thereby, monitoring of pathogenic *V. parahaemolyticus* in shellfish and diarrheic patients is crucial.

Among the potentially pathogenic vibrios occurring naturally on fish and shellfish, *V. parahaemolyticus* is the most widespread (FEHD, 2005). Endogenous marine species of *V. cholerae* can also be isolated from fish during cholera outbreaks (FEHD, 2005). It has been suggested that vibrios are the most common bacterial causative agents in food poisoning resulting from the consumption of shellfish (FEHD, 2005).

5.1. FISH PATHOGENIC BACTERIA AS POTENTIAL CAUSAL AGENTS FOR DISEASE IN HUMANS

Viruses, bacteria, fungi and parasites in fish may cause disease or food-borne infections in humans. Under normal conditions, practically no infectious agents which cause disease in fish also infect humans. Nevertheless, under certain conditions, bacteria which cause fish diseases may also infect humans, without necessarily being regarded as a major human health problem (Hastein et al., 2006). Bacteria represent a major and important group of microorganisms because of their frequent occurrence and activities that may have a negative impact on fish quality (Hastein et al., 2006). Generally, seafood from cold waters harbours lower numbers of potentially pathogenic microorganisms than seafood from warmer waters (Hastein et al., 2006). The presence of human pathogenic bacteria in fish and fish products may also be attributed to contamination during processing (Hastein et al., 2006). Several bacteria are, however, reported to cause infection and mortality in both fish and humans and these represent a particular hazard, caused either by handling infected fish on fish farms or in grocery stores or by the ingestion of raw or inadequately processed infected fish and/or contaminated fish products (Hastein et al., 2006).

5.1.1. *Hafnia alvei*

Hafnia alvei, a Gram-negative, facultative anaerobic bacterium of the family Enterobacteriaceae, is found in natural environments, such as sewage, soil and water, but is also a gastro-intestinal commensal (Hastein et al., 2006). This bacterium is not usually considered pathogenic but has occasionally been reported to cause disease in fish as well as terrestrial

animals and humans (Padilla et al., 2005; Hastein et al., 2006). *Hafnia alvei* has been associated with epizootic haemorrhagic septicaemia in rainbow trout (Hastein et al., 2006); kidney pathology in cherry salmon (*O. masou*) (Hastein et al., 2006); and mortalities in brown trout (*S. trutta* L.) in freshwater aquaculture (Hastein et al., 2006), but not in marine aquaculture (Padilla et al., 2005; Hastein et al., 2006). In humans, *H. alvei* has been associated with several disease conditions, such as: septicaemia, gastroenteritis, meningitis, pneumonia, wound infections (Hastein et al., 2006). However, so far, there have been no reports of the bacterium transferring from fish to humans. Nevertheless, in some regions there may be a combination of marine fish farming and human activities, such as disposal of sewage in areas where people swim, which may lead to the transfer of *H. alvei* between humans and fish and vice versa (Hastein et al., 2006). Based on challenge experiments with *H. alvei* in gilthead seabream (*Sparus aurata* L.), Padilla et al. (2005) concluded that, although the gilthead seabream seemed to have a considerable resistance to experimental infections, the bacterium could remain viable in the fish without clinical signs for some three months. This means that aquaculture and human activities should take place in separate areas as farmed fish may represent a risk for the transfer of *H. alvei* to humans (Hastein et al., 2006).

5.1.2. *Streptococcus iniae*

The genus *Streptococcus* includes many species that can cause disease in different hosts, including fish in sea, brackish and fresh water as well as in mammals and humans (Hastein et al., 2006). *Streptococcus iniae* has been described as a cause of disease in both fish (mad fish disease) and people (Facklam et al., 2005; Hastein et al., 2006). It is a Gram-positive, beta-haemolytic bacterium that was first isolated from diseased Amazon freshwater dolphins (*Inia geoffrensis*) (Hastein et al., 2006), but was later described in cultured fish species, such as rainbow trout (*O. mykiss*), tilapia, channel catfish (*Ictalurus nebulosus*), Japanese flounder (*Paralichthys olivaceus*) and yellowtail (*Seriola quinqueradiata*) (Hastein et al., 2006).

In fish, infection with *S. iniae*, previously described as *S. shiloi* and *S. difficile* (Hastein et al., 2006), is characterised as a septicaemic disease which may become chronic (Hastein et al., 2006). In humans, disease caused by *S. iniae* was not described until 1995 to 1996, when the bacterium was isolated from a group of patients in Canada who had handled fresh whole tilapia from infected farms (Hastein et al., 2006). This was despite the fact that the bacterium had been identified as early as 1991 (Facklam et al., 2005). The disease condition in humans, characterised

principally by septicaemia, cellulitis, endocarditis, meningitis and pneumonia, has been particularly associated with people of Asian ethnicity, caused by their handling live and freshly killed fish (mainly tilapia) contaminated with *S. iniae* during food preparation (Facklam et al., 2005; Hastein et al., 2006). The bacterium is most often introduced through wounds and abrasions in the skin (Hastein et al., 2006). In the reported cases from Canada, the affected patients were elderly and many had poor health and compromised immune systems. The risk of healthy humans acquiring disease is minimal (Hastein et al., 2006).

5.1.3. *Mycobacterium species*

Several *Mycobacterium spp.*, such as *M. marinum*, *M. chelonae* and *M. fortuitum*, have been reported in both fish and humans (Hastein et al., 2006). Fish with mycobacteriosis pose a particularly significant threat of transmitting the infection to humans and thus may well become hazardous to human health (Hastein et al., 2006). Mycobacteriosis is a chronic disease reported in seawater, brackish water and freshwater fish species, in aquaculture and aquariums as well as from the wild (Hastein et al., 2006). Human infection with *M. marinum* has been reported in many countries since 1951, yet it is still considered rare (Hastein et al., 2006). It is mainly associated with granulomatous skin lesions, especially lesions acquired by aquarists when cleaning fish tanks (Hastein et al., 2006). The infection usually enters through open wounds or abrasions exposed to contaminated water in which infected fish have been kept or while processing fish (Hastein et al., 2006). In the USA, rockfish infected with *M. marinum* are believed to cause skin problems in humans (Hastein et al., 2006).

5.1.4. *Enterobacteria*

The studies about the human pathogenic bacterial load, particularly enterobacteria in fresh water is scanty in literature (Emikpe et al., 2012). The total aerobic bacteria and enterobacteria load in African Catfish (*Clarias gariepinus*) and Nile Tilapia (*Oreochromis niloticus*) randomly sampled from different aquatic environments in Ibadan, Southwest Nigeria by Emikpe et al. (2012). The high microbial load of the wild catfish and wild tilapia in their study (Emikpe et al., 2012) may be due to mass pollution of the environments where the fish were caught. *Escherichia coli* is implicated in newborn meningitis and infantile diarrhea, *Salmonella paratyphi* is the causative agent of paratyphoid fever in humans, who are the only reservoir of this organism (Adebayo-Tayo et al., 2006). *Enterococcus sp.* has been implicated in human infections like pharyngitis, scarlet fever and pneumonia (Adebayo-Tayo et al., 2009).

5.1.5. *Vibrio Species*

Centre for Disease Control reported in 1976 that the genus *Vibrio* were distributed in the tropical sea water and in the aquatic environment. This genus includes 37 species among which only 11 species are pathogenic to human causing disease (Rajapandiyana et al., 2009). *Vibrio* spp. are Gram-negative, facultatively anaerobic motile curved rods with a single polar flagellum. Among the members of the genus, 11 species have so far been reported to be pathogenic to humans, where eight of these may be associated with foodborne infections of the gastrointestinal tract. Most of these foodborne infections are caused by *V. parahaemolyticus* and *V. cholerae*, and to a lesser extent by *V. vulnificus* (FAO/WHO, 2005). The following paragraphs highlighted the characteristics of these three vibrios. Among the vibrios, *V. cholerae* is of most concern because of its ability to cause cholera. *V. cholerae* can be divided into serogroups on the basis of the O antigen. Of the more than 200 *V. cholerae* serogroups that exist, only O1 and O139 are associated with the epidemiological features and clinical syndrome of cholera (FEHD, 2005). However, organisms of *V. cholerae* serogroups other than O1 and O139 (non-O1 non-O139 serogroups) have been associated with sporadic cases of foodborne outbreaks of gastroenteritis, but have not spread in epidemic form (Heymann, 2004). The most important virulence factor associated with *V. cholerae* O1 and O139 serogroups is the cholera toxin. Non-O1 non-O139 serogroups are generally nontoxicogenic (FEHD, 2005). There are only limited reports available in the world regarding the pathogenicity of *V. vulnificus* (Rajapandiyana et al., 2009).

5.2. PRINCIPAL HUMAN DISEASE RESULTING FROM INGESTION OR CONTACT WITH FISH AND SHELLFISH

Two major concerns are observed here: 1) those mainly affecting the consumers and 2) those causing occupational diseases among people employed in the fishing industry.

5.2.1. Diseases mainly affecting Consumers

5.2.1.1. Bacterial

1) Bacterial infections (e.g., salmonellosis (003) typhoid fever (001); paratyphoid fever (002); *Vibrio parahaemolyticus* infection (005.8); and shigellosis (004).

2) Bacterial intoxications (e.g., botulism (005.1) and staphylococcal food poisoning (005.0)

3) Bacterial intravital intoxication (e.g., cholera (000), *Clostridium perfringens* food poisoning (005.2)).

4) Parasitic (e.g., clonorchiasis (121.1), paragonimiasis (121.2), diphyllorhynchiasis (123.4), and anisakiasis).

5) Viral (e.g., infectious hepatitis (070).

6) Intoxication due to chemical poisons (e.g., chronic mercury poisoning (Minamata disease).

7) Intoxication due to biotoxins (e.g., paralytic shellfish poisoning, tetraodon poison, and ciguatera poisons

8) Allergic reactions following ingestion

9) Undetermined etiology (e.g., agents causing summer diarrhoea) (Adedeji and Adetunji, 2004; Adedeji, 2012).

5.2.1.2. Parasitic diseases

1) Several helminths inhabiting fish as larval stages are capable of causing diseases in human beings if they are ingested (Adedeji and Adetunji, 2004; Adedeji, 2012).

2) Most of the fish-borne helminthoses of man have a limited geographical distribution (Adedeji and Adetunji, 2004; Adedeji, 2012).

3) The food habits of the people in areas where such helminths are found are the main factors influencing the incidence of these diseases (Adedeji and Adetunji, 2004; Adedeji, 2012).

4) Man becomes infected only if he ingests raw, insufficiently cooked, or improperly processed (Adedeji and Adetunji, 2004; Adedeji, 2012).

5) Trematodes: Over 40 species of trematodes belonging to the genera *Opisthorchis*, *Clonorchis*, *Metrorchis*, *Pseudamphistomum*, *Metagonimus*, *Clinostomum*, *Paragonimus*, *Heterophyes* etc. *Opisthorchis*, *Clonorchis*, *Paragonimus*, are the most important they are wide spread and can cause serious diseases (Adedeji and Adetunji, 2004; Adedeji, 2012). They parasitized the bile ducts, liver and intestine. *Paragonimus* may invade the lungs; go to ectopic sites including the brain and heart (Adedeji and Adetunji, 2004; Adedeji, 2012).

6) Cestodes: Plerocercoids of pseudophyllidean tapeworm species, among which the genus *Diphyllorhynchium* is the most important, may reach man through consumption of raw fish (Adedeji and Adetunji, 2004; Adedeji, 2012). If raw fish muscle or roe containing the living plerocercoids reaches the intestine of the definitive mammalian host, the plerocercoids develop into the adult tapeworms that generally cause debility and anaemia (Adedeji and Adetunji, 2004; Adedeji, 2012). Plerocercoids of species other than *Diphyllorhynchium* are incapable of maturing to the adult tapeworm in man but migrate into the skin or subcutaneous tissues causing the condition known as sparganosis. Water containing proceroids, or infested copepods and the meat of other animals are more frequent sources of this disease than fish (Adedeji and Adetunji, 2004; Adedeji, 2012).

7) Nematodes: Migrant larvae of several nematodes genera (*Anisakis*, *Ancylostoma*,

Bunostomum, Contraecum, Dioctophyme, Gnathostoma, Toxocara, Angiostrongylus and Capillaria (Adedeji and Adetunji, 2004; Adedeji, 2012)

8) Anisakis: The larvae occur in many marine fishes. Man is infected by eating raw or improperly processed fish. Slightly salted raw herring is the most commonly reported source of infection. There is no obvious reaction in man when the first larvae migrate from the gut into the abdominal cavity, but subsequent penetration of other larvae at the same site induces a severe reaction as a result of the previous sensitization. This allergic reaction causes localized enteritis described as eosinophilic phlegmonous enteritis (Adedeji and Adetunji, 2004; Adedeji, 2012).

9) *Angiostrongylus cantonensis* Is a common parasite of the lungs of rats in the Pacific Islands and South-East Asia. This parasite has been shown to cause eosinophilic meningitis in man. Its usual intermediate host is a slug or land snail, and occasionally the larva can pass through a paratenic host such as freshwater prawn, land crab, and possibly bonito from which man is infected (Adedeji and Adetunji, 2004; Adedeji, 2012).

10) *Capillaria philippinensis*: The nematode has been recognized as causing a syndrome called intestinal capillariasis in man. It is a disease characterized by intractable diarrhoea with malabsorption due to atrophic changes in the intestinal epithelium produced by the parasites (Adedeji and Adetunji, 2004; Adedeji, 2012). The infection is believed to be caused by eating raw freshwater fish harbouring the eggs of the parasites. It is not known, however, whether the fish act as intermediate hosts or paratenic hosts. An unusual feature of the infection is that all stages of the parasites can be seen in the same infected person and therefore autoinfection is possible (Adedeji and Adetunji, 2004; Adedeji, 2012).

5.3. FISH RELATED FOOD BORNE ILLNESS AND DISEASES

The quality of our fish is of major concern to the food processors, consumers and public health authorities and provisions of safe, wholesome and acceptable fish and its product as food to consumers and control of microorganisms is essential to meet these objectives (Adebayo-Tayo et al., 2012b). According to the Center for Food Safety and Applied Nutrition in Washington (2001 cited by Adebayo-Tayo et al., 2012b), most fish related food borne illness are traced to *Salmonella*, *Staphylococcus spp.*, *Escherichia spp.*, *Vibrio parahemolyticus*, *Clostridium perfringens*, *Clostridium botulinum E*, and *Enteroviruses* (Yagoub, 2009; Adebayo-Tayo et al., 2012b). Microorganisms are found mostly on the skin, gills, operculum and intestines of live and newly caught fish. The microbial loads vary enormously in

the different parts of the fishes and reported the normal range of 10^{-2} - 10^7 on skin surfaces (Adebayo-Tayo et al., 2012b). The potential of seafood to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Adebayo-Tayo et al., 2012b).

5.3.1. *V. parahemolyticus* food borne illness

V. parahemolyticus ranked first as the most common causative agent of food poisoning outbreaks in Hong Kong in recent years (FEHD, 2005). Among all the food groups, seafood is the most frequently incriminated food which caused *V. parahemolyticus* food poisoning outbreak. It accounted for 56.7% of the total number of confirmed cases in Hong Kong (FEHD, 2005). Oysters pose high risk of *V. parahemolyticus* food borne illness due to their ability to concentrate pathogenic vibrios and toxins during the filter feeding process (Zhuang et al., 2007; Shen et al., 2009). The study by Merwad et al. (2011) clarified an infection rate of 2% for *V. parahemolyticus* in oysters in Egypt. Nearly similar findings of 2.04% in clams and 2.7% in mussels were recorded in Italy (Baffone et al., 2000). Slightly lower infections of *V. parahemolyticus* were cited in mussels by Colakoglu et al. (2006), where they reported 1.2% in Turkey. On the other hand, higher percentages were recorded in previous studies: Pinto et al. (2008) reported 32.6% for mussels in Italy; Blanco – Abad et al. (2009) reported 11.2% for mussels in Spain and Kirs et al. (2011) reported 94.8% for oysters in New Zealand. The higher reports of *V. parahemolyticus* in these studies may be associated with the growing of bivalve mollusks in uncontrolled waters subjected to contamination and their peculiar characteristic of filtering large amounts of water. *Vibrio parahemolyticus* may spread into humans orally via contaminated mollusc shellfish particularly oysters (Drake et al., 2007; Merwad et al., 2011) leading to development of gastroenteritis with diarrhea (Cho et al., 2008; Merwad et al., 2011) accounting for 60-80% of cases, wound infections in 34% and 5% have septicemia (Merwad et al., 2011). Although few data exist on *V. parahemolyticus* infections in human, a notable increase in its worldwide incidence has been reported (Merwad et al., 2011). Other studies detected *V. parahemolyticus* in stool of diarrheic patients: Fuenzalida et al. (2007) recorded 19 patients in Chile as a result of mussel consumption. In addition, Lee et al. (2003 cited by Merwad et al., 2011) isolated two *V. parahemolyticus* strain from patients stool with acute gastroenteritis and edible mollusk abalone and their implication as the first evidence of laboratory acquired zoonosis.

5.3.2. *Vibrio vulnificus* food borne illness

Vibrio vulnificus is a notorious sea-food borne pathogen with a high mortality rate. This is

ubiquitously present in marine environments, particularly in tropical water (Rajapandiyani et al., 2009). *Vibrio vulnificus* is a leading cause of seafood-related deaths in the United States (Han et al., 2011). *Vibrio vulnificus* is one of the emerging food and waterborne zoonotic bacteria that represents a human health hazard (Canigral et al., 2010). This pathogen causes gastroenteritis and primary septicemia due to consumption of contaminated oysters, while skin and soft tissue infection results from handling contaminated shellfish or from exposure of open wounds to sea water (Horseman and Surani, 2011). High prevalence of *V. vulnificus* has been identified in the coastal and estuarine environment during warm weather due to high temperatures in the sea (Rajapandiyani et al., 2009). During hot weather up to 25% of death rate have been reported in USA, Europe and Asia, due to exposure of wounds to sea water and handling of seafood products (Guagliandolo et al., 2005; Rajapandiyani et al., 2009). Colakoglu et al. (2006) reported higher percentage of 16.6 for *Vibrio vulnificus* in their study. However, previous studies cited irrelevant higher reports: Canigral et al. (2010) cited 10% in oysters and Kirs et al. (2011) cited 17.2% in oysters. Regarding the zoonotic significance, *V. vulnificus* are usually acquired through ingestion of shellfish or through contaminating open wounds during swimming, crabbing, shellfish cleaning and other marine activities as was previously sustained and are implicated in epidemic human gastroenteritis (Ballal et al., 2010). For the zoonotic hazard, 252 cases of *V. vulnificus* infection were recorded of which 116 cases followed consumption of crabs (Barton and Ratard, 2006; Merwad et al., 2011).

5.3.3. *V. fluvialis* Food borne illness

Regarding the public health hazard, *V. fluvialis* was reported in a human case of severe watery diarrhea and bacteremia in Taiwan (Lai et al., 2006; Merwad et al., 2011). Also, *V. fluvialis* was implicated in an outbreak of food poisoning and gastroenteritis in India during 1981 with an infection rate of 64.28% (Merwad et al., 2011). Also, consumption of raw shellfish among immunocompromised patients is a risk factor for severe *V. vulnificus* infection (Merwad et al., 2011). Infection due to *V. fluvialis* most commonly present as gastroenteritis and diarrhea (Merwad et al., 2011).

With respects to *V. fluvialis*, Merwad et al. (2011) reported higher infection rates in oysters (16%), followed by crabs (14%) and shrimps (12%). However, lower infection rates were cited in other studies. Gopal et al. (2005) cited by Merwad et al., (2011) recorded 4.6% in shrimps and Hidalgo et al. (2008) obtained 3.7% in clams. The variations in the incidence of *V. fluvialis* in shellfish may be accounted for the differences in water contamination levels in

many geographic areas (Merwad et al., 2011). In addition, higher reports of *V. fluvialis* in the study by Merwad et al. (2011) were associated with presence of planktons which are a tool for survival and distribution of these bacteria in aquatic environments as was advocated by Gugliandolo et al. (2005). Merwad et al. (2011) reported the total infection rate of *V. fluvialis* in stools from diarrheic patients to be 8%.

5.3.4. *V. hollisae* Food borne illness

Thirty three cases of human infection with *V. hollisae* have been described after eating raw oysters (Merwad et al., 2011). The study by Merwad et al. (2011) showed an infection rate of 2% for *V. hollisae* in diarrhoeic patients. Otherwise, higher infection rate (18.8%) was cited in adult patients.

5.3.5. *V. furnissii* Food borne illness

Concerning the public risk, *V. furnissii* has been linked with infantile diarrhea and diarrheal disease from 16 patients in Brazil (Merwad et al., 2011). In the study by Merwad et al. (2011), the overall infection rate of *V. furnissii* in diarrheic patients was 2%.

5.4. FOOD BORNE INTOXICATIONS CAUSED BY FISH CONSUMPTION

5.4.1. *Clostridium botulinum*

Clostridium botulinum type E is a strict anaerobic bacterium that may occasionally be present in fish (Hastein et al., 2006). This bacterium is a recognised commensal in fish in fresh water, as well as in sea water. Under optimal growth conditions, the bacterium may produce a potent neurotoxin in processed fish (Hastein et al., 2006). To avoid problems, it is important to control the factors that can prevent the growth of this organism in fish and fish products, such as temperature and salt concentration. The anaerobic bacterium *C. botulinum* has been reported to cause intoxication in farmed fish, as well as terrestrial animals and humans (Hastein et al., 2006). Thus, *C. botulinum* may become an important hazard to the food safety of aquaculture products (Hastein et al., 2006). The condition in pond-cultured fish is usually called botulism or bankruptcy disease and, as in humans, it is due to the potent neurotoxin produced by the bacterium. In countries where fermented fish (both farmed and wild salmonids) is a speciality, unhygienic production conditions may result in intoxication and death in humans (Hastein et al., 2006).

5.4.2. Histamine fish poisoning

Scombrototoxic fish poisoning, also known as scombroid or histamine fish poisoning, is caused by bacterial spoilage of a limited number of fish species (Hastein et al., 2006). These comprise mainly: mackerel (*Scomber scombrus*), bonito (*Sarda* spp.), various tuna species (*Thunnus* spp.), swordfish

(*Xiphias gladius*), common dolphinfish (mahi-mahi) (*Coryphaena* spp.). As bacteria break down fish proteins, by-products, such as histamine and substances blocking histamine breakdown, may build up in fish. The human tolerance limit for histamine is 10 mg per 100 g. In general, there is no risk of histamine poisoning in well-iced fish (Hastein et al., 2006).

5.4.3. Allergies to fish and seafood

Eating fish may produce severe allergic reactions. Allergies to fish, shellfish and mussels are among the most common food allergies triggered by immunoglobulin-E antibodies, and allergic reactions to seafood antigens may produce severe symptoms, including angio-oedema and anaphylaxis. These symptoms do not differ from allergic reactions to any other type of food (Hastein et al., 2006). Even though allergens are more or less species-specific, there is a high degree of cross-reactivity among different fish species. This means that a patient who is allergic to one fish species is at a high risk of being allergic to other species (Hastein et al., 2006).

There are no specific symptoms for allergic reactions to food. Consequently, after ingesting seafood, the clinical manifestations of an allergic reaction do not differ from allergic reactions to any other type of food (Hastein et al., 2006). An allergy to fish and shellfish often becomes evident during the first year of life but, in general, presents later than an allergy to eggs and milk (Hastein et al., 2006). While many children outgrow their allergies to eggs, cows milk, wheat and soy, they may continue to be hypersensitive to fish and shellfish in later life (Hastein et al., 2006). There is no evidence supporting the contention that the prevalence of fish allergy has anything to do with the level of fish intake, despite comments made in the literature. Studies on fish allergy in Reykjavik (high consumption) and Uppsala (low consumption) showed that, although the population in Reykjavik ate two to three times as much fish as that of Uppsala, there were no significant differences in prevalence between these two populations (Hastein et al., 2006).

Fish consumption in Norway is among the highest in Europe; the median value being about 65 g per day (Hastein et al., 2006). However, the prevalence of seafood allergy in the Norwegian population is low (< 1%). The importance of seafood allergy to the health of the Norwegian population is marginal (Hastein et al., 2006). The first isolated allergen from fish is the calcium-binding protein, parvalbumin. Since patients usually react to both raw and cooked fish, it is assumed that the allergen is heat resistant. However, recent data indicate that some individuals react only to raw fish, while others react

only to cooked, suggesting the existence of additional allergens (Hastein et al., 2006). The lowest dose of fish reported to produce an allergic reaction is 5 mg. Established thresholds for food allergens are important tools for food production and labelling (Hastein et al., 2006). However, at present there are insufficient data available to establish such thresholds. Consequently, no values have been established for food allergens in European Union (EU) legislation (Hastein et al., 2006).

5.5. ZOONOTIC AGENTS IN FISH

- Transmitted between animals and humans
- Zoonoses are common
- Animals part of everyday life
- Recognize the risk factors

In general, true zoonotic agents associated with fish, crustaceans and molluscs are few. Many commensal and pathogenic bacteria, viruses, fungi and parasites associated with fish have temperature growth limits that will not support their development in humans. However, there are some exceptions. In the following section, bacterial and parasitic agents which can cause disease in both fish and humans are discussed (Hastein et al., 2006).

5.5.1. Zoonotic Pathogens

a. Mycobacterium

i. Organisms: *Mycobacterium* spp. (most often *M. marinum* & *fortuitum*).

ii. Clinical Signs

1. Animals – multifocal skin sores in amphibians and nodules in fish (CCARE, 2012).

2. Humans – Skin sores at site of infection; disseminated disease in immunosuppressed individuals.

iii. Transmission: Contamination of tiny scratches on the hand or skin (CCARE, 2012).

b. Fish Handler's Disease or Erysipelas

i. Organisms: *Erysipelothrix rhusiopathiae*

ii. Clinical Signs

1. Animals – Asymptomatic or diarrhea.

2. Humans – localized skin sore with pain and swelling, may lead to more widespread skin or systemic infection.

iii. Transmission: Contamination of tiny scratches on the hand or skin (CCARE, 2012).

c. Salmonellosis

i. Organisms: *Salmonella* spp.

ii. Clinical Signs

1. Animals – Asymptomatic.

2. Humans – Diarrhea, nausea, vomiting, abdominal pain.

iii. Transmission: Accidental ingestion of contaminated water/environment (CCARE, 2012).

TABLE 1: ZOONOSES OF FISH, AMPHIBIANS AND REPTILES

PATHOGEN	TRANSMISSION	ANIMAL DISEASE	HUMAN DISEASE
<i>Salmonella spp.</i>	<ul style="list-style-type: none"> • direct contact, handling, and ingestion of animal and/or water • remains virulent in tap water for 3 months and pond water for 4 months 	<ul style="list-style-type: none"> • fish, amphibian and reptile carriers rarely show any clinical disease • intermittent shedding 	<ul style="list-style-type: none"> • abdominal pain, acute gastroenteritis, bloody mucoid diarrhea, nausea, vomiting, fever • meningitis, osteomyelitis, urinary tract infections • increase prevalence and severity in immunocompromised individuals
<i>Aeromonas spp.</i>	<ul style="list-style-type: none"> • puncture wounds, lacerations, and ingestion 	<ul style="list-style-type: none"> • ulcerative stomatitis in snakes • fatal hemorrhagic septicemia in snakes and fish • common isolate of fish skin ulcers 	<ul style="list-style-type: none"> • wound infections, fever • diarrhea • septicemia if immunocompromised
<i>Campylobacter spp.</i>	<ul style="list-style-type: none"> • handling and ingestion of animal and/or contaminated water 	<ul style="list-style-type: none"> • fish, amphibian, and reptile carriers rarely show any clinical disease 	<ul style="list-style-type: none"> • diarrhea, acute gastroenteritis, nausea, vomiting, cramps, fever
<i>Klebsiella spp.</i> <i>Enterobacter spp.</i>	<ul style="list-style-type: none"> • direct contact, handling 	<ul style="list-style-type: none"> • fish and reptile carriers rarely show any clinical disease • pulmonary infections in snakes 	<ul style="list-style-type: none"> • urinary tract infections, septicemia
<i>Yersinia spp.</i>	<ul style="list-style-type: none"> • handling fish and reptiles 	<ul style="list-style-type: none"> • enteric “red-mouth” disease 	<ul style="list-style-type: none"> • acute painful gastroenteritis • mesenteric adenitis, nephritis, arthritis
<i>Mycobacterium spp.</i>	<ul style="list-style-type: none"> • handling, puncture wounds, scratches and/or inhalation 	<ul style="list-style-type: none"> • affects fish and reptiles • granulomatous disease affecting skin, subcutis, oral mucosa, lungs, liver, spleen, gonads, bones, and/or CNS (“fish tank granuloma”) • hemorrhages, exophthalmos, and skeletal deformities in fish • ulcerative stomatitis in snakes 	<ul style="list-style-type: none"> • circumscribed cutaneous granulomatous disease at infection site • immunocompromised individuals may develop disseminated respiratory disease, lymphadenitis, arthritis, osteomyelitis and/or tenosynovitis
Zygomycosis Phycomycosis Mucormycosis	<ul style="list-style-type: none"> • inhalation, ingestion, or inoculation with spores 	<ul style="list-style-type: none"> • saprophytic fungi are common isolates from fish, amphibian, and reptile gastrointestinal tracts • may produce upper respiratory disease and pneumonia 	<ul style="list-style-type: none"> • upper respiratory infections and conjunctivitis may lead to meningitis • dermatitis or subcutaneous infection if wound contamination • gastritis or enteritis if ingested
<i>Aspergillus spp.</i>	<ul style="list-style-type: none"> • direct contact, inhalation 	<ul style="list-style-type: none"> • isolated from skin, pulmonary, and systemic lesions of reptiles 	<ul style="list-style-type: none"> • immunocompromised patients are highly susceptible to disseminated disease • bronchopneumonia, disseminated infections (thyroid, brain, myocardium), and/or hypersensitivity
<i>Candida spp.</i>	<ul style="list-style-type: none"> • direct contact, inhalation 	<ul style="list-style-type: none"> • isolated from pulmonary and hepatic lesions of reptiles and skin lesions of fish 	<ul style="list-style-type: none"> • immunocompromised patients are susceptible to hematogenous spread to eyes, kidneys, bones • white plaques on oral mucosa, skin-fold dermatitis
<i>Cryptosporidium</i>	<ul style="list-style-type: none"> • no know transmission to humans 	<ul style="list-style-type: none"> • isolated from reptiles and fish 	<ul style="list-style-type: none"> • immunocompromised patients are highly susceptible to severe, persistent diarrhea
Gnathostomiasis	<ul style="list-style-type: none"> • handling or ingestion of contaminated water 	<ul style="list-style-type: none"> • infected fish shed infective nematode larvae into water • amphibians and reptiles may be transport hosts 	<ul style="list-style-type: none"> • nausea, salivation, pruritus, edema, urticaria, and stomach discomfort • larvae may migrate to other organs leading to localized inflammation and/or specific organ disease
Mites	<ul style="list-style-type: none"> • direct contact with infested animal 	<ul style="list-style-type: none"> • heavy infestations on reptiles may lead to severe anemia, lethargy, and death 	<ul style="list-style-type: none"> • papular, vesicular, or bullous lesions with variable pruritus

Sources: Acha and Szyfres (1989), Nemetz and Shotts, Jr. (1993), Johnson-Delany (1996); Cornell Center for Animal Resources and Education (CCARE, 2012).

5.5.2. Detection of *Vibrio* species in Fish

From zoonotic point of view, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. hollisae* and *V. furnissii* were isolated from stool of diarrheic patients in a study by Merwad et al. (2011), and associated with consumption of undercooked shellfish. Some zoonotic vibrios were also detected in water samples analyzed by Merwad et al. (2011) with 5% infection rate for *V. vulnificus*, *V. alginolyticus* and *V. fluvialis*, while that of *V. damsella* was 10%.

Otherwise, low incidence of *V. fluvialis* (0.6%) was reported by Gopal et al. (2005). Also, the infection rate of *V. alginolyticus* in water in that same study by Merwad et al. (2011) contrasted other studies cited by Masini et al. (2007) cited 28.5% and Hassanin (2007) cited 60%. Comparing the prevalence of *V. vulnificus* reported by Merwad et al. (2011) in water, lower percentages of 2 and 3.7 were detected by Masini et al. (2007) and Gugliandolo et al. (2005), respectively. However,

higher infection rates of 12.5% and 32% for *V. vulnificus* were cited in previous reports by Canigral et al. (2010). According to Maugeri et al. (2006) and Merwad et al. (2011), the different results of *Vibrio* infections in sea water may be attributed to differences in level of contamination of investigated geographic areas.

Previous studies determined the occurrence of *V. parahaemolyticus* in shellfish in different geographic areas over the world: Gopal et al. (2005 cited by Merwad et al., 2011) reported it in shrimp in India; Hassanin (2007) in Egypt, and Kirs et al. (2011) reported it in oyster in New Zealand, whereas Colakoglu et al. (2006) in Turkey; and Blanco-Abad et al. (2009) in Spain. Many literatures reported other pathogenic *Vibrio* in different seafood: Hidalgo et al. (2008) found *V. alginolyticus*, *V. fluvialis* in molluscan shellfish in Spain. In Egypt, Merwad et al. (2011) identified *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. hollisae*, *V. furnissii*, *V. mimicus*, *V. alginolyticus*, *V. damsella* from shrimps, crabs and oysters.

The differences in infection rates of patients with *Vibrio* spp. may obey to variation in cultural food habits and geographic areas. Thermostable direct hemolysin gene (*tdh*) has been recognized as primary virulence factor in pathogenic *V. parahaemolyticus* (Pinto et al., 2008; Merwad et al., 2011). Based on studies conducted in different regions of the world, generally 0.2 to 3% of environmental *V. parahaemolyticus* isolates are potentially pathogenic based on presence of *tdh* gene (Nordstrom et al., 2007; Merwad et al., 2011). In the current study by Merwad et al. (2011), *tdh* gene was detected in 1 out of 2 *V. parahaemolyticus* isolates (50%) from stool of diarrheic patients. Their finding (Merwad et al., 2011) was nearly in accordance to Robert – Pillot et al. (2004), who cited that 46% of *V. parahaemolyticus* isolates from patient stool was *tdh* + in France. However, each microbial isolate from human stool was *tdh* (100%) in Chile (Merwad et al., 2011).

In a study by Wafaa et al. (2011), *Vibrios* were isolated from 52.0% of tested seafood with the highest percentage (88.0%) from oyster. The results from the study by Wafaa et al. (2011) confirmed that bacterial contamination in seafood products is common, and suggest that routine examination of such products for pathogenic agents would be advisable. While in Iran, a report showed that only 2.1% of studied shrimp samples harbored *Vibrio* spp. Isolated *Vibrio* spp. were *V. parahaemolyticus*, *V. damsela*, *V. alginolyticus* and *V. fluvialis*, that the authors described as indigenous to the marine environment and shrimps (Hosseini et al. 2004). Yang et al. (2008) revealed 251 isolates of *V. parahaemolyticus* from 1293 seafood samples (19%) collected from the 25 sites in China, during July to October in 2007, while Ji et al. (2011), reported 58.6%

of 239 samples from different sources were positive for *V. vulnificus* in 10 Chinese cities from June to September 2009.

Previous studies recorded variant distributions of *tdh* positive *V. parahaemolyticus* of shellfish origin: Pinto et al. (2008) reported 33.3% in mussels in Italy and Messelhauser et al. (2010) reported 18.18% in shrimps in Germany. This is compared with other studies targeting *tdh* gene in *V. parahaemolyticus* isolates from oysters where 44% of pacific oysters from Alaska (Nordstrom et al., 2007), 44-56% of Eastern oysters from Mexico (Zimmerman et al., 2007), 20% of Eastern oysters from Chesapeake bay (Parveen et al., 2008), 3.4% of oysters from India (Ragunath et al., 2009) and 3.4% of oysters from New Zealand (Kirs et al., 2011) have been found *tdh V. parahaemolyticus*. Thereby, detection of *V. parahaemolyticus* isolates bearing *tdh* gene from patients stool and oyster constitutes a public health hazard, where this microbial infection may cause food poisoning and gastroenteritis (Merwad et al., 2011). Messelhauser et al. (2010) also pointed that PCR assay is a time saving and a reliable solution for detection of pathogenic *V. parahaemolyticus*. Shellfish acts as an important food vehicle for some zoonotic vibrios, of which these microorganisms are implicated in outbreaks of food poisoning and diarrhea in humans. *V. parahaemolyticus* carrying *tdh* gene in oyster and diarrheic stool using PCR could be useful as basis for a preventive consumer protection policy. Recent studies has recommended further investigation for other virulent genes in pathogenic *V. parahaemolyticus*.

5.5.3. Detection of *Salmonella* species in some seafood

Sea foods are prone to bacterial contamination and could cause health risk to consumers (Wafaa et al., 2011). Wafaa et al. (2011) reported *Salmonella* in 10.0% of samples consisting 14.0%, 8% and 8% from shrimp, oyster and mussel respectively. In the Wafaa et al. (2011) study, *Salmonella* was recovered from 10% of seafood samples, 14% of shrimp samples, and 8% of each of oyster and mussel samples. In India, Kumar et al. (2008) detected *Salmonella* in 18.9% of naturally contaminated shrimp samples and 21.4% of crab, clam, mussel and oyster samples. Much higher results were obtained by Shabarinath et al. 2007, who examined 100 fish and shellfish samples obtained from the market and fish-landing centre in India, where *Salmonella* was detected in 70% of fish, 59% of shrimp and 30% of oyster samples. The potential source of *Salmonella* contamination in seafood farms is likely due to poor water quality, farm runoff and fecal

contamination from wild animals or livestock (Wafaa et al., 2011). In addition to poor distribution, retail marketing, handling, and preparation practices, high stocking densities and high water temperature may be responsible for increased *Salmonella* contamination of shrimp (Wafaa et al., 2011). In the Wafaa et al. (2011) study, the relatively low recovery of *Salmonella* obtained from samples could be attributed to the fact that mussel are cleaned from dirt and plankton and salted by addition of sodium chloride before they are sold. That comes in concordance with the results of Mansour *et al.* (1998 cited by Wafaa et al., 2011), who suggested that risk of food poisoning due to *Salmonella* from consumption of Om El Kholoul is reduced by addition of salt and lemon before consumption.

Several authors reported the occurrence of *Salmonella* at wholesale markets, importers and distributors, where they isolated *Salmonella* from utensils (2%) and floor swab samples (4%) (Sivapalasingam *et al.* 2004 cited by Wafaa et al., 2011). As regards isolated *Salmonella*, seven different *Salmonella* serotypes were identified (*Typhimurium*, *Derby*, *Typhi*, *Paratyphi A*, *Paratyphi B*, *Infantis*, and *Abortus equi*). *S. Typhimurium* and *S. Derby* were the predominant serotypes (40.3% and 26.7% respectively) in the study by Wafaa et al. (2011). In concordance to Wafaa et al. (2011) findings, Kumar *et al.* (2009) reported that *S. Weltevreden*, *S. Rissen*, *S. Typhimurium* and *S. Derby* were found to be the most predominant serovars in seafood. Similarly the most frequent serotypes in imported seafood were commonly involved in food borne illness in the U.S. (Wafaa et al., 2011). *S. Typhimurium* is the most *Salmonella* serotype that has ubiquitous host range (Busani *et al.* 2004 cited by Wafaa et al., 2011). Isolation of *Salmonella* serovars from live molluscan shellfish from marine environments has been reported from Galicia region of Spain (Wafaa et al., 2011).

5.6. MAIN CONTRIBUTING FACTORS OF FISH RELATED FOOD BORNE ILLNESS AND DISEASES

The objectives of most recent studies have been to determine: (i) the occurrence and distribution of *V. cholerae* in local waters and its association with environmental factors including the *E. coli* count; (ii) the occurrence of *V. cholerae* in live fish kept in fish culture zones and its association with other parameters (FEHD, 2005). While vibrios are natural habitants of seawater, it is not surprising that the majority (56.7%) of *V. parahaemolyticus* food poisoning outbreaks are caused by the consumption of seafood in a study carried out in Hong Kong. Among all the seafood items, crustaceans, including crab, shrimp/prawns and lobster, represented the highest percentage (28.7%) of incriminated seafood involved in *V. parahaemolyticus* outbreaks in Hong Kong. Of the 313 confirmed *V.*

parahaemolyticus food poisoning outbreaks due to consumption of seafood during 1999 to 2003 in Hong Kong, inadequate cooking was the main contribution factor which accounts for 59.7% of the cases, and this was followed by contamination by raw food which accounts for 23.6% of the cases (FEHD, 2005).

Nearly 60% of all the *V. parahaemolyticus* food poisoning cases by seafood occurred in 1999 to 2003 were due to the consumption of inadequately cooked food (FEHD, 2005). The second and third major contributing factors for *V. parahaemolyticus* food poisoning was due to the consumption of food contaminated by raw food and the consumption of contaminated raw food respectively (FEHD, 2005). Thorough cooking and avoidance of cross contamination hold the key to successful prevention of *V. parahaemolyticus* food poisoning (FEHD, 2005). These two factors (consumption of inadequately cooked food and consumption of food contaminated by raw food) contributed to more than 80% of all the cases. Other factors included other reasons for cross contamination, improper storage, inadequate reheating and food prepared too far in advance. Following the isolation of *V. cholerae* in fish tank water of two retail outlets selling live seafood in Hong Kong in 2003, there were calls to further strengthen the control the microbiological quality of fish tank water and abstraction of seawater for keeping live seafood (FEHD, 2005). The limited number of local study on ecology of *V. cholerae* suggested that the non-O1 non-O139 serogroup were isolated in both local seawater and seafood samples (FEHD, 2005). However, these studies were limited by small sample numbers and limited coverage of sampling locations (FEHD, 2005).

6. PREVENTION AND CONTROL MEASURES

Depending on the sources and other environmental factors, a wide range of variation in distribution of microflora in fish has been reported (Emikpe et al., 2011). The study Emikpe et al. (2011) clearly showed variation in bacterial load in fish of different sources. Therefore, precaution should be taken to prevent water contamination during harvesting as well as post harvest handling of fish.

According to the study by Emikpe et al. (2011), the safety of the public then depends on the improvement of sanitation within the metropolis by provision of public toilets, and enactment of effective policy for the collection and disposal (management) of municipal solid waste as these would drastically reduce the pollution of running water and rivers with human and domestic waste.

The sanitary conditions under which fishes are reared or cultured in ponds should be improved by following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things (Emikpe et al., 2011).

The farmers should embrace standard operating practices as applicable to fish farming. The workforce should be educated on the maintenance of good hygienic practices, and should be provided with necessary working and safety equipment (Emikpe et al., 2011).

The microbial load of fish can also be improved through regular disinfection of catching gears or working equipment, and brief immersion of caught fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public (Emikpe et al., 2011). The public should be enlightened on the inherent danger that may accompany handling fresh fish or consumption of improperly cooked fish (Emikpe et al., 2011).

6.1. SAFE WORK PRACTICES

A document released by Cornell University provides information about potential zoonotic exposure while working with aquatic animals or their products (e.g. tissue samples). The infectious agents listed in this review are not all inclusive, but provide the most common zoonotic agents seen in aquatic animals (CCARE, 2012). The safe work practices are provided as suggestions for staff and researchers who work with animals, in animal facilities, or with animal products (CCARE, 2012).

6.1.1. Good Personal Hygiene (Hygiene-Hand Washing)

- i. Wash hands after working with animals or animal products and when leaving animal facilities.
- ii. Do not eat, drink, or use tobacco products in animal facilities (CCARE, 2012).

6.1.2. Personal Protective Equipment (PPE)

- i. Wear disposable gloves during procedures that increase the likelihood of exposure to zoonotic agents (e.g. handling of aquatic animals or cleaning holding tanks/aquariums) (CCARE, 2012). Exercise increased caution when handling sick animals (i.e. animals showing clinical signs such as skin lesions and lethargy) or when you have an exposed break in skin (e.g. cut or scratch) (CCARE, 2012).
- ii. Use proper PPE for work setting as appropriate (e.g. gown, facemask, and protective sleeves). Maintain dedicated protective clothing and footwear while working with animals or in animal facilities. Do not wear the same protective clothing outside of animal facility (CCARE, 2012).

- iii. Use face masks/goggles when appropriate (i.e. activities where splashing of water may occur) (CCARE, 2012).

6.1.3. Animal Care

- i. Isolate sick or infected animals when possible.
- ii. Handle and care for sick or infected animals last (CCARE, 2012).

6.1.4. Cleaning and Disinfection

- i. Maintain clean, dry, and uncluttered animal areas and workspace.
- ii. Disinfect laboratory work surfaces after each use. Use only disinfectants approved by facility managers and that are suitable for the potential agents identified in this information sheet (CCARE, 2012).

- iii. Dispose of deceased animals, animal products, items contaminated by animal products, contaminated bedding, and laboratory waste in a facility approved manner (CCARE, 2012).

6.1.5. Proper Sharps Handling

- i. Work only with one uncapped needle at a time and immediately dispose of after use in sharps receptacle.
- ii. Avoid recapping needles whenever possible (CCARE, 2012).

6.1.6. Medical Attention

- i. Contact Occupational Medical officers for medical evaluation if you suspect any exposure, or if you develop any symptoms associated with infection with zoonotic agents (e.g., fever, malaise, diarrhea, abdominal pain).

- ii. Alternatively, see your own personal health care provider if any injury or potential exposure to a zoonotic agent occurs.

- iii. Notify the principal investigator or supervisor and complete an accident and injury report (CCARE, 2012).

6.2. HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP)

6.2.1. History

The concept of HACCP as a food safety system started in 1959 when NASA asked the Pillsbury Company to develop a system for ensuring that food prepared for astronauts was virtually 100 percent safe (Russell et al., 2004). The traditional endproduct testing would not satisfy this requirement, because so many products would have to be tested to satisfy statistical requirements that little would be left for the astronauts (Russell et al., 2004). This dilemma actually raised a couple of questions about food processing in general, namely: 1) because companies at the time did not carry out extensive end-product testing, just how safe was our food supply? and 2) what might the industry do, using new technology, to approach the

100 percent safe level for all consumers, not just astronauts? (Russell et al., 2004).

The researchers concluded that the best approach to food safety would be to develop a preventive system which would require control over the raw materials, processes, environment, personnel, storage, and distribution early in the system (Russell et al., 2004). Combined with adequate record keeping, such a system could virtually eliminate the need for *routine* end-product testing. Such testing could be relegated to *verification* that a particular process is operating correctly. The HACCP system was introduced in 1971 during the National Food Protection Conference (Russell et al., 2004). In 1985 the National Academy of Sciences recommended that the HACCP system be adopted by all food regulatory agencies and that it become mandatory for all food processors. Since that time, HACCP has been adopted for seafood, red meat, pork, poultry, fresh fruits and vegetables, and other food processes (Russell et al., 2004).

HACCP has been endorsed by most of the countries to which aquaculture producers would be shipping, namely, the European Union, Canada, Australia, New Zealand and Japan, as well as by the United Nations through its Codex Alimentarius program (Russell et al., 2004). Seafood processors must keep in mind that HACCP *does not replace* GMPs or guard against fraudulent practices (Russell et al., 2004). Inspectors will continue to evaluate plants for proper sanitation, employee hygiene, nonsafety food standards, and even economic fraud (e.g., species mislabeling, incorrect size counts, etc) (Russell et al., 2004).

6.2.2. HACCP and aquaculture

HACCP Programs are designed to prevent unsafe foods from reaching the consumer. Although producers of aquatic products are exempt from HACCP-related regulations, the processors of all aquaculture products will list *receiving* or *pre-harvest* as a CCP in their HACCP plans (Russell et al., 2004). Therefore, it is the responsibility of the producer to provide the processor with information concerning chemical contaminants and aquaculture drugs so that the processor can comply with his plan. Aquaculture producers who engage in any form of processing, such as eviscerating or heading, are considered processors and must follow the procedures to determine what, if any, HACCP plans they might need (Russell et al., 2004).

The HACCP concept is built upon the following steps (Russell et al., 2004):

1. Conduct a hazard analysis.
2. Determine the critical control points (CCPs) in the process.
3. Establish critical limits.
4. Monitor each CCP.
5. Establish corrective actions.
6. Establish verification procedures.

7. Keep records and documentation.

Therefore, environmental chemical contaminants and pesticides should be considered a significant hazard and addressed at the processing step (the Critical Control Point) where a preventive measure can be used to prevent, eliminate, or reduce the likelihood of occurrence to an acceptable level (Russell et al., 2004). As previously mentioned, for aquaculture products this processing step is either pre-harvest or receiving, and the suggested preventive measures are as follows:

1. **Pre-harvest** as a CCP: On-farm visits by the processor to collect soil, water and/or fish samples to be analyzed for chemical contaminants and pesticides that are reasonably likely to be present in the growing area (Russell et al., 2004).

2. **Receiving** as a CCP: A. Receipt of producer's lot-by-lot certification of harvesting from uncontaminated waters. If this method is chosen the processor should keep in mind that inspectors will expect to see a verification procedure appropriate for such a "self-reporting" system (Russell et al., 2004).

Seafood processors and growers who are also processors (by definition above), must have a trained individual to carry out the following tasks: 1) develop the HACCP plan; 2) reassess and modify the HACCP plan and hazard analysis; and 3) review HACCP records within a week following their recording (Russell et al., 2004). The regulation defines a trained individual as one who has "successfully completed training in the application of HACCP principles to fish and fishery product processing that is at least equivalent to that received under a standardized curriculum recognized as adequate by the USFDA, or who is otherwise qualified through job experience to perform these functions (Russell et al., 2004). Job experience will qualify if it has provided knowledge at least equivalent to that provided through the standardized curriculum (Russell et al., 2004)."

7. CONCLUSION

It is well known that fish, particularly oily fish, are an important source of long chain fatty acids (LC n-3 polyunsaturated fatty acid or PUFA), reducing the risk of cardiovascular diseases, as well as having beneficial effects on foetal development (Hastein et al., 2006). Seafood is also a valuable source of certain minerals, vitamins and protein. However, balanced against this are the possible detrimental effects of contaminants found in certain fish species (Hastein et al., 2006). Scientific publications, as well as the national and international press, have questioned if the presence

of certain contaminants and residues represents a health risk to fish consumers (Hastein et al., 2006). In these discussions, the main emphasis has been on the chemical assessment and possible health risk of consuming wild or farmed fish, while little or no consideration has been given to the nutritional value (Hastein et al., 2006). In an effort to bring together the nutritional and toxicological considerations, food safety authorities in several countries have requested their relevant scientific committees to weigh the nutritional benefits against the possible risks of fish consumption, and such benefit-risk assessments have recently been performed in the UK and EU (Hastein et al., 2006). In Norway, a similar assessment is being completed by the Norwegian Committee on Food Safety (Hastein et al., 2006). However, it is important to understand the mechanisms and interactions between nutrients and contaminants in seafood if researchers are to give sound scientific advice on the amount and type of seafood that should be recommended to promote health and maximise safety in different groups of the population (Hastein et al., 2006). At present, there is no agreed methodology for taking both the risks and benefits of seafood into account in a quantitative way. The organisation EFSA advised that a framework should be developed which allows such a quantitative comparison, based on a common scale of measurement (EFSA, 2005).

Owing to the potential hazard of some pathogens, it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health. It is therefore recommended that people should be educated on the unhealthy implication of water pollution as this goes a long way in contaminating the aquatic animals (Udeze et al., 2012b). Proper processing, storage, and handling procedures should be cultivated. Adequate processing methods before consumption should be employed. Fish that have shown signs of spoilage should not be sold to the public so as to boost the health standard of the populace. The populace should also be educated on the proper measures to be embarked on sewage and effluents disposal and not to the rivers as this would pollute the water and cause health risk (Adebayo-Tayo et al., 2012c; Udeze et al., 2012b).

Because of the availability of safe water supply to the population and a reasonably high standard of sanitation locally, cholera no longer appears in an epidemic form in Hong Kong (FEHD, 2005). However, since *V. cholerae* are widely distributed in temperate and tropical aquatic environment, in particular estuarine waters and that cholera is endemic in this part of the world, cholera still appears in Hong Kong as sporadic diseases (FEHD, 2005). To protect animal and human health, internationally agreed maximum permitted

levels have been set for several chemical contaminants in both feed and food (Hastein et al., 2006). National and international monitoring programmes exist to ensure that the levels present are acceptable. In addition, aquaculture industries are using hazard analysis critical control point principles to ensure the acceptable quality of their products (Hastein et al., 2006). If fish and fish products contain values of environmental contaminants above the accepted international levels, this will almost certainly have a significant impact on international trade. Importing countries will introduce bans on fish and fish products. They have already done so due to contaminants such as cadmium, dioxins and malachite green (Hastein et al., 2006). Good manufacturing practices should always be observed by the trade to minimise the risk of cholera and vibrio food poisoning associated with the consumption of seafood products. Hygienic qualities of fish tank water in particular the source water for keeping live seafood is also important (FEHD, 2005).

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