Chapter one

Introduction

In a world where more than70% of the planet is covered with water, aquatic foodsmay provide an essential component of the global food toimprove nutrition, health, and well-being ofhumans(Tacon and Metian, 2013).

Fishes accounts for approximately 17% of the global animal protein intake globally, the production of fish compete the growth of world population(FAO, 2014).

Fish is one of the best sources of proteins, vitamins, minerals and essential nutrient required for supplementing both infant and adult diets. (Abdullahi *et al*; 2001).

Fish is a source ofunsaturated fats, called omega-3 fatty acid, which affectcardiac functions including hemodynamic and arterialendothelial function
(Wolfe, 2010).

Fish is an excellentsource of high quality protein, contains the essentialamino acids that are necessary for human health(Hoyleand Merritt, 1994).

Fish are extremely susceptible to microbial contamination because of their soft tissues and aquatic environment.

Contamination results mainly rupturing of fish intestines during poor processing or unhealthy washing .million of bacteria many of them potential spoilers are present in the surface slime on the gills and in the intestines of live fish ,although the flesh itself is normally sterile bacterial growth and invasion on the fish are prevented by the body's natural defense system during life ,but after death the defense system break down and the bacteria multiply and invade the flesh**.**(Abolagba and uwagbai; 2011).

The common human pathogenic diseases that transmitted from fishes caused by patients' contact with fish, aquatic environment, dietary habits, and immune system status of the exposed individuals are fairly common. (Akoachere *et al*., 2009).

The natural habitat of fish is extremely susceptible to pollution from domestic, industrial and agricultural discharges. Therefore, fish and other aquatic life forms are vulnerable to all environmental hazards. (Raufu *et al*., 2014).

The other factorsmay include handling, processing and transport of fishes,hypoxia, sudden changes in temperature, orother stressful conditions. (Olsviketal. 2013).

Salmonellaspp. (enteric bacteria) may present in fish and fishery products due to fecal contamination and bacterial contamination during storage, processing, and preparation for consumption. In dense populations of cultured food, aquarium fishes or fish farm the epidemics of bacterial diseases are common. Susceptibility to such outbreaks commonly is associated with organic loading of the aquatic environment and poor water quality.(Francis Floyd, 2011).

The factor that may have contributed to thecontamination of fishes with Salmonella, originated fromterrestrial sources such as untraditional utilization ofcattle and poultry feces as fertilizer or manure onFarmland located close to the canal, river or pond.Consequently, during the rainy season the top soilwashed into the water reservoir enhance the fish andenvironmental contamination. These have mainlycontributed to the growth of human and fish microbes.The untreated sewage water when enter to the lakes orfish farm through runoff or storm water contaminated thefishes. The transportation of fishes in contaminatedfishing boat, or containers and washing of fish withpond or lakes water may also lead to aquatic environmentand food contamination.(Raufu *et al*., 2014).

Most bacterial pathogens of fishes can be diagnosed by isolation of the organism in pure culture from infected tissues and identification of the bacterial agent

(Toranzo*etal*. 2005). XU *et al*. (2007) and Law *et al*. (2015).

Objectives of this study are to:

1- Determine the bacterial counts of Nilefish's wastes and workers in Elmurada market.
2- Detection of salmonella in fishes wastes and workers in Elmurada market.
3- Evaluate the efficacy of thermal versus chemical treatment to control of salmonella.

Chapter two

Literature review

The River Nile is one of the longest rivers all over the world. Due to its lengthCharacteristics, and its path through many cities and villages, the inland fisheries of Sudan are based on the Nile river tributaries, contributing over 90% of the estimated production potential of the country. the Sudd swamps in the south and the manmade lakes on the White Nile, the Blue Nile, Atbara River and the main Nile River count as the major fishing localities with respect to fish resource magnitude and exploitation thrust. the commercially important fish are Nile perch (Lates niloticus), Bagridae catfish (Bagrus bayad), Silver catfish (Bagrus docmac), Nile tilapia (Oreochromus niloticus), Carp fish (Labeo spp.), Barbs fish (Barbus binny), Mormyrus fish (Mormyrus spp.), Nile Distichodus (Distichodus spp.), Tiger fish (Hydrocyon spp.) and Nile robber (Alestes spp.). there are many other species.water quality of the Nile basin and its channels and lakes has demonstrated a trend of lower water quality in the downstream basins compared with those located upstream. This is mainly due to the following: the all-year round continuous flow of the river from south to north which dilutes existing pollutants and contaminants and carries them downstream. less industrialization of upstream countries compared to the downstream ones. neither water multi-use nor recycling processes are common practice in upstream countries compared with the downstream ones. Low population densities and less modernized sewage systems at upstream countries compared with the downstream ones. In addition to the above, the main impact of human activities in the areas surrounding the upstream Nile river water bodies may result from the uncontrolled drainage and discharge of agriculture fertilizers, insecticides, herbicides and untreated sewage into the Nile basin. no commercial value , however, in these upper parts of the Nile basin, the existing civil wars and the absence of hygiene have resulted in periodic devastating epidemics such as cholera and other infectious diseases that may also negatively influence fisheries production either directly or indirectly.

More than 30.000 known species, fish form the biggest group in the animal kingdom that is used for the production of animal-based foods. About 700 of these species are commercially fishes and used for food production. Further, some 100 crustacean and 100 molluscan species (for example mussels, snails and cephalopods) are processed as food for humans in fish industry. (Oehlenschläger and Rehbein, 2009).

Some fishery product is processed in a modern fish industry which is a technologically advanced and complicated industry in line with any other food industry, and with the same risk of product being contaminated with pathogenic organisms (Huss, 1994).

Nowadays, the use of food wastes as animal feed is an alternative of high interest, because it stands for environmental and public benefit besides reducing the cost of animal production. (Samuels *et al*., 1991; Westendorf *et al*., 1998; Myer *et al*., 1999; Westendorf, 2000).

2.1. Fish waste:

Fish wastes produced during fish processing operation can be divided into two parts.

2.1.1. Solid wastes:

Include skin, viscera or intestine, fish heads, and carcasses (fish bone).solid waste can be recycle in fish meal plants or it can be treated as municipal waste.

2.1.2. Liquid wastes:

Include blood water and brine from drained storage tanks, and water discharges from washing and cleaning. This waste may need holding temporarily, and should be disposed of without damage to the environment. How liquid waste should be disposed from fish processing operation depends on the content levels in the waste of solid and organic matter, as well as nitrogen and phosphorus content, it also

depends on an assessment of parameters such as acidity levels, temperature, odour and biochemical oxygen demand and oxygen demand***(http://en.m.wikipedia.org/wiki/fish-processing).***

Fish waste management has been one of the problems having the greatest impact on the environment. Fish farming detrimental effects on the marine environment in particular have become an issue of public concern. In many countries, numerous Directives, Decisions and Regulations were voted in an attempt to minimize the environmental impact of fisheries within the frame of Integrated Coastal Management. Treated fish waste has found many applications among which the most important are animal feed, biodiesel/biogas, dietic products (chitosan), natural pigments (after extraction), food-packaging applications (chitosan), cosmetics (collagen), enzyme isolation, Cr immobilization, soil fertilizer and moisture maintenance in foods(hydrolysates).(Ioannis*et al*., 2008).

2.2 Uses of fish waste:

Food industry wastes are an important environmental contamination source. Research has been carried out in order to develop methods to convert these wastes into useful products (Perea *et al*., 1993; Kristinsson and Rasco, 2000; Larsen *et al*., 2000; Guerard *et al*., 2001; Coello*et al*., 2002; Laufenberg et al., 2003).Probably, Morethan 50% of the remaining material from the total fishcapture is not used as food and involves almost32 million tons of waste (Kristinsson and Rasco, 2000).

2.2.1 Animal feed:

Nowadays, the use of food wastes as animal feed is an alternative of high interest, because it stands for environmental and public benefit besides reducing the cost of animal production (Samuels *et al*., 1991; Westendorf*et al*., 1998; Myer *et al*., 1999; Westendorf, 2000). Offal from the fishing industry could be used as a feed

ingredient, as it represents a valuable source of high-quality protein and energy (New, 1996; Gabrielsen and Austreng, 1998).

2.2.2 Biodiesel/biogas:

Biodiesel fuel, acquired from the oils and fats of vegetables and animals, is a substitute for, or an additive to, diesel fuel derived from petroleum (Alcantara *et al.*, 2000). however, during the early 1980s, engine tests showed that the combustion of vegetable oils caused durability problems related to incomplete combustion such as nozzle coking, engine deposits, ring sticking and crankcase lubricant contamination (Dunn and Bagby, 2000**).**

2.2.3 Natural pigments:

Carotenoids are responsible for the color of many important fish and shellfish products. Most expensive seafood, such as shrimp, lobster, crab, crayfish, trout, salmon, redfish, red snapper and tuna, have orange-red integument and or flesh containing carotenoid pigments (Haard, 1992). the grading or pricing of shrimp, salmon, rockfish and snapper is directly related to the intensity of red hue (Sacton, 1986).

The recovery of chemical components from seafood waste materials, which can be used in other segments of the food industry, is a promising area of research and development for the utilization of seafood by-products. Researchers have shown that a number of useful compounds can be isolated from seafood waste including enzymes, gelatin and proteins that have antimicrobialand antitumor capabilities. Chitosan, produced from shrimp and crab shell, has shown a wide rangeof applications from the cosmetic to pharmaceutical industries ***(http://ift.confex.com/ift/2001/techprogram/ paper_6188.htm).***

2.2.4 Miscellaneous uses:

Mohan *et al*. (1993) utilized fishmeal waste as an attractant for economically important flies of agricultural crops, such as sorghum shoot fly (Atherigona soccam Rond.), moringa fruit fly (Gitona sp.) and the Indian uzifly (Exorista bombycis (Louis) attacking mulberry silkworm. The waste fishmeal material was Dried, powdered, moistened in polyethylene bags with a piece of cotton dipped in an insecticide (Dichlorvos 76 sc, Amvac Chemical Corp. of Los Angeles, CA, USA) and placed in fields. The observations indicated that female flies were attracted and nearly 50% of them were with eggs.

Offal from the fishing industry could be used as a feed ingredient, as it represents a valuable source of high quality protein and energy. (New, 1996; Gabrielsen and Austreng, 1998).

Oils from fish offal are also used extensively in the food industry as raw materials and ingredients (Jacobs *et al*., 1997). Composting experiments of fish offal (heads, skin, viscera and skeleton) from rainbow trout and wood by-products were conducted by Laos *et al*. (1998). (Ioaniss Arvanitoyannis and Aikaterin kassaveti; 2006).

2.3 Fish and fishery products contamination:

Seafood contamination occurs naturally from the environment where fish are harvested, during harvesting, processing or during food preparation.

Cross contamination may occur during harvesting, processing or during food preparation. Where bacteria are transferred from raw fish and or contaminated surfaces and or from utensils to hygienically safe seafood**.**(Wekell *et al*., 1994).

During processing contaminated water may also introduce microorganisms including pathogens into the food. In some cases the levels present in the food may not be critical as to pose a health hazard to consumers. Improper methods of handling (poor general and or personal hygiene) and distribution (time temperature abuse)**.**(Wekell *et al*., 1994).

Salmonellais responsible for more than 40.000 cases of food-borne illness every year. The incidence of Salmonellainfections has raised dramatically since the 1980s, leading to high medical costs, a loss of wages for workers who become ill, and a loss of productivity for the companies whose workers do become ill. In all, these financial losses can cost more than $3.6 billion each year. Salmonellainfections have long been a concern to scientists, doctors, and the U.S. Food and Drug Administration (FDA)(Brands, 2006).

Salmonellais causing a public health problem associated with fish and fishery products. A monitoring of Salmonellahas been suggested as a measure of fish quality. Also, risk management decisions should take into account the whole food chain from primary production to consumption, and should be implemented in the context of appropriate food safety infrastructures, for instance regulatory enforcement, food product tracing and traceability systems. In the fish processing chain managing risks should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during food preparation, storage and transport, retail and catering(Popovic *et al*., 2010).

2.4 Salmonella:

Salmonellais a member of the Enterobacteriaceace, Gram negative, motile, with Peritrichous flagella and nonperforming rods (the rods are typically 0.7-1.5 μm x 2.5 μm in size). Salmonellais a facultative anaerobic (can grow with or without oxygen) catalase positive and oxidase negative bacteria. Salmonellais not included in the group of organisms referred to as coli forms.(Huss and Gram, 2003; Adams and Moss, 2005; Erkmen, 2007; Lawley *et al*., 2008).

These mesophilic organisms are distributed geographically all over the world, but principally occurring in the gastrointestinal tracts of mammals, reptiles, birds, and insects and environments polluted with human or animal excreta.

(Huss, 1994, Huss and Gram, 2003; Saeed and Naji 2007).

Survival in water depends on many parameters such as biological (interaction with other bacteria) and physical factors (temperature). More than 2,500 different types of Salmonellaexist, some of which cause illness in both animals and people. Some types cause illness in animals but not in people. The various forms of Salmonella that can infect people are referred to as serotypes, which are very closely related microorganisms that share certain structural features. Some serotypes are only present in certain parts of the world (Brands, 2006).

Salmonellabelongs to Enterobacteriaceaefamily. They are common waterborne Bacterium which may be apparently present in the tissues of normal fishes (Newaj-Fyzul *et al*., 2008). First the Salmonellawas isolated from a pig, suffering from hog cholera, by an American scientist Dr. D. E. Salmon in 1985. Salmonellaspp. may be present naturally in tropical aquatic environments (Musefiu *et al*., 2011).

The environmental stresses such as high temperature and poor water quality mainly contribute to the start and severest of Enterobacteriaceaeinfections in fishes (Zheng *et al*., 2004)**.**

Members of the genus Salmonellaare ubiquitous, found in all organisms including humans (Lotfy *et al.*, 2011).

The Salmonellaspp. is carried through fish farmed, fish meal, and fresh fish. When the fishes are caught in contaminated areas with fecal pollution, processed and distributed under unsanitary conditions and slightly cooked. (Norhana *et al.*, 2010).

2.5 Salmonella in fish and fishery product:

Salmonellahas been isolated from fish and fishery product, though it is not psychotropic or indigenous to the aquatic environment (Mol *et al*., 2010).

The relationship between fish and Salmonellahas been described by several scientists; some believe that fish are possible carriers of Salmonellawhich are harbored in their intestines for relatively short periods of time and some believe that fish get actively infected by Salmonella. The organism was never recovered from the flesh of the fish, but was isolated from viscera and epithelium. (Pullela, 1997).

Most outbreaks of food poisoning associated with fish derive from the consumption of raw or insufficiently heat treated fish and cross-contamination during processing and about 12% of the food borne outbreaks related to consumption of fish is caused by bacteria including Salmonella.(Huss *et al*., 2000; Aberoumand, 2010).

2.6 Salmonella in freshwater fishes:

Salmonellain freshwater fishes has been usually related to the fecal contamination of water from where fish were harvested (Mhango *et al*., 2010). Fishes work as a passive carrier of Salmonellathat may excrete Salmonellawithout apparent symptoms and represent no clinical disease. The high prevalence of Salmonellain catfish was reported by Wyatt *et al.* (1979b). This high prevalence rate was attributed to thehigh temperature in pond water because high temperature promotes the growth rate of the organism (Wyatt *et al.*, 1979a). Salmonellawas isolated from the intestines of Silver Carp by Bocek *et al.* (1992).

The prevalence of Salmonella isolated from freshwater lake, farmed andMarket fishes were 31%, 5% and 10 to 28%, respectively.(Mohamed Hatha and Lakshmanaperumalsamy, 1997).

2.7 Sources of Salmonellacontamination in fish and fishery products:

Aquatic environments are the major reservoirs of Salmonella. Therefore, fishery products have been recognized as a major carrier of food-borne pathogens

(Kamat *et al*., 2005; Upadhyay *et al*., 2010).

Pathogenic bacteria associated with fish and fishery product can be categorized into three general groups:

(1) Bacteria (indigenous bacteria) that belong to the natural micro flora of fish (Clostridium botulinum, pathogenic Vibriospp., Aeromonas hydrophila).

(2) Enteric bacteria (no indigenous bacteria) that is present due to fecal contamination (Salmonellaspp., Shigella spp., pathogenic Escherichia coli, and Staphylococcus aureus).

(3) Bacterial contamination during processing, storage or preparation for consumption (Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Salmonella spp.) (Lyhs 2009).

Information from literature indicates that fresh fish, fish meal, oysters, farmed and imported frozen shrimp and forelegs can carry Salmonellasp., particularly if they are caught in areas contaminated with fecal pollution (prior to harvest and during harvest) or processed, packed, stored, distributed under unsanitary conditions and consumed raw or slightly cooked.(Kumar *et al*., 2003; Kamat *et al*., 2005, Mol *et al*., 2010; Norhana *et al*., 2010).

2.8 Some pathways of contamination of aquaculture systems with Salmonella:

2.8.1 Non-point water run-off:

During rainfall events, increased run off of organic matter into ponds may occur and can contaminate the aquaculture system.

2.8.2 Animals (domestic animals, frogs, rodents, birds, insects, reptiles, etc.):

A variety of animal waste has been shown to be potential sources of Salmonella. Animal waste can be introduced directly through bird droppings or frogs living in ponds or indirectly through runoff.

2.8.3 Fertilization of ponds:

In some aquaculture systems animal manures are used in ponds to stimulate the production of algae. The use of non-composted manures can lead to production systems been contaminated with Salmonella.

2.8.4 Contaminated feed:

Improperly stored feed or feed prepared on a farm under poor hygienic conditions can be a source of Salmonella.

2.8.5 Contaminated source water:

The water used in grows out ponds, cages or tanks can be contaminated with Salmonellathrough wildlife runoff, untreated domestic sewage, discharge from animal farms, etc.

2.8.6 on farm primary processing:

Aquaculture products can become contaminated with Salmonellathrough the use of unsanitary ice, water, containers, and poor hygienic handling practices.
(FAO, 2010).

For example, for shrimp processing industry the information from literature indicates that the principal sources of Salmonellacontamination are culture ponds, coastal water used for handling and processing of seafood.(Hariyadi *et al*., 2005; Shabarinath *et al*., 2007; Upadhyay *et al*., 2010).

Similarly, Pal and Marshall (2009) reported that the potential sourceof Salmonella contamination in farm-raised catfish is likely due to poor water quality, farmrunoff, fecal contamination from wild animals or livestock, feed processing under poorsanitary conditions or distribution, retail marketing, and handling or preparation practices.(Ray *et al*., 1976).

Reported that the potential hazard in cooked fishery product is cross contamination of the cooked products with raw fishery product which might occur under commercial processing condition. Thus, good sanitation practices on the unloading docks and during transport to the processing facility are essential for preventing product contamination. The use of contaminated ice or uncleaned holding facilities may also contribute to the product contaminant load.(Gecan *et al.*, 1988).

As a result, many factor including inadequate supplies of clean water, inadequate sanitary measures, lack of food hygiene and food safety measures have been responsible for increased incidence of food borne salmonellosis**.**(Shabarinath *et al.*, 2007).

Deep-sea fish are generally Salmonellasp. free but susceptible to contamination post-catch. Water temperature has been proposed as playing an important role in the long-term survival of Salmonellain the environment.(FAO, 2010).

In raw seafood products mainly from tropical climates, there is a high prevalence of Salmonellawhereas low prevalence or absence can be common in temperate regions. (Millard and Rocklif, 2004)**.**

2.9 Control of Salmonellain fish and fishery products

Since most of fish products, with the exception of cold smoked fish, sushi, and a few specialty products such as spiced, salted, or pickled fish, is expected to be cooked prior to consumption, the presence of microbiological pathogens should not present a human health hazard. (Flick, 2008)**.**

The aquaculture farm is the first link in the food safety continuum and controls must be in place and implemented throughout the food safety chain. The experts agreed that good hygienic practices during aquaculture production and biosecurity measures can minimize but not eliminate Salmonellain products of aquaculture.

2.10 Control measures to minimize the risk of Salmonellacontamination of aquaculture products:

According to FAO 2010 there are important measures to minimize the risk of contamination of aquaculture products there are.

2.10.1 Farm location:

Farms should be secured from the entry of wild and domestic animals that may lead to the contamination of aquaculture products with Salmonella.

2.10.2 Farm layout, equipment and design:

Farm design and layout should be such that prevents cross contamination Equipment such as cages, nets and containers should be designed and constructed toallow for adequate cleaning and disinfection Septic tanks, toilet facilities and bathrooms or showers should be constructed and placedSo drainage does not pose a risk of contamination of farm facilities.

2.10.3 Source water:

Farm source water should be free from sewage contamination and suitable for aquaculture production Farms should have settling ponds or waste water treatment in place to condition the output water prior to discharge.

2.10.4 Ice and Water Supply:

Potable or clean water is available and used in sufficient amount for harvest, handling and cleaning operations; Ice should be manufactured using potable water and produced under sanitary conditions Ice should be handled and stored under good sanitary conditions which precludes the risk for contamination.

2.10.5 Harvesting:

Harvesting equipment and utensils easy to clean and disinfect and kept in clean condition. Harvesting is planned in advance to avoid time/temperature abuse. Aquaculture products should be hygienically handled. Records on harvesting are maintained for traceability.

2.10.6 on farm post-harvest handling:

Utensils and equipment for handling and holding of aquaculture products is

Maintained in clean condition. Aquaculture products are cooled down quickly and maintained at temperatures approaching that of melting ice. Operations such as sorting, weighing, washing, drainage, etc., are carried out quickly and hygienically. All additives and chemicals (disinfectants, cleaning agents, etc) used in post-harvest aquaculture products should be approved by the national competent authority.

2.10.7 Transport of aquaculture products from farm:

Transport is carried out in easy to clean and clean facilities (boxes, containers, etc.). Conditions of transport should not allow contamination from surroundings (e.g. dust, soil, water, oil, chemicals, etc.). Aquaculture products are transported in containers with ice or with, in sufficient amounts to ensure temperature around 0ºC (approaching that of melting ice) in all products and during the whole period of transport.

2.10.8 Employee health:

Staff should be medically fit to work and should be screened regularly to determine carriers of Salmonella. On the other hand, a number of studies have been carried out to develop methods to control contamination of proceed fishery products. They are sub-divided into physical or chemical approaches. (Norhana *et al*., 2010).

Proteus is a genus of gram negative; bacilli are widely distribution in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine, human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections.

Chapter three

Materials and methods

3.1. Study area:

This study was carried out in Elmurada market for fishes (Omdurman locality) during the period January to March 2017.

3.2. Sampling method:

On total samples were taken from three (organs 45 {skin15, gill 15, and intestine 15}).Of Nile fishes (Heterotis niloticus, tilapia nilotica, Gymnarchus niloticus, Synodontis schall, Hydrocynus vittatus).and from (workers15 {hands 3, utensils 3, cutting board 3, wipes 3, and table surfaces 3}). By sterile swabs. Then swabs were transferred in ice box to the microbiology laboratory of collage of veterinary medicine of Sudan University of sciences and technology to examination.

Immediately after rich to lab samples was undergo to procedures of isolation for salmonella and enumeration for total count of bacteria.

3.3 Isolation and identification procedures:

Using prepared peptone water, nutrient agar, macconkey agar. The plates were incubated at 37℃ for 24 hours well isolated colonies obtained from agar medium different broth cultures of gram negative and gram positive bacteria were constantly sub cultured into agar from time to time ,incubated at 37℃ for 24 hours and stored at 4℃ .

3.4 Preparation of samples:

On the arrival is the laboratory, samples were immediately were inoculated into liquid broth and incubated over night then diluted with normal saline then one ml was streaked with sterile loop on solid media (nutrient agar) and incubated at 37℃ for 24 hours.

For isolation and identification of gram negative organisms were inoculated in Macconkey agar and incubated aerobically at 37℃ for 24 hours.

3.5 Examination of cultures:

Examination of all cultures on solid media was performed by detection of growth, Pigmentation; colonial morphology as well as changes in the media.

Plates that showed visible growth were subjected to further bacteriological tests while those did not show visible growth were incubated for further 48 hours and discarded if no growth was detected.

3.6 Purification of cultures:

The primary isolation was sub culture on nutrient agar. The sub culture was repeated several times until pure colonies were obtained.

3.7 Culture media:

3.7.1. Solid culture media:

3.7.1.1 Nutrient agar:

The medium was prepared by take 28 gram of the powder were added to one liter of distilled water and brought to boil to dissolve the powder completely .it is sterilized by auto calving for 15 minutes at 121℃. Then poured aseptically as 18-20 ml in Petri-dishes.

3.7.1.2 Macconkey medium:

Fifty five grams of Macconkey agar were added to one liter of distilled water and brought to boiling until dissolved completely .then sterilized by auto cleaving at 121℃ for 15 minutes.

Then it was aseptically distributed in sterile petri-dishes as 15- 20 ml portion and left to solidify.

3.7.2 Liquid culture media:

3.7.2.1 Peptone water:

Peptone water was prepared by taken ten grams peptone and 5 grams NACL were dissolving by heating in one liter distilled water the medium was distributed in test tubes (5 ml) and sterilized by autoclaving at 115℃ for 15 minutes. The stock was preserved in the refrigerator.

3.8 Sterilization:

3.8.1 Hot air oven:

This method was used for sterilization of clean glass containers which were wrapped in paper and the temperature was 160 ℃.

3.8.2 Sterilization by red heat:

The method was used for sterilization wire loops, straight wire and tissue forceps it was done by vertical as possible until it become red –hot.

3.8.3 Sterilization by auto-cleaving:

This method was used for sterilizing of culture media and for materials that could not with stand the dry heat .the temperature was 115℃ – 121℃ for 15-20 minutes**.**

3.9 Methodology of viable bacteria cell count:

Serial dilution was used, plating and counting of live bacteria to determine the number of bacteria in a given population was used serial dilutions of a solution containing unknown number of bacteria were made .the total number of bacteria in the original solution was determined by the counting the number of colony forming unit represented a bacterium that was present in the diluted sample. The number of colonies forming units (CFUs) is divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per ml that were present in the original solution.

3.10 Serial dilution:

Five small, sterile test tubes werelabeled 1 through 5 and then 9 ml of normal saline was added to each test tube. (0.5)ml of the original solution was pipette into test tube1. bacterial suspension was mixed thoroughly (using the vortexes) before proceeding to the next step. 0.5ml of the diluted bacterial suspension was pipette into the second test tube this process repeated until to the 5^{th} tube to obtained dilution of 1×10^{5}.

3.11 Biochemical tests:

3.11.1 Gram stain:

Using sterile wire loop a part of isolate colonies was taken and spread on microscopes slides to make thin smears. They were fixed with heat and placed in staining rack. they were covered by crystal violet for two minutes and washed off by tap water, then decolorized with acetone for few seconds and wash off by tap water, then covered with carbol fuchsine for thirty seconds .finally the stained smears were examined under oil immersion lens (100).the gram positive and negative organisms shape and arrangement of organisms were identified according to.

3.11.2 Oxidase test:

Tetra methyl- pheynlen – diamine dehydro chloride was prepared as 1% aqueous solution. Filter paper of 50×50 millimeter size were impregnated in reagent before and dried at 50 ℃ .a sterile platinum loop was used to spread the isolation colony on oxidase paper. color change (violet)indicate appositive reaction.

3.11.3 Catalase test:

Using sterile glass rod a part of isolation colony was emulsified in one drop of hydrogen peroxide on a clean slide. gas bubbles indicated positive reaction.

3.11.4Kovacs reagent:

This reagent was prepared as describe by. five grams of p-dimethylamine benzaldehyde were dissolved in 75ml amylalhol by warming in water bath. after the mixture was cooled. 25ml of concentrated hydrochloride acid were added. It is used for indol test.

3.11.5 Motility test:

The isolation was studied for motility by Craigie technique**.** In which the bacteria was inoculated into a central tubes containing semi solid agar placed in test tube using straight wire .after incubation at 37 ℃ for 24 hours the tubes were examined for migrating of bacteria outside the tube .

3.11.6 Kliger Iron agar:

Weight 49 of powder and mix with 1liter of distilled water. bring to boil with frequent stirring to dissolve completely. dispense into tubes and sterilize for 15 minutes at 121℃. cool in a slanted position such that slopes are formed over deep butts approx. 3 cm in depth, appearance reddish brown agar pH: 7.4±0.2 .

3.11.7 Simon citrate agar:

Weight 24 grams of powder, disperse in 1 liter of distilled water .allow to soak for 10 minutes, swirl to mix then heat to dissolve the agar and solids. Dispense into tubes or bottles then sterilize by autoclaving at 121 ℃ for 15 minutes. Allow to set as slope, appearance green, opalescent PH: 6.9 ± 0.2 (Hyde and Denton, 2002).

3.11.8 Urease test:

A slope of urea agar medium was inoculated with organism , then incubated and examined after 24 hours , and daily for 7 days for change in the color of the medium to pink indicating positive result ,while yellow color indicating negative result.

3.11.9 Indol test:

Peptone water medium was inoculated with test culture and incubated at 37℃ for 48 hours. One ml of Kovac ́s reagent was run down the side of the tube where no color appears on the reagent layer within a minute in positive samples.

3.12 Treatments:

Two treatments was carried out in this study to control of salmonella

Physical method: thermal approach (heating or cooking treatment) where salmonella exposure to high temperature in short time 90 ℃ for 15 minutes. After heating treatment, salmonella inoculated on differential media (Macconkey agar) then incubated in incubator at 37 ℃, overnight. Result showed absence of salmonella.

Chemical method: salting treatment are used in this study (NACL salt), divided into two concentrations (4% and 8% per 100 ml distilled water). Salmonella placed in salt solution for two hours, after that inoculated on differential media (Macconkey agar), then incubated in incubator at 37 ℃, overnight. Result showed that Salmonella absence in concentrations, 4% and 8% NACL.

3.13 Statistical analysis:

Data were collected and stored in Microsoft Excel and then analyzed by using SPSS program version 16.0 all bacteria counts were converted to log 10 CFU cm^{-2} for analysis using ANOVA one way .statistical significance was set at statically significant (P-value <0.05).

Chapter four

Results

4.1 Bacterial count for fishes:

Results showed that, there is no significant different between all means. where the maximum number was in Synodontis schall 2.12±0.045CFU/g, while the minimum number was in Hydrocyanic vittatus2.03±0.079CFU/g.

Table (1)Total bacteria count for fishes

Bacterial count	CFU/g	N
Heterotis niloticus	2.09±0.078	9
Tilapia nilotica	2.07±0.030	9
Gymnarchus niloticus	2.03±0.031	9
Synodontis schall	2.12±0.045	9
Hydrocyanic vittatus	2.03±0.079	9
Over all mean	2.07±0.083	45

4.2 Bacterial count for organs:

Results of bacterial count for fish organs wereshown in table (2).

Table (2)Total bacteria count for organs

Bacterial count	CFU/g	N
Skin	2.05 ±0 .082	15
Gill	2.06 ± 0.071	15
Intestine	2.10 ± 0.093	15
Over all mean	2.07 ± 0.083	45

4.3Bacterial count for workers:

Results of bacterial count for workers were shown in table (3).

Table (3) Total bacterial count for workers		
Bacteria count	CFU/g	N
Hands	1.98±0.33	3
Utensils	1.84±0.46	3
Cutting boards	1.89±0.11	3
Wipes	2.02 ±0.045	3
Table surfaces	2.07±0.073	3
Over all mean	1.96±0.24	15

4.4Salmonella:

Salmonella was showed form all fish spp, fish organs and workers. 45 samples were taken, (15 were positive while 30 were negative),the highest percentage of salmonella were in Heterotis niloticus and Gymnarchus niloticus,(15.6%),(6%) respectively while the lowest percentage were in Hydrocyanic vittatus was (2%).

The total number of salmonella in fish organs in this study was 15 (skin (6), gill (4), and intestine (5).

Fifteen samples were taken from workers, results showed (13) of these samples was infected with salmonella. (Hands2, utensils 3, cutting boards 2, wipe 3, table surfaces 3).

Table (4) Occurrence of salmonella in fish species			
	Salmonella		Total
	Positive	Negative	

Fish species	n	%	n	%	n	%
Heterotis Niloticus	7	15.6	2	4.4	9	20
tilapia nilotica	2	4.4	7	15.6	9	20
Gymnarchus niloticus	3	6.7	6	13.3	9	20
Synodontis schall	2	4.4	7	15.6	9	20
Hydrocynus Vittatus	1	2.2	8	17.9	9	20
Total	15	33.3	30	66.7	45	100

Table (5) Occurrence of salmonella in the different fish spp

Fish species	Salmonella		Infected organs			Total
	Positive	Negative	skin	gill	Intestine	
Heterotis Niloticus	7	2	3	1	3	9
Tilapia nilotica	2	7	-	2	-	9
Gymnarchus niloticus	3	6	1	1	1	9
Synodontis schall	2	7	1	-	1	9
Hydrocynus vittatus	1	8	1	-	-	9
Total	15	30	6	4	5	45

Table (6) salmonella in organs.

Organ	(N)	Positive%	(N)	Negative%
Skin	6	13.3	9	20
Gill	4	8.9	11	24.4
Intestine	5	11.1	10	22.2
Total	15	33.3	30	66.7

Table (7)

Type of sample	Salmonella		Total
	Positive	Negative	
Hands	2	1	3
Utensils	3	0	3
Cutting board	2	1	3
Wipes	3	0	3
Table surfaces	3	0	3

Table (8) Salmonella in workers (%)

workers	Positive	Negative	Total
Hands	13.3	6.7	20
Utensils	20	0	20
Cutting boards	13.3	6.7	20
Wipes	20	0	20
Table surfaces	20	0	20
Total	86.7	13.3	100

4.5 Treatments to control of salmonella:

Results of physical method showed that disappearance cells of salmonellaon high temperature (90 ℃), In Chemical method, Salmonella cells absence after immersed in NACL salt.

Chapter five

Discussions

Bacterial growth is the main cause of problems of health and economical losses therefore it is logical to use bacterial number as index of fish quality. In this study the total number of bacterial count for five Nile fish species(Heterotis niloticus, tilapia nilotica , Gymnarchus niloticus, Synodontis schall, Hydrocyanic vittatus was $2.09\pm.078\times10^5$CFU/g, $2.07\pm.030\times10^5$CFU/g, $2.03\pm.031\times10^5$CFU/g, $2.12\pm.045\times10^5$CFU/g, $2.03\pm.079\times10^5$CFU/g,respectively. This numbers was acceptable limit mentioned by SSMO (Sudanese standards and metrology organization, SDS357) which was 5×10^5 -10^6 CFU/g for fish products.

In the present study the total bacterial load in skin, gill, and intestine were 2.05 ± 0.82, 2.06 ± 0.71, $2.10\pm0.932\times10^5$ CFU/g respectively which was less than Jannat *et al* (2007). Who found that the total bacteria count in skin and scale,gill,muscles, and intestine were 9.4×10^9 ,8.5×10^7 , 6.0×10^7 ,and 9.0×10^7 CFU/g-^{1}respectively.

However, in this study the fish samples of different species were contaminated with bacteria load, this might be due to the contamination of water farm where the fishes were cultured or might be due to secondary contamination during the time of handling as well as transporting of fishes in boats, cars to markets or during storage with contaminated Ice.

According to FAO (1979), good quality fish should have count of total bacteria less than 10^5 per gram. This study within the acceptable limit recommended by food and agricultural organization.

This indicates slightly human health risk due to consumption of Nile fishes

Therefore precautions should be taken to prevent contamination during harvesting as well as post- harvest handling of fishes.

Percentage of salmonella in fish organs in this study were (13.3%, 8.9%, and 11.1%) for skin, gill, and intestine. Respectively this result less than (Nwiyi and Onyeabor, 2012Elhadi, 2014) whose found the percentage of salmonella in skingill,and intestine were(66.7 %,50%,20%) in tilapia nilotica respectively.
And less than Budiati *et al.,* 2011).who found the percentage of salmonella in skin, gill, and intestine were (60%, 40%, 20%) in catfish, respectively.
The presence of bacterial load and salmonella in the fish samples collected from fish market may be due to heavy load sewage disposal into the river Nile water.

This untreated and improper way of sewage disposal system and wrong attitude toward aquatic environment is one of the main sources for microbial water contamination which results in the accumulation of these bacterial pathogenic species in the commercial fishes.

Unhygienic fish handling practices of these infected fishes of cross contamination via kitchen utensils or by handling and inadequate cooking may further contribute to the spread of these pathogens. Hence, we are in urgent need to implement programs such as HACCP as part of good manufacturing practices (GMP) and sanitation standard operation procedures (SSOP) to monitor the quality of the fishes.

Chapter six

Conclusion and Recommendations

61 Conclusion:

Isolation of Salmonellafrom fishes is an indication of contamination of the river Nile by pathogens.

This study provides vital data that are critical for assessing and controlling the risk associated with the presence ofSalmonella in the river Nile fishes, also during harvesting, handling, processing.

Thermal approach the best method to control of salmonella, but in the other hand high degrees of temperature leads to protein denaturation.

Chemical approach is good to control of salmonella, but not for all pathogens because some pathogens it have able to tolerant highchemical concentrations.

6.2 Recommendations:

Fish wastes should be properly processed before uses as animal's feeds.

Handlers or workers in markets must be maintaining high degree of personal hygiene, cleanliness and, were suitable protection clothing.

Quality control measures should be introduced for fish markets.

Apply hazard analysis for critical control points (HACCP) application system for all segments or sectors of food chain from primary production into final consumption point.

Moresearches, long time, and an increase sample of fishes and workers in markets for this work.

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