Sudan University of Science and Technology College of Graduate Studies

Assessment of Bacterial Contamination and Risk factors in Fish Farms in Khartoum State

تقييم التلوث البكتيرى وعوامل الخطر في مزارع الاسماك بولاية الخرطوم

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الآيــــــــة

قال الله تعالي:

بسم الله الرحمن الرحيم

لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِن نَّسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِن قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنتَ مَوْلَانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ ﴿٢٨٦﴾

سورة البقرة ـ الآية 286

Dedication

. . . .

To soul of my father and mother

…………………

To my kind unfailing support sister

………………….

To my brothers

…………………

To my small family , husband and sons

…………………

For those who were in my heart and are still there

…………………

Thank you a lot for your support

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

ABSTRACT

This study was conducted in the period from August to November 2016 , in fish farms in Khartoum State aim they to investigate the bacterial contamination in their farms . The critical points that associated with environment in this farms were also conducted using questionnaire survey to under stand on the personal hygiene of the workers .

Samples (swaps) (n=150) were collected from two sites , the gills and skin of the fish , the isolates were two types of gram negative bacteria e.coli and salmonella .Bacteriological analyses were performed using accredited methods . Salmonella found in 60 isolate (40%) and e.coli had 50 isolate(33.3%) and the mixed sample which contain salmonella and e.coli together about 40 isolate (26.7%) .From this study salmonella had the highest prevalence in Khartoum Bahri , whereas e.coli recorded the highest prevalence in Omdurman . The gills sample had the highest number of bacteria . In this study , the total plate count for all samples ranged between 7.00 to 7.18 this number was not accepted limit mentioned by SSMO which had limit 5 for fresh fish products . Violations of hygienic practices were also observed . The study provided background data that could help in applying HACCP in fish farms to ensure safe fish products for human consumption . Furthermore , the critical control points were differenced where hygienic violations occur to help fish farmers correcting the deviation in their included . The bacterial contamination problem should be resolved by the use of good

manufacturing procedures and the strict application of sanitary practices .

ملخص الدراسة

تمت هذه الدراسة فى مزارع اسماك بولاية الخرطوم فى الفتره من شهر اغسطس حتى نوفمبر 2016 م . تم الكشف عن التلوث البكتيرى فى هذه المزارع بالاضافه لعلاقه العوامل البيئة المحيطة بهذه المزارع بمدى السلامه الحيويه لهذه المزارع .

من خلال هذه الدراسه تم اخذ 150 عينه من اسماك البلطى المستزرعة فى هذه المزارع حيث عزلت العينات فى المعمل و كانت النتيجة ان نسبة السالمونيلا فى هذه العينات كانت 60 بنسبه 40% من مجمل العينات بينما كانت نسبة ال ايكولاى 50 بنسبه 33.3% وكانت العينات الخليط التى تحوى نوعى البكتريا 40 بنسبه 26.7% . واخذت هذه العينات من الخياشيم و الجلد .

ووجد من خلال الدراسه ان نسبة السالمونيلا كانت اعلي فى الخرطوم بحرى بينما نسبه الايكولاى اعلي فى ام درمان . وعينات الماخوزه من الخياشيم كانت نسبه التلوث البكتيرى فيها عاليه مقارنه مع عينات الجلد .

بالنسبة للعد الكلى للبكتريا وجد ان البكتريا فى كل العينات تتراوح نسبها بين 7.00 الى 7.18 والتى تعد نسبه امراضيه حسب التصنيف العالمي و المحلي للنسبه الطبيعيه الممكن تواجدها فى الاسماك والتي لا تزيد عن 5 .

نتج من خلال تحليل نتايج الاستبيانات التى وزعت علي العاملين فى هذه المزارع وغيرها من مزارع استزراع الاسماك بالولايه ان هذه المزارع تفتقر لعدم وجود ابسط مقومات النظافه والصحه الشخصيه للعاملين مع انعدام التدريب والتاهيل المناسب لمهامهم والالمام بقواعد ضبط الجوده و سلامه الاغذيه لتفادى المشكلات الصحيه التى تؤثر مباشره على المستهلك .

INTRODUCTION

Sudan is one of the largest countries in Africa with an area of 1861500 km. The contribution of fisheries to the GDP is currently marginal , however Sudan is endowed with water resources and lands that can support vigorous capture fisheries and aquaculture (FA0,2014) .

Sudan's capture fisheries production was estimated to be about 34000 ton in 2012 ,29000 ton from inland water catches and 5000 ton from marine catches . The aquaculture sector is still incipient and the annual production was estimated at 2000 ton in 2012.Capture fisheries activities are centered around the River Nile and its tributaries, seasonal flood plain and four major reservoirs, fresh water fish culture is primarily based on the pond culture of the Nile Tilapia Oreochromis niloticus (FAO, 2014).

For all this reason the production of fish be important also availability and variety of water sources in Sudan , indicates that there is great potionial for production of fish . The most important ways of exploit these resource in aquaculture, is to establish modern fish farms that rely scientific methods in operation and apply production requirements and management of large quantities , which will contribute effectively to the achievement of food security for the population .(FAO,2004) .

In Sudan over 95% of catch of fish obtained from inland fisheries on the Nile. Three main sectors can be identified : subsistence ,artisanal and

commercial fisheries .Subsistence fishing is found partially all of the inland

waters , but especially in the isolated areas of South where the Nile

seasonally over spill . The artisanal sector exist alongside subsistence activities, principally on Jebel Aulia and lower reaches of the White Nile before confluence with blue Nile . The artisanal fisher man owns boat of traditional design .Commercial activities are little developed and practiced by private , semi private and co operative sector . The marine catch in the Red Sea is less else compare to that from the Nile (FAO,2004).

With more than 30,000 known species , fish farm constitutes the biggest group in the animal-based   foods .About 700 of these species are commercially fished and used for food production (Oehlen Schlager +Rehbein ,2009) .

The vast majority of outbreaks of food related illness are due to pathogenic microorganisms rather than to chemical or physical contaminant (Lelieveld et al 2009) .

Objectives of the study

General objective of this study was to acquire knowledge on assessment of bacterial contamination in selected fish farms located Khartoum state

The specific objectives of the study were :

1- isolate bacteria that associated with contamination of value chain in fish farms in Khartoum state .

2- provide background data that could help in applying HACCP in fish farms to ensure safe fish products for human consumption .

3- determine the critical control point where hygienic faults are present .

CHAPTER ONE

LITERATURE REVIEW

1.1 Fish

Fish are aquatic vertebrate animals that are typical ectothermic (previously cold blooded) .They have a stream- lined bodies that allow them to swim rapidly . They extract oxygen from water using gills or an accessory breathing organ to enable them to breathe atmospheric oxygen .Fish have two sets of paired fins , usually one or two (rarely three) dorsal fins ,anal fin and tail fin . It has jaws , skin that is usually covered with scales , and lays egg that are fertilized internally (Helfiman ,et al 1997)

1.2 Important of fish

Fish is regarded as a healthier meat option due to the high content of long chain polyunsaturated fatty acids such as omega -3 fatty acids , which are associated with improving health and preventing disease of old age(FAO,2010).

Furthermore , fishes accounts for approximately 17% of the total animal protein intake globally and the production of fish was reported to increase , and thus contribute to cover protein demands of the world population (FAO,2014).

Fish is a vital source of food for people and contributes about 60% of the world's supply of protein . Sixty percent (60%) of the developing countries

divide 30% of their annual protein from fish (Abisoye et al,2011) . This shows that fish is the most important source of high quality protein ,providing approximately 16% of the animal protein consumed by the world's population (FAO,1997) .In Africa ,fish supplies 17% of protein and it is one of the cheapest sources of protein (Calucas and ward ,1996).African people are generally less fish flesh eaters compared to people from other continents , and the national average fish consumption is lower (7 kg /person /year in Africa generally ,1.2 kg /person /year in Sudan) , as reported by the General Administration of Fisheries of the Sudan ,GAFS (GAFS, 2004) . By comparing fish intake in Africa , including Sudan , with that of the rest of the world , the best ways for increasing the fish intake is to increase the fish production and improve the microbiological quality .Hence ,producing enough quantity of high quality fish and thus increasing the fish consumption as an important protein source .

The advantage of fish as food comes from the fact that fish is easily digested and has high nutritional value. Fish should be regarded , not only as food , but also as a source of income in the small holder farming sector (Smith and yoshida ,2000).

In developing countries , despite the low consumption of fishes ,it contributes to 180 kilocalories per capita per day ,in a few countries with a developed fish preference (Lokuruka ,2009) fish is a source of un saturated

fats , called omega-3 fatty acid ,which affect cardiac functions including

hemody namics and arterial endothelial function (Wolfe,2010). Fish is an excellent source of high quality protein, contains the essential amino acids that are necessary for human health (Hoyle and Memitt,1994).

1.3Source of fish

Capture fisheries and aquaculture supplied the world with about 142 million tons of fish in 2008 of this 115 million tons was used as human food (FAO,2010) . In Sudan , there is a considerable scope for development of large scale fish cultures in land waters which unlike many parts of the world, are still free from pollution (George , 2006).

1.4Aquaculture of fish

Aquaculture is the rearing of aquatic organisms under controlled or semi controlled conditions to maximize the output and achieve the highest possible efficiency of resource underutilization (Beveraga et al,1994) .Fish farming initiation in the Sudan goes back to 1950 in connection with the development of Jonglie canal .Introduction of fish culture in ponds was not an urgent matter in Sudan due to availability of red meat and fish from return waters ,towards the late fatties the first experimental fish farm came in to existence at Alshagara , located 11 km south of Khartoum on the eastern

bank of the White Nile river (Geroge ,1975)

1.5 pathogens of fish

 Fishery products , which are of great importance for human nutrition worldwide and provide clear health benefits , can act as a source of food

borne ,pathogens and may be a potential source of infection (Kromhout et al 1985). Fish are extremely susceptible to microbial contamination because of their soft tissues and aquatic environment . Several of these microbes are potential spoiler and are present in the surface slime, on the gills and intestines of live fish . Although the flesh of healthy fish itself is normally sterile because bacterial invasion and growth is prevented by the body's natural defense system during life, but after death the defense system breaks down and the bacteria multiply and invade the flesh . Microbial activities play a large part in the spoilage of fish (Eyo ,2001).

Pathogens from fish can be transmitted to humans through both active and passive contact and may cause food borne diseases such as , dysentery , typhoid , fever , salmonellosis and cholera . The practice of livestock – fish farming needs to be placed in perspective with the likely health risk (FAO ,2003) . The use of different kinds of live stock manure in fish production may increase the level of pathogenic rural community (Musaiger and D,souzi , 2008) .

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) .

1.6 Bacterial Diseases

Aquaculture health management is vital to successful industry .The lack of effective disease prevention and control are the main limiting factors of the realization of highly stable fish production. Successful control of bacterial disease of culture fish is a major management and economic problem for the

aquaculture industry .Disease fish are vehicles for human infection and deaths by septicemia (Veenstra etal 1992). Moreover of all fish diseases, bacterial diseases are widespread, and can be of serious concern in fish farming. Bacterial diseases was found responsible for heavy mortality in both wild and cultured fish (Hudson,1990) .Disease caused by bacteria are often chronic than acute and vary also in causing a high percentage of death which is highly induced by environmental stress (Olufemi ,1998)   .The possible economic losses a fish farmer may suffer in the event of disease occurrence are mortality growth reduction during and after an outbreak , treatment or prevention expenses and loss of investors' confidence . Loss or damage to broad stock may have major consequences on the genetic pool , increase in the time required for the fish to reach market weight –size and therefore postponement or loss of the opportunity to sell fish .Moreover , damage to wild population may result not only in the loss of a resource , but also decrease biodiversity and a shift in the ecological balance (Cameron and Douglas , 2002).Fish contamination can be linked to raw material , personnel , processing tools such as forklifts through leakage ,opening in building and pests .Some pathogens may even established in the processing plants from niches where they can survive for a long period of time

(Adebayo et al ,2012) .

Bacterial disease are responsible for heavy mortality in both wild and cultured fish species (Richards and Robert ,1978).

There are often bacterial species that are facultative pathogenic for both fish and man , and these bacterial may be isolated from fish without apparent

symptoms of disease . The infective source may be fish kept either for food or as comment (Acha et al ,2003).

Human infectious by the fish pathogens are usually through contact with infected fish while handling or with water or other constituents of an aquatic environment (Acha et al ,2003) .According to Lyhs (2009) , Pathogenic bacteria associated with Fish and Fishery product can be categorized in to three groups : (a) Bacteria (indigenous bacteria) that belong to the natural micro flora of fish (ex .pathogenic Vibrio spp) , (b) Enteric bacteria (non indigenous bacteria) that are present due to fecal contamination (Salmonella spp , E.coli) (c) Bacterial contamination during processing , storage or preparation for consumption (Bacillus cereus). The quick and exact identification of bacterial pathogenesis is equally important for quality declaration and to tracing bacterial pathogens in the food supply (Germini etal ,2009) .

1.7 Normal bacteria

 The ranges of the normal bacterial load of the surface slime , gills and intestine of fish have previously been reported to be 10^2 -10^7 , 10^3 -10^7 cfu/cm respectively .(Yagoub ,2009 ,Adebayo et al 2012).

1.8. Hazard of Aquaculture

Food safety hazard in aquaculture included contaminants such as environmental pollutants, fish disease , and hygienic aspects including microbial agents (Hastein etal ,2006) .

The hazard factors during handling of fishes may include catching, slaughtering and processing for consumption (Hastein etal,2006).

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

Infection due to microbial contamination does not usually result in a disease but environmental stresses may upset the balance between the potential pathogens and their hosts (Igbal et al , 2012) .

The use of different kinds of livestock manure in fish production may increase the level of pathogenic bacteria causing a Public health risk to the rural community (Musiger and D Souza 2008) .

1.9. Types of fish in Sudan

Tilapia is the common name for 70 species of perch-like fish (family lichlidae) native to the fresh waters of tropical Africa and is considered as an aquaculture fish in the Africa continent(Trewaves ,1983, Stiasany ,1991)

.The intensive farming of tilapia (Orerochromis niloticus) is rapidly

expanding and tilapias (including all species) are the second most widly farmed fish in the world with annual production exceeding 2 million tons in 2005 (FAO, 2007) . Moreover, the O. niloticus has many outstanding advantages such as easy to culture, high growth rate , easy breading high fibrils protein , good taste ,white cotton meat like sea bass fish , high nutrition value and having more omega-3 than other wild fresh water fishes and wild estuarine fishes (Aquatic Animal Research Centre Choroen Pokphand ,1999) .

Culture of tilapia in Africa and Sudan is based mostly up on un improved populations of Nile Tilapia (Orerochromis niloticus) (Kamel , 2001).

Tilapia is an important aquaculture production for food supply . Globally , fish and fish products are the most important source of protein and it is estimated that more than 3O% of fish for human consumption comes from aquaculture (Hastein etal,2006) .

1.10..Salmonella SPP

Salmonella can be disseminated as a result of water currents underground springs and rain runoff carrying contaminated material (Abdalmonem et al ,1990) . Human infections by these fish pathogen are usually through contact with infected fish under handing process   ,water or other constituents of fish life environment (Acha et al ,2003) . This pathogenic organism has been isolated from freshwater fish such as Tilapia nilotica (D,Aoust et al ,1992) .

1.10.1 Description of Salmonella

Salmonella is a member for the family Entero bacteriaceace , gram negative , motile with peritrichous flagella and non- spore forming rods (the rods are typically 0.7-1.5 pm and 2.5 pm in size) .Salmonella is a facultative anaerobic (can grow with or without oxygen)   (Huss and Gram ,2004 ,Adams& Moss ,2005 ,Erkmen ,2007 ,Lawelay et al ,2008) .

Salmonella spp is a rod shaped , pathogenic bacteria of water bodies in warm climate zones , which pose great risk to human health (Heinitz ,2000)

They are common water borne bacterium , which may be apparently present in the tissues of normal fishes (Newaj –Fyzul et al ,2008) .

1.10.2 The environment of Salmonella:

Salmonella survival in water depends on biological factors (micro and macro invertebrate) and physical factor (e.g, temperature) .Salmonella spp were found to be present in all parts of the fishes (Cahill, 1990) .

1.10.3 Pathogenicity of Salmonella

Salmonella cause a wide range of human diseases such as enteric fever , bacteremia and gastroenteritis . Gastroenteritis has the greatest adverse effect on children's growth and development (Black et al , 1984) .

The majority of 1.3 billion annual cases of salmonella cause human

Gastro enteritis ,through the ingestion of under cooking eggs ,shall fish and

fish (Awuor et al ,2011) .The major reservoirs of the salmonella spp are aquatic environment , however , fish and fishery products have been renovated as a carrier of food borne pathogens (Upadhyay et al ,2010) .

1.10.4 Types of Salmonella

More than 2500 different types of salmonella exist , some of which cause illness in both animals and humans ,some types cause illness in animals but not in human .(Brand ,2006) .

1.10.5 Sources of Salmonella contamination in fish

Aquaculture product can be contaminated with Salmonella through the use of unsanitary ice , water ,containers and poor hygienic handling practices (FAO,2010) . The U.S food and Drug Administration (FDA) data showed

that Salmonella was the most common contaminant of fish and fishery products (Allshouse et al 2004) .

The presence of Salmonellae as entero pathogens in fish farms may reflect the un satiafactory hygienic conditions during catching , handling , distribution and marketing of the fish . The presence of considerable numbers of Salmonellosis indicates un satisfactory hygienic measures doing catching and distribution of the fish (Valdivia et al 1997) .

1.10.6 Escherichia Coli

Escherichia coli (e.coli) is a commons al microorganism whose niche is the

mucous layer of the mammalian colon .This bacterium is the most abundant

facultative anaerobe of the human intestinal micro flora (Anon,2000) . Escherichia species are Gram negative , rod shaped , non spore forming bacteria belong to the family Entero bacteriaceas .

1.10.7The pathogenicity of e. coli

E. coli is a classic example of enteric bacteria causing gastro enteritis . According to Chattopadhyay (2000) e.coli and other coliform bacteria should not be present on fresh caught fish .

The contamination of food of fish origin with pathogenic e.coli occurs probably during handling of fish and under the production process (Ayuto et al , 1999 , Asai et al , 1999) . Thampuran et al (2005) reported that the microbial quality of the Tilapia indicated that all tissue samples except for muscle tissues were mostly contaminant by e.coli , which was often encountered in high numbers .

In Sudan Hanadi (2008) reported the presence of e.coli in gills and intestines of Oreochromis niloticus and Clarias spp fishes . Furthermore , Al-Harbi (2003) detected E.coli in intestines of Tilapia raised in farm and correlated them with pigeon droppings . All the work in e.coli supported that fish obtain it from the surrounding environment .

1.10.8 The environment of e.coli

E. Coli is widely distributed in the intestinal tracts of warm - blooded

animals (Aquatic Animal Research ,1999). Morever , e. coli is often

non pathogenic, although different strains may cause diseases in gastro intestinal, urinary or central nervous systems (Arvanitoyannis and Hadjicostas ,2001).

The quality of fresh fish is strongly determined for bacterial micro biota (Claucas and Ward ,1996) . The e.coli is considered as the reliable indicator of fecal contamination in small and large numbers , and proper hygienic practices (Eze et al 2011) .

1.10.9 History of HACCP

Food safety and inspection services (FSIS) developed the regulatory proposal that became the pathogen reduction /HACCP systems rule (Hulebak and Schlosser, 2002). Subsequently, as a means of safe food production, HACCP principles were adopted worldwide as given in codex aliment Arius commission (1997) and the National Advisory Committee on Microbiological Criteria for Food (NACMCF,1992) .Hazard Analysis and Critical Control Points became a mandatory program for approximately

4000 sea food processors in December 1997 and for foreign processors that ship seafood to the United State (FDA,2001)

1.11 Importance of HACCP

HACCP is management system in which food safety is addressed through analysis and control of biological ,chemical and physical hazards from raw

material , production , procurement and handing to manufacturing

,distribution and consumption of the fished product (NACMCF ,1992) .

1.11.2 The need for HACCP:

In order to successfully implement HACCP in food supply system, authorities responsible for food safety should be aware of system such as HACCP .In a survey conducted to find out whether HACCP was more effective strategy than their current or other methods industry groups had used to secure food hygiene , 41% strongly agreed , 50% agreed, while only 9% did not think that the strategy was more effective than their current provision (Ehiri et al ,1997).

1.11.3 Hazards in food safety

The regulation defines a food safety hazard as any biological, chemical or physical factor that may cause a food to be unsafe for human consumption (USDA,1997). Historically consumers have mostly been concerned with chemical hazards such as pesticide residues and heavy metal contamination. However , microbiological contaminants and allergens have been the recent focus on public health officials concerns. The HACCP system addresses and controls all significant hazards associated with a particular product

(Goodrich et al ,2005) . There are three categories of hazards that are considered in a HACCP plan. These are physical, chemical and biological. All types of hazards can enter a food product at any stage during processing (Harris ,1999) .

1.11.4. Biological hazards

Biological hazards include food poisoning bacteria such as Salmonella , E. coli that are hazardous because they can survive inadequate cooking ,grow to harmful levels in stored food given the right conditions and spread from raw foods to ready eat foods known as cross-contamination . (McSwane et al, 2000).

The numbers and types of bacteria vary from one food or animal species to another, from one geographic region to another, and with production and slaughter or harvesting methods. During production, processing packaging, transportation, preparation, storage and service , any food may be exposed to

bacterial contamination. Although biological hazards may also be due to parasites or zoonotic disease processes (USDA,1997) .Microbial cells have a growth cycle of five phases : lag phase (adaptation period) ,logarithmic growth phases (bacteria multiplication) , stationary growth phase (slowdown of growth) , accelerated death phase (rapid death of microbial cells) and reduced death phase (slowdown of death rate) (Marriott ,1997) .

Examples of biological hazards are disease causing bacteria (Mycobacteria) , (McSwane et al , 2000) . Quantitative scientific assessments of the risks from micro-organisms in foods and water on the basis of dose-response relationships and exposure assessment, customarily

carried out for chemical contaminants, have been developed for some pathogens, particularly in drinking water. Two particular difficulties have to be mentioned for the quantification of microbiological hazards associated with the consumption of foods: the determination of the minimal effective dose and the complicated kinetics of bacterial survival, growth and death in foods which necessitate greater care in contaminations (Untermann ,1998) .

1.11.5 The Seven Principles of HACCP

The HACCP system consists of seven principles .These principles make up the Codex standard which has become the reference for international food safety and identified as the base line for consumer protection under the Agreement on Sanitary and Phyto sanitary Measures agreed at the General Agreement on Tariffs and Trade (GATT) negotiation in 1995 (Slatter ,2003).

There are seven principles of HACCP:

1- Conduct hazard analysis .
2- Determine the critical control point .
3- Establish critical limits .
4- Establish monitoring procedure to control the (CCP) .
5- Establish corrective action when monitoring indicates the CCP is not controlled .
6- Put in place procedures for verification confirms that HACCP is working effectively .
7- Retain documentation for all procedures and records . (Pearson and Dusion , 1995) .

CHAPTER TWO

MATERIALS AND METHODS

2.1.**Study area**

This study was carried out in Khartoum state in central Sudan , in the period of August to November (2016) .In which samples (n=150) were collected from three localities Khartoum , Omdurman and Khartoum Bahri .

2.2.1.Sampling

Atotal of 150 swaps samples were collected from fish farm and were distributed as follows among the selected fish farms : 50 from Alshagara fish farm , Alshagara Fisheries Research Station in Khartoum , 50 from Ahmeds farm in Omdurman and 50 from NaserAldin farm in Bahri . Selection of the farms was based on the willingness of the owners to participate in the study , which means not all the farms have the same chance for being selected , this is known as non-probability sampling methods as described by Thrusfield (2007) .

Swabs were taken aseptically , preserved in sterile bags and transported immediately under complete aseptic condition to Sudan University of Science and Technology ,College of Veterinary Medicine Laboratory for bacteriological analysis . Samples were opened under sterile condition using sterile blades .

2.2.2.Sample preparation

In the lab 5 ml Nuturint Broth was added to all samples and put in an incubater overnight . The primary cultures were examined for bacterial growth , then smeared for gram stain to identify bacterial shape .After that special media (EMB,XLD) were prepared for sub culture .

2.2.3. Sterillization

2.2.3.1. Sterillization by Hot oven

Was used for sterilization of clean glass containers which were wrapped in paper or put in stainless steel cans , temperature at 160 c for hour .

2.2.3.2. Sterillization by flame

This was used for sterilization of straight wire and forceps , it was done by holding the object over flame as near and vertical as possible until it become red .

2.2.3.3. Sterillization by Autoclaving

Was used for sterilizing culture media and plastic wares , the temperature was 121c for 15 minute . . (Cowan and Steel ,1993)

2.2.4. Preparation of bacteriological media

 Culture media used for bacteriological investigation were prepared according to the formulae presented by the manufacturers . Powder bases of these media : XLD , EMB , Nutrient agar and Nutrient broth were weighted as follows 6,7 grams ,3,5 grams ,2,8 grams , 1,3 grams then dissolved in 100 ml distilled water , and autoclaved at 121 c for 15 minutes .Then put to cool at 45 c , and poured in Petri dishes . After dispensed prepared media was convered at 4c in refrigerator till being use.

2.2.5. Procedure to identify bacteria

The media used in tests was pure culture inoculated in sterile Pasteur pipette with in saline suspension , then put the desiceated medium in each tube , which was filled by bacterial suspension .

2.2.6. Biochemical reactions

After incubation of bacterial suspension in a humidity chamber for 24 hours at 37c ,a drop of bacteria suspension added to do the tests , oxidase test , catalase test , motility test , indole test ,nitrate reduction and urease test . .(Cowan and Steel ,1993 ,Monica ,2006) .All reaction were recorded in Result Sheets .

2.2.7. The total plate count

The plate count agar was prepared by dissolving 32 g of nutrirnt agar in 400 ml distilled water then sterillized by autoclaving in 121 c for 15 minute .

Counting was done according to plate count method .

2.2.8. The isolates of Escherichia coli

The isolates of Escheria coli appeared on EMB as green color . Gram negative non-spore farming rods were seen under microscope . Biochemical reactions were done .

2.2.9. The isolates of Salmonella spp

The isolates of salmonella appeared in XLD grew as pink color with black centre . Biochemical tests and gram stain was carried out .

CHAPTER THREE

RESULT

3.1. Bacteria isolated

This study was carried out in fish farms in Khartoum state to determine the certical points that can influence the quality of fish to consumption for humens . The result in this study refered to using either the screening tests or questionnaires survey .One hundred and fifty samples were taken from skins and gills of fish , the isolates were two types of gram negative bacteria, e.coli and salmonella spp which in (table ,1) . It is found that e.coli had a number of 50 isolate (33.3%) in all sample where as salmonella were 60 isolate (40%) from the total , and the mixed samples which contained salmonella and e.coli together was 40 isolate (26.7 %) . The salmonella was the highest prevalence in Khartoum Bahri this might be due to precipitation , environmental conditions prevailing especially feeding habits and workers . E.coli recorded higher isolation in Omdurman may be due to environment habitats and feeding habits, the supplementary or prepared feeds from non-conventional sources were supplied to increase growth rate of cultured fish . Poultry dropping was also used as organic fertilizer to accelerate plank tonic growth in the ponds which is considered primary food of fish ,there are many bacteria present in poultry dropping including both pathogenic and non pathogenic species .

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3.2. The differentiation media

 The media were used to identify bacteria in the laboratory was XLD media and EMB media these was explain in (Table 4) prepared according to the formulae presented by the manufacturers method .

3.3. The biochemical reactions

The biochemical reactions that done to isolate bacteria were the primary test

and secondary tests (Table 5) .

3.4. The Total plate count

In this study the gills had the highest number of bacteria, the total plate count (TPC) for all the fish samples ranged between 7.00 and 7.18 (table 2) out of the 150 fish samples analysis .

3.5. The critical point of environment

The critical points that associated with environment in fish farms were investigated using questionnaire survey , as shown in(table 3) .

The main results showed that the farm workers in thier farms their age in mean over 40 and they had simple training percent (64.7%) of the total number . Also we find that the fish size went to sell in the markets was medium in the most finding percent (79.4%) from the total . The production in more farms about 5 ton in the year(47.1%), fish ponds arranged between 2-5 in most farms .The workers whom had uniform for their work (55.9 %) , also who did hand washing was less(5.9%) . The untensic wash was low percent(35%) ,and while did sometimes(50%) . The distribution of fish in

Khartoum state is most common (85.3%) , and the fish cooling was less than selling without cooling(47.1%). Fish transport before cleaning was most uses (64.7%) , and after cleaning was (34.3%) . The most way to preserve fish was freezing (67.6%), also the prefered season was summer . Workers who bath after work was(73.5%), and who diseased was low (17.6%) and no nail cutting was(61.8%) .

Table (1)Number of gram-negative bacteria isolated from examined fishes

locality	No. examined	Sal-No	Sal-percent	E.coli-No	E.coli-percent	mixed-No	mixed-percent
khartoum	50	15	30%	17	34%	18	36%
Omdurman	50	18	36%	19	38%	13	26%
Khartoum bahree	50	27	54%	14	28%	9	28%
Total	150	60	40%	50	33.3%	40	26.7%

No-number ,percent-percent ,sal-Salmonella

.

Table (2) The mean count of bacteria isolate from gills and skins of examined fish :

Locality	No . of sample	No 0f bact	Percent %	Mean+St.Dv	observ
khartoum-g	25	25	16.7 %	6.89 ±o.20	NS
khartoum-s	25	25	16.7%	6.92 ± 0.23	NS
Omdurman-g	25	25	16.7%	7.27 ± 0.24	NS
Omdurman-s	25	25	16.7%	7.19 ±0.09	NS
Khartoum bahree-g	25	25	16.7%	7.24 ± 0.18	NS
Khartoum bahree-s	25	25	16.7%	7.27 ± 0.17	NS

(g) gill –(s) skin –(NS) no significant

Table (3) : knowledge ,practice and attitude of workers towards safeties in fish farm in Khartoum state (questionnaire survey) :

Factor	Frequency	Percent %
1-Sex male	33	97.1
Female	1	2,9
2-Age 20- 30	7	20.6
31-40	10	29.4
> 40	17	50
3- Training simple	22	64.7
High	12	35.3
4- Education literate	23	67.6
illiterate	11	32.4
5- Fish size large	3	8.8
Medium	27	79.4
Small	4	11.
6- Fish pond one	6	17.6
2-5	15	44.2
>6	13	38.2

Table (3) continue :

7- Production <5	16	47.1
6-9	5	14.7
>10	13	38.2
8-Uniform yes	19	55.9
No	15	44.1
9-Hand washing yes	2	5.9
No	2	5.9
Missing system	30	88.2
10-Untensil wash yes	12	35.3
No	5	14.7
Some times	17	50.0
11-Sellplace instate	29	85.3
Outstate	5	14.7
12-Fish cooling yes	16	47.1
no	18	52.9
13-Fishtransport after	12	35.3
Before clean	22	64.7
14-Roten fish unclean	34	100

Table(3) continue :

15-Fish preserve chillin	8	23.5
Freezed	23	67.6
Salted	3	8.8
16-Preferseason	17	50.0
Summer	11	32.4
Winter	6	17.6
Autoum		
17-Bath after yes	25	73.5
No	9	26.5
18-Diseased yes	6	17.6
No	28	82.4
19-Nail cutting yes	13	38.2
No	21	61.8

CHAPTER FOUR

DISCUSSION

The bacteria pathogens that were isolated and identified include e.coli , salomenlla spp which indicated public health hazards and concern , the isolation of these groups of organisms indicated fecal and environmental pollution and these support the finding of Yagoub et al (2009) who isolated pathogenic and potential pathogenic organisms from tap water that originated from Nile River . This also confirms the finding of Koutsoumanis and Nychas (2000) , Gonzalez-Podriguez et al (2001) and Herrera et al (2006) who isolated similar organisms from fish and fish products . E ,coli was isolated from gills and skins of samples fish in the present investigation this is in agreement with previous study in Sudan by Hnadi (2008) who reported the presence of e.coli in gills and intestines . Also in this study salmonella was isolated from both gills and skins of examined fish this is in agreement with Brands et al (2005) , and Amaglani et al (2011) . High prevalence of salmonella in fish attributed to the poor and un hygienic , handling practices and feeding habits . In this investigation indicated these fishes could be a source of infection and the possibility of transmission of these pathogens to worker in fish farms and consumers , generally human contract fish –borne bacterial disease through ingestion of contaminated

 fish tissue infection or by contaminated water and injection of the organism

in to puncture wounds the transmission of others bacterial species has not been documented the potiontial for human infection does exit among individual who handle diseased fish (Stoskdpf ,1993) .

According to published microbiological guidelines as cites by Gilbert et al (1996) the results suggest that microbiological quality of the fish examined is unacceptable levels .

The total number of bacterial count for fresh fish was ranged between 7.00 and 7.18×10^5 for salmonella and 7.08 to 7.16×10^5 for e.coli , this number was not acceptanse since the limit mentioned by SSMO (Sudanese Standards and Metrology Organization ,SDs357) which was 5×10^5 cfu/g for fresh fish products . This results is differ from the finding of Kapute et al (2013) who found that the total bacterial count reach 2.1×10^5 . This means that Salmonella the highest prevalence in the visited farms .

In this study , the critical points depend on the environment habitats and the other point of the feeding of fish .This shows the importance of control the pathogenic bacteria which might cause serious infection leading to considerable economic losses in fishes when environmental condition altered in ponds and fish's resistance was reduced .

CONCLUTIONS

Many critical points related to fish or the environment were observed in fish farms in Khartoum State .

It can be concluded that the gram negative bacteria contain e.coli and salmonella were isolated from the samples collected from different farms of fish in Khartoum state . Also the hazards of environment factor were poor hygiene condition during different practices , handling and distribution of fish , be most pollution for rearing fish and efficiency of the products .

More over , the control for all points mentioned were as follow :

Access increasing the knowledge of the workers , technical and trained , improvement of general hygiene conditions in the environments in the farms during different practice .

RECOMMENDATIONS

1-An attention should be made for increasing awareness of the workers more extensions and training and sufficient information a bout hazards and risks involved .

2-Investigate bacteria of fish and needed to follow it in fish ponds to avoid the death of fish .

3-Encourage more studies and research in fish for food safety .

4- Apply HACCP system in fish farm to avoid the contamination and safe human health .

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TABLES

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5- Fish size large	3	8.8
Medium	27	79.4
Small	4	11.
6- Fish pond one	6	17.6
2-5	15	44.2
>6	13	38.2
7- Production <5	16	47.1
6-9	5	14.7
>10	13	38.2
8-Uniform yes	19	55.9
no	15	44.1

9-Hand washing yes	2	5.9
No	2	5.9
Missing system	30	88.2
10-Untensil wash yes	12	35.3
No	5	14.7
Some times	17	50.0
11-Sellplace instate	29	85.3
Outstate	5	14.7
12-Fish cooling yes	16	47.1
no	18	52.9
13-Fishtransport after	12	35.3
Before clean	22	64.7
14-Roten fish unclean	34	100
15-Fish preserve chillin	8	23.5
Freezed	23	67.6
salted	3	8.8
16-Preferseason	17	50.0
Summer	11	32.4
Winter	6	17.6
Autoum		

17-Bath after yes	25	73.5
No	9	26.5
18-Diseased yes	6	17.6
No	28	82.4
19-Nail cutting yes	13	38.2
No	21	61.8

Table (4) The differentiation media that we used to isolate bacteria in the laboratory :

Media	Salmonella	E.coli	Mix
EMB	Dark pink	Green	Green / Pink
XLD	Black	Yellow	Yellow / Black

Table (5) : The biochemical reactions that down to isolates bacteria

Test	E. coli	Salmonella
1- Gram stain	-ve	-ve
2- Oxidase	-	-
3- Catlase	+	+
4- M	+	-
5- OF	+	+
6- Suger	+	+
7-Indole	+	-
8- Citrate	-	+
9- Urease	+	-
10- KIA		
Buff	Y	y
Sloope	Y	red
Gas	+	+
H$_2$S	-	+

(+) positive , (-)negative ,F fermentative , M motility

بسم الله الرحمن الرحيم
إستبيان حول تطبيق نظام تحليل المخاطر في مزارع الأسماك

الإسم :

النوع : ذكر ☐   أنثى ☐

العمر:

المهنة :

نوع التدريب :

مستوى التعليم : ملم بالقراءة ☐   غير ملم بالقراءة ☐

العنوان :

حجم الأسماك المصادة :

صغيرة ☐   متوسطة ☐   كبيرة ☐

عدد الأحواض بالمزرعة:

تقدير الإنتاج السنوي:

هل تستعمل ملابس ومعدات معينة عند الصيد ؟

نعم ☐   لا ☐

هل يتم غسل الأيدي قبل أو بعد الصيد ؟

نعم ☐   لا ☐   أحيانا ☐

هل يتم غسل الصناديق و الأواني المستعملة بعد عملية الصيد ؟

نعم ☐   لا ☐   أحيانا ☐

هل يتم نقل الأسماك و توزيعها ؟

داخل الولاية ☐   خارج الولاية ☐

هل يتم تبريد الأسماك بعد خروجها من المياه مباشرة ؟

نعم ☐   لا ☐

يتم نقل الأسماك :

بعد نظافتها ☐   من غير تنظيف ☐

أكثر الأسماك تلفا أو خسارة:

الأسماك المنظفة ☐   الأسماك غير المنظفة ☐

أي الطرق أنجح في حفظ الأسماك ؟

تبريد ☐   تجميد ☐   تمليح ☐

أي الفصول أنسب لإنتاج الأسماك ؟

الصيف ☐   الشتاء ☐   الخريف ☐

هذا الإستبيان بغرض البحث العلمي