Sudan University of Science and Technology

College of Animal Production Science and Technology

Department of Fisheries and Wildlife Science

Isolattion and identification of pathogenic bacteria infections From *O.niloticus and C.gariepinus* Collected from fish Hatchery

A Thesis Submitted in Partial Fulfillment of the Requirement of the B.Sc. Degree in Fisheries and Wildlife Science (Honor)

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بسم الله الرحمن الرحيم

قال تعالى:

"وما يستوي البحران هذا عذب فرات سائغ شرابه وهذا ملح أجاج ومن كل تأكلون لحما طريا وتستخرجون حلية تلبسونها وترى الفلك فيه مواخر لتبتغوا من فضله ولعلكم تشكرون "

صدق الله العظيم

سورة فاطر الآية (12)

Dedication

To our Mothers and Fathers for their encouragement and supporting us

And blessing.

And to whom we are always indebted to our brother, sister and friend.

And to our big brother Ayman Al-Amin who has not tired of our help.

Acknowledgment

All our greatest thank first to Allah, the most Merciful who gave us the health strength and patience to conduct this study. Grateful thanks to our Supervisor; Professor; Fathia Abdulhamed Khogali College of Science and Technology of Animal Production Department of Fisheries and Wildlife Science for her guidance and provision of scientific knowledge. Finally our thanks to all member of Microbiology lab and fish hatchery.

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Abstract

The main objectives of this study was to determine the presence of Pathogenic bacteria species Counted and isolated, identified pathogenic bacteria in *O. niloticus* and *C. gariepienus*, and which organ(s) was more susceptible to bacterial infection. Six fish samples were collected randomly three Nile tilapia *O.niloticus* and three catfish *C.gariepienus* from fish hatchery, all fish were weighed using sensitive balance, then External observations on fish were also performed. Samples were inoculated medium were observed for the presence of bacterial growth after 24 hours incubation. the bacterial colonies present were sub-cultured onto (MckA) medium and incubated at 30 C in an incubator for 24 hours. Data were analyzed by SPSS version 2.1 The result from this research shows that the bacterial abundance varies in the six segments of the fishes analyzed, the skin, gills, gonad, spleen, liver and viscera. The bacterial occurrence in all sample was high (salmonella, 30.5, Psdomonus 11.1 , Protieus 8.3 and E.coli 0.0, 8.3) in tilapia and catfish respectively.

Keywords: bacterialcount, present,idintifiction, *O.niloticus C.garpienus.*

الخلاصة

كان الهدف الرئيسي من هذه الدراسة لعزل الأنواع البكتيرية وعدها, ومن ثم تحديد البكتيريا المسببة للأمراض في البلطي والقرموط ومعرفة أي الأعضاء الداخلية والخارجية أكثر إصابة بكتيرية. تم جمع ستة عينات عشوائيا ثلاثة أسماك من البلطي نيلي وثلاثة عينات من اسماك القرموط الأفريقي من مفرخ الأسماك, وتم وزن جميع الأسماك باستخدام الميزان الحساس, وسجلت الملاحظات الخارجية على الأسماك, تم تحضين العينات في الحاضنة لمدة 24 ساعة. وتم تزريع البكتريا في وسط ماكونكي, وحضنت في درجة 37 درجة مئوية في الحاضنة لمدة 24 ساعة وتم تحليل النتائج بواسطة برنامج التحليل الإحصائي, رقم الاصدار 2.1. ودلت النتيجة من هذا البحث على أن وفرة البكتريا تختلف بين أجزاء جسم الأسماك (الجلد, الكبد, المناسل, الطحال, والكبد). وكانت نسب البكتريا في جميع العينات للبلطي والقرموط على التوالي كالأتي : (السالمونيلا 30.5, سودومونس 11.1 ,بروتيوس 8.3 , الإشريشية القولونية 0.0, 3.8)

كلمات مفتاحيه: العد البكتيري, النسبة, التعرف على البكتيريا, البلطي النيلي, القرموط الإفريقي.

CHAPTER ONE

INTRODUCTION

Fish is important to human populace in trade and economy; it is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem **(Osuigwe and Obiekezie, 2007).** As the human population inevitably increases, the demand for fish as source of protein will grow **(Abolarin, 1996).** In recent times, there has been tremendous increase in the development of fish farming and culture attributable to the increased need for affordable animal protein especially in the tropics **(Davies et al., 2006).**

The world is full of bacteria; in fact, our world would not exist as we know it without them. In 1884 a Danish physician, Christian Gram, discovered that bacteria could be separated into two groups, gram-positive and gram-negative. Using a particular staining process, the bacteria could be determined either gram-positive or negative, depending on whether they retained (positive) or lost (negative) a violet color during this process. Most bacteria that cause disease in marine fish are gram-negative. Ones most commonly associated with these infections are of the genus Pseudomonas and Vibrio, as well as Mycobacteria **(Toranzo *et al*., 2005)**.

Infectious diseases of cultured fish are the most notable constraints on the expansion of aquaculture and the realization of its full potential **(Klesius *et al*., 2000; Robertand Moeller, 2012).** Bacterial pathogens are the most serious disease problem in fish production causing 80% of mortalities **(Austin and Austin, 1993).**

Bacterial disease is the most common infectious problem of fishes. Collectively, only water quality problems exceed bacterial diseases in the area of fish morbidity and mortality. The majority of bacterial infections

are caused by Gram-negative organisms including the following pathogenic genera: *Aeromonas, Citrobacter, Edwardsiella Flavobacterium (Flexibacter), Mycobacterium, Pseudomonas, and Vibrio. Streptococcus,* a Grampositivegenus, has been shown to cause disease infishes. Bacterial organisms may be the primary causeof disease, or they may be secondary invaders, takingadvantage of a breach in the fish's integument orcompromise of its immune system. The majority ofbacterial fish pathogens are natural inhabitants of theaquatic environment, whether it is freshwater or marine. (**Robert and Moeller, 2012)**.

Bacterial species isolated from fish could have serious health issues for humans who consume or ingest them. Some of the bacteria species that are isolated from the gut of fish are faecal coliforms; and this is especially characteristic of farms where there is little or no biosecurity. Contamination of hands and surfaces during cleaning and evisceration of fish is a common route for pathogenic infection in humans **(Adedeji *et al.*, 2011).**

Effective water management in a fish holding facility is one of the important factors contributing to the success of fish culture **(Hossain *et al.*, 2006).Ahmed *etal.* (2009).**

Found that seasonal variations in pH, temperature and dissolved oxygen play important roles in the multiplication of pathogens thus leading to diseases in fish. Low or rapid changes in water temperature, rapid or prolonged depression of pH, low alkalinity and low dissolved oxygen are seasonal aggregations of fish diseases **(Lilley *et al.*, 1992)**.

Objectives:

1. To isolate and identify pathogenic bacteria from *O. niloticus* and *C. gariepinus*.
2. To determine bacterial count in *O. niloticus* and *C. gariepinus*.
3. To isolate pathogenic dentify the percentage of bacterial species in *O. niloticus* and *C. gariepinus*, and which organ(s) was more susceptible to bacterial infection.

CHAPTER TWO
LITERATURE REVIEW

2.1 Bacterial:

Common noun bacteria singular bacterium constitute a large domain of prokaryotic microorganisms. Bacteria have a number of shapes, Ranging from spheres to rods and spirals. Bacteria were among first life forms to appear on earth. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of earth's crust. bacteria also live in symbiotic and parasitic relationship with plants and animals. Most of bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory. The study of bacteria in known as bacteriology, a branch of microbiology.

2.2 Bacteria associated with fish diseases:

2.2.1 *Edwardsiella* species:

Edwardsiella species are Gram-negative, rod-shaped bacteria. *Edwardsiella tarda* and *E. ictaluri* produced two different diseases **(Noga, 1996).***Edwardsiella tarda* causes septicemia in warm water fish, particularly in eels and catfish. It is widely disseminated in aquatic animals, pond water and mud, occurrences that provide ready opportunities to re-infect cultured fish. Infected fish processed for human consumption are a source of this organism, which can cause gastroenteritis in humans. *Edwardsiella ictaluri* causes a septicemia in catfish, and is a highly contagious disease with serious effects on the commercial culture of catfish in the southern USA **(Noga, 1996).** It is an important zoonotic disease of humans in which it is a serious cause of intestinal disease. In humans, it has also been implicated in meningitis, liver abscess, wound infections; most commonly, however, this organism causes gastroenteritis. Catfish fillets in processing plants are often

contaminated with this organism that may spread to humans by the oral route **(Noga, 1996).**

2.2.2 *Yersinia ruckeri*:

Yersinia ruckeri is Gram-negative, non spore-forming, straight rodshaped bacterium; widespread in fresh-water environments **(Noga, 1996).***Yersinia ruckeri* is the cause of enteric red mouth (ERM) disease, a condition that has been known since the 1950s. ERM has been a problem mainly for cultured rainbow trout, but all salmonids and some other species of fish are affected. Losses may be relatively low in chronic infections or can become much higher if water conditions are poor, or if fish are exposed to stresses such as handling. In its early stages in salmonids, ERM disease resembles aeromonad and vibrio infections, with darkening of the dorsum, lack of appetite and lethargy. Internally, there are tiny hemorrhages on the viscera, enlargement of the spleen, and necrosis of the inner surface of the intestine and a mucoid exudate. In the more chronic condition, the abdomen is distended, there may be unilateral or bilateral exophthalmia, and hemorrhage in the eyes. The latter causes darkening of the fish because of the induced blindness which leads to lack of control of melanin pigment **(Noga, 1996).**

2.2.3 *Flexibacter* species:

Bacterial gill disease (BGD) is caused by a variety of bacteria including *Flexibacter columnaris, F. psychrophilus, Cytophagapsychrophila*, and various species of *Flavobacterium*. Currently, a suggested name for this agent is *Cytophaga columnaris*, although it has also been called *Flexibacter columnaris* **(Noga, 1996).** Whereas *F. columnaris* infects fish only in fresh water environments, *F. maritimus*, an organism that seems to be less infectious than *F. columnaris*, infects fish in marine environments, where it produces salt-

water columnaris disease. Both mortality and acuteness of disease increase with temperature **(Noga, 1996).**

2.2.4 *Aeromonas* species:

Aeromonas species are Gram-negative, non spore-forming, rodshaped bacteria. *Aeromonas hydrophila* is a common inhabitant of healthy fish and the aquatic systems (Kaper *et al*., 1981); it is also an established opportunistic pathogen infecting fish under physiological or environmental stress **(Groberg *et al*., 1978).** According to **Noga (1996),** motile Aeromonas infection (MAI) is likely the most common bacterial disease of fresh water fish, all of which are probably susceptible. Motile aero monads can also inhabit brackish water, but they decrease in prevalence with increasing salinity. Pathogenic *Aeromonas sobria* has been identified as causative agent of ulcerative fish disease in farmed European perch **(Goldschmidt *etal*., 2008).***Aeromonas hydrophila* was isolated from the wounds infection of 11 brackish-water fish in Bangladesh.. *Aeromonas hydrophila* was isolated from skin lesions of three fish species collected from several locations in West Bengal **(Pal and Pradhan, 1990).Kasing *et al*. (1999)** isolated *Aeromonas hydrophila* from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds.

2.2.5 *Pseudomonas* species:

Pseudomonas species are Gram-negative, rod-shaped, non spore forming

Bacteria. Distributed widely in nature and found in soil and in water. *Pseudomonas* spp is commonly associated with fish eggs, skin, gills, and intestines. In Sudan **Hnadi (2008)** reported the presence of *Pseudomonas* spp in gills and intestines of *Oreochromis niloticus* fishes. **Mohammed (1999)** isolated *Pseudomonas aerugenosa* from apparently healthy *Oreochromisniloticus* fish and diseased fish. **Tripathy *et al*. (2007)**

isolated 10 *Pseudomonas aeruginosa* 6 from intestine of freshwater fish and 4 from pond sediment.**).Lönnström *et al*. (1994); Berthe *et al.* (1995)** and **Doménech *et al.* (1997)** isolated *Ps. anguilliseptica* from eye, kidney, Spleen, liver and ascetic fluid of Baltic herring. However, secondary occurrence of pseudomonades was found to be rather occasional in several culture and wild fish species of Southeast Asia **(Boonyaratpalin, 1989).** Later on, even colonies of *P. aeruginosa* were detected on the surface and muscle lesions of several UDS afflicted fish species including the channids **(Kar *et al*., 1990).**

2.2.6 *Citrobacter freundii*:

Kasing *et al*. (1999) isolated *Citrobacter freundii* from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds. *C. freundii* causes abnormal inflammatory changes in the intestine of trout and inflammatory and necrotic changes in the internal organs of cyprinids. *C. freundii* represents approximately 29% of all opportunistic infections **(Whalen *et al.,* 2007)**, it does not generally cause disease in healthy human hosts. Therefore, in patients with a suppressed immune system, *Citrobacter* species are known to cause a wide variety of nosocomial infections of the respiratory tract, urinary tract, blood and neonatal meningitis **(Whalen *et al.,* 2007).**

2.2.7 *Enterobacter* species:

It is a Gram-negative rod that belongs to the Entero bacteriaceae family. *Enterobacter* species were reported from the liver of *Labeo rohita* **(Naqvi *et al.,* 1990). Kasing *et al*. (1999)** isolated *Enterobacter aerogenes* from intestine of four freshwater fish species belonging to the family *Cyprinidae* reared in experimental ponds. An enteric bacterium from the kidneys of moribund fish was isolated and identified as *Enterobacter cloacae*. Fish were experimentally infected by this isolate and the organism was re-isolated from the kidneys of the moribund fish.

This study revealed that human enteric bacteria which are considered as nonpathogenic to fish may become pathogenic **(Thillai *et al.*, 2008).** *Enterobacter* species are emerging as important human pathogens, particularly among hospitalized patients **(Gallagher, 1990).***E. cloacae* and *E. aerogenes* are the most frequently isolated species associated with human diseases (Sanders *et al.*, 1997).

2.2.8 *Escherichia* species:

Escherichia species are Gram-negative, rod-shaped, non spore forming bacteria belongs to the Entree bacteriaceae family, widely distributed in nature, and constitute a part of digestive flora of mammals, and birds. **Aydin *et al*. (1997)** reported mass mortality with gross clinical and histopathological abnormalities in a number of organs of rainbow trout after inoculation of *Escherichia vulneris.* In Sudan **Hnadi (2008)** reported the presence of *Escherichia coli* in gills and intestines of *Oreochromisniloticus* and *Clarias* spp fishes. **Naqvi *et al*. (1990)** reported the presence of *Escherichia* from the liver of *Labeo rohita*. **Kasing *et al*. (1999)** isolated *Escherichia coli* from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds.

Del Rio-Rodriguez *et al.* (1997) reported the use of *E. coli* laden feed to infect rainbow trout intestines, and **Al-Harbi (2003)** detected *E. coli* in intestines of and tilapia raised in farm correlated them with pigeon droppings. **Guzman *et al.* (2004)** found two fish species that harbored *E. coli* from a river contaminated by sewage effluent. Their work also supported the view that fish obtain *E. coli* from the environment.

Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious food poisoning in humans **(Vogt and Dippold, 2005)**

2.2.9 *Staphylococcus* species:

Staphylococcus species are Gram-positive, non spore-forming cocci. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. In Sudan **Hnadi (2008)** reported the presence of *Staphylococcus* spp in gills and intestines of *Synodontis* spp and *Clarias* spp fishes**. Kasing *et al*. (1999)** isolated *Staphylococcus* spp from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds. *Staphylococcus aureus* has long been recognized as one of the major human pathogens responsible for a wide range of afflictions from minor infections of the skin to wound infections, bacteremia, infections of the central nervous system, respiratory and urinary tracts, and infections associated with intravascular devices and foreign bodies. Most *S. aureus* strains are opportunistic pathogens that can colonize individuals, without symptoms, for either short or extended periods of time, causing disease when the immune system becomes compromised **(Oliveira *et al*., 2001).**

2.2.10 *Streptococcus* species:

Streptococci are Gram-positive cocci, non spore-forming bacteria, usually arranged in pairs or chains. Streptococcal septicemia has occurred sporadically and as epizootics among cultured fresh-water and salt-water fish in many parts of the world. *Streptococcus iniae* mainly cause disease in tilapia, hybrid striped bass and rainbow trout **(Stoffregen *et al*., 1996).** The known cyprinid species that are affected include golden shiner and blue minnow (experimental). However, *S. iniae* is a serious problem in some operations rearing tilapia. Signs of this illness vary among different species of affected fish.

2.2.11 *Enterococcus* species:

Enterococcus species are Gram-positive, non spore-forming cocci. *Enterococcus* species can be isolated from the water, plants and from the excretion of animals and humans as commensal microorganisms. They are responsible for considerable economic losses in cultured yellowtail fish **(Kusuda and Salati, 1993),** turbot **(Toranzo *et al*., 1995)** and tilapia. *Enterococcus faecium* and *E. faecalis* were the predominate species isolated from fish in the integrated farms, whereas *E. casseliflavus* and *E. mundtii* isolates were most prevalent in traditional fish farms **(Petersen and Dalsgaard, 2003).** Clinical signs and pathological manifestations are similar to streptococcosis and consist of exophthalmia, muscular haemorrhages, acute bronchitis, and supportive inflammation in the eyes and necrosis of the spleen and kidney. In man infections caused by the genus Enterococcus (most notably *Enterococcus faecalis*, which accounts for ~80% of all infections) include urinary tract infections, bacteremia, intra-abdominal infections, endocarditis and post-operative complication of cataract surgery **(Huycke *et al*., 1998).**

2.2.12 *Micrococcus* species:

Micrococci are Gram-positive cocci usually arranged in tetrads or irregular clusters **(Smith *et al*., 1999).** *Micrococcus luteus* exists in the normal microbial flora of intestines of fresh water fish, and is considered a pathogenic bacterium of fish. *M. luteus* infection has been observed, and fish were experimentally infected in the United Kingdom **(Austin and Stobie, 1992).** Micrococcus that is opportunistic pathogens, have been associated with necrotic ulcers which are thought to be the secondary infections leading to death in severely ulcerated fish **(Lilley *et al*., 1991).** Micrococcus is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in human hosts with compromised immune systems, such as

HIV patients **(Smith *etal.*, 1999)**. Recently, this organism was recognized as an opportunistic pathogen and has been implicated in recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration, and cavitations pneumonia in immunosuppressed patients **(Smith *et al.*, 1999).**

2.2.13 *Bacillus* species:

Bacillus species are Gram-positive, large rod-shaped, spore-forming bacteria, widely distributed in the environment mainly because of their highly resistant endo-spore. **Ananthalakshmy *et al.* (1990)** isolated *Bacillus* species from 4 fish species through study of quantitative and qualitative distribution of histamine-forming bacteria associated with fish. **Kasing *et al*. (1999)** isolated *Bacillus* spp from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds. In Sudan **Hnadi (2008)** reported the presence of *Bacillus mycoides* in gills and intestines of *Synodontis* spp. *Bacillus cereus* is a causative agent of both gastrointestinal and nongastrointestinal infections. Enterotoxins, emetic toxin (cereulide), hemolysins, and phoshpolipase are known as potential virulence factors of *B. cereus* (**Anja *et al*., 2000).**

1.2.2.14 *Mycobacterium* species:

Mycobacterium species are Gram-positive, long rod-shaped, non spore-forming and acid-fast bacteria. Most of these agents are free-living in soil and water, and some species cause disease in animals and humans. *Mycobacterium* infections of fish are really tuberculosis of a number of species. The disease affects a wide range of fresh water and marine species of fish, and particularly aquarium fish **(Noga, 1996).**

CHAPTER THREE

Materials and methods

3.1 Study area:

Microbiology lab in Sudan University of Science and Technology, College of Animal Production Department of Fisheries and Wildlife.

3.2 Reagents and indicators

3.2.1 Indicators:

- Petri dishes - Swabs
- Tubes - Auto clave
- Reffregrator - Miro pipet
- Loop – gas stove – Slides
- Flask – Measuring tube

3.2.2 Reagents:

- Normal saline – mackonky agar
- Minitol agar – nutrient agar
- Peptone – nutrient broth
- Urea base – kovac's reagent.

3.3 Sterilization:

- 4.1 – Steaming , by using the auto calve to sterilizing the solutions or the liquid reagents
- 4.2 – Dry sterilization , by using the incubater " incubating room"
- Gas stove: using to sterilizing the loops in culturing or the tubes or media top before covering it.

3.4 Collection of samples.

Six fish samples were collected randomly three Nile tilapia (*O. niloticus*) and three catfish (*C. gariepienus*) from fish hatchery, all fish was weighed using sensitive balance, External observations on fish were also recorded.

3.5 Water sample

Two samples of water have been collected from each pond one from calaris lazira water and the other sample from tilapia water. We put the water on swabs. By using swabs, six samples were taken from each fish from (skin, gills, kidney, gonads, intestine and liver).

3.6 Preparation of media.

Petri dishes are prepared and sterilized in the sterilization oven.

The blood agar was prepared and dissolved in 200ml of normal saline and placed in the autoclave. After an hour it was extracted and poured into the dishes and placed in the refrigerator until it was cohesive. Some media are prepared for the bacterial count, by nutrient agar base.

3.7 bacterial isolation from fish & debris at sampling site.

Peptone water used as feeder for the batteries in all swabs, and placed on the Incubating room. The samples were cultured onto mackonky agar base by using sterile wire loop. The inculpated mackonky agar media were incubated at room temperature 22 c .

The broth samples were mixed by using micro petite . one hundred micro liters of the broth was then dropped onto medium and streaked with a sterile loop, the incubated medium was the incubated at 30 c for 18-24 hours .

After extracting the nutrient broth from the incubator ,we prepared the tubes to reduce the number of bacterial colonies.

For each sample, 5 tubes were prepared and filled with a normal saline of 9ml per tube. With the micro petite , 1ml of the first sample was taken from the nutrient broth and placed in the first tube and mixed with the normal saline. 1ml was taken from the first tube and placed to the second tube and so on for all the 4 and 5 tubes.

Form the fifth tube of each sample 1ml took from it and dropped 5 drops on the nutrient agar medium and linked the 5 drops by the loop. And all

the medium placed at the incubator for 24h. next day all of the colonies of each medium were counted .

3.8 Subculture of fish samples & debris bacterial colonies from (McK.A) Medium.

Inoculated medium were observed for the presence of bacterial growth after 24 hours incubation. the bacterial colonies present were sub-cultured onto (MckA) medium and incubated at 30 C in an incubator for 24 hours.

3.9- Gram staining & catalas and API test:

The gram stain is deferential stain commonly used in the the microbiology Laboratory that differentiates bacteria on the basis of their cell wall structure.

3.10- Procedure:

By using a sterile inoculating loop, 1 drop of sterile water added to the slide. Prepare a mixed smear of E.Coli & Staphylococcus.

- Air dry and heat fix.
- Cover the smear with crystal violet for 1 min.
- Gently wash off the slide with water.
- Adding gram's Iodine for 1 min.
- Washing with water.
- Decolorize with 95% ethanol. this the tricky step.stop decolorizing with alcohol as soon as the purple color has stopped leaching off the slide, immediately wash with water.
- Smear covered with safranin for 30 second.
- Wash the slide with water.
- The slides were examined on the microscope to see the smear by using the oily lens and the result recorded.

3.11 Data Analysis:

Results were analysis by SPSS program use cross tap version 21.

CHAPTER FOUR

Results

The results obtained showed that there are four types of bacterial species found in the two fishes. Results were analyzed statistically and are tabulated as follows and one graph for percentage: Table 4.1 shows the type of bacterial species found and in what percentage.

Table (4.1) Bacterial species frequency and percent in different organ of tilapia sampled from fish hatchery

Bacterial species	Percent
Salmonella	58.3
Psdomonus	11.1
Proteus	22.2
E.coli	8.4

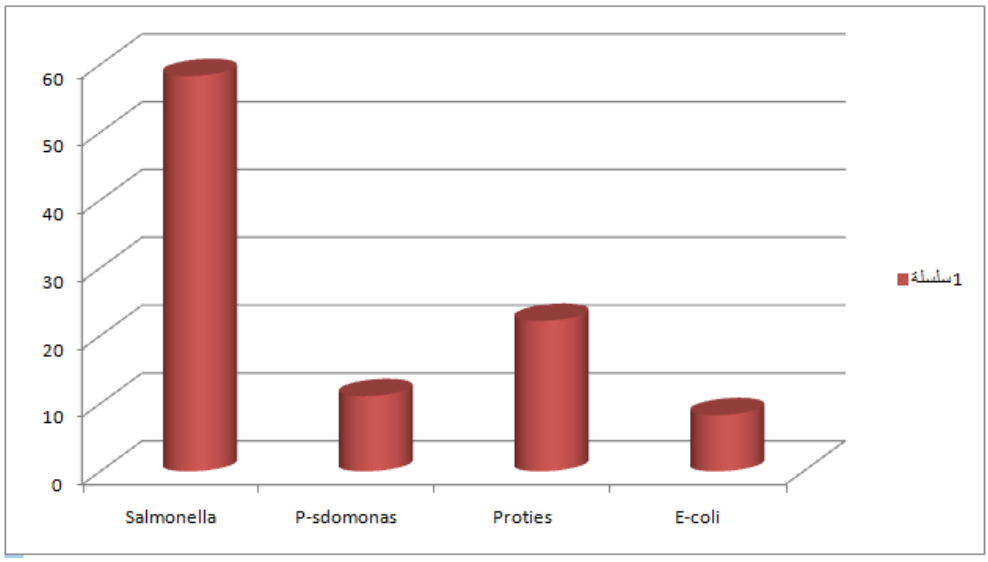

Figure (4.1) Bacterial species frequency and percent in different organ of tilapia sampled from fish hatchery

The bacterial occurrence in the different organs of *O.niloticus* is shown in table 4.2 And the frequency of occurrence for each species is illustrated in table 4.3

Table (4.2) Bacterial species occurrence in different organ of tilapia sampled from fish hatchery

Bacterial species	**Organ**				
	Skin	**Gill**	**gonad**	**Liver**	**Viscera**
Salmonella	2	2	1	1	2
Proteus	0	1	1	1	1
Psdomonus	1	0	1	1	0
E.coli	0	0	0	0	0

Table: (4.3) Frequency of occurrence of bacterial isolates from *O.niloticus* sampled

Bacterial species	**Number of occurrence**	**% occurrence**
Salmonella	11	30.5
Psdomuns	4	11.1
Proteus	3	8.3
E.coli	0	0.0

Table 4.4 shows the bacterial occurrence in the different organs of catfish *C. gariepienus*

Bacterial species	**Organ**				
	Skin	**Gill**	**gonad**	**Liver**	**Viscera**
Salmonella	0	1	2	2	3
Proteus	1	1	1	0	0
Psdomonus	0	1	0	0	0
E.coli	2	0	0	1	0

Table: (4.5) Frequency of occurrence of bacterial isolates from catfish *C.gariepienus* sampled from hatchery

Bacterial species	Number of occurrence	% occurrence
Salmonella	11	30.5
P.sdomuns	3	8.3
Proteus	1	2.7
E.coli	3	8.3

Table: (4.6) The result of bacteria in water for both Tilapia *O.niloticus* and Catfish *C.gariepienus*

Fish Water	Bacteria Species
Tilapia	Salmonella
Catfish	Salmonella

CHAPTER FIVE
DISCUSSION

The result from this research shows that the bacterial abundance varies in the six segments of the fishes analyzed, the skin, gills, gonad, spleen, liver and viscera. The bacterial occurrence in all sample was high (salmonella, 30.5, Psdomonus 11.1, 8.3, Proteus 8.3 and E.coli 0.0, 8.3) in tilapia and catfish respectively. This might be attributed to the high ambient temperature in the pond and feed where it was caught which is close to optimum for many mesospheric bacteria. As bacterial Abundance in fish might increase with the increase of water temperature **(Douglas, D., 2007).**

The E.coli bacteria had the lowest bacterial population compared to the Salmonella, P.sdomonus and Proties.

Base on the percentage frequency of occurrence, *Salmonella* spp. showed the higher frequency of occurrence of 30.5% in *O.niloticus and C. gariepienus* The presence of Salmonella spp. Indicates faucal contamination of water from which the fishes were harvested.

Salmonella spp. has been reported to cause enteritis and systematic disease. **(Adams *et al*., 1999)** has demonstrated that fish and fish products are only occasionally associated with Salmonella and that filter feeding shell fish harvested from polluted water have been identified as higher risk products.

The percentage frequency of occurrence of P.sdomuns 11.1, 8.3%, Proteus 8, 3, 8.3% and E.coli 0.0, 8.3%. this result was agreement with finding by **Okpokwasili and Alapiki (1990)** who confirmed that bacteria flora associated with a Nigerian water culture include the genera, Bacillus, Lactobacillus, Staphylococcus, Escherichia, Micrococcus, Proteus and others.

Conclusively, this research has brought to highlight those bacterial species associated with fresh Tilapia *O.Niloticus* fish and catfish *C. gariepienus* and has shown that they are potentially pathogenic to humans. Hence adequate measures should be taken in processing the fish before consumption.

CHAPTER SIX

Conclusion and recommendation

6.1 Conclusion

The present study concluded that the highest incidence of bacteria were salmonella proportion (58.3%) for both catfish *C.gariepienus* and tilapia *O.niloticus*, Proteus were the second appearance of bacteria (22.2%), Psdomonus were the third appearance of bacteria (11.1%) , E.coli showed lowest percentage of appearance (8.4%).

The salmonella bacteria in viscera of tilapia percentage are (37.5%), and liver and gonads (25%), gills (12.5) and zero salmonella bacteria appearance in skin.

6.2 Recommendations

- Further study was needed to determine fungi infestation in *O.niloticus* collected from fish hatchery ponds.
- Further study were needed to determine parasites infection in *O.niloticus* collected from fish hatchery ponds
- Further study were needed to determine water quality parameters in water collected from fish hatchery ponds.

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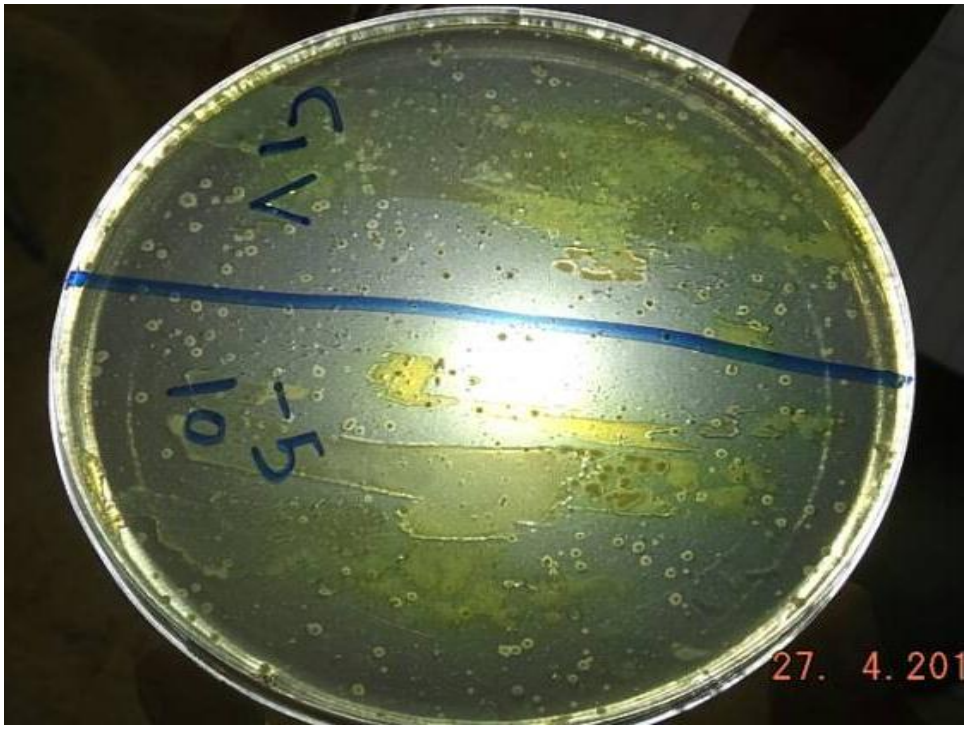
Plates(1): The medium of mackonky agar base to identify the lactose and none lactose bacteria.

Plate(2): The medium of nutrient agar base for bacterial count



Plate(3) and (4): Shows the bacilli bacteria gram negative under microscope .