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Endo Microbial Fauna of Tilapia spp. *(Oreochromis niloticus)* found in a Flowing Canal at Eden Garden and Park Utako, Abuja

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Abstract

A research conducted on the endofauna of fresh water Tilapia spp., (*Oreochromis niloticus*) found in a flowing canal or stream at Eden garden and Park, Opposite Chida hotel Utako Abuja, Nigeria. Sections of the skin, gills and intestine of twenty randomly selected fish were aseptically removed by means of sterile scalpel and pair of sterile scissors. Four (4 g) of each sections were homogenized in cml of sterile distilled water, which served as the original stock culture. A Serial dilution of 10^9 was carried out, and surface plated on nutrient agar. A total of eighteen (18) bacteria specie were isolated and identified. Twelve (12) bacteria were identified to the specie level and six (6) to the genus level. Eleven (11) were Gram positive namely. *Bacillus spp.,, Staphylococcus spp.,, Streptococcus spp., Lactobacullus spp., Clostridium spp., Enterobacter aerogenes, Bacillus moratorium, Bacillus pumillus, Bacillus alvei, Bacillus licheniformis, Staphylococcus saprophyticus* and Seven (7) Gram negative bacteria namely; *Escherichia coli, Klebsiella pnemoniae, Pseudomonas alcaligenes Salmonella* spp., *Listeria monocytogenes, Serratia mercescens* and *providential stuertii*. The frequency of occurrences of the isolated Bacteria indicated that *Bacillus alvei* had the highest frequency occurrence (12.72%), while *Salmonella* spp., had the least frequency occurrence (1.82%). The mean viable count from each sections of the samples revealed 50.1 × 10^7 $Cfug^{-1}$ from the gills, 29.8 × 10^8 $Cfug^{-1}$ from the isolates were found to be of medical importance to man.

Keywords: *Salmonella* spp; Tilapia; *Serratia mercescens*; Nitrogenous

Introduction

Fish has been one of the main foods for humans for many centuries and still constitute an important part of the diet in many countries [1]. In Nigeria, the short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group [2]. As a result, there is a considerable increase in the demand for fish being the cheapest source if animal protein [3].

The advantages of fishing as food are its easy digestibility and high nutritional value [1]. These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria [4]. Fish flesh naturally contains very low levels of carbohydrates and these are further depleted during the death struggle of the fish [4]. This has two important consequences for spoilage. Firstly, its limits degree of post morten acidification of the tissue so that the ultimate pH of the muscles is 6.2-6.5 [4]. Disease breaks out in fish tank very quickly and you have to first identify the type of disease before you can take action.

The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to Deal with ref. [5]. Bacteria can enter the fish through the gill or skin or it can stay on the surface of the body [5].

There are four types of bacterial infections namely:

- Bacterial gill disease: The gills are the primary target.
- Systematic bacterial disease: Bacterial invades the fish body and damages internal organs.
- Bacterial body ulcers: Lesions on the fish body that can be shadow or deep and Fin rot: Most likely resulting from environmental stress [5].
- Secondly, the absence of carbohydrate means that bacteria present in the body will immediately resort to using the soluble pool of readily assimilated nitrogenous material, producing off – odour [4].

Shell fish such as Tilapia have a particular large pool of nitrogenous extractives and are even more prone to raid spoilage, a factor which accounts for the common practice of keeping them alive until immediately prior to consumption [4]. The speed with which a product spoils is also related to the initial microbial load in the product; the higher the count, the sooner spoilage occurs [4].

The fresh water or rivers and lakes have a complex flora of micro-organisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources [4]. The scale of human activities has had a detrimental effect in coastal waters. Many shell fishes used for food out particles from large volume of waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be consecrated by the filter feeding activities of shell fish [4]. Also during handling of the commodity, the natural flora of the environment may be contaminated with organisms associated with man such as members of the Enterobacteriaceae and *Staphylococcus aureus* which can grow well at 30-37°C [6].

By monitoring the bacteria contents of fish organs, the quality of fish can be measured since these will affect the storage life and quality of the fishery products [7]. In order to provide a predictive capability for possible disease outbreaks and provides an opportunity to design preventive management actions, detailed information of the bacterial load and types of bacteria associated with the organs of apparently

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healthy Tilapia fish is needed. An attempt is made in this paper to investigate the bacterial micro flora associated with fresh Tilapia fish, caught at Eden garden and part Utako, Federal capital territory Abuja, Nigeria.

Literature Review

Nigerian aquatic environment

Majority of aquatic food (fish) that are commercially important in Nigeria are harvested in coastal and offshore waters estuaries e.g.; Lagoons and creeks, river and lakes.

Pathogenic bacteria and viruses as known as to be discharged in large numbers into the sea through sewage out falls in Nigeria, this pose a potential health risk to consumers of fish and shell fish breed in adjacent waters [8]. Studies by Nwakwu [9] showed a higher degree of microbial pollution in Lagos Lagoon with counts of micro-organisms above the acceptable standard of ten coliforms per 100 mL. It is estimated that at least 25% of Nigerian Inland water are polluted [10].

Micro flora of fish

The Microbial flora inhabiting the alimentary canal of fish is part of a complex ecosystem the stability of the ecosystem is due to factor that is in part, host related and in part microbial. The result of interaction between host and microbe is an ecosystem comprise of thousands of niche, each inhabited by the species or strain of microbe most aptly suited to that location the niche dweller has successfully completed for that particular site [11].

Many factors may predispose for bacterial flora of fish some of these include common sources as poor water quality over - crowding [12]. Also Environment of fish, it feeding habit the temperature of the water and season of the year influence bacteria flora of fish [13]. Many factors are responsible for the bacterial micro flora of fish some of the important factors include the environment of the fish its structure, the Temperature and nature of water, Transportation of harvested to sale outlets and the season of the year. Fish is known to harbour bacteria in the skin in the gills, the alimentary canal and the eggs. Of these, the alimentary canal tends to harbour the greatest number [14]. Growth and survival of micro-organisms on fish has been recognised to be influenced by nutrient status of fish. Physiological attributes of contaminated micro - organisms are affected by extrinsic factors like Temperature, Gas, Environment, Relative humidity and processing factor [15].

Fish from cold water gradually have an abundance of Gram negative organisms are predominant in fish from topical waters [16].

Pseudomona, *Alternomanas*, *Moraxella*, *Acinetobacteria, Vibrio*, *Flavobacterium* and *Cytophage* are most of the organisms belonging to Gram negative genera whereas the Gram positive organism most often belong tos the genera, *Mcrococcus* and *Bacilus* [17]. Some of the organism from cold water marine source like species of *Vibro*, *Klebsiella* and *Staphylococcus* could be pathogenic. The guts of marine fish have reported to support population of *Acromobacter, Alcaligenes, Flavobacterium*, *Bacillus* and *Micrococcus* [18].

Fresh water fish often carry high number of Coryne forms bacteria. Sometimes, members of the Enterobacteriaceae, including *Salmonella* spp are encountered in fresh water fish. On the other hand halophilic organism such as *Vibro parachaemolyticus* are encountered in marine fishes. It has been shown that bacteria in fish from cold fresh water are likely to be Psychrophilic and capable of growing in temperature range of 0-30°C [14].

As a corollary, Liston [19] has shown that Mesosphilic bacteria are more prevalent in fish from warm fish water.

The season at which fish is caught has a direct relationship with the bacteria flora found in the fish found out that bacteria count varies with sampling period or time of the year [20].

Normal flora of fish

Fish plays a very role in human diet, supplying some the nutrients which other food materials area deficient. Many factors are responsible for the environment of the fish, its structure, and temperature, nature of the water and the season of the year [17].

The major bacteria that have been identified in different species of fish are mostly dominated by Gram negative bacteria such as the genera: Flavobacteria, Pseudomonas, Achromobacter and less frequently *Vibro* spp., and members of Enterobacteriaceae while Gram positive bacteria such as: *micrococcus, Bacillus* and *clostridium* spp occur variable and in small number [14]. Report has also been made on the occurrence of *Staphilococcus, Escherichia coli, Streptococcus* spp., and *Citrobacterspin* many species of some fish [21].

Reay and Shewan [22] showed that bacteria are found on the external and internal surface of fish stored in refrigerated rooms. The types of bacteria found on the skin of iced and fresh fish directly determined by the bacteria present in the environment present the environment of storage as reported by Shewan et al [17]. Bacteria are mostly found on the skins, gills, intestinal tracts and eggs of fish. Liston [23] demonstrated the variation that occurs in the microbial flora of fish species at different periods of the year. It has been reported that the skin may carry 10^2-10^6 bacteria per cm^3 on the skin surface [19]. The intestinal counts may be very or moderately high ranging from 10^1-10^8/g depending on whether the fish is feeding or not [24]. The gills may harbour as much as 10^6 aerobic organism/g of the gills tissue [25] and eggs up to 10^3-10^6 bacteria/g [26]. During spoilage, skin counts of fish may increase comparably to 10^7 organism/cm^3 or more also with gill [15].

On the other hand, poor handling and processing of fish also lead to their contamination by organisms in addition to micro bio flora [23]. Thacher and Clark [24] reported that when fish have more than 10^6 micro-organisms per Gram, it is considered to have a very short potential shelf-life or even be at incipient spoilage.

Pathogens associated with fish

The occurrence of various pathogenic bacteria is important to fish processor who design processing and handling procedure to exclude, eliminate or inhibit them. Shewan [18] reported that the entire pathogenic organism associated with fish is contained through contamination from environmental sources with the possible exception of lactose positive *Vibrio* spp such as *Vibro cholera* common among the pathogenic organism are: *Salmonella* spp., *Shigella* spp., *Edwardsiella tardi, Plesiomonas, Shigellosides, Vibrio* [14]. Edwardsiella was isolated from fresh samples only and these suggest ingigeous contamination of catfish and *Yesina* spp., have also been reported in fish [25].

Lewis et al. [25] has reported that *Salmonella Typhi mutican* persist in warm water sea fish and fresh water species for 30 days and some of the fish actually showed signs of salmonellosis. Liston [19] determine two species of pathogenic bacteria that occur naturally namely the *Clostridium botulinum* type E produces toxin. However, they do not appear to grow and produce toxins in living fish but are carried passively.

Staphylococcus aureus, Enterococci and *Escherichia coli* are the common organism obtain due to contamination by mishandling during capture and processing of fish and low levels of these organisms are found on iced fish as they are offloaded from the boats [26].

Fish borne infection

Andrew et al. [27] reported public health problems as a result of pathogens micro-organism which are passively transferred by fish. The problems of contamination of fish with pathogenic organisms usually results from mishandling or inadequate processing after landing [14]. Both fresh and raw fish obtained from waters polluted with sewages rarely contains pathogenic organisms of man than other *Clostridium botulinum* and *Vibrio parahaemolyticus* [28]. Staphylococcal and Streptococcal food borne illness are due to contamination of fish on a fishing vessels or in the processing plant [19].

Among the human pathogens found on the fish (gills, intestine and environment) are *Salmonella* spp, *Shegella* spp, *Clostridium botulinum* and *Staphylococcus* and *Escherichia coli* [29]. Shell fish have been the cause of outbreaks of poisoning. However, Fananya, et al. [21] proposed in action limit of 15 *E. coli*/g of fish. People who consumed Mackerel (*Scrombroid* spp.,) have been documented to show allergic reactions and it is considered due to the levels of histamine in the flesh. The formation of histamine by the action of bacteria histamine decarboxylase has been described [27]. The species responsible for this are *E. coli, Proteus morganii* and *Clostridium welchii.* Some of these pathogenic organisms belong to the family Enterobacteriaceae [30].

Enterobacteriaceae

The Enterobacteriaceae are a large heterogeneous group of Gram negative rods whose natural habitat is the intestinal tract of human and animal. The family includes many general such as *E. coli, Shigella, Salmonella, Enterobacter, Klebsiella, Proteus* and others [31]. They are Gram positive, non-sporing rods often motile, with peritrchate flagella, easily cultivated on ordinary laboratory media. They are aerobic of facultative anaerobes, all species ferment glucose with the acid formation and gas both aerobically and an aerobically. All reduce nitrates to nitrates. Oxidize negative, catalyses positive.

Long ago in 1893, it was pointed out by Thaobald Smith that certak in important groups of organism pathogenic to man and animal different from most of the non-pathogenic forms in family to ferment lactose and the genera *Salmonella* and *Shigella* were created for these non-lactose fermenters. The lactose fermenters were for the most part found to be normal inhabitants of the intestinal tract of man and higher animal [32]. Those that cause primary and opportunistic infections in human belong mainly to the following genera:

- Non lactose fermenting such as *Salmonella* spp, *Shigella* spp., *Proteus* spp., *Serratia* spp., *Edwardsiella* spp, *Morgenella* spp.
- *Lactose fermenting such as: E. coli, Klebsiella* spp, *Citrabacter* spp., *Entrobacter* spp.,

Enterobacteria possess a wide variety of antigens which are used in serotyping particular *Salmonella, Shegella* and *E. coli* cross reactions however can occur due to a sharing of an antigens.

Antigens present are O antigens found in the bacteria cell wall, K antigens which are polysaccharide antigens and H antigens which are flagella protein antigens possessed by motile Enterobacteria [31].

Salmonellae

They are Gram negative motile bacilli which characteristically fail to ferment lactose and are pathogenic for man or animals by the oral route. The different species are closely related antigenic ally. They area 2 species in the *Salmonella* namely: *Salmonella enterica* divided into 6 subspecies and *Salminella bongoli.* More than 90-95% of all *Salmonella* that are found to be pathogenic in animals and human fall into *Salmonella enterica* subspecies enteric I namely:

- *Salmonella enteric* sub species *enteric serotype enteritidis*
- *Salmonella enteric* sub species *enteric serotype typhimurium*
- *Salmonella enteric* sub specie *enteric serotype samara*
- *Salmonella enteric* sub species *enteric serotype typhi.*

Morphology and identification

Salmonellae are Gram negative, non-spore forming *bacili* which vary in length. Most species are motile with peritrichous flagella except *Salmonella pullorum* and *Salmonella gallinarium. Salmonella* grow rapidly on ordinary media but do not ferment lactose, sucrose or salicin. They form acid and usually gas from a method of differentiating various species but this is not as reliable as antigen analysis [32].

Isolation

It is usually isolated from specimen using;

1. Differentia medium cultures such as Eosin methylene blue agar, Mac agar or De Oxychloate medium.
2. Selective medium cultures, these medium cultures include *feacalis*, *Salmonella-Shigella* agar, Hectoen enteric agar [33].

Pathogenesis

Disease causing salmonella have recently been classified into single species, *Salmonella entrica* which has numerous strains. *Salmonella typhi* is well known strains that cause typhoid fever. *Salmonella* food borne disease is most frequent reported; one from human is *Salmonella strain typhimurium.*

The following diseases are cause by *Salmonella.*

1. Enteric fever (typhoid and paratyphoid) with bacteraemia cause by *Salmonella typhi* and paratyphi A, B, C.
2. Diarrhea disease (enterocolitis), this can be caused by many Salmonella serotypes. In developing countries, *S. typhimurium* and *S. enteritidis*are common cause.
3. *Bacteraemia* non *typhi Salmonella* particularly *S. typhimurium* and less septicaemia in young children in developing countries [31].

Antimicrobial Sensitivity

Antimicrobials with activity against *Salmonella typhi* include chloramphenicol, co-trimaxazole and ampicillin. Chloramphenicol resistant strains, however, have been reported from developing countries. *S typhimurium* multi-drug resistance is causing a major public health problem in several developing countries and other part of the world where the incident of salmonellosis transmitted from animals to humans has increased greatly [29].

Escherichia coli

E. coli is a Gram negative usually motile rod. Inactive strains are non-motile. A minority of the strains are usually capsulated. There are at least 5 sub-group of *E. coli* namely;

Enteropathogenic *E. coli,* Enterotocigenic *E. coli,* Enteroinvasive *E coli,* Enteroaggeagateive *E. coli,* Enterohaemorrhagic *E. coli.*

Each strains has different characteristic as follows:

1. Enterohaemorrhagic *E. coli* cause life threatening hemorrhagic diarrhea often without fever.
2. Enteroxigenic *E. coli* cause watery diarrhea due to the production of plasmid mediated toxins in infants and adults.
3. Entroinvasive *E. coli* cause dysentery, fever and colitis with blood, mucus and pus cell.
4. Enteroaggreagaive *E. coli* cause chronic watery diarrhea and vomiting.
5. Enteropathogenic *E. coli* cause vomiting, fever and prolong diarrhea mainly in infants [29].

Morphology and Identification

E. coli are motile or non-motile organism. It ferments mannitol usually with gas production. Most strain acidify lactose promptly and form gas from it at 37°C and 44°C, it gives a positive methylene reaction, a negative Voges-Proskauer reaction and do not grow in Koser's citrate medium. Most strains fail to hydrolyze urea and non-deainate phenylalanine. No blackening of Kilgler's H_2S medium, no growth in Muller KCN medium. Glucose is not oxidized and possesses lysine and glutamic acid decarboxylase. It do not liquefy gelatin.

Isolation and identification

E. coli are aerobic and facultative anaerobes. Optimum temperature for growth is 36-37°C with most strains going over the range of 18-44°C. A selective and differential media is used for isolation. It produces a metallic sheen a=on Eosin methylene blue agar, smooth pink colonies on Mac agar and yellow colonies in CLED agar. Kigler's iron agar, most strains of *E, coli* produce an acid deep and an acid slope with gas production and no H_2S blackening.

Pathogenesis

Antimicrobials that are used to treat *E. coli* urinary and other infections include lactose with activity against Gram negative organisms such as sulphonimides, trimethoprim, cotrimoxazole, nalidixic acid, nitrfurantoin, tetracycline, ampiciline, amoxicillin, cephalosporin and amino glycosides. Plasmid – mediated antibiotic resistance however is common. In treatment of *E. coli* diarrhea, the use of antibiotics is general only of minor importance, rehydration of the patient is always the most important measure taken [29].

Shigelae

Shigellae are common negative, non-sporing and non-capsulated rods. Unlike salmonella, many other *Entrobacteria* are characterized by their somatic antigens. The *Shigella* group is generally considered to consist of four (4) main sub-groups namely:

Sub-group A: these are organisms which do not usually ferment mannitol members of these sub–groups are described as serotypes of *Shigella dysenteries.*

Sub-group B: this is a group of serologically interrelated organisms which usually ferment mannitol and are referred to as *Shigella Flexner.*

Sub-group C: this group of mannitol fermenters resembling *Shigella flexner* biochemically but differing from it antigenic ally referred to as *Shigella boydii.*

Sub-group D: the only member of this sub-group is *Shigella sonnei*a late lactose fermenting organism [32].

Morphology and Identification

Shigella is non-motile organism with gas production restricted to members of one serotype. Usually no fermentation of lactose if it occurs usually delayed. Indole production is variable and adonitol, inositol and salicin very rarely fermented. Give a positive methyl-red and a negative Voges-Proskauer reaction and do not grow in Koser's citrate medium. It do not hydrolyze urea, deaminate phenylalanine, blackening Kligler's H_2S medium, grow in Muller cyanide medium, utilize malonate, oxidize gluconate or liquefy gelatin, it produce glutamic acid decarboxylase.

Isolation of *Shigella* spp.

Shigellae are aerobes and facultative anaerobes. They grow between 10-45°C with an optimum temperature of 37°C a selective medium is required to isolate *Shillega* spp. It produces non lactose fermenting pale coloured colonies and deoxychocolate agar and MacConkey agar. It produces on XLD agar a red-pink colourless with black centres [31].

Pathogenesis

Shigella spp., causes bacillary dysentery or shigellosis with *Shigella dysenteriae* serotype I being most virulent. It is also known to cause acute gastro-enteritis. Chessbrough [31] reported that it has been estimated that annually there are 164.7 million episodes of shigellosis throughout the world of which 163.2 million occur in developing countries with 1.1 million deaths (61% involving children under 5 years). *Shigella dysenteriae* causes major epidemics of bacillary dysentery in developing countries with high loss of life among young children.

Antimicrobial Sensitivity

Only patients with severe shigellosis required antimicrobial therapy. Drug resistant strains are becoming widespread serotype I epidemics causes by multi-drug resistant strain are being increasingly reported from developing countries. Jawetz [31] shows that resistance is common to ampicillin, cotrimoxazole and more recently nalidixic acid.

Pseudomonas aeruginosa

This group is comprised of Gram negative motile rods which produce water soluble pigments that diffuse through the medium. They occur widely on soil, water, sewage and air. It is frequently in small number is the normal intestinal flora. Its prevalence there increases greatly when coli form organizers are suppressed [31].

Identification and Morphology

P. aeruginsa are rod shaped organism, motile by means of polar flagella. They are negative non-sporing strictly aerobes which attach glucose and several other carbohydrates oxidative. It frequently produces a watery pigment which diffuses through the medium. It is catalyse positive, oxidize positive and produce ammonia from origins, many strains liquefy gelatin one species is pathogenic for man that is *Pseudominas aeruginosa.*

Isolation of *Pseudomonas aeruginosa*

On blood agar, *P. aeruginosa* produces large spreading colonies which are often haemolytic and usually pigment producing. The pigment diffuses into the medium giving it a dark greenish blue colour. It also produces pale coloured colonies on MacConkey agar and green colonies on CLED agar medium.

Pathogenesity

Most infections are often difficult to eradicate due to *Pseudomonas aeruginosa* being resistance to many antimicrobials. Infections caused include: urinary infections which usually following catheterization or associated with chronic urinary disease. Respiratory infection in immune compromise person, skin infections and external ear infections.

Antimicrobial Sensitivity

Pseudomonas aeruginosa is resistant to most of the commonly used antibiotics. Antimicrobial that usually shows activity against *Pseudomonas* include amino glycosides, ploymyxin and some penicillin and cephalosporin [31].

Klebsiellae

Klebsiellae are Gram negative, non-motile usually capsulated rods. The members of these groups are diverse in their cultural and biochemical character and in their habitat. The organisms included in the genus *Klebsiella* fall into two groups namely:

First group consists of non-motile, capsulated bacilli. Among these are the organism seen in animal faeces and in water include *Klebsiella aerogens*.

Second group consists of motile organisms which are less common capsulated and generally liquefy gelatin example include *Klebsiella cloacar* [31].

Identification and Morphology

They are motile or non-motile Gram negative bacilli which ferment glucose and mannitol with the production of acid and usually also of gas. It characteristically gives a negative methyl-re, a positive Voges-Proskauer reaction but a small number of strains of respiratory origin are methyl-red positive and Voges-Proskauer negative. No blackening of Kligler's H_2S medium, they do not deaminate phenylalanine. They do not produce re pigments and some strains liquefy gelatin. They are mostly found in the bowel and respiratory tract of man and animals and in soil and water [32].

Isolation of *Klebsiella* spp.

Klebsiellae are aerobes and facultative anaerobes which produces larges grey whole usually mucoid colonies in blood agar. Most *Klebsiella* spp are lactose fermenting producing mucoid colonies in CLED medium.

Pathogenesity

Some strains of *Klebsiella* cause chest infections and occasionally severe bronchial pneumonia with lung abscesses which is characteristic of sub species *Klebsiella pneumonia.*

Antimicrobial sensitivity

Klebsiella are often beta-lactamases and are resistant to ampicillin. Cephalosporin and amino glycosides are used to treat *Klebsiella* infection. Some *Klebsiella* strains show multiple drug resistance.

These enteric bacteria and others are common pathogenic organisms obtained due to contamination of fish and its surrounding. Out of 200 fishes screened from A.B.U dam, the following organisms were present based on percentages: *Aeromona* spp 47%, *E coli* 27%, *Pseudomonas aeruginosa* 18%, *Plesimonas shigelloides* 9%, *Shigella* spp 3%, *Salmonella typhi* 3% and *Acinetobacter* spp 1% [33].

Materials and Methods

Sample collection

Twenty (20) samples of Tilapia spp (*Oreochromis niloticus*) were collected from Eden garden and park located opposite Chida Hotel, Utako Abuja, federal capital territory Nigeria. The fishes were collected using a cast net and transferred immediately into clean water from the same source as that of the canal. (This was done in other to keep the fishes alive and healthy for laboratory work). The fish sample was then taken to the biological science laboratory of the University of Abuja for analysis.

Laboratory measurements: The weight of each fish sample was measured to the nearest Gram using a manual weighing balance (Zhengya weighing apparatus), while both the total and the standard length were measured to the nearest (cm) using a meter rule [34].

Sex determination: The sexes of the *Oreochromis niloticus* spp., were determined by examining the genital papilla located on the ventral side just before the anal fin. The male genital papilla have only one opening while the female has two openings. Eggs exit through a separate oviduct and only urine passes through the urinary pore, Tilapia fish-farming (2013).

Preparation of stock culture: Section of the gills, skin and intestine of twenty samples of fish were specially removed by means of a sterile scalpels and a pair of scissors and razor blade and kept in sterile petri dishes. Four Grams each of these sections was pounded with morter and pestle. Homogenization was carried out to obtain uniform distribution of cells through stock culture.

Enumeration isolation and identification of bacteria: Sixty serial dilutions of the original stock culture from gills, skin and intestine were prepared. Each dilution was platted on the solidified freshly prepared nutrient agar and spread using a sterile glass rod and incubated at 37 for 24 h after which the colony that developed on the plate were counted. Those counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC). Distinct colonies from each plate were then picked by means of a sterile wire loop and sub cultured onto a freshly prepared nutrient agar medium contained in sterile plates. This was done with view to obtaining pure growth. The plates were incubated at 37 for 24 h.

Characterization of pure isolate was performed and involved colonial characters, cell micro morphology, mobility test and biochemical test, glucose, sucrose test, mobility test, indole test, urease test, hydrogen sulfide production, gas production, methyl red test, Voges Praskaure test, coagulase test, and spore staining. This test was done to identify isolates to generic level as contained in Chessbrough [35] and Cowan and Steel [36].

Result

The results from Table 1 shows the total viable count of bacterial isolates from gills, skin and intestine of twenty (20) sample of tilapia spp (*Oreochromis niloticus).* The mean total viable count revealed 50.1 $\times 10^2$ cfug^{-1} from the gills, 29.8 $\times 10^8$ cfug^{-1} from the intestine and 21.8 $\times 10^{-1}$ cfug^{-1} from the skin. A range of total viable count from the three site analysed revealed 3.0-8.0 $\times 10^7$ cfug^{-1} from the gills, 1.2 -3.0 $\times 10^8$ cfug^{-1} from the skin. The mean count computed for each fish part sampled shown that gills had the least count of 25 $\times 10^7$ cfug^{-1} and intestine had the highest count of 2.98 $\times 10^8$ cfug^{-1}.

The Table 2 indicate the biochemical characteristic used to identify the bacterial species isolated. Table 3 showed the frequency

of occurrence of bacterial isolates as well as their percentage in the twenty (20) analysed fishes samples from the table, *Bacillus alvei* has the highest occurrence (it occurred 7 times) with a percentage occurrence of 12.73%. While *Salmonella* spp., is the least only once with a percentage occurrence of 1.82%.

Discussion

The result from this research shows that the bacterial load varies in three segments of fishes analysed namely; skin, gills and intestine. The bacterial load in all samples was high. Gills 25×10^7 cfug^{-1} and intestine 2.98×10^8 and may be attributed to the high ambient temperature in the canal were the fishes was caught which is close to optimum for many Mesophilic bacterial. Bacteria load in fish might increase with increase of water temperature [37] reported in intestinal bacteria load of tilapia fish has 5.5×10^9 cff. this count is comparable to the result in this research at similar temperature. The high bacteria count on the skin may be attributed to contamination by genuinely aquatic species as well as those that communicate the commodity (fish) during handling. The gills had the lowest bacterial population compared to the intestine and skin. According to Trust [25], the number of bacterial associated with gills are actively maintained at low level, there by enable it to keep the bacterial number low, and therefore afford it some degree of protection against bacterial invasion by the gills micro flora [38].

			Counts in cfg^{-1}
Bacterial isolates	**Total counts**	**Range**	**Mean (SD)**
Gills	50.1×10^7	$3.0\text{-}8.0 \times 10^7$	25.0×10^7
Intestine	29.8×10^8	$1.2\text{-}3.0 \times 10^8$	2.98×10^8
Skin	21.3×10^8	$1.1\text{-}2.9 \times 10^8$	2.13×10^8

Table 1: Total viable counts of bacterial in cfug^{-1} of tilapia fish sampled from Abuja (Eden garden canal).

Bacterial isolates	Colonial characters	Cell micromophology
1. Bacillus spp.	Rhizoid, Raised edge, undulate und transparent	Appeared Gram positive rod
2. Escherichia coli	Rhizoid, flat or effuse	Gram Negative rod
3. Staphylococcus spp.	Circular, Flat or effuse, lobate opaque and creamy	Cells are cocci with appearance in cluster
4. Streptococcuss spp.	Irregular, raised edge, low convex, entire, transparent and milky	Cells are cocci with appearance in chain
5. Lactobacillus spp.	Circular, flat, Rhizoid Rough transparent and whitish	Appeared as Gram positive rod.
6. Clostridium spp.	Circular, Raised, Lobate, Rough, Opaque and Milky	Cells appeared as small rod
7. Enterobader aerogenes	Circular, Raised, Lobate, smooth, transparent and whitish	Cells appear as long rod of Gram Negative.
8. Klebsiella pneumonia	Irregular, convex Lobate, smooth transparent and whitish.	Cells appear as small rod Gram Negative.
9. Pseudomonas alkali	Circular, Raised, Lobate Smooth, Milky and translucent	Cells appear as small rod Gram Negative.
10. B. megatarium	Large Milky colonies with rough edges.	Appeared as positive rod.
11. B. Pumillus	Colonies appear pale and large in size	Gram positive rod
12. B. Alvei	Colonies present with pinkish colour, small and large	Gram positive rod.
13. B. licheniformis	Produces translucent colonies with rough edges	Gram positive and appear scattered.
14. S. saprophyticus	Colonies white with streaks of yellow with large edges.	Cells are cocci with appearance seen in cluster.
15. Salmonella spp.	Colonies are grayish white smooth translucent and convex.	Gram Negative rod
16. L. monocytogenes	Small drop like colonies showing blue green indexes	Cells appeared as coccobacillus and in cluster.
17. S. mercescens	Produces discrete translucent colonies	Gram Negative rod.
18. P. stuartii	Colonies appeared colourless with rough edge	Gram Negative rod.

Table 2: Biochemical Characterization used to identify the bacterial Species isolated.

S\No	Bacterial isolate	Number of occurence	% occurence
1	*Bacillus* spp	3	5.45
2	*Escherichia coli*	3	5.45
3	*Staphylococcus* spp	3	5.45
4	*Streptococcus* spp	2	3.64
5	*Lactobacillus* spp	2	3.64
6	*Clostridium* spp	3	5.45
7	*Enterobacter aerogenes*	2	3.64
8	*Klebsiella pnemoniae*	2	3.64
9	*Pseudomonas alcaligenes*	4	7.27
10	*Bacillus moratorium*	4	7.27
11	*Bacillus pumillus*	3	5.45
12	*Bacillus alvei*	7	12.73
13	*Bacillus licheniformis*	3	5.45
14	*Staphylococcus saprophyticus*	4	7.27
15	*Salmonella* spp.	1	1.82
16	*Listeria monocytogenes*	3	5.45
17	*Serratia mercescens*	3	5.45
18	*Providential stuertii*	3	5.45
	Total	55	100%

Table 3: Frequency of occurrence of bacterial isolates.

Base on the percentage frequency of occurrence, *Salmonella* spp., showed the least frequency of occurrence of 1.82%. The presence of *Salmonella* spp indicates faecal contamination of the water from which the fishes were harvested. The percentage frequency of occurrence of *Bacillus alvei* is 12.73% the presence of the isolated organism was not surprising since according to Draser [39], fish lives in water habitat full of microorganism. OkpoKwasili and Alapoki confirmed that bacteria flora associated with Nigerian water culture include the genera, *Bacillus, Lactobacillus, Staphylococcus, Escherichia, Micrococcus, proteus* and others. *Bacillus* spp. are implicated in causing a wide range infectious disease including abscosses, bacterimia/septicimia, wound and food borne infectons, ear infections endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis and respiratory tract infection. *Providential stuarti* is also reported to cause enteritis and systematic disease Adam [4] 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. *Listeria monocytogenes* is widespread in the environment and humans can be exposed to the bacteria in various ways though many persons remain symptomless [36]. Subpopulation who could develop the disease which sometimes can be life threatening include pregnant women, new born and infant and adults with a compromised immune system [40]. *Listeria monocytogenes* produces a series of toxins haemolytic, lipolytic, hemorrhagic and pyrogenic which are involved in the disease process [41].

Five forms of *Listeriosis* can be caused by infections with *Listeria monocytogenes:* Pregnancy infections, granulomatosis infantiseptica, sepsis meningoencephalitis and focal infections. The bacterium can also invade the eyes and skin cause conjunctivitis and skin lesions [42]. *Escherichia coli* has been reported to bloody diarrhea. It also causes severe anemia of kidney failure which can lead to death. Others strains of *E. coli* can cause urinary tract infections or other infections. *Enrobacter aerogenes* is reported to cause lower respiratory tract infections which include asymptomatic, colonization, *tracheobronchitis, pneumonia,* lung abscess and empyema with other respiratory pathogens, chronic obstructive pulmonary diseases, diabetes mellitus, alcohol abuse, malignancy and neurological diseases are risk factors for the acquisition of lower respiratory tracts infections. *Klebsiella pheumaniae* has been reported to caused different types of healthcare – associated infections, including pneumonia, blood stream infections, wound or surgical sites infections and meningitis.

Pseudomonas alcaligene has been reported as a rare opportunistic human pathogen. A whole genome sequence of *palcaligenes* strain MRY 13-0052 was isolated from a bloodstream infection in a medical institution in Japan in 2013 and was resistant to broad. *Spectrum cephalosporins* and *monobactums*. Whole genome shotgun (WGS) sequence of strain MRY 13-0052 was performed using the Roche 454 pyrosequencing platform (500 bp insert size). It is reported that *Lactobacillus* causes respiratory gastrointestinal and urogenital tract infections. They are commonly non-spore forming and lack motility. They are commonly used to ferment food and as probiotics. They may also be used as a treatment for infantile and adult diarrhoea, candidal vaginitis and antibiotic associated diarrhoea. Streptococci are Gram positive aerobic organisms that cause many disorders, including pharyngitis, pneumonia wound and skin infections, sepsis and endocarditis. *Staphylococcus* spp are facultative anaerobic which are Gram positive. It is reported to be the common cause of food poisoning as they can be produced by bacteria growing in improperly stored food items. The most common sialadenistis is caused by *staphylococci* as bacterial infections.

Conclusion

This research has brought to light those bacterial species associated with fresh water Tilapia fish and has shown that they are potentially pathogenic to humans. Hence, adequate measures should be taken in processing the fish before consumption.

Recommendations

This research has brought to light some bacterial species associated with fresh water Tilapia spp and their potential to cause pathogenic infections to humans. So therefore, it is recommended that fresh water fish consumers should properly process and or preserved their fish properly before consumption. Public enlightenment campaign should be organised to enlighten the public on the susceptibility of fresh water fish to bacterial infection and their pathogenic effect to man or humans.

According to Adedeji et al. [43], in Nigeria, the greatest challenges encountered by fish farmers have to do with pollution of water bodies from industrial activities by the mining and petrochemical industries. Also over exposure of land to erosional forces such as; wind, rain, flood run and froze action etc., should be put to a check and if possible stopped in other to preserve the vitality, viability and usefulness of water and water products in our environments or society as a whole [44-56].

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