CHAPTER ONE

1.0. INTRODUCTION

1.1. Background of the study

In many countries, fish are consumed and are considered to be good source of dietary protein. The fish provide high-quality essential proteins (Claudious *et al*., 2019). The word "fish" are generally defined as aquatic vertebrate that are typically cold-blooded, covered with scales and equipped with two sets of paired fins and several unpaired fins that use gills to obtain oxygen from humbler of skeletal called Fin-Rays (Adebayo-Tayo *et al.*, 2012). Fish and fish products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Danba *et al*., 2014). The high demand for fish has resulted in the increase in the number of fish ponds in Nigeria. Individual farmers, organized groups and institutions have developed, constructed fish ponds and started fish farm oblivious of the cost (Sawere & Oghenekowhoyan, 2019).

The fisheries and aquaculture sector significantly expanded in the past decades and total production, trade and consumption reached an all-time record of 179 million tonnes in 2018 (FAO, 2020). The top fish producers are China and Indonesia accounting for almost 50 percent of total global capture production (FAO, 2020). Over the past 35 years, aquaculture production in Nigeria has grown 12 percent per year (compared to the world average of 8 percent), from a little over 6,000 metric tons in 1980 to nearly 307,000 metric tons in 2016 (WorldFish, 2018). Nigeria is the largest aquaculture fish producer in sub-Saharan Africa, accounting for 52 percent of the total farmed fish production in the region (WorldFish, 2018).

Aquaculture has become a fast growing sector compared to other animal food production sectors (Romero *et al*., 2014). Food and Agricultural Organisation (2007), estimated that fisheries and aquaculture supplied the world with about 110million metric tons of food fish per year. Fish aquaculture has remained an important financial source, boosts the economy of many developing countries and served as an important source of food, nutrition, income, and livelihoods for many people around the globe (Jamal *et al*., 2019). Fishes are reared in different water culture media or confinement such as concrete, earthen or plastic ponds. Concrete and earthen ponds have been the widely used culture system for fish (Fakorede *et al*., 2019). Fishes reared in these environments are contaminated by both

pathogenic and opportunistic microorganisms. Microorganisms exist in the aquatic environment and enter into the digestive tract of fish and make commensal intestinal microbiota which provides a suitable environment for them (Yasin *et al*., 2018). The contamination of these culture systems has been attributed to poor water quality, high stocking densities and the use of animal manure and contaminated feed (Mukwabi & Okemo, 2019). Water is the natural habitat of fishes and other aquatic animals; it is, therefore, of great importance to study water quality while studying fish production especially when done in an artificial setting (Sawere & Oghenekowhoyan, 2019). Pollution of water is measured by assessing the physicochemical parameters of water. Physico-chemical analysis is the prime consideration to assess the quality of water for its best utilization like drinking, irrigation, fisheries, and industrial purpose. It is helpful in understanding the complex processes, interaction between the climatic and biological processes in the water (Raj & Sevarkodiyone, 2018). A sharp drop or increase within these limits has adverse effects on the body functions of the fish. Good water quality is essential for survival and growth of fish (Kumar *et al*., 2017). The pollutants of fish pond water include; residual food, faecal matter, pathogenic bacteria, viruses and parasites, suspended solids, drugs and disinfectants (Wairimu *et al*., 2019). Pond waste water if disposed untreated can, therefore, alter water quality in the receiving water. These microorganisms in fish and fish ponds portend grave consequences for public health (Li *et al*., 2019). Some of these microorganisms possess resistant determinant which enhances their potentials for infecting consumers. For instance, *Escherichia coli* are known to survive well in aquatic environments, and they are highly adept at horizontal gene transfer, a notorious vehicle for antibiotic resistance dissemination (Fakorede *et al*., 2019).

The number of infections caused by antibiotic resistant bacteria is rising worldwide (Claudious *et al*., 2019). Resistant pathogens are capable of undermining effective health outcomes and prolonging hospitalization of patients (Fakorede *et al*., 2019).

The growth of aquaculture industry is hampered by unpredictable mortalities, many of which are caused by environmental damages and pathogenic microorganisms. Diseases have been attributed to biological production bottlenecks in aquaculture hence necessitating the use of conjuvants such as drugs and antibiotics in health management strategies (Biswas *et al*., 2019).

Clarias gariepinus (Burchell, 1822) is a species of great economic importance in Africa and South-East Asia, especially as a food fish and vital in the local sustainability of

the aquaculture activity. Their aquaculture attributes include ability to withstand handling stress, disease resistance, high growth rate, fecundity and palatability. There is acute reduction of these species in inland natural water bodies in Nigeria because of the over-exploitation methods of indigenous fishers that destroy the habitat and fisheries resources. The effort made by the Nigeria government to conserve and propagate the aquaculture of this species is being hindered, since there is little information available to producers, on the species ecology and disease issues in Nigerian waters (Ikpi & Offem, 2011).

The use of antibiotics has accompanied the growth in aquaculture. It is used for prophylaxis and for treatment in fish farming. The intensive use of antibiotics poses serious environmental and health risks. Its effects are directly linked to food safety, occupational health hazards and antimicrobial resistance. Environmental risks include residue accumulation, aquatic biodiversity toxicity, microbial community selection for antibiotic resistance and the emergence of multi- antibacterial resistant strains (Lulijwa *et al*., 2019).

Probiotics have been widely used in human and veterinary medicine. In fish aquaculture, they are used because they constitute the normal flora of fishes as well as the ease of using them as powders to protect the fishes (Assefa & Abunna, 2018). Desirable characteristics for the selection of potential probiotics include (i) no harm to the host; (ii) acceptance by the host through ingestion, and colonization and proliferation within the host; (iii) ability to reach target organs where they can work; and (iv) no virulent resistance or antibacterial resistance genes (Kesarcodi- Watson *et al.,* 2008). Some yeast strains already in use in fish probiotherapy are *Saccharomyces cerevisiae, Candida tropicalis, Rhodotorula rubra inter alia*. Yeast supplemented diets stimulate growth, feed efficiency, blood biochemistry, survival rate, and non- specific immune responses (Harikrishnan *et al*., 2011).

This research is directed towards assessing fish farming practices, microbial populations, and their possible effects on fish health and the effect of *Saccharomyces cerevisiae* in the control of fish infections as opposed to the use of conventional antibiotics.

1.2. Statement of the problem

In Anambra State, fishes are reared in artificial ponds, wherein symbiotic relationships exist among commensals such as bacteria, fungi and algae; this symbiosis has the tendency of causing opportunistic infections which can negatively affect the well being of fishes.

Feeding and sanitary practices such as feeding method, type of water used and delayed water changing period are the most common factors that predispose fishes to opportunistic infections, thus the need to check the microbial quality of these practices.

Bacterial infection of fishes impacts on their health condition and poses risks to fish consumers.

The use of conventional antibiotics as control measures against morbidity and mortality in fish farming results in increased antibiotic resisitance, which is presently a global public health issue, hence the need for a more biological means of control.

Improperly processed fishes cause several disease conditions on human consumers, while farmers exposed to infected fish pond water are liable to getting infected by opportunistic pathogens. There's resultant economic loss, acute and chronic human diseases and death if these parameters are not taken care of.

1.3. Aim of the study

The aim of the study is to assess the impact of bacteria on the health status of fishes from fish ponds in three senatorial zones of Anambra state.

1.4. Objectives of the Study

The objectives of the study are to:

1. Isolate and identify bacterial organisms present in the fish and water samples
2. Determine the seasonal bacterial loads of the fish ponds
3. Ascertain the sanitary and feeding practices of fish farmers and their effects on fish health using a questionaire
4. Evaluate the antibiogram of selected antibiotics on bacterial isolates
5. Determine the physico-chemical parameters of the fish pond water.
6. Measure the pathogenic effects of the bacterial isolates on the weight, haematology and blood chemistry profiles of fish and albino mice.

7. Examine the histological effects of the bacterial isolates on vital organs of the fish and albino mice.
8. Compare the use of antibiotic (chloramphenicol) and probiotic (*Saccharomyces cerevisiae)* in the control of possible infections elicited by the bacterial isolates in the fishes.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Nutritional and Economic Value of Fish

Fish is a cold-blooded animal whose body temperature is constantly influenced by its environment. It is an important and commonly used protein dietary source across the globe. It is the most nutritive ingredient in the food chart of human beings (Pramanick *et al*., 2019), that meet up around 60% of the world's demand for high-quality protein (Kousar *et al*., 2020). Fish contains high nutritional quality including low-fat content, cholesterol, saturated fat, and high levels of proteins, polyunsaturated fatty acids, and minerals such as calcium, sodium, potassium, phosphorus, and magnesium (Fakorede *et al*., 2019). Food and agriculture organization's (FAO) state of world fisheries and aquaculture report estimates that fish now provide 6.7% of all protein consumed by humans globally, passing the 20kg per capita and year mark for the first time (FAO, 2016). In Nigeria, fish is a stable source of animal protein compared to other animal protein sources such as beef, mutton, pork and poultry. The high consumption of fish also stems from the fact that it is generally accepted without any religious bias (Aliyu *et al*., 2018). Fish oils are the only concentrated source of eicosapentanoic acid (EPA) and decosahexaenoic acid (DHA). These fatty acids play a major role in the development and maintenance of brain, and prevention of different pathologies mainly the cardiovascular diseases, and also psychiatric disorders such as stress, depression and dementia (Bourre, 2005).

2.2. Aquaculture

Aquaculture according to FAO (1997) is "the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants in selected or controlled environments". Aquaculture is an ancient occupation of man in which its fast growth due to rapid development has given birth to modern equipment and technology leading to its intensification and commercialization (Akanmu, 2018). Today, aquaculture is growing rapidly than all other animal food-production sectors (Ntsama *et al*., 2018). It is becoming a business and occupying a priority list in the world, remedying the noticeable decrease in capture fisheries (Adeshina, *et al*., 2016). Aquaculture in the world has been practiced in many countries and is the only sustainable solution to the declining natural fish stocks as a source of food (Bene *et al*., 2016) and is projected to contribute over 50% of the total

fisheries production by 2020 (Wairimu *et al*., 2019). In 2014, global aquaculture production surpassed the production of capture fisheries (Dat *et al*., 2019). Aquaculture fish production in Nigeria has grown from 0.1% to 0.4% from 1995 to 2016 (FAO, 2018). The FAO estimates an increase in fish production in Nigeria by 2030 to about 18.2% while export will increase by about 6.6% (FAO, 2018). These figures show the importance of fish to nutrition and food security. The safety and quality of breeding fish, as an important food source, are, therefore, of clinical importance in aquaculture. In aquaculture, a high density of fish is often maintained in a single pond to increase production efficiency (Endo and Wu, 2019). The rapid development and intensification of culture practices have been associated with serious disease outbreaks and economic losses (Chi, *et al*., 2017).

The advent and anticipation of aquaculture is to enhance food production, income generation and good health for the people (Idowu *et al*., 2017). Recent reports on fish meat safety have heightened consumers fear regarding fish products (Fakorede *et al.,* 2019). However, disease outbreak is considered to be a significant constraint to the development of aquaculture, causing huge economic losses per year worldwide (Kumar & Roy, 2017). The growth of aquaculture industry is hampered by unpredictable mortalities, many of which are caused by environmental damages and pathogenic microorganisms (Biswas *et al*., 2019). Fish especially from aquaculture has also been reported to be capable of harbouring pathogenic bacteria and the level of its prevalence depends on many factors such as sanitary condition of the farm, nature and quality of the feed, quality of the water and eealth status of the farmers.

2.3. Fish Farming Practices

Aquaculture farm systems and techniques: There are different systems and techniques used in aquaculture for fish production. We have three major systems based on feeding methods; extensive, intensive and semi-intensive.

In extensive system, the fish feeding is based on natural foods like phytoplanktons and zooplanktons ie, no supplementary feeding is required. Intensive system is the one in which the fish are fed with external food supply, whereas, in semi-intensive, the fish are fed with supplementary feed in support of the natural food supply.

The various techniques used are as follows:

2.3.1. Flow through systems, ponds, cages, tanks and recirculating systems: A Flow-through system, also known as a raceway, is an artificial channel used in aquaculture to culture aquatic organisms. Raceway systems are among the earliest methods used for inland

aquaculture. A raceway usually consists of rectangular basin or canal constructed of concrete and equipped with an inlet and outlet. A continuous water flow-through is maintained to provide the required level of water quality, which allows animals to be cultured at higher densities within the raceway (Mirzoyan *et al*., 2010). Freshwater species such as trout, catfish and tilapia are commonly cultured in raceways (Gupta & Acosta, 2004). Fish Cage aquaculture refers to the rearing of aquatic species, within enclosures in natural waterways. The fish cages are placed in lakes, bayous, ponds, rivers or oceans to contain and protect fish until they can be harvested (Ozigbo *et al*., 2014). The method is also called "off-shore cultivation" when the cages are placed in the sea. Fish are stocked in cages, artificially fed, and harvested when they reach market size. Open systems are being implemented in a wide range of environments including freshwater rivers, brackish estuaries and coastal marine regions. Floating mesh cages are anchored to the seafloor and vary in size depending on the scale of operation and the species cultured.

2.3.2. Fish tanks: Fish farming can also be carried out in outdoor or indoor concrete or plastic tanks. Tanks can be in form of small aquaria (glass or plastic), canvas or large fibreglasses. Production tanks vary in size and shape, however, round tanks between 5,000 to 10,000 liters are most commonly used (Ozigbo *et al*., 2014). Tanks need to be non corrosive, therefore, plastic or fiberglass is recommended. Smooth round tanks with a conical shaped bottom are considered advantageous as this will assist with waste solids disposal during draining.

2.3.3. Fish pond: A Fish pond is a controlled pond, artificial lake, or reservoir that is stocked with fish and is used in aquaculture for fish farming, or is used for recreational fishing or for ornamental purposes. Mostly earthen ponds are used for culture of carps, tilapia, catfishes and sea bass (Ozigbo *et al*., 2014).

2.3.4. Recirculating aquaculture system (RAS): This is essentially a closed system and involves fish tanks and filtration and water treatment systems. The fish are housed within tanks and the water is exchanged continuously to guarantee optimum growing conditions. Water is pumped into the tanks, through biological and mechanical filtration systems and then returned into the tanks. Not all water is 100% exchanged however, as it is difficult to ensure that all waste products are converted or removed by the treatment process. Most culture systems recommend at least 5% to 10% water exchange rate per day depending on

stocking and feeding rates (Ozigbo *et al*., 2014). RAS occupies a very small area and allow the grower to stock fish at high densities and produce high yields per unit area. These systems are very intensive and, therefore, require a high level in management of stock, equipment and water quality. They provide a predictable and constant environment for growing fish. RAS can be expensive to purchase and operate. For this reason, it is usually only economically viable to farm high value species in these systems.

2.4. Fish Pond Preparation

Before a pond can be stocked with new fish, the excessive wastes, which accumulate in the pond during the previous farming, must be removed and the soil and water conditioned or upon a newly constructed pond, the following preparations have to be taken into consideration: cleaning, liming, and eradication of predators, fertilization, and aeration.

2.4.1. Cleaning

There are two methods of cleaning; the drying method and wet method. The drying method occurs where the ponds can be completely dried like a concrete pond. Whereas, the wet method takes place in ponds like earthen ponds in which the water in the pond cannot be completely dried.

2.4.2. Liming

Once the pond is cleaned, it is then filled with water and left overnight before flushing out to remove debris and elevate the pH. This process should be repeated until the pH of the water remains above 7, and only then the lime is applied. The types of lime to be used depend on the water pH. It is recommended that agricultural lime ($CaCO_3$) or dolomite [$CaMg(CO_3)_2$] should be used in a pond with water pH near neutral and the hydrated lime [$Ca(OH)_2$] should be used in a pond with water pH below 5 (KAU, 2013) The amount of lime to be used should be carefully calculated to avoid inducing an excessively high water pH, which may increase ammonia toxicity and result in the mortality of the fish stocked. When the pond is properly limed and filled with water, the average water pH should be between 7.5-8.5 with daily fluctuation of less than 0.5.

2.4.3. Eradication of Predators

After liming, the pond should be filled with water to the maximum depth through a screen with fine mesh to prevent the predators and competitors from entering the pond. Some chemicals should be used to eradicate these animals in the pond before stocking. Predators can be killed by the application of tea seed powder at the rate of 20-30ppm (KAU, 2013). After the application of tea seed, the pond should be left for 3 days before the fry, fingerlings and post larvae can be stocked. Hypochlorite, either calcium or sodium salt, is currently used

at 15-20% (60% active ingredient) to eliminate both vertebrates and invertebrates. The pond must be cleaned prior to the application of hypochlorite since hypochlorite may react with the organic matters and produces the toxic organochlorine compounds. Hypochlorite should be applied after the pond is filled to the maximum height and left for 3 days to allow the hatching of planktonic organisms. After the hypochlorite application, the pond should be aerated and the application of lime and fertilizer should be conducted on day 3, then stock the pond with fish on day 7. During the first month, water must not be added to the pond, unless the water quality is poor, to prevent the introduction of competitors and predators (Ibrahim *et al*., 2014).

2.4.3. Fertilization

The pond must be fertilized with either organic or inorganic fertilizer to stimulate the plankton bloom in order to provide shade to the pond bottom and utilize the nitrogenous and phosphate wastes within the pond. The shade will also prevent the growth of harmful benthic algae. The sun dried chicken manure is the most common organic fertilizer to be used in amounts of 200-300kg/ha. The manure must be soaked in water for 24h before it is spread over the surface of the water. Inorganic fertilizers, such as urea (46% N) and compound fertilizers like, ammonium phosphate (16:20:0) or those with N:P:K combination of (16:16:16) can be used at 20-30kg/ha (Ozigbo *et al*., 2014). The fertilizer must be dissolved in water before it is spread over the water surface to avoid precipitation of the fertilizer onto the pond bottom, which will enrich the soil and accelerate the growth of benthic algae (KAU, 2013).

2.4.4. Aeration

A 0.5-1.0ha pond would require four aerators installed at the corners of the pond, approximately 3-5m from the bottom of the dike and positioned at an angle that will encourage the maximum water flow within the pond. The type of aerator to be used depends on the depth of the water. One horsepower paddle wheel aerators should be used in ponds of less than 1.2m water depth and the 2 HP (horsepower) paddle wheel aerators should be used in ponds deeper than 1.2m. The aerators should be switched-on 24h before the post larvae are stocked to allow enough time to create the current and clean up the feeding area (KAU, 2013).

2.5. Microorganisms associated with fish

2.5.1. Initial microflora

The initial microbial population of fish reflects the microflora of the environment at the time of capture or harvest, and is subsequently modified by the ability of different

microorganisms (mainly bacteria) to multiply in the sub-environments provided by the skin/shell surface, gill areas, and the intestinal content. The muscle tissue and the internal organs are normally sterile: microorganisms can be found on skin, on chitinous shell, on the gills of fish as well as in the intestinal tract. However, the ecology of these associations present in multiple microhabitants in fish remains elusive, especially on the microbial assembly in fish external (skin and gill) and internal (stomach and intestine) niches, and the relationship with the rearing environment (Zhang *et al.*, 2019a).

Microbial loads depend on water conditions and temperatures. During storage, the microflora changes owing to different abilities of the bacteria to tolerate the preservation conditions. After capture or slaughter and death, finfish is usually stored in ice, favouring a temperature dependant change in microflora composition. Psychrotrophic bacteria normally represent microbial population of fish and shellfish from cold waters, while in fish from temperate waters an increased load of mesophilic bacteria could be observed. Both psychrotrophic and mesophilic microorganisms grow easily at temperatures ranging between 25 and 30 degrees. The microorganisms present are mainly halotolerant rather than strictly halophilic, revealing an optimal growth at sodium chloride concentrations of 1-3%. This is due to the use of the ice to maintain the products, which determines a decrease of salinity during storage, favouring the survival and growth of halotolerant species. One example of the effect of the salinity in selecting microflora population is in the intestinal tract where halotolerant *Vibrio* spp. are often reported as dominant marine species; Lactic Acid Bacteria are also frequently isolated from fish intestine. Microorganisms isolated from skin and gills are typically aerobic, in particular *Vibrio* spp. In warm water fishes, the predominant microflora is represented by a higher proportion of Gram-positive cocci (Micrococci) and *Bacillus* spp., but even by Gram negative bacteria. The microflora of temperate waters include genera like *Psychrobacter*, *Moraxella*, *Pseudomonas*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Aeromonas* and *Cytophaga* but also *Micrococcus* and *Corynebacterium*. *Psychrobacter-Acinetobacter-Corynebacterium* and *Micrococcus* dominate crustaceans' microflora while *Vibrio* is the main genus isolated from molluscs. *Listeria monocytogenes* occurs generally in the environment and especially could be isolated from fish caught or culture close to land with agricultural run-off. It is often isolated from ready to eat seafoods like cold-smoked salmon (Bernardi *et al.*, 2011). Improper hygiene and handling might lead to contamination of ready-to-eat fish and eventually affect the health of consumers (Okonko *et al.*, 2008).

2.6. Fish Spoilage

Fish spoilage is a complex process, in which physical, chemical and microbiological mechanisms are implicated (Adebayo-Tayo *et al*., 2012). The quality of the freshly caught fish and its usefulness for further utilization in processing is affected by the fish capture method. Unsuitable fishing method does not only cause mechanical damage to the fish but also creates stress and the conditions which accelerate fish deterioration after death. Fish is highly susceptible to deterioration without any preservative or processing measures (Adeyeye, 2016). Fish spoilage is due to autolysis, oxidation, dehydration and bacterial activity. The high water content, neutral pH and the high total number of autolysis enzymes in fish tissue influence the susceptibility to spoilage. There is a rigor mortis phase before the actual spoilage. Rigor mortis is caused by the enzyme activity in fish. After dying, the fish blood circulation and oxygen supply to the cells stop. However, the enzymes of the flesh keep functioning by creating lactic acid from the flesh glycogen. After the glycogen is worn out, enzymes stop functioning and the fish becomes rigid and hard. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness, whereas microbial activity is responsible for the obvious spoilage, thereby establishing product shelf life and resulting in heavy economic loss (Adeyeye, 2016).

2.6.1. The effects of spoilage on fish

At the time of rigor mortis, the fish is relatively fresh. Fresh fish has a mild smell, glossy eyes and red gills. The spoilage affects the appearance, smell, taste and texture of the fish. Spoiled fish is no longer rigid, but limp. The eyes of the fish are bleary, its gills are pale and the smell of the fish is unpleasant and rotten. Fish tends to become slimy and sticky on the surface. The intestines of the spoiled fish will burst even if the fish is preserved at 0 °C and drain out of the blood water which contains both protein and oil (Ikape & Cheikyula, 2017). The metabolites of the microbes diffuse into the fish and cause the spoiling effects. This can evoke acids, NH_3, harmful amines, H_2S or even botulin, depending on the microbiota.

2.6.2. Enzymatic and chemical spoilage

The spoilage of raw fish is first caused by enzymatic autolysis followed by microbiological spoilage. In autolysis, the enzymes and ATP-related compounds disintegrate and tissues become soft. These degradation products operate as microbial nutrient: enzymes disperse into surrounding tissues, leading to intestinal or abdominal peritoneum puncture. Autolytic activities by endogenous enzymes results in products that initially cause loss of the characteristics fresh odour and taste of fish and softens the flesh (Mahmud *et al*., 2018). The

fish fat is mostly composed of polyunsaturated fatty acids, especially fatty fish like salmonoids, which become oxidized and fester easily. Oxidation and hydrolysis develop faulty odours and flavours in the fish as well. Both enzymes and microbes in the fish intestines and tissues hydrolyze fatty acids. Stringing, filing, light and heat accelerate the hydrolysis. On the other hand cooling, antioxidants, the removal of oxygen and freezing, except on fatty fish, decelerate the hydrolysis. There are a lot of non-protein nitrogen compounds in fish. These include among others trimethylamineoxide (TMAO), amino acids and nucleotides. TMAO is an odourless nitrogen compound that many bacteria can convert it to trimethylamine (TMA). The latter is the usual compound in spoiled fish that causes bad smell.

2.6.3. Microbiological spoilage

Many variables influence the rate of spoilage and microbial growth in fish. Live fish is normally considered to be sterile, but microorganisms are found in varying numbers on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish (Pal *et al*., 2016). Microbes get into the flesh from the surrounding environment and the fish intestines after the fish have died. It is therfore very important how the fish is preserved until it is consumed. Microbes have various optimal conditions to extend their growth. The fish condition (fish diseases), species, season (time of spawn), nourishment, environment, the temperature, fishing manner (stress level) and handling all affect the rate of microbial growth. A small fish has a high pH after death. Thin skin and high fat content increase the rate of bacterial growth. In addition, fish fillets have inferior preservability to the whole fish, because the flesh is more exposed to bacteria. The microbes of fish are situated in the slime layer of skin, gills and intestines. Since the spoiling starts from the intestines, cleaning the fish is very critical to minimize contamination. Spoiling and draining of the blood also reduces the enzymes, bacteria and bacterial breeding grounds and, therefore, slows down the rate of spoiling. Microbial spoilage causes slime, faulty odours, flavours and colours and eventually the fish becomes inedible (Jay, 2000).

High microbial load of wild catfish and wild tilapia were observed due to pollution of the environment in which the fish were caught (Pal *et al*., 2016). The biota of fresh fish is reflective of the water they grow in. Fresh and warm-water fishes have different microbiota from salt water or cold-water fishes. Warm-water fish tend to have more of a mesophilic and gram-positive biota and cold-water fish have mostly gram-negative bacteria in their biota. Rainbow trout is expected to have more gram-negative and psychrophile or psychrotroph bacteria because of its cold-water surroundings (Laaksonen, 2008). Commonly appearing

bacteria genera are *Pseudomonas*, *Flavobacterium*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Photobacterium*, *Vibrionaceae*, and *Aeroemonadaceae*. Gram-positive organisms such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, and *Corynebacterium* can also be found in rainbow trout. Bacteria need nourishment, warmth, moisture, the suitable acidity and oxygen to grow. Because of these terms, food products are naturally good breeding grounds for bacteria. On the other hand, the competition between bacteria limits their growth in foods. When all water and nutrients have run out, they can no longer multiply. Some of the conditions can be influenced. Still the existence of bacteria cannot be entirely removed, but only reduced by controlling the conditions of handling and preservation (Laaksonen, 2008).

2.6.4. Specific Spoilage Organisms (SSOs)

Only a part of the fish spoilage microflora participates in the spoilage process: the SSOs are typically present in low number and constitute a small fraction of the total microflora (Gram & Dalgaard, 2002). The identification of SSOs is determined by the comparison of the sensory and chemical characteristics of spoiled products with those isolated from the spoilage microflora. The ability to produce off-odours and spoilage microorganisms are at the base for the identification of a SSO. The spoilage potential of a microorganism is the ability of a pure culture to produce the metabolites associated with the spoilage of a particular product (Gram *et al.,* 2002). The spoilage activity of a microorganism determines the production of metabolic compounds. The qualitative ability to produce off-odours (spoilage potential) and the quantitative ability to produce spoilage metabolites (spoilage activity) are essential in the identification of a SSO (Gram & Dalgaard, 2002). The cell concentration of SSO could be called the "minimal spoilage level" and the concentration of the metabolite that corresponds to spoilage can be used as an objective chemical spoilage index (CSI).

Different SSOs could be found in different seafoods and may be a single species. SSO are different depending on the typology of fish and the conditions of storage. For example, *Shewanella putrefaciens* is the specific spoilage bacteria of marine temperate water-fish stored aerobically in ice, while *Pseudomonas* spp. are the specific spoilers of iced stored tropical freshwater fish and together with *S. putrefaciens* the spoilers of marine tropical fish stored in ice.

2.6.5. Microbial Contamination of Fish Gills, Gut and Skin

The gills, skin surface and gut of fish provide the first point of contact in microbial contamination. Millions of bacteria are found on the skin surface, on the gills and in the gut of living fish (Huss *et al*., 2004). It is suggested that microbial contaminants found in these

three regions are usually present in the fish at the time of capture. The gills, gut and the skin regions of a dead fish are dominated by pathogens such as *E. coli*, *Vibrio* species, *Listeria* species and *Staphylococcus aureus* (El- Shafai *et al*., 2004). Adams and Moss (2000), revealed that microbial contamination in fish is mostly caused by bacteria that have the ability to proliferate in sub-environments provided by the skin surfaces, gills and alimentary canals. They found that Gram-negative bacteria such as *Pseudomonas*, *Shewanella*, *Psychrobacter*, *Vibrios*, *Flavobacterium* and *Cytophaga* were mostly present in the gills, gut and skin surface of the fish.

2.6.5.1. Fish gills

Fish gills are used for respiration; however, they also provide an easy access to bacteria and harbour a variety of microorganisms (Robert & Stadler, 2000). The gills consist of threadlike structures called filaments. Each filament contains a network of capillaries that allow a large surface area for exchange of gases. Fish respire by pulling oxygen rich waters through their mouths and pumping it over the gill filaments. Because their large surface area contacts the external environment, the gills are sensitive to even minor chemical or physical changes in the surroundings and are the target organ for many contaminants carried in water (Strzyzewska *et al*., 2016). Furthermore, when these microbes are provided with favourable conditions for growth, they get established on the gills.

2.6.5.2. Fish skin surface

The skin surface is a good source of microbial contamination for investigation since it is in direct contact with the environment. The flesh of the fish is an excellent substrate for growth of a wide range of microorganisms. However, studies have indicated that the subsurface of live fish is bacteriologically sterile, as the immune system prevents bacteria from growing in the flesh. Bacteria gain entry only when the fish dies and proliferate freely (Huss & Gram, 2004). It has been demonstrated that a high load of bacterial microflora dominated by *Vibrios* was detected on fish skin (Snoussi *et al*., 2006). Adams and Moss (2000), reported that the numbers of microorganisms on fish skin ranged from 10^2-10^7 colony forming units (c.f.u.)/cm², suggesting that skin surfaces carry a substantial number of bacteria.

2.6.5.3. Fish gut

Food is usually present in the gut of the fish when caught and it may carry microbial contaminants. Also powerful digestive enzymes of the digestive tract occasionally leak and penetrate the flesh of the dead fish. The leaked enzymes contribute to the breakdown of protein in the flesh and favours microbial growth resulting in the deterioration of fish stocks.

Huss and Gram (2004), have shown that these digestive enzymes have the ability to penetrate the flesh of frozen fish. This further proves that bacteria are able to establish themselves on the outer and inner surface of the live fish (skin and the gills) and have the ability to gain entry again into the inner parts of the fish such as in the gut. The occurrence of bacteria on fish gills and the gut ranges from 10^3-10^9 c.f.u./g, suggesting that even though internal organs of freshly caught fish are usually free from contaminants, all fish could be carrying some levels of bacteria (Adams & Moss, 2000). Microorganisms such as *Bacillus, Micrococcus* and *Corynebacterium, Psychrobacter, Moraxella, Pseudomonas, Actinobacter, Shewanella, Flavobacterium, Cytophaga* and *Vibrios* usually dominate the gills, gut and the skin surface of fish and the incidence of these microbial agents in fish are vastly affected by the geographical location (temperate and cold waters) [Austin, 2002].

2.7. Factors Affecting Microbial Growth in Fish

2.7.1. Geographical location (origin and quantity)

The number and variety of microorganisms in fish are determined by the food origin, quantity and quality (Nickelson *et al*., 2001). It is anticipated that fish which consume more food (heavy feeders) have a higher level of bacteria as compared to nonheavy feeders. The geographical location of the catch, the season, and method of harvest, handling and storage are some of the factors contributing to fish deterioration (Nickelson *et al*., 2001). Fish, whether they are pelagic (surface to mid-water) or demersal (bottom) feeders will influence the numbers and types of microorganisms present on freshly caught seafood (Safaeian & Khanzadi, 2018).

2.7.2. Improper handling

Fish can be further contaminated by handling onboard, at the docks and at markets after landing, particularly where they are exposed for sale and are subjected to contamination with human pathogens by birds and flies (Adams & Moss, 2000). Studies carried out by Harwood *et al*. (2004), have demonstrated that microbial contamination of fish was mainly caused by lack of sanitary procedures in handling fish during transit from the sea to the market and also during the sale of fish. In addition, findings have also demonstrated that the initial microflora on the surface of fish is directly related to the water environment, while the microflora in the gastrointestinal tract corresponds to the type of food and condition of the fish (Ganguly & Prasad, 2011).

2.7.3. Improper storage conditions

After capture, fish are commonly stored in ice or refrigerated sea water before they are transported to land (Adams & Moss, 2000). Hence, it is important that a clean cooling agent is used, as re-use of ice and other cooling agents will lead to a rapid buildup of microorganisms and accelerated spoilage of the stored fish (Adams & Moss, 2000).

2.7.4. Time and temperature

Other factors responsible for elevated bacterial contamination in fish include temperature and time. Temperature and time control are two important factors that need to be considered when fish are stored and handled as they are responsible for multiplication of microorganisms. The true storage temperature is not only the final market box temperature, but the temperature history of the product (Huss *et al*., 2004). Ambient temperature during harvest, the delay in refrigerated storage and fluctuation in temperature during storage are three factors that determine the presence of initial spoilage microflora in fish (Nair *et al*., 2007). When temperature is below minimum required by microorganisms, they either remain in a lag phase or dies slowly. Exposure of fish to direct sunlight should be avoided (Ikape & Cheikyula, 2017).

2.8. Factors Contributing to Microbial Fish Contamination

Intrinsic factors arise from the metabolism feeding, and other activities within the aquatic environment. These are essentially environmental stressors ie factors that cause stress to fish in their cultural environment.

These environmental stress factors include poor water quality, Overcrowding, Improper fish nutrition, and Aquatic vegetation (Ikeogu *et al*., 2010).

2.8.1. Poor water quality

Water is essential in the life of all living organisms from the simplest plant and microorganisms to the most complex living system known as human body (Kumar *et al.*, 2017). But due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity, it is highly polluted with different harmful contents (Raj and Sevarkodiyone, 2018). Water quality analysis is one of the most important aspects in water studies.

Water quality is determined by various physico-chemical and biological factors, as they may directly or indirectly affect its quality and consequently its suitability for the distribution and

production of fish and other aquatic animals (Sikoki & Veen, 2004). Fish productivity depends on the physico-chemical characteristics of the water body (Sandoval *et al.*, 2017). A sharp drop or increases within these limits have adverse effects on the fish's body functions (Kumar *et al.*, 2017).

The intensive production of fish can cause a poor quality of pond water (Sandoval *et al*., 2017). So, good water quality is essential for survival and growth of fish. Water quality is not constant; varies with the time of the day, season, weather conditions, water source, soil type, temperature, stocking density, and feeding rate and culture systems (Kumar *et al*., 2017). For a successful aquaculture venture, the dynamics and management of water quality in culture media must be taken into consideration (Kumar *et al*., 2017).

2.8.1.1. Color

Color is an important parameter for any aquatic water body and indicates the purity of the water (Kumar *et al*., 2017). National Agricultural Extension and Research states pale color, light greenish or greenish waters are suitable for fish culture (Bhatnagar & Devi, 2013). Delince (1992), stated that the abundance of phytoplankton and zooplankton is responsible for the determination of the color of an aquatic body and green, bluish green/brown greenish color of water indicates good plankton population hence, good for fish health (Kumar *et al*., 2017).

2.8.1.2. Water temperature

The temperature of an organism is defined as the level of hotness or coldness in the body of a living organism either in water or land (Shobana *et al*., 2014). Fish is a cold blooded animal, so its temperature is dependent on the temperature of its environment. It changes with the temperature of the surroundings. The temperature changes affect the metabolisms and physiology of fishes and so its productivity. The optimum water temperature for fish survival has been reported to be between 20-30^{0}C (Kumar *et al*., 2017). Water temperature may depend on the seasons, geographic location and sampling time (Prameena and Sheeja, 2016).

2.8.1.3. pH

pH is the negative logarithm of hydrogen ion concentration (Delince, 1992). It is the level of acidity or alkalinity of a solution. The survival and growth of fish are also dependant

on pH of the water. The pH of freshwater ponds can fluctuate considerably both daily and seasonally. These fluctuations are due to photosynthesis and respiration by plants and animals. Fish are known to have an average blood pH of 7.4, therefore, pond water with pH within this average is optimum (Kumar *et al*., 2017).

2.8.1.4. Electrical conductivity

Electrical conductivity (EC) is a useful tool to evaluate the purity of water. It is dependent on the ionic concentration and water temperature. A total load of salts in a water body is directly related to its conductivity. Conductivity is also regarded as an indication of its freshness or otherwise of a water body (Acharya, *et al*., 2008). Fishes differ in the ability to maintain osmotic pressure (Sikoku & Veen, 2004), therefore, the optimum conductivity for fish production differ from one species to another (Kumar *et al*., 2017).

2.8.1.5. Turbidity

Turbidity is a measure of the ability of water to transmit the light that restricts light penetration and limit photosynthesis (Stevens, 2007). It consists of suspended particles in water and is usually affected by factors such as clay particles, dispersion of plankton organism, particulate organic matters as well as pigment caused by decomposition of organic matter. Turbidity and the appearance of water are important considerations in pond aquaculture (Kumar *et al*., 2017).

2.8.1.6. Dissolved Oxygen (DO)

Dissolved Oxygen is the measure of the amount of gaseous oxygen dissolved in an aqueous solution (Tara *et al*., 2011). It is an important parameter in water quality assessment and reflects the physical and biological processes of aquatic life. Oxygen is needed by fish and other aquatic organisms, and levels of DO vary daily and seasonally and depends on so many factors such as temperature, salinity, water movement, light penetration (Kumar *et al*., 2017).

2.8.1.7. Bio-chemical Oxygen Demand

This is used to assess the organic load in a water body. It is the measurement of total dissolved oxygen consumed by micro-organisms for biodegradation of organic matter (Stevens, 2007). It depends on temperature, the extent of biological activity, the concentration of organic matter and microbial population such as bacteria and fungi (Priyanka *et al*., 2013).

2.8.1.8 Alkalinity

Water alkalinity is a measure of its capacity to neutralize acids. It is a measure of buffering capacity of the water (Elayaraj *et al*., 2016). The optimum alkalinity for fish productivity is between 25 – 100 mg /L (Bhatnagar & Devi, 2013).

2.8.1.9. Nitrate

Nitrate is thought to be produced by autotrophic *Nitrobacter* combining oxygen with nitrite in the converter and on the walls of the pond (Sajitha & Viayamma, 2016). It is important that the level of nitrate in a pond is controlled to avoid eutrophication. Nitrates are, however, not harmful to fish (Kumar *et al*., 2017).

2.8.1.10. Salinity

Salinity plays an important role in the growth of culture organisms through osmo-regulations of body minerals from that of the surrounding water. It is a major driving factor that affects the density and growth of aquatic organisms' population (Kumar *et al*., 2017). Fresh and Salt water fish species generally show poor tolerance to large changes in water Salinity (Meck, 1996).

2.8.2. Over crowding

Overcrowding leads to declining water quality as crowded culture systems often have low dissolved oxygen levels, declining pH values, high nitrates and ammonia that rise daily after feeding. In addition to lowering the fish's resistance by poor water quality, crowding also increases the chances that pathogens find susceptible hosts. Also what should not have constituted a problem in a healthy aquatic system could be disastrous in an overcrowded system. Overcrowding encourages cannibalism (Ikeogu *et al*., 2010).

2.8.3. Improper Fish Nutrition

Improper fish nutrition in the form of underfeeding, feeding unbalanced diet leads to improper growth and organ development as well as weak immune system which predispose fish to diseases (Ikeogu *et al*., 2010). Improper fish nutrition may cause nutritional diseases. Examples of nutritional diseases in catfish include "broken back disease" caused by vitamin C deficiency and "no blood disease" related to folic acid deficiency (Floyd, 2005). Nutritional diseases can be avoided through proper feeding practices (Idowu *et al.*, 2017).

2.9. Treatments of Fish Diseases

In most cases, healthy fish have ability to withstand considerable environmental changes and thus can resist diseases. Since most disease causing agents are usual component of pond water environment, disease problem is inevitable in aquaculture production (Govind *et al*., 2012). However, the development of a disease problem in a culture system calls for a quick and effective response to detect and identify the cause of the disease at an early stage, for appropriate treatment and control of potential transmittable fish disease. The first response of fish to disease is abnormal behavior. A simple routine health check on fish stocks especially their feeding and swimming behaviours is essential (Idowu *et al*., 2016) for early detection of possible disease problem. Also, check on pond fish body surface, eyes, fins scales and gills for distortion in forms and structures (such as discoloration, ulceration, protrusion, swollen and disintegration), or presence of surface parasites. When these symptoms are detected, the involvement of professional assistance is necessary for further follow up (laboratory or internal examination) on the situation and recommendation of appropriate remedial actions (Idowu *et al*., 2017).

Ideally any therapeutic treatment of an infectious disease should be based on a correct diagnosis including identification of the pathogen involved before any treatment takes place (Chi *et al.* 2017). Consequently, a wide range of anti-microbial, including antibacterial (antibiotics), pesticides, hormones, anesthetics, pigments, minerals and vitamins, are used to avoid or control diseases in aquaculture (Seo *et al*., 2015; Kumar & Roy, 2017).

2.10. Fish as an Aetilogic Agent for Human Infection

Contamination of seafood by these pathogens is also a contributing factor towards human morbidity (Ripabelli *et al.*, 2004). These bacteria gain access to the human body by direct contact with infected fish during improper handling, or being in contact with other constituents of the fish environment (Acha & Szyfres, 2003). Individuals can be infected by fish bites and fish fins which contribute to microbial contaminants gaining entry (Novotny *et al*., 2004).

2.11. Fish as carriers of food-borne diseases

Fish are also known to be responsible for a significant percentage of food-borne diseases worldwide (Croci & Suffredini, 2003). It has been reported that in the United States, 10-19% of food-borne illnesses involved consumption of fish and seafood, whereas in Japan 70% of food-borne illnesses have been attributed to consumption of raw fish (FAO/ WHO,

2001). The true incidence of seafood-borne diseases worldwide is still unknown, as there is no surveillance system in the developing countries (Karunasagar *et al*., 2005).

2.12. Antibiotic use in Aquaculture

The use of antimicrobials in aquaculture basically started with the work of Gutsell (1946), who recognized the prospective use of antibiotics (sulphanamides for combating furunculosis). An antibiotic is an agent that kills microorganisms or stops their growth (Burnett-Boothroyd & McCarthy, 2011). Antibiotic can be grouped according to the microorganisms they act primarily against. For example, antibacteria are used against bacteria and antifungals are used against fungi. It is the common practice to use antibiotics as a prophylactic/therapeutic measure especially when fish development is hindered. This is also meant to treat the microbial infections in stored water in tanks.

The use of antibiotics and other veterinary compounds in aquaculture are meant to be assured and approved by different authorities (Phu *et al*., 2015; Chi *et al*., 2017). Effective quality assurance and regulatory authoritative systems of antibiotics and other compound usage in aquaculture is essential to ensure farmer's access to quality drugs, allowing for effective disease treatment and the provision of safe foods to consumers with no antimicrobial residues (Chi *et al.*, 2017). Well known mechanisms of action of antibiotic as presented in Table 1 include; interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of intermediary metabolic pathways and disruption of the cytoplasmic membrane (Tenover, 2006).

Table 1: Groups of Antibiotics, their target sites and mode of action

Drugs	Target site	Mode of action
β-lactam antibiotics (e.g penicillins, cephalosporins, monobactams etc)	Cell wall of bacteria	Prevents the cross-linking of small peptide chains in peptidoglycan
Tetracyclins, macrolides, lincomycin, and chloramphenicol	Protein synthesis	Interacts with bacterial ribosome and inhibits its function
Quinolones, pyrimethamine, sulfonamides etc	Nucleic acid synthesis	Inhibition of nucleic acid synthesis
Polymyxins, azoles, and polyenes (e.g fluconazole, amphotericin B)	Cell membrane	Inhibition of cell membrane function

Source: (Hooper and Gordon, 2001)

The usage of antibiotics in the fish and shrimp aquaculture has become a major problem. This seepages through pond bottom and discharges as wastes and effluents mixing into runoff water and covering coastal mangroves destroying important natural habitats for shrimps, fishes and to humans (Jatindra *et al*., 2009). The studies of fish farms have shown that the majority of antibiotics added in feed are not assimilated by fish but go into the environments (Weston, 1996). Le and Munekage (2004), also reported that antibiotics residues may cause harmful effect on ecosystems. Thus the accumulation of these antibiotics in sediments will definitely alter the natural functions that might take place in the environment. It is for this reason, that most of the importing countries have completely banned the use of certain antibiotics.

2.13. Problems associated with antibiotic use in aquaculture

2.13.1. Antibiotic resistance

The aquatic environment has more direct and intimate contact with human life due to the wide consumption of fish and shrimps. Thus it may transfer antibiotic resistance posing threat to public health and ecosystems. The antibiotics used are often non- biodegradable and remain in the aquaculture environment for long periods of time. This encourages the growth of bacteria, which can survive in the presence of these antibiotics, acquiring a resistance that is passed onto consequent generations. The danger is, these bacteria can be transferred to human and animal pathogens, leading to increased infectious disease in fish, animals and humans alike (Vignesh *et al*., 2011).

The continuous and indiscriminate usage of antibiotics in the aquaculture farms has led very serious consequences. It paved way for the outbreak of mutagenic, multi-drug resistant microbial strains which can cause infectious diseases (Vignesh *et al*., 2011). Marine fish farms are surrounded by a wide range of marine ecosystem like fishing, and other marine food processing units. Some of the wild fishes feed from leftover food pellets which are medicated with antibiotics and obviously it passes to the fishes and a research has confirmed that wild fishes capture around aquaculture areas ingest the pellets. Residues of tetracycline and quinolones were reported in wildfishes captured in these aquaculture areas (Tendencia *et al*., 2001). Apart from antibiotic resistance, these antibiotics get accumulated in the tissues of the fishes and shrimps. The evolution of resistance is an inevitable consequence of antibiotic use (Stokes, 2001) and this consequence is perhaps the most important implication of antibiotic use in aquaculture for a variety of reason (Salyers *et al*., 2002). The use of

Oxytetracycline (OTC) in aquaculture has been shown to cause a seasonal shift in bacterial species towards enterobacteriaceae and is associated with antibiotic resistance (Wollen- berger *et al*., 2000) samples taken from gills and intestines of wild and commercial fishes captured near fish farming activities have shown high frequencies of multiple antibiotic resistance (Rhodes *et al.,* 2000). Also the resistant strains of bacteria require even higher levels of dosing to treat diseases leading to more antibiotic reaching the natural environment (Bruun *et al*., 2003). The scientific evidence is that use of antibiotics in food producing animals can lead to resistance in intestinal bacteria and this resistance can then be transmitted to the general population causing treatment- resistant illness. In fish farming aquaculture mariculture the widespread use of antibiotics for treating bacterial diseases has been associated with the development of antibiotics resistance in *Aeromonas hydrophila, Aeromonas. salomonicida, Edwardsiella tarda, Edwardsiella icttaluri, Vibrio anguillarum, Vibrio.salmonicida, Pasteurella piscida* (Miranda *et al*., 2018).

2.13.2. Aquaculture as a source of antibiotic resistance in human pathogens

Genes exchange for resistance to antibiotics between bacteria in the aquaculture environment and bacteria in the terrestrial environment, including bacteria of animals and human pathogens has been shown (Sorum, 2006). For example, strong epidemiological and molecular indication exists showing that fish pathogens such as *Aeromonas* can transfer and share determinants for resistance to antibiotics with pathogens such as *Escherichia coli* isolated from humans (L'Abee-Lund and Sorum, 2001). The antibiotic resistance determinants of *Salmonella typhimurium* DT104 are encoded by a transmissible genetic element in the chromosome that contains a resistance gene for florfenicol, an antibiotic extensively used in aquaculture in the Far East (Angulo, 2000). Moreover, the DNA sequence of the transmissible element harboring these antibiotic resistance determinants has an important DNA sequence similarity to a plasmid of *Pasteurella piscicida*, which is also a fish pathogen. This process demonstrates the potential role of carrying of antibiotic-resistant bacteria as an alternative mechanism responsible for the spread of antibiotic resistance determinants from the aquatic environment to the terrestrial environment. The existence of antibiotics in the aquatic environment can result in the appearance of resistance among human pathogens forming part of its microbiota. The widespread transmission of antibiotic resistance determinants between bacteria of the aquatic and terrestrial environment has been recently revealed by the emergence of plasmid-mediated quinolone resistance among human Gram-negative pathogens (Nordmann and Poirel, 2005). Thus, the commonality of antibiotic

resistance determinants and of genetic elements between aquatic bacteria, fish pathogens and bacteria from the terrestrial environment powerfully supports the idea that antibiotic usage in aquaculture will influence the appearance of resistance in bacteria of other places, including resistance in pathogens able to produce a variety of human and animal diseases (Poirel *et al*., 2005).

2.13.3. Antibiotics and their effects on fish stress responses

The use of wide range of antibiotics in aquaculture has well-known positive effects on the control of bacterial infections; however, some side effects that affect both the fish and the environment are linked with excessive use. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products (Saglam & Yonar, 2009). The adverse effects of the antibiotics are known to cause damage to developmental, cardiovascular, and metabolic systems, as well as in altering anti- oxidant and immune responses, in fish (Yang *et al*., 2020). The widespread occurrence of naturally resistant bacteria in the aquatic environment could also contribute to the passage of antibiotic resistance genes (Wamala *et al*., 2018). Unfortunately, there are only a few studies that analyze the side effects of antibiotic use on fish themselves. There is evidence that some antibiotics can persuade nephrotoxicity (Hentschel *et al*., 2005), but the most well recognized side effect is immunomodulation (Tafalla *et al*., 2002). In the case of nephrotoxicity, a study conducted by the Bonventre group (Hentschel *et al*., 2005), determined that, as in rats and humans, gentamicin, an aminoglycoside antibiotic induces acute renal failure in fish. Their results showed that gentamicin induced pericardial edema in a time-and dose-dependent manner, which resulted in the fish being unable to maintain fluid homeostasis.

2.14. Use of probiotics in Aquaculture

Probiotics are used in aquaculture as alternative to antibiotics. Probiotics are microbial dietary adjuvant that beneficially affect the host physiology by modulating mucosal and systemic immunity, as well as improve nutritional and microbial balance in the intestinal tract (Nayak, 2010). These biological agents have been utilized for disease control, supplements to improve growth, and in some cases as a means of replacing antimicrobial compounds in aquaculture (Akanmu, 2018). Probiotics have proven to inhibit the growth of pathogens through production of antagonistic compounds, competition for attachment sites, nutrients, and alterations of enzymatic activity of pathogens, immune-stimulatory functions,

and nutritional benefits such as improvement in digestibility and utilization in feed (Khalafalla, 2013). Hence, the concept of utilizing probiotics in animal feed, particularly, poultry and fish, is fast gaining acceptance (Swavnendu *et al*., 2010).

Probiotics has five important characters which indicate the potential or efficiency of the probiotic in context to its use in the aquatic environment.

- Effectiveness in application
- Non-pathogenic and non-toxic
- Existing as viable cells preferably in large numbers
- Capable of surviving and metabolizing in the gut environment example resistance to low pH and organic acid
- Stable and capable of remaining viable for periods under storage and field conditions (Pramanick *et al*., 2019).

2.14.1. Probiotic Mechanisms of Action

2.14.1.1. Competition for adherence

Probiotic bacteria bind with the binding sites in the intestinal mucosa and form a physical barrier which prevents the connection of pathogenic bacteria (Pramanick *et al*., 2019). Probiotic bacteria compete with invading pathogens for binding sites to epithelial cells and the overlying mucus layer in a strain-specific manner. Surface layer proteins purified from *L. helveticus* R0052 inhibited enterohemorrhagic *Escherichia coli* O157:H7 adherence and the subsequent rise in permeability, without altering the growth of the pathogen (Johnson-Henry *et al*., 2007). *Saccharomyces boulardii* secretes a heat-labile factor which has shown to be responsible for the decreased bacterial adherence.

2.14.1.2. Immune modulation

Some probiotic bacteria stimulate the immune system by increasing the production of antibodies, and also activation of macrophages, T cell proliferation and interferon production (Pramanick *et al*., 2019). Immunomodulatory effects and clinical health benefits of probiotics have been attractive in the treatment of various degenerative diseases.

The immunomodulatory effect of probiotics is attributed to the release of cytokines, including interleukins (ILs), tumor necrosis factors (TNFs), interferons (IFNs), transforming growth factor (TGF), and chemokines from immune cells [lymphocytes, granulocytes,

macrophages, mast cells, epithelial cells, and dendritic cells (DCs)] which further regulate the innate and adaptive immune system (Azad *et al*., 2018). *Lactobacillus casei* have been shown to augment total and pathogen-specific secretory IgA levels upon infection in mice by stimulating B cell class switching to IgA (Galdeano and Perdigon, 2006). Specific antibodies against *L. casei* were not produced, indicating the non-responsiveness of the gut immune system to this beneficial bacterium. In infant rabbits pretreated with *L. casei*, morbidity of subsequent EHEC (Entero-Hemorrhagic *E. coli*) infection was reduced due to increased mucosal levels of anti-EHEC and anti-Shiga toxin IgA antibodies compared with controls (Ogawa *et al*., 2001). *L. casei* down-regulated the transcription of a number of genes encoding pro-inflammatory effectors such as cytokines and chemokines and adherence molecules induced by invasive *Shigella flexneri*. This resulted in an anti-inflammatory effect that appeared mediated by the inhibition of the NF-κB pathway.

2.14.1.3. Competition for nutrients

Probiotic bacteria utilize the nutrients and competition for nutrients can play an important role in the composition of the microbiota of the intestinal tract or ambient environment of the cultured aquatic organisms (Pramanick *et al*., 2019). This situation occurs with *Clostidium difficile*, a potentially pathogenic organism that is dependent upon monosaccharides for its growth. Probiotic organisms in sufficient numbers can utilize most of the available monosaccharides, which results in the inhibition of *C. difficile* (Khalighi *et al*., 2016).

2.14.1.4. **Barrier function**

Probiotics are capable of influencing many of the components of epithelial barrier function either by decreasing apoptosis of intestinal cells or increased mucin production. The intestinal epithelial barrier is thus the main defense mechanism against infection and inflammation, and the disruption of its integrity is one of the primary causes of several intestinal disorders (Liu *et al*., 2020). *Lactobacillus rhamnosus* GG was able to prevent cytokine-induced apoptosis in intestinal epithelial cell models by inhibiting tumor necrosis factor (TNF) (Yan and Polk, 2006). *Lactobacillus* species have been shown to increase mucin expression *in vitro* in human intestinal epithelial cells, thus blocking pathogenic *E. coli* invasion and adherence. *Lactobacillus rhamnosus* GG has been shown to prevent inflammation and programmed cell death of the lining intestinal epithelial cells and shown to exert mitogenic effects and enhancing mucosal regeneration (Caballero-Franco *et al*., 2007).

2.14.1.5. Interference with quorum sensing signaling

Bacteria communicate with each other as well as with their surrounding environment through chemical signalling molecules called auto-inducers. This phenomenon is called quorum sensing (QS) (Miller & Bassier, 2001). The use of this cell-to-cell signaling mechanism facilitates the regulation of important traits of enteric microbes that allow them to successfully colonize and/or start infection in their host. Prevalent in nature, QS networks provide bacteria with a method to gather information from the environment and make decisions based on the Intel. With its ability to autonomously facilitate both inter- and intraspecies gene regulation, this process can be rewired to enable autonomously actuated, but molecularly programmed, genetic control (Wang *et al*., 2020). Medellin-Pena *et al.* (2007), demonstrated that *Lactobacillus acidophilus* secretes a molecule that inhibits the quorum sensing signalling or directly interact with bacterial transcription of *E. coli* O157 gene, involved in colonization and thus, bacterial toxicity is opposed. Miller and Bassier (2001), stated that gram positive and gram negative bacteria use QS communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation (Al Mamum *et al*., 2019).

2.14.2. Feed probiotics

The concept behind the composition of feed probiotics is to apply the beneficial bacterial strains in feed using binders such as eggs and cod liver oil to obtain the beneficial microbial effects with more efficacies and at less environmental cost. The majority of commercial preparations contain *Lactobacillus* or *Saccharomyces cerevisiae*, nitrifying bacteria, Streptococci, *Roseobacter* and *Bacillus* sp. (Arıg *et al*. 2013).

Beneficial effects of regular use of probiotics in fish feed in the UK and other European countries have been reported (Cerrato, 2000). In aquaculture, probiotics can also be encapsulated in feed (Ramos *et al.,* 2013) or in live food such as rotifers and Artemia (Mahdhi *et al.,* 2011). Another efficient application of probiotics to aquatic animals is through bioencapsulation or infusions in diets. Preparation of probiotic diets has been demonstrated by Yassir *et al.* (2002). According to the FAO and WHO guidelines, probiotic organisms used in food must be capable of surviving passage through the gut. They must have the ability to resist gastric juices and exposure to bile (Senok *et al*. 2005). In addition, probiotics must be able to proliferate and colonize the digestive tract to be safe, effective and maintain their effectiveness and potency for the duration of the shelf life of the product

(Senok *et al*. 2005). The benefits of inclusion of bacterial strains into feed ingredients include improvements in feed values, contributions to enzymatic digestion, inhibition of pathogenic microorganisms, anti-mutagenic and -carcinogenic activity, growth-promoting factors and enhanced immune response. Purwandari and Chen (2013) studied the effects of probiotic *Bacillus subtilis* on intestinal microbial diversity and immunity of the Orange-spotted grouper *Epinephelus coioides*. The innate cellular response and respiratory burst activity of the supplemental groups were significantly higher compared to the control at 10 and 20 days after feeding and even more significant at 30 days. Probiotic *B. subtilis* increased the intestinal microbial diversity by stimulating the bacterial populations of *Paenibacillus sp., Lactobacillus oeni* strain 59 b and *Methylacidiphilum infernorum* strain V4, which are beneficial for *E. coioides*. The best dose of the probiotic *B. subtilis* based on the growth performance, innate cellular responses and microbial profile of fish intestines is 0.1 %, which showed equal efficacy as the 1 % diet. In this way, the use of feed probiotics in aquaculture has opened the window onto the possibility of sustainable commercial aquaculture.

Table 2: Main Mechanisms of Probiotics.

Modification of the intestinal microbiota	**Enhancement of barrier function**	**Immuno-modulation**
Decrease luminal pH	Increase mucus production	Effects on epithelial cells
Secrete antimicrobial peptides	Enhance barrier integrity	Effects on dendritic cells
Inhibit bacterial invasion		Effects on monocytes
Block bacterial adhesion to epithelial cells		Effects on lymphocytes - B cells lymphocytes - NKcells - T cells and T cell redistribution.

Source: (Ng *et al.*, 2009)

Feed probiotics were found to provide better growth results, an improved feed conversion ratio and enhanced appetite than had been experienced in aquaculture previously (Chae-Woo *et al.*, 2009). El-Dakar *et al*. (2007), demonstrated a marked (73–78 %) reduction in the feed cost of rabbit fish (*Siganus rivulatus*) culture only through BiogenR (a mixture of probiotics and prebiotics) supplementation. There is need to conduct studies relating to

probiotic resistance to antibiotics and the possibility of transmission of genetic elements to other microorganisms in the fish GIT, and thus to humans when consuming the aquaculture product (Munoz-Atienza *et al*., 2013).

2.15. Source of Nutrients and Enzymatic Contribution to digestion

Probiotic microbes have a beneficial effect in the digestive processes of aquatic animals. Sakata (1990) reported that bacteroides and *Clostridium sp*. have contributed to the host's nutrition, especially by supplying fatty acids and vitamins. Besides, some probiotics may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors (Prieur *et al.,* 1990, Pramanick *et al.,* 2019).

2.16. Influence on Water Quality

Probiotics have a role to improve the water quality of the aquaculture ponds by turnover of organic nutrients in the ponds (Pramanick *et al*., 2019). Ammonium and nitrite, toxic metabolites originating in the feces, underused feed and waste in aquatic systems can result in enormous economic loss. Recently, different kinds of probiotics have proven effective in ammonia nitrogen degradation. Accordingly, these eco-friendly additives can contribute to improve water quality (Jahangiri & Esteban, 2018).

2.17. Application of Probiotics in Aquaculture

Probiotics can be administered through:

- Feeding
- Injection and
- Directly to water

2.17.1. Application in Feed

Probiotcs are generally used in the fish feed by directly mixing with the feed ingredients or by spraying the prepared feed (Fuller, 1989; Pramanick *et al.,* 2019). Supplementation to the diet is the most widely used administration method. Generally, probiotics and cell wall components (parabiotics) are applied in the feed, added to the entire tank or pond water to confer protection against infection (Ringo, 2020). They have the capacity of surviving while passing through the gut and should have the ability to flourish and settle in the gut which should be safe and effective for the host species. The commonly

used probiotics in aquaculture are *Lactobacillus* sp., *Bacillus* sp. and *Saccharomyces cerevisiae* (Akter *et al.,* 2016). The administration via feeding (dry feed) definitely has limitations during early larval stages due to immature digestive tracts of fish in that stage of development (Jahangiri & Esteban, 2018).

2.17.2. Application through Injection

Probiotics are applied by injecting to fish species through intraperitoneal route to decrease the mortality rate (Yassir *et al.,* 2002). Futhermore, injection which is applicable to larvae, results in a high level of stress (Jahangiri & Esteban, 2018).

2.17.3. Application to Culture Water

Direct application of probiotics in the water exhibits beneficial effect on fish health by changing the microbial composition of the water and sediment. Among the probiotic bacteria, *Bacillus* spp., *Aerobacter* sp., *Nitrobacter* sp. and *Saccharomyces cerevisiae* (yeast) have an important role in water quality improvement (Akter *et al.,* 2016). Direct addition of probiotics to the rearing water can be applied from the first day of hatching in incubators (Jahangiri & Esteban, 2018).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Description of Study Area:

The study area is Anambra State, which is one of the five eastern States of Nigeria and covers an area of 4,416km^2 (Ojiako *et al*., 2015). Anambra state lies at Latitude of 6^0 1^1 28.6608"N and longitude 7^0 4^1 45.9336" E. It has an annual population growth rate of 2.21% per annum and experiences warm humid tropical climate, with average rainfall between 1520 and 2020 mm per annum (NBS, 2011; Ifeka & Akinbobola, 2015). Its mean annual temperature is 32.8^{0}C and a relative humidity of 73$^\alpha$ – 89$^\alpha$ (Statistical Year Book, 2011). Ministry of Agriculture, (FADAMA unit), Awka was visited for the list of registered fish farms in Anambra State. From the list, twenty farms were randomly selected from three senatorial zones of Anambra State (Anambra South, Anambra North and Anambra Central). The three senatorial zones have a total population of 5,466,200: Anambra South (2,024,400) with 7 Local Government Area and 67 towns, Anambra Central (1,943,300) with 7 Local Government Area and 57 towns and Anambra North (1,498,500) with 7 Local Government Area and 43 towns (Statistical year book, 2011). Residents are mainly traders, farmers, poultry farmers, fish farmers and civil servants.

3.2. Description of the sampled fish ponds

All the sampled fish ponds were open concrete ponds having different dimensions, with capacity to contain 100 – 1000 fishes per time. The sources of water for running the pond were bore holes which were sunk within the farm. The waste fish pond water was mostly removed fortnightly through an opening properly channeled to drainage system.

3.3. Questionnaires

Questionnaires were developed to obtain information on the socio-demographic, physical and physiological health conditions of the fishes, sanitary practices used by fish farmers, and their impact on fish health as well as the management of the ponds. Copy of the questionnaire is attached in Appendix 1.

3.4. Sample Collection

3.4.1. Collection of fish sample

A total of 720 fishes (360 fishes each in dry and rainy seasons respectively) with weight ranging from 125.97±1.05g to 137.13±0.19g of the genus *Clarias gariepinus* (African catfish) were collected from the 20 fish farm sites (18 fishes from each zones in dry and rainy seasons respectively). The fishes were collected in batches and were identified by Dr Ikele of the Department of Zoology, University of Nigeria, Nsukka. The fishes were aseptically collected with the help of fish handlers and transported in a sterile polythene bag to the laboratory for further examination. All samples collected were processed and analysed within 3h of collection.

3.4.2. Collection of fish pond water

A total of 480 fish pond water samples were collected from 20 fish farm sites namely: Priny farms, Agabah farms, Iyom Idiogo fish farms, Ikenwa and sons Industries Ltd, Orient writers and Udokamma fish sellers Co-operative society in Anambra North Senatorial zone; De Rock farm, Okata Okoli farms, Medosky farms, fish farmers Oraeri multipurpose Co-operative, Nia Agro Investment Nigeria, Aworonze fish Hatchery and Nursery farm, Meniks farms Ltd, Muokebe farms in Anambra South Senatorial zone; Errys Integrated farm, New Age fish farm, Ecoworld Agro Ventures, Greenfield and fish farm Investment Limited, Jipet fish farm and Adika farms, in Anambra Central Senatorial zone all located in the three senatorial zones in Anambra state, during the dry and rainy seasons.

Fish pond water samples were collected using sterile screw-capped containers between the periods of May to October 2016 (Rainy season) and November to April, 2017 (Dry season). 120 water samples were collected in dry and rainy seasons respectively and sampling was done at two points - at the surface of the pond and depth of 30cm below the water surface. The water samples were transported in a box containing ice packs to the laboratory for microbiological and physico-chemical analysis.

3.5. Preparation of Stock Cultures from Fish

The fishes were observed, to note external symptoms and weight of each fish was measured using weighing scale of 0.01g sensitivity. Fish gills and liver were harvested

aseptically using sterile dissecting blades and forceps, and were transferred into properly labeled sterile containers for microbiological analysis.

One gramme each of the dissected liver or gill was macerated using mortar and pestle and placed in 9ml of sterile distilled water. This was vigorously shaken for 1 min and allowed to stand for 5min. This served as the stock culture for fish analysis.

3.6. Isolation and enumeration of bacteria in Fish sample

A 10-fold serial dilution of the stock culture from gills or liver of the fishes was prepared. A 0.1ml of 10^{-4} dilution was inoculated onto sterile nutrient agar plate using spread plate technique and incubated at 37^0C for 24h. Developed colonies were counted and expressed as colony forming unit per gramme of the gill or liver. Colonies of the bacteria were subcultured to obtain pure cultures which were inoculated into freshly prepared nutrient agar slants and stored at 4^oC for further tests.

3.7. Isolation and enumeration of bacteria in water samples from fish ponds

A 1 ml aliquot of fish pond water sample was serially diluted in 9ml of sterile distilled water, and 0.1ml aliquot of 10^{-4} dilution spread plated on sterile nutrient agar plate. The plate was incubated for 24h at 37^oC, and thereafter colony counts of the bacterial growth were recorded. Colonies of the bacteria were subcultured to obtain pure cultures which were inoculated into sterile nutrient agar slant and stored at 4^oC for further tests.

3.8. Biochemical Characterization of the Bacterial Isolates

The bacterial isolates were characterized based on their morphological and biochemical characteristics as described by Noel and John (1984). Biochemical tests carried out include Gram reaction, motility test, indole test, methyl red, Voges-Proskaeur test, citrate utilization test, catalase test, oxidase test and sugar fermentation tests. Selected isolates were further identified based on 16S rRNA sequencing at University of Illinois, Chicago Sequencing Core (UICSCQ), USA.

3.8.1. Gram Reaction

A thin smear of the isolate on a clean slide was air dried and heat-fixed. The smear was stained using crystal violet, followed by addition of lugols iodine. The smear was rapidly decolourized with alcohol and then counterstained with safranin. The slide was dried and

viewed under the oil immersion microscope (X100). Purple colour indicates Gram positive bacteria while red colour indicated Gram negative bacteria (Cheesbrough, 2006).

3.8.2. Motility Test

A straight needle was used to touch a colony of a 24 hr culture growing on agar medium. This was stabbed once to a depth of only one third in the middle of semi-solid agar medium and incubated at 37^{o}C. This was examined daily for up to 7 days. Diffused zone of growth flaring out from the line of inoculation indicated a positive result (Cheesbrough, 2006).

3.8.3. Indole Test

The isolate was inoculated into different test tubes containing peptone water and incubated at 37^{0}C for 48h. Few drops of Kovac's reagent (Amyl or isoamyl alcohol, para-dimethylaminobenzaldehyde and concentrated hydrochloric acid) were added. A red colour in the alcohol layer indicated a positive result (Cheesbrough, 2006).

3.8.4. Methyl Red Test

About seven drops of methyl red solution were added to 5m1 of a 5-day old culture of the isolate inoculated in glucose-phosphate broth. Red colour change indicates a positive test while yellow colour indicated a negative test (Cheesbrough, 2006).

3.8.5. Voges-Proskauer Test

A mixture of 3m1 of 5% alpha-naphthol dissolved in absolute alcohol and lml of 40% potassium hydroxide were added to 5m1 of a 5- day old culture of the isolate in glucose-phosphate medium. Bright pink or red colour indicated a positive test whereas a yellow colour indicated negative (Cheesbrough, 2006).

3.8.6. Citrate Test

The isolate was inoculated into Simmon's citrate agar slant and incubated for 24-96h at 37°C. A colour change from green to deep blue indicated a positive result (Cheesbrough, 2006).

3.8.7. Catalase Test

A few drops of 3% hydrogen peroxide were added to a thick emulsion of the isolates on a clean slide and observed for effervescence. Effervescence indicated a positive result (Cheesbrough, 2006).

3.8.8. Coagulase Test

An emulsion of a 24h old culture of the isolate was made on a clean grease-free slide and a loopful of human plasma added to it. Clumping within 10sec indicated a positive result (Cheesbrough, 2006).

3.8.9. Oxidative - Fermentative Test

Two test tubes containing 10m1 each of the oxidative — fermentative basal medium and 10% glucose were inoculated with 24h culture of the isolate by stabbing. Bromothymol blue was added as an indicator. One of the test tubes was added lml of sterile paraffin oil (fermentative) while the other (oxidative) was without paraffin. Both tubes were incubated at 37°C for 14 days. A change in colour from green to yellow in both tubes indicates the isolate is both oxidative and fermentative. Colour change in tube with paraffin only indicates fermentative isolate while a change in colour in tube without paraffin indicates an oxidative isolate (Cheesbrough, 2006).

3.8.10. Sugar Fermentation Test

Sugars such as glucose, sucrose, lactose and maltose were added in peptone water in 1% (w/v). Two drops of Bromothymol blue were added as indicator. Inverted Durham tube was inserted into each of the tubes with the tubes containing the broth. The sugar solutions were sterilized by autoclaving at 115^0C for 15 min. 200µl of a 24h broth culture of the bacterial isolates was inoculated in each tube and then incubated at 37^0C for 24h. Colour change from green to yellow indicated a positive result and presence of bubbles in the inverted Durham tubes indicated gas production (Cheesbrough, 2006).

3.9. Antibiotic Susceptibility of Bacterial Isolates

Susceptibility of the test isolates to conventional antibiotics was carried out using the Kirby-Bauer disc diffusion susceptibility testing method (Bauer *et al*., 1966). A loopful of 24h old culture of the test isolate was inoculated into a test tube containing 4ml trypticase soy

broth and incubated for 5hr to produce a bacterial suspension. Afterwards, 0.1ml of standardized bacterial suspension (1.0 McFarland standard) was spread inoculated onto sterile Mueller Hinton agar plate. The plate was covered and allowed to dry. Standard antibiotic discs –Vancomycin (30µg), Trimethoprim (75µg), Ciprofloxacin (5µg), Gentamycin (10µg), Streptomycin (10µg), Oxytetracycline (10µg), Ampicillin (10µg) and Erythromycin (15µg) were properly placed aseptically on the surface of the inoculated plate using a sterile forcep to ensure even contact with the medium. The test was carried out in duplicates. The plate was incubated at 37^0C, and after 24h, the zone of growth inhibition around the antibiotic discs were measured. The result was interpreted as resistant or susceptible according to the Clinical and Laboratory Standards Institute guidelines (2017). Percentage susceptibility (PS) was calculated thus;

$$PS\ (\%) = \frac{\text{No. of susceptible organisms to an antibiotic}}{\text{Total No. of organisms tested}} \times 100$$

3.10. Molecular Characterization of Selected Isolates

Genomic DNA from bacterial cells was extracted using an automated DNA extraction device, the Maxwell16 instrument (Promega), implementing the Maxwell® 16 Tissue DNA Purification Kit according to the manufacturer's protocol. The extracted genomic DNA was subjected to PCR amplification with the primer set 27F (AGAGTTTGATCMTGGCTCAG) / 1492R (GGTTACCTTGTTACGACTT) amplifying nearly the entire bacterial small subunit ribosomal RNA gene, using DreamTaq Green PCR Master Mix (2X) (ThermoFisher). PCR-amplified DNA was purified AMPure XP beads (0.6X) to remove unused primers and dNTPs. Amplified genomic DNA was sequenced on an ABI 3730xl capillary sequencer using the 27F primer to initiate the sequencing reaction. Sequence data was trimmed to remove poor quality bases, and sequences analyses were performed using the NCBI BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the SILVA on-line aligner (https://www.arb-silva.de/aligner/), and the software package MEGA (https://www.megasoftware.net/) (Kumar *et al*., 2016).

3.11. Physico-Chemical Analysis of the Water Sample from Fish Pond

3.11.1. Temperature

The temperature of the water sample from fish pond was measured at site of collection using a mercury-in-glass thermometer calibrated in degree Celsius. The

thermometer was gently inserted into the water sample and allowed to stand for 2min. The temperature read was recorded in ˚C (APHA, 2012).

3.11.2. pH

The pH of the water sample was determined using a pH meter (HI 8314 membrane pH meter). The pH meter was first standardized by dipping the electrode of the meter into a buffer of known pH and then inserted into 50ml of the water sample contained in a beaker. The pH was read and recorded accordingly (APHA, 2012).

3.11.3. Determination of Electrical Conductivity

The electrical conductivity of the water sample depends on the presence of ions, proteins and organic acids in the water samples. Electrical conductivity was determined at 20^0C using a conductivity meter (Model DDS-307). The conductivity cell containing the electrode was first inserted into deionized water with specific electric conductivity to zero the conductivity meter. Afterwards, the conductivity cell was inserted in sufficient volume of the sample and allowed for 2min. The electrical conductivity of the sample was read and recorded in microsiemens per centimeter (µS/cm) (APHA, 2012).

3.11.4. Turbidity Determination

Turbidity of the water sample was determined by photometric method using HACH DR/2010 Spectrophotometer. A 25ml aliquot of deionized water was used as blank to zero the spectrophotometer prior to sample reading. The fish pond water sample was vigorously shaken and 25ml was used to read on the spectrophotometer at a wavelength of 860nm (APHA, 2012).

3.11.5. Determination of Alkalinity

A 50ml volume of water sample was placed in a volumetric flask and 3 drops of phenolphthalein indicator was added to it. The mixture was titrated against 0.02N sulfuric acid at pH 8.3. Positive result was indicated by clearing of the pink colour of the phenolphthalein indicator (Sawyer *et al*., 2000).

$$\text{Phenolphthalein Alkalinity (mg/L)} = \frac{(A1 \times N \times 50{,}000)}{V}$$

Where A1 = Volume of Sulfuric acid used in ml;

N= Normality of acid used to titrate; V = Volume of Sample used in ml.

3.11.6. Nitrite Determination

The nitrite content of the water sample was estimated by sulphanilamide method (APHA, 2012). A 50ml water sample in a 250ml conical flask was mixed with 1ml of sulphanilamide solution and allowed to stand for 3min. 1ml of N-1 naphthyl ethylene diamine hydrochloride solution was added and the optical density of the solution measured in a spectrophotometer at 543nm.

3.11.7. Phosphate Determination

A 50ml of the water sample was placed in a 100ml polyethylene bottle. To the sample, 5ml of the mixed reagent (250ml sulfuric acid solution, 100ml ascorbic acid solution, 50ml potassium antimonyl- tartarate solution and 100ml ammonium molybdate solution) was added and mixed homogenously. The absorbance of the sample was read in a spectrophotometer at a wave length of 885nm. A reagent blank was prepared using de-ionized water in place of the 50ml water sample and the process earlier described was performed (Nawar, 2016).

3.11.8. Sulphate Determination

A 250ml water sample in a conical flask was adjusted to a pH 5 adding drops of 1N HCl. The solution was heated to boiling while stirring slowly and 4.0% (w/v) of Barium chloride solution containing 4.0% (v/v) ethanol, and 2.4% (v/v) nonyl phenol polyethyleneoxy ether added slowly until precipitation was complete. After precipitation, 2ml of $BaCl_2$ solution was added further and the precipitate digested at 80^0C overnight. The precipitate was filtered through an ashless filter paper and washed with warm distilled water until it is free of chloride as indicated by testing with silver nitrate-nitric acid reagent. The precipitate was placed in a pre-weighed crucible along with the filter paper, and then dried. The crucible was kept in a muffle furnace and ignited at 800^0C for 1h, cooled in a desiccator and the Barium sulphate precipitate weighed (Zarate *et al*., 2011).

$$S0_4 \text{ in mg/L} = \frac{\text{Weight (mg) of } BaS0_4 \text{ x } 411.6}{\text{volume (ml) of sample}}$$

3.11.9. Biological Oxygen Demand (BOD) Determination

A 10ml aliquot of the water sample was diluted in 90ml distilled water and dispensed into BOD volumetric flask. To the solution, 22.5g/L of $MgSO_4.7H_2O$ and 2ml alkali-iodide-azide reagent were carefully added to avoid the formation of air bubbles. 2ml of $CaCl_2$ and $FeCl_3.6H_2O$ were also added in the same manner. The bottle was closed and the sample mixed by inverting many times. A brownish cloud in the solution indicates the presence of oxygen. The brown precipitate was allowed to settle out to the bottom, and then 2ml of concentrated H_2S0_4 carefully added without forming air bubbles. The flask was closed and the solution mixed to dissolve the precipitate. The flask was kept in BOD incubator for 5 days at 20^0Cand 50ml of the water sample titrated against 0.025N sodium thiosulphate to a pale yellow colour. Addition of 2ml of starch solution to the titrate gave a blue color and the titration continued until a clear sample was obtained and the reading noted. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used (Vlab, 2012). The initial and final burette reading were recorded as i and f and the dissolved oxygen (DO) for 5 days were calculated thus;

$$DO_1 = \frac{\text{f x titrant volume}}{\text{Volume of sample}} \times 8000$$

$$BOD = \frac{DO_1 - DO_5}{0.05}$$

Where, DO_1 is dissolved oxygen for day 1; DO_5 is dissolved oxygen for day 5

3.12. Pathogenicity Testing of the Bacterial Isolates on Healthy Fish and Mice

3.12.1. Experimental Animals:

A total of 370 of 10week old fishes (with weight ranging from 125.97±1.05g to 137.13±0.19g) and 370 three month old adult albino mice (measuring from 342-362g) were used for the experimental study. The animals were procured and housed in cages, comprising of eight mice per six groups in the animal house of Zoology Department, at University of Nigeria, Nsukka, and were fed with commercial feed and sterile water *ad libitum*. One group out of the six groups served as control. Each group was placed in triplicates. The fishes were similarly treated but placed in bowls.

3.12.2. Inoculum Preparation:

The five bacterial isolates selected based on their antibiotic susceptibility tests were identified as *Lysinibacillus sphaericus, Pseudomonas aeruginosa, Serratia marcescens, Bacillus subtilis* and *Paenalcaligenes suwonensis* were used for inoculum preparation. Aliquots of 24h old culture of the test organisms in nutrient broth was used to obtain a 0.5 Mc Farland standardized stock culture, which was used for further analysis (Tankeshwar, 2016).

3.12.3. Animal inoculation:

A 0.1ml (3.2×10^4 cells/ml) of the inoculum preparation of the isolate was administered on different groups of the albino mice through oral and subcutaneous routes, while fishes were challenged through oral route only.

The control animal was inoculated with the same volume of phosphate buffer saline and all the animals were observed for 14 days, for the appearance of clinical signs such as gross morphological changes, pathological signs and symptoms (Pascual *et al*., 2009).

3.12.4. Effect of the bacterial isolates on the experimental animals

On the infected mice, the hematology, blood chemistry profile and mortality rate were examined. On the infected fishes, the hematology and blood chemistry profile, bacterial load in the gill and liver and mortality rate were examined.

Some of the infected animals (fish and mice) were sacrificed after the 14^{th} day of inoculation for histological examination of the internal organs to determine the possible damages to the invaded tissues and organs.

3.12.5. Hematological Analysis

3.12.5.1. Hemoglobin determination:

The haemoglobin was determined using the cyanmethaemoglobin method (Wintrobe, 1967). A 0.02ml of the blood from the animal was added into 5ml of Drabkins solution in a test tube, mixed properly and allowed to stand for 5min. A blank was prepared following the same process and using distilled water in place of the sample. The test sample was read against the blank spectrophotometrically at 540nm and the absorbance readings recorded. The hemoglobin concentration calculated thus:

$$\frac{\text{Abs of test sample}}{\text{Abs of Standard}} \times \text{Concentration of Standard} \times \frac{\text{Dilution factor}}{1000}$$

3.12.5.2. Evaluation of red blood cell count:

The erythrocytes count was determined using microscope Neubauer counting chamber, after diluting the blood with Hayems fluid at the ratio of 1: 200. Total numbers were reported as 10^6 mm^3 (Wintrobe, 1967).

3.12.5.3. Evaluation of total white blood cell count:

A 0.02ml of the blood sample of the mice in a test tube was added 0.038ml of Turk's solution. The mixture was allowed to stand for 2min and an aliquot used to fill the counting chamber of the already charged Neubauer chamber. The Neubauer chamber was allowed to stand for 2min, for the cells to settle and the underside of the chamber cleaned, placed under the microscope and viewed using x10 objective lens. The blood sample of the fish was similarly treated (Cheesbrough, 2006).

3.12.5.4. Evaluation of differential counts:

Differential leucocytes was estimated by dropping fresh blood of the mice onto one end of a clean grease-free slide, placed on a horizontal surface using a spreader, a little narrower than the slide. The drop of blood was smeared on the slide, allowed to air-dry and then stained immediately using Leishmann technique. About 10 drops of Leishmann stain was added to the dried smear and after two minutes, 20 drops of distilled water, of pH 6.8, was added, and the mixture allowed standing for another 10min. The stain was washed off, allowed to dry and the different cells examined under oil immersion objective lens (x100), using binocular microscope. The blood sample of the fish was similarly treated (Wintrobe, 1967).

3.12.6. Blood Chemistry Determination

3.12.6.1. Determination of serum aspartate aminotransferase (AST)

A 5 ml blood sample of the animal in a sterile tube was allowed to clot. The test tube was centrifuged and the resulting supernatant, the serum, was transferred into another test tube. A 0.1ml aliquot of the serum was pipetted into a test tube, mixed with 0.5ml of the AST assay buffer and incubated for 1h at 37^0C. A 0.5ml aliquot of a chromogen solution was mixed with the sample solution, allowed to stand for 20min at 20^0C and then, 5.0ml of 0.4N NaOH added. The solution was allowed to stand for another 10 min at room temperature and the activities of AST in the serum were determined by reading the optical density (OD) in a spectrophotometer at 520 nm using green filter. A blank was prepared following the same process and using distilled water in place of the sample (Reitman & Frankel, 1957).

3.12.6.2. Determination of serum alanine aminotransferase

The serum alanine aminotransferase was determined following the method of Reitman and Frankel (1957). The blood of the fish was collected through the caudal peduncle and that of the mice through the retrobulbar plexus of the median canthus and transferred into specimen tube without EDTA. The blood (5ml) was allowed to clot, centrifuged and the resulting supernatant, the serum, transferred into another test tube and immediately refrigerated to maintain the high level of enzyme activity until further analysis. A 0.1ml aliquot of the serum was pipetted into a test tube, mixed with 0.5ml of ALT assay buffer and incubated for 30min at 37^0C. A 0.5ml of the chromogen solution was mixed with the sample solution, allowed to stand for 20min at 20^0C and 5.0ml of 0.4N NaoH added. The sample solution was allowed to stand for another 5min at room temperature and the absorbance read against a blank at 546nm in a spectrophotometer. The blank was prepared following the same process and using distilled water in place of the sample.

3.12.7. Histological examination of the fish and mice organs

The organs obtained from the fishes and mice were fixed in 10% neutral formalin, embedded in paraffin, sectioned, stained by hematoxylin and eosin, and examined by optin microscopy.

3.12.8. Plating of the internal organs of the infected fish on selective media

A 1.0g portion of gill, liver, intestine and subcutaneous tissues of the infected fishes were mashed and inoculated onto different selective media agar plates (Appendix ii), and incubated at room temperature for 24h. This was to demonstrate that the diseases and changes observed were actually caused by the bacterial isolates inoculated into the test animals (Koch's postulate) [Vincent, 2010].

3.12.9. Treatment of the infected fishes with probiotic and a conventional antibiotic

Saccharomyces cerevisiae, a probiotic fungus, was used for treatment of the infected fishes, while a conventional antibiotic, chloramphenicol, served as control (Cruz *et al.*, 2012). A two week treatment of the fishes infected with the bacterial isolates was carried out and the effects of the treatment on hematological profile, blood chemistry profile, weight of the fish and bacterial load in the internal organs measured.

3.12.9.1. Administration of *Saccharomyces cerevisiae*

The method of Hai (2015) was used for preparation and administration of the probiotic. The commercial *Saccharomyces cerevisiae* obtained from Alpha Pharmacy and stores, Agbani, Enugu , was administered during their feeding by incorporating 1.0g of the stock into 1kg of the fish feed after different concentrations (0.5-2.0g/kg) of the probiotic was prepared.

3.12.9.2. Preparation and administration of Chloramphenicol

The lethal concentration of chloramphenicol was first determined by trying different concentrations (2.5mg/L – 60mg/L) of the antibiotic on bacterial isolates. The concentration of chloramphenicol that achieved LC_{50} (30mg/L) was administered to the fishes as feed additive.

3.12.10. Statistical Analysis

Univariate and One way analysis of variance were used to analyze the data obtained using SPSS version 27.

CHAPTER FOUR

4.0. RESULTS

4.1. Isolation and enumeration of bacteria in Fish sample

The mean bacterial counts obtained from the gill and liver of the fish samples during dry and rainy seasons are shown in Figs.1 and 2. The counts obtained from the gills ranged from $1.3 \times 10^3 - 7.9 \times 10^3$cfu/g in dry season and $1.3 \times 10^3 - 9.6 \times 10^3$cfu/g in rainy season, while the counts obtained in the liver during dry and rainy season ranged from $1.7 \times 10^2 - 7.0 \times 10^2$cfu/g and $2.6 \times 10^2 - 6.3 \times 10^2$cfu/g respectively. Univariate analysis of variance of the bacterial counts (Appendix iii), shows that there was significant difference in the counts obtained between the gills and liver, among the three senatorial zones and during dry and rainy season (p value < 0.05). The mean estimates revealed that bacterial count in the gill was higher than the count in the liver during the rainy season than the dry season and highest in Anambra North and least in Anambra south (Appendix iii).

Out of the 720 fish samples collected from the fish farms, a total of 165 bacterial isolates belonging to the genera *Vibrio, Aeromonas, Pseudomonas, Lactobacillus, Staphylococcus, Microbacterium, Serratia, Proteus, Bacillus, Streptococcus, Citrobacter* and *Micrococcus* were isolated (Table 3). *Bacillus* sp. had the highest occurring bacterium with occurrence (18.2%), while *Vibrio* sp had the least occurrence (1.2%) [Table 4].

4.2. Isolation and enumeration of bacteria in water sample from fish pond

Table 5 shows the total bacterial count in the water sample from the fish ponds, collected from three senatorial zones in Anambra state during the dry and rainy season. During the dry season, the mean bacterial count obtained in the fish pond water ranged from $2.1 \pm 0.95 \times 10^6$ to $22.6 \pm 0.81 \times 10^6$ cfu/ml and $20.6 \pm 0.81 \times 10^6$ to $44.8 \pm 0.49 \times 10^6$ cfu/ml during the rainy season. The range of mean bacterial count observed in dry and rainy season in the three senatorial zones were as follows: $2.1 \pm 0.95 \times 10^6$ to $21.1 \pm 0.66 \times 10^6$ cfu/ml during dry season and $20.6 \pm 0.81 \times 10^6$ to $32.1 \pm 1.85 \times 10^6$ cfu/ml in rainy season, all in Anambra North; $13.3 \pm 0.29 \times 10^6$ to $22.6 \pm 0.81 \times 10^6$ cfu/ml and $19.2 \pm 1.04 \times 10^6$ to $44.8 \pm 0.49 \times 10^6$ cfu/ml during dry and rainy seasons in Anambra South. For Anambra Central, the range was $10.3 \pm 0.42 \times 10^6$ to $15.0 \pm 1.0 \times 10^6$cfu/ml and $22.0 \pm 05 \times 10^6$ to $38.9 \pm 0.25 \times 10^6$cfu/ml during dry and rainy season respectively. The univariate analysis of the bacterial counts obtained in the fish ponds in the three senatorial zones (Appendix iv) shows that there is significant

difference in the counts obtained during the dry and rainy season in all the ponds and in the three senatorial zones (p value< 0.05).

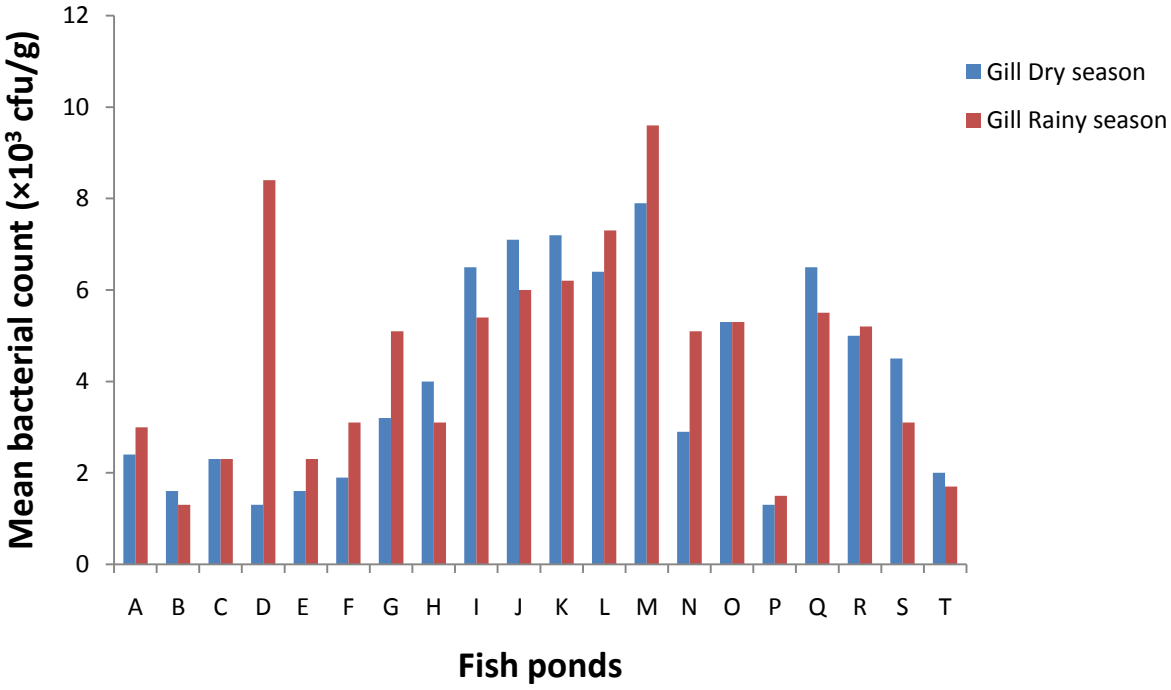

Fig.1: Mean seasonal bacterial count in gills of fish samples

Key

A – Priny farms; B – Agabah farms; C – Iyom Idiogo fish farms; D – Ikenwa and Sons Industries Ltd; E – Orient Writers; F – Udokamma fish sellers co-operative society; G – De Rock farm; H – Okata Okolie farms; I – Medosky farms; J – Fish farmers Oraeri multipurpose co-operative; K – Nia Agro Investment; L – Aworonze Fish Hatchery and Nursery Farm; M – Meniks farms; N – Muokebe farms; O – Errys Integrated farm; P – New Age fish farm; Q – Ecoworld Agro Ventures; R – Greenfield and fish farm Investment; S – Jipet fish farm; T – Adika farms

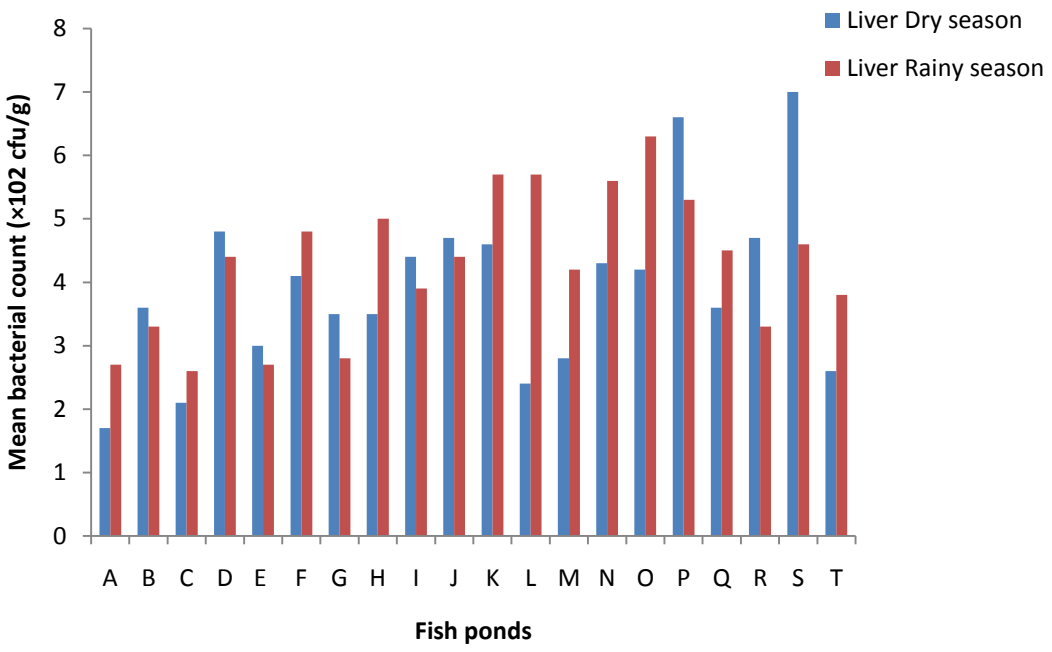

Fig. 2: Mean seasonal bacterial count in the liver of fish samples

Key

A – Priny farms; B – Agabah farms; C – Iyom Idiogo fish farms; D – Ikenwa and Sons Industries Ltd; E – Orient Writers; F – Udokamma fish sellers co-operative society; G – De Rock farm; H – Okata Okolie farms; I – Medosky farms; J – Fish farmers Oraeri multipurpose co-operative; K – Nia Agro Investment; L – Aworonze Fish Hatchery and Nursery Farm; M – Meniks farms; N – Muokebe farms; O – Errys Integrated farm; P – New Age fish farm; Q – Ecoworld Agro Ventures; R – Greenfield and fish farm Investment; S – Jipet fish farm; T – Adika farms

Table 3. Biochemical test of bacteria isolated from the fish samples

	Isolates											
Biochemical tests	**1F^{g}**	**2F^{g}**	**3F^{g}**	**4F^{l}**	**5F^{lg}**	**6F^{lg}**	**7F^{lg}**	**8F^{lg}**	**9F^{lg}**	**10F^{lg}**	**11F^{lg}**	**12F^{lg}**
Gram reaction	-ve	+ve	+ve	-ve	ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve
Shape	Rod	Cocci	Cocci	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Motility	+	-	-	+	+	+	-	+	+	+	+	-
Indole	+	-	-	+	-	-	-	+	-	-	-	-
Methyl Red	-	-	-	-	-	-	+	+	-	+	+	-
Voges Proskaeur	+	-	+	-	-	+	+	-	+	-	-	-
Citrate	+	-	+	+	+	+	+	-	+	+	+	-
Catalase	-	-	+	+	+	+	+	+	+	+	+	-
Oxidase	+	-	+	+	+	-	-	-	-	-	-	-
Coagulase	-	-	-	-	-	-	+	-	-	-	-	-
Sugar fermentation												
Lactose	+	+	-	+	+	+	+	+	-	+	-	+
Mannitol	+	-	-	+	+	+	+	+	+	+	-	-
Sucrose	+	+	-	+	+	+	+	+	+	+	-	+
Arabinose	-	-	-	+	+	-	-	-	-	+	-	+
Maltose	+	+	-	+	+	+	+	+	+	+	-	+
Glucose	+	+	+	-	-	+	+	+	+	+	+	+

Key:

1F = *Vibrio* sp., 2F = *Streptococcus* sp, 3F = *Micrococcus* sp., 4F = *Aeromonas* sp., 5F = *Pseudomonas* sp., 6F = *Bacillus* sp.,

7F = *Staphylococcus* sp., 8F = *Microbacterium* sp., 9F = *Serratia* sp., 10F = *Citrobacter* sp., 11F = *Proteus* sp., 12F = *Lactobacillus* sp.

Isolates with superscript ‘g’ were isolated from the fish gill only, isolate with superscript ‘l’ was isolated from the fish liver only, while isolates with superscript ‘lg’ were isolated from both fish liver and gills.

Table 4. Percentage occurrence of the bacteria isolated from the fish samples

Organism (Genera)	Frequency of occurrence	Percentage occurrence(%)
Vibrio sp.	2	1.2
Aeromonas sp.	5	3.0
Pseudomonas sp.	20	12.1
Lactobacillus sp	15	9.0
Staphylococcus sp.	28	17.0
Microbacterium sp.	10	6.1
Serratia sp	10	6.1
Proteus sp.	5	3.0
Bacillus	30	18.2
Streptococcus sp.	15	9.0
Citrobacter sp.	18	10.9
Micrococcus sp.	7	4.2
Total	165	100

$$\text{Percentage occurrence (\%)} = \frac{\text{frequency of occurrence}}{\text{Total no of samples screened}} \times 100$$

Table 5: Mean seasonal bacterial counts of organisms isolated from water samples of the fish pond

Senatorial Zone	Pond	Mean Bacteria counts ($\times 10^6$cfu/ml) Dry Season	Rainy Season
Anambra North	A	17.3±0.70	27.9±0.61
	B	21.1±0.66	22.4±0.36
	C	13.1±1.25	32.1±1.85
	D	2.1±0.95	20.6±0.81
	E	20.8±0.75	24.8±1.04
	F	11.3±0.61	23.7±0.61
Anambra South	G	14.2±3.33	19.2±1.04
	H	19.5±0.50	37.6±0.35
	I	13.3±0.29	40.5±0.50
	J	13.5±0.50	31.2±1.08
	K	21.0±1.32	35.9±0.36
	L	22.6±0.81	30.9±0.26
	M	22.0±0.50	44.8±0.49
	N	19.9±0.78	36.8±0.70
Anambra Central	O	11.8±0.26	30.0±0.45
	P	12.1±0.15	38.9±0.25
	Q	15.0±1.00	22.0±0.50
	R	13.5±0.45	23.9±0.32
	S	10.9±0.36	36.2±0.76
	T	10.3±0.42	38.3±0.42

Key: Counts represent Mean±Standard deviation
A – Priny farms; B – Agabah farms; C – Iyom Idiogo fish farms; D – Ikenwa and Sons Industries Ltd; E – Orient Writers; F – Udokamma fish sellers co-operative society; G – De Rock farm; H – Okata Okolie farms; I – Medosky farms; J – Fish farmers Oraeri multipurpose co-operative; K – Nia Agro Investment; L – Aworonze Fish Hatchery and Nursery Farm; M – Meniks farms; N – Muokebe farms; O – Errys Integrated farm; P – New Age fish farm; Q – Ecoworld Agro Ventures; R – Greenfield and fish farm Investment; S – Jipet fish farm; T – Adika farms

Out of the 480 water samples collected from the fish ponds, a total of 15 genera of bacterial organisms were observed. Isolates belonged to the following genera: *Enterococcus, Pseudomonas, Bacillus, Staphylococcus, Enterobacter, Paenalcaligenes, Lysinibacillus, Serratia, Streptococcus, Citrobacter, Micrococcus, Proteus, Lactobacillus, Acinetobacter* and *Escherichia. Micrococcus* sp was isolated only during the rainy season while *Escherichia, Lysinibacillus* and *Paenalcaligenes* sp were isolated only during the dry season (Table 6). *Staphylococcus* sp. had the highest percentage occurrence (14.0%) while *Escherichia.* sp had the least percentage occurrence (1.7%) [Table 7].

Table 6. Biochemical test of bacteria isolated from the fish pond water samples

Biochemical tests	Isolates														
	1W^{dr}	**2W^{dr}**	**3W^{dr}**	**4W^{dr}**	**5W^{dr}**	**6W^{d}**	**7W^{d}**	**8W^{dr}**	**9W^{dr}**	**10W^{dr}**	**11W^{r}**	**12W^{dr}**	**13W^{dr}**	**14W^{dr}**	**15W^{dr}**
Gram reaction	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Shape	Cocci	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod	Rod
Motility	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Methyl Red	-	-	-	+	-	-	-	-	-	+	-	+	-	-	+
Voges Proskaeur	+	-	+	+	+	-	+	+	-	-	+	-	-	-	-
Citrate	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+
Catalase	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+
Oxidase	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-
Coagulase	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation															
Lactose	+	-	+	+	+	-	-	-	+	+	-	-	+	-	+
Mannitol	+	+	+	+	+	-	-	+	-	+	-	-	-	-	+
Sucrose	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
Arabinose	-	-	+	-	+	+	-	-	-	+	-	-	+	+	+
Maltose	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+
Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: 1W= *Enterococcus* sp.,2W = *Pseudomonas* sp, 3W = *Bacillus* sp.,4W = *Staphylococcus* sp., 5W = *Enterobacter* sp., 6W = *Paenalcaligenes* sp., 7W = *Lysinibacillus* sp., 8W = *Serratia* sp.,9W = *Streptococcus* sp., 10W = *Citrobacter* sp., 11W = *Micrococcus* sp.,12W = *Proteus* sp., 13W = *Lactobacillus* sp.,14W = *Acinetobacter* sp. and 15W = *Escherichia* sp.

Table 7: Percentage occurrence of the bacteria isolated from water samples of the fish ponds

Organism	Frequency of occurrence	Percentage occurrence (%)
Enterococcus sp	15	5.2
Pseudomonas sp	32	11.2
Bacillus sp	38	13.3
Staphylococcus sp	40	14.0
Enterobacter sp	28	9.8
Paenalcaligenes sp	8	2.8
Lysinibacillus sp	10	3.5
Serratia sp	20	7.0
Streptococcus sp	15	5.2
Citrobacter sp	16	5.6
Micrococcus sp	12	4.2
Proteus sp	10	3.5
Lactobacillus sp	22	7.7
Acinetobacter sp	15	5.2
Escherichia sp	5	1.7
Total	286	100

$$\text{Percentage occurrence (\%)} = \frac{\text{frequency of occurrence}}{\text{Total no of samples screened}} \times 100$$

4.3. Antibiotic Susceptibility Tests of the Bacterial Isolates

Table 8 shows the antibiotic susceptibility tests of the bacterial isolates. *Staphylococcus* sp, *Enterobacter* sp, *Micrococcus* sp, *Escherichia* sp and *Microbacterium* sp showed 100% susceptibility to the antibiotics tested, while *Serratia* sp had the least susceptibility (62.5%) to the antibiotics tested. Percentage susceptibility of the tested bacterial isolates was highest with chloramphenicol, vancomycin, ciprofloxacin and trimethoprim (94.4%), and least with erythromycin (72.2%).

4.4. Molecular Characterization of Selected Isolates

Five bacterial isolates selected based on their antibiotic susceptibility tests were identified following 16S rRNA sequencing technique as *Lysinibacillus sphaericus* G39, *Bacillus subtilis* BB1, *Serratia marcescens* Db11, *Pseudomonas aeruginosa* PA01 and *Paenalcaligenes suwonensis* UN24. The phylogenetic tree showing the evolutionary relatedness of the bacterial isolates is shown in Fig.3.

Table 8: Antibiotic susceptibility test of the bacterial isolates showing zones of inhibition

	Zones of inhibition (mm)								
Organism	**Ampicillin**	**Erythromycin**	**Chloramphenicol**	**Gentamicin**	**Vancomycin**	**Ciprofloxacin**	**Trimethoprim**	**Oxytetracyclin**	**% susceptibility**
Enterococcus sp	6	12	50	18	0	15	22	16	87.5
Pseudomonas sp	8	0	32	0	28	20	CI	13	75
Bacillus sp	32	2	32	21	CI	0	0	28	75
Staphylococcus sp	45	36	10	25	16	25	21	10	100
Enterobacter sp	16	18	30	12	30	50	20	CI	100
Paenalcaligenes sp	CI	0	35	8	50	13	20	0	75
Lysinibacillus sp	8	0	12	0	13	50	22	CI	75
Serratia sp	0	0	45	0	50	38	26	CI	62.5
Streptococcus sp	16	28	0	45	CI	12	16	50	87.5
Citrobacter sp	38	0	56	20	37	22	24	16	87.5
Micrococcus sp	26	12	28	6	12	10	16	8	100
Proteus sp	0	28	37	48	CI	26	20	18	87.5
Lactobacillus sp	16	CI	15	27	40	10	26	0	87.5
Acinetobacter sp	22	26	12	58	66	19	28	0	87.5
Escherichia sp	18	14	26	30	10	22	16	20	100
Vibrio sp	26	38	10	25	52	12	22	0	87.5
Aeromonas sp	22	12	40	0	26	30	10	18	87.5
Microbacterium sp	16	32	10	26	38	CI	12	18	100
% Susceptibility	88.9	72.2	94.4	77.8	94.4	94.4	94.4	77.8	

Key: CI = complete inhibition; 0= No inhibition

WHO Standard:

0-6= Resistant, 7-10 = Moderate and 11- above = Susceptible

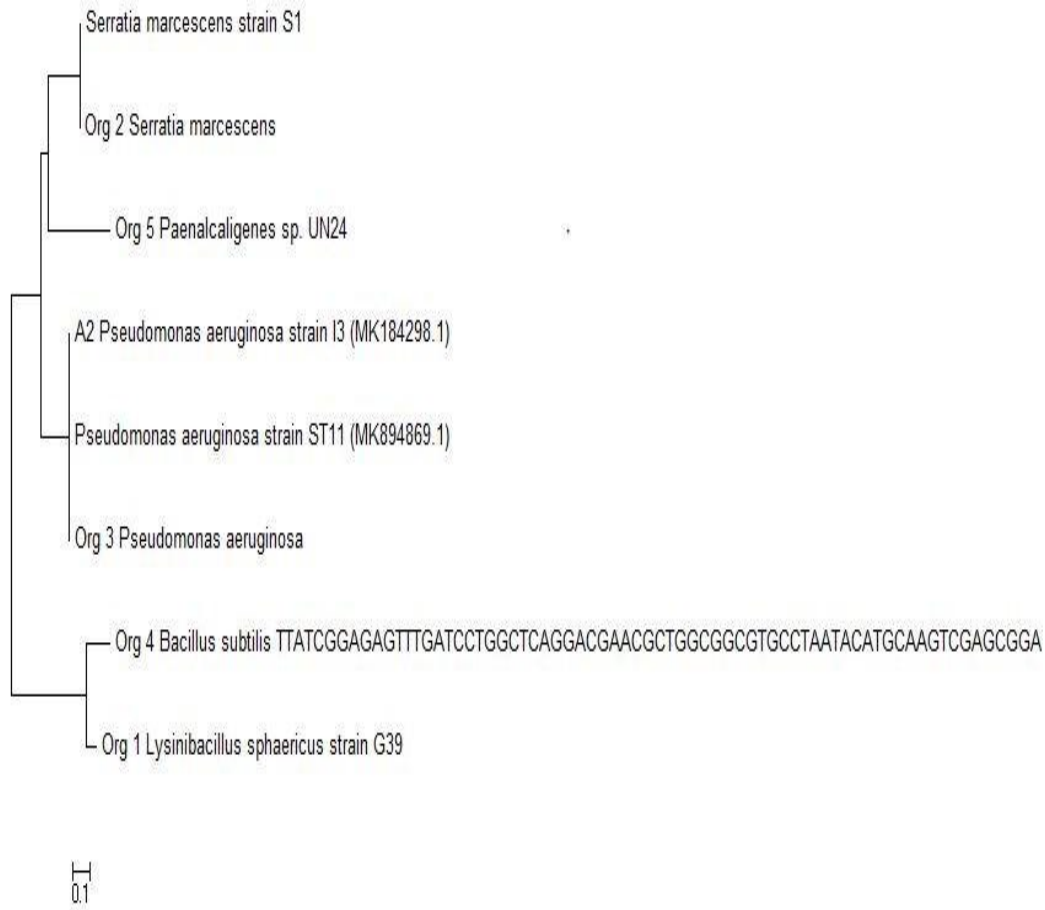

Fig.3: Phylogenetic tree showing evolutionary relatedness of the bacterial isolates.

4.5. Questionnaires

4.5.1. Assessment of Sanitary Practices used by Fish Farmers and their Impact on fish Health

Information gathered from the respondents' answers to the questionnaire is shown in Appendix v - vii. All the examined ponds used for fish aquaculture in the 3 senatorial zones are made of concrete, and 80% of them rear African catfish (*Clarias gariepinus*), while 20% in addition to *Clarias gariepinus,*also rear *Clarias* x *Heterobranchus* hybrid (Heteroclarias), *Heterobranchus bidorsalis* and *Clarias nigro-digitatus.* Also 35% of the ponds breed 100-500 fishes per pond, 50% have 500-1000 fishes while 15% has a population of more than 1000 fishes per pond (Appendix v).

Appendix vi shows that 100% of the farmers use commercial feed and source their water supply from borehole. While 10% of the farmers wash and change spent pond water weekly, 88% change their water fortnightly, and 2% wash and change spent water monthly. From appendix vii, it was observed that 85% of fish infection and death occurred during rainy season, while 15% occurred during dry season. Physiological and health conditions observed on the fishes show that 86% of the ponds had white fluffy patches on the fish, 64% blood spots on the fish skin, 12% descaling of the fishes and 54% complete tail loss when the fishes are infected.

4.6. Physico-chemical Analysis of the Water Samples from the Fish pond

Table 9a and 9b show the physico-chemical analysis of the fish pond water samples collected from 20 different fish farms in the 3 Senatorial zones in Anambra state. The temperature and pH values of the samples ranged from 27.1^0C-28.5^0C during the rainy season and 28.1^0C-32.1^0C in dry season, and 7.3- 8.0 in rainy seasons and 6.4-7.7 in dry seasons respectively. There were notable variations in the conductivity, turbidity, alkalinity, nitrite, phosphate and sulphate values. Priny Farms had the highest conductivity of 64 µS/cm, Nia-Agro had the highest turbidity of 10.9NTU, Errys Integrated farm had the highest alkalinity of 137.3mg/L, Orient writers had the highest nitrite value of 2.9mg/L, Aworonze fish Hatchery and Nursery farm had the highest phosphate value of 2.3 mg/L while Ikenwa and Sons Industries Limited had the highest sulphate value of 12.1mg/L. BOD values of the pond

waters ranged from 1.4 -2.7mg/L in rainy season and 1.1- 3.04mg/L in dry season, and and DO values ranged from 6.2 – 12.0mg/L in rainy season and 6.2-10.4mg/L in dry season respectively (Table 9)**.**

Table 9a: Physical characteristics of the water samples from the fish ponds

	Names of the fish pond	Temp (^{0}C)		pH		Conductivity (µS/cm)		Turbidity (NTU)	
		Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Anambra North	Priny farms	27.1±0.1	29.0±0.1	8.0±0.2	7.7±0.2	54.0±2.0	61.0±3.1	8.3±0.3	9.7±0.5
	Agabah farms	27.3±0.3	28.1±0.1	7.7±0.4	6.6±0.4	51.0±3.6	59.7±1.5	6.0±0.3	9.6±0.4
	Iyom Idiogo fish farms	28.0±0.2	30.3±0.6	7.7±0.7	6.4±0.2	47.3±3.1	56.7±5.0	7.0±0.2	9.3±0.6
	Ikenwa and sons Inds ltd	27.4±0.3	29.1±1.1	7.8±0.2	6.5±0.2	41.3±4.2	50.7±8.3	6.0±0.2	7.4±1.1
	Orient writers	28.2±0.3	29.6±0.5	7.7±0.4	6.8±0.5	45.3±4.2	57.3±4.2	9.7±0.1	9.7±0.5
	Udokamma fish sellers	28.2±0.2	29.6±0.8	7.5±0.6	6.9±0.1	42.0±5.3	49.0±2.6	6.4±0.2	7.7±1.3
Anambra South	De Rock Farm	28.2±0.3	31.3±0.6	7.8±0.2	6.8±0.1	40.0±9.2	43.3±6.5	5.5±0.6	6.3±0.9
	Okata Okoli farms	28.2±0.1	31.9±1.7	7.9±0.1	6.9±0.2	45.3±4.2	43.3±1.5	6.7±0.4	7.6±0.4
	Medosky farms	28.5±0.1	30.4±1.4	7.6±0.5	6.7±0.3	47.0±5.6	50.7±3.1	5.5±0.3	7.0±0.2
	Fish farmers Oraeri Co-op	28.5±0.2	31.4±1.5	7.7±0.2	6.9±0.3	52.7±11.0	54.7±4.2	8.4±0.6	8.3±1.1
	Nia Agro Investment	28.3±0.1	29.9±1.8	7.3±0.2	7.0±0.2	52.0±5.3	57.0±4.6	9.8±0.7	10.9±1.0
	Aworonze fish Hatchery	28.1±0.1	29.3±0.6	7.3±0.4	6.7±0.3	44.3±5.1	47.3±3.1	8.0±0.1	8.7±0.2
	Meniks farms	28.1±0.1	30.4±1.6	7.5±0.5	6.6±0.3	43.3±4.7	46.7±4.2	8.2±0.2	9.7±0.4
	Muokebe farms	28.0±0.2	32.1±0.9	7.4±0.2	6.4±0.4	52.0±5.3	55.3±4.7	7.9±0.3	8.4±1.8
Anambra Central	Errys Integrated farm	28.2±0.1	30.1±0.9	8.0±0.1	6.9±0.8	44.7±7.6	43.0±4.6	7.9±0.9	9.6±0.5
	New Age fish farm	27.2±0.3	29.9±1.8	7.5±0.1	6.9±0.2	42.7±6.4	47.3±3.1	8.7±0.4	8.9±1.1
	Ecoworld Agro Ventures	27.2±0.4	28.9±0.9	7.4±0.4	6.9±0.4	46.0±7.2	40.7±3.2	7.1±0.3	7.1±0.3
	Green field & fish farm invest	27.6±0.3	31.4±2.1	7.4±0.2	6.5±0.2	44.7±11.0	50.0±2.0	8.1±0.2	9.3±0.6
	Jipet fish farm	27.4±0.4	29.3±1.1	7.3±0.4	6.8±0.4	46.0±10.6	58.3±2.5	8.3±1.5	9.6±0.7
	Adika farms	28.2±0.2	31.0±1.0	7.3±0.4	6.8±0.2	40.0±2.0	40.3±2.5	7.6±0.7	8.6±0.6
	WHO Limit	20-30^{0}C	20-30^{0}C	6.5-9	6.5-9	20-1500	20-1500	5-25	5-25

Table 9b: Chemical characteristics of the water samples from the fish ponds

	Names of the fish pond	Alkalinity (mg/l)		Nitrite (mg/l)		Phosphate (mg/l)		Sulphate (mg/l)		BOD (mg/l)		DO (mg/l)	
		Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Anambra North	Priny farms	118.3±15.5	112.7±7.0	1.9±0.2	2.1±0.2	2.1±0.2	2.2±0.2	8.1±0.3	9.7±0.6	2.2±0.3	3.0±0.2	8.5±0.4	8.6±0.1
	Agabah farms	117.3±17.5	118.0±7.0	2.2±0.1	2.6±0.2	1.8±0.2	2.0±0.2	9.1±0.5	10.4±0.4	1.9±0.2	1.8±0.1	7.3±0.5	7.7±0.2
	Iyom Idiogo fish farms	104.0±7.2	94.0±8.7	2.5±0.2	2.9±0.1	1.4±0.2	1.2±0.2	9.4±0.4	9.2±0.4	2.7±0.2	2.4±0.3	7.4±0.2	7.2±0.1
	Ikenwa and sons Inds ltd	118.7±13.8	92.0±5.3	2.2±0.3	2.4±0.2	1.1±0.3	1.0±0.2	10.0±0.5	12.1±0.3	2.4±0.4	2.7±0.1	6.2±0.1	6.2±0.1
	Orient writers	127.0±23.1	132.0±2.0	2.9±0.2	2.9±0.1	1.6±0.1	1.6±0.3	8.4±0.2	9.0±0.2	1.4±0.2	1.2±0.2	10.1±0.3	8.3±0.1
	Udokamma fish sellers	124.7±5.5	123.3±4.9	2.1±0.5	1.8±0.2	1.2±0.7	0.9±0.1	6.2±0.2	6.5±0.1	2.1±0.2	2.3±0.3	8.5±0.3	7.5±0.1
Anambra South	De Rock Farm	120.3±4.5	101.3±3.1	2.2±0.5	1.9±0.8	1.7±0.1	1.5±0.3	6.9±0.3	8.1±0.3	1.8±0.1	1.6±0.1	9.3±0.3	8.5±0.2
	Okata Okoli farms	123±10.1	120.0±8.5	1.6±0.3	1.9±0.1	1.8±0.2	1.9±0.1	9.9±0.3	10.1±0.5	1.4±0.1	1.1±0.1	9.4±0.3	8.5±0.5
	Medosky farms	108.7±6.7	100.0±2.0	1.7±0.2	2.3±0.4	1.0±0.1	1.1±0.1	8.0±0.21	8.2±0.3	1.4±0.1	1.2±0.2	9.3±0.2	9.2±0.1
	Fish farmers Oraeri Co-op	129.7±2.5	122.7±5.0	2.0±0.2	2.4±0.5	1.0±0.2	1.2±0.2	6.3±0.1	8.5±0.3	2.3±0.2	2.2±0.2	12.0±0.4	10.4±0.2
	Nia Agro Investment	121.7±17.2	120.0±1.0	1.8±0.1	2.0±0.2	1.7±0.1	1.8±0.1	9.9±0.3	11.2±0.4	3.2±0.1	2.6±0.3	7.3±0.6	6.9±0.4
	Aworonze fish Hatchery	100.3±2.5	104.7±6.4	1.4±0.3	1.4±0.1	2.1±0.5	2.3±0.3	8.9±0.2	10.6±0.4	2.5±0.5	2.6±0.3	9.9±0.6	9.6±0.2
	Meniks farms	110.0±9.2	106.7±4.2	1.6±0.2	2.0±0.3	0.8±0.3	0.9±0.3	6.1±0.1	7.4±1.0	2.4±0.5	2.3±0.2	9.4±0.4	8.6±0.1
	Muokebe farms	107.0±12.3	101.3±6.1	1.1±0.1	1.3±0.4	1.8±0.2	2.1±0.1	6.8±0.2	7.1±0.2	1.9±0.1	1.7±0.1	6.3±0.2	6.6±0.3
Anambra Central	Errys Integrated farm	137.3±7.4	130.0±2.0	1.6±0.1	1.6±0.1	1.9±0.1	1.8±0.2	9.9±0.2	10.0±0.3	2.1±0.2	2.1±0.1	6.7±0.5	6.8±0.1
	New Age fish farm	135.3±3.1	133.7±5.7	1.5±0.1	1.6±0.1	1.6±0.1	1.7±0.2	8.5±0.7	9.5±0.5	2.4±0.3	2.4±0.2	10.1±0.1	10.1±0.2
	Ecoworld Agro Ventures	134.0±4.6	120.0±14.8	2.0±0.2	2.3±0.2	0.9±0.3	1.1±0.6	8.0±0.1	8.8±0.2	1.6±0.1	1.6±0.1	6.9±0.3	6.7±0.4
	Green field & fish farm invest	105.3±7.2	109.3±16.3	1.4±0.1	1.8±0.2	0.9±0.2	1.2±0.1	9.1±0.2	10.3±0.6	1.7±0.1	1.7±0.1	9.7±0.6	9.5±0.3
	Jipet fish farm	114.7±5.7	108.0±6.0	1.2±0.1	1.3±0.1	1.3±0.1	1.4±0.2	8.9±0.3	10.1±0.5	2.7±0.2	2.6±0.1	6.6±0.4	6.3±0.3
	Adika farms	127.0±4.4	118.7±9.1	1.3±0.8	1.2±0.4	1.2±0.1	1.2±0.1	7.2±0.4	8.3±0.8	2.4±0.3	2.2±0.1	8.3±0.3	8.2±0.1
	WHO Limit	25-100	25-100	0.03-2	0.03-2	0.03-3	0.03-3	250	250	10	10	5-9.5	5-9.5

4.7. Pathogenicity Testing of the Bacterial Isolates on Healthy Fish and Mice

4.7.1. Effects of oral administration of bacterial isolates on hematology of fishes

The effects of the oral administration of the bacterial isolates on the healthy fish as presented in Table 10 show some observable changes in the hematological profile of the fishes. Exposure of the fishes to the different bacterial isolates had significant effects on the hemoglobin level (p value < 0.05). A continuous decrease in the hemoglobin level was observed in the fishes exposed to *L.sphaericus, P. aeruginosa* and *S.marcescens* throughout the experimental period. No decrease in the control group. For the groups inoculated with *B. subtilis* and *P. suwonensis* there was a rise in hemoglobin level during the first week and then a decrease in the second week (Table 10). Although there's significant difference in the effects of the bacterial isolates on the hemoglobin of fishes, *Pseudomonas aeruginosa* and *Lysinibacillus sphaericus* showed no significant difference (p = 0.746) [Appendix viii].

Effects of the bacterial isolates on packed cell volume of the fishes are presented in Table10. Continous decrease in the packed cell volume (PCV) was observed in all the groups. Although the PCV level increased in group D and E by week 1, they decreased by second week of exposure. Statistically, the effects of the bacterial isolates on the PCV level of the fishes were significantly different during the experimental period (p value < 0.05) [Appendix ix].

The Red blood cell (RBC) level of the fishes decreased throughout the experimental period in group B, C, D and F. Group A and E experienced slight increase within the first week of exposure, but decreased markedly by the second week. *Bacillus subtilis* had the highest effect on the red blood cell value of the fishes (Appendix x).

An observable increase in white blood cell (WBC) levels was observed in the fishes after 2 weeks exposure to the bacterial isolates (Table 10). *Lysinibacillus sphaericus* had the highest effect on the white blood cell of the fishes. *Paenalcaligenes suwonensis* and *Bacillus subtilis* fell under the same subset as well as *Pseudomonas aeruginosa* and *Serratia marcescens,* showing no significant difference (p value > 0.05) between their respective effects in the subsets [Appendix xi].

A decrease was observed in the lymphocyte levels in the first week in all the groups except the group inoculated with *P. suwonensis* (group E), and then subsequent increase in

the second week. The effects of the bacterial isolates on the lymphocyte level of the exposed fishes were statistically significant (p value < 0.05) [Appendix xii].

A significant difference on the monocyte levels of the fishes was observed upon exposure of the fishes to the bacterial isolates. *Lysinibacillus sphaericus* and *Bacillus subtilis* had the same effect on the monocyte level of the fishes at p value 0.433, while the effects of the other isolates vary (Appendix xiii).

Table 10: Effects of the bacterial isolates on hematology profile of the fish

Group	Bacterial isolate	Before inoculation	1 week	2 weeks
Haemoglobin level (g/dl)				
A	*L.sphaericus*	8.73± 0.42	7.48± 0.75	6.57± 0.65
B	*P. aeruginosa*	9.28± 0.45	7.58± 0.68	5.27± 0.57
C	*S. marcescens*	9.03± 0.31	8.50 ± 1.18	7.77±0.76
D	*B. subtilis*	8.37± 0.38	10.27±1.04	7.07±0.86
E	*P. suwonensis*	9.17± 0.50	10.45±0.95	8.47±0.78
F	Control	9.37± 0.13	9.15±0.84	9.13±0.86
Packed cell volume (%)				
A	*L.sphaericus*	28.03±1.05	25.72±0.54	24.33±0.75
B	*P. aeruginosa*	28.08±0.87	24.18±1.09	17.30±0.70
C	*S. marcescens*	30.13±0.96	26.33±0.98	24.20±0.42
D	*B. subtilis*	25.33±0.70	29.24±0.77	23.30±0.75
E	*P. suwonensis*	28.83±1.21	29.00±0.90	24.79±0.45
F	Control	29.00±0.30	34.00±0.27	34.00±0.81
Red blood cell (10^6/mm^3)				
A	*L.sphaericus*	2.35±0.48	2.53±0.40	1.37±0.71
B	*P. aeruginosa*	2.67±0.62	2.37±0.64	1.56±0.33
C	*S. marcescens*	2.33±0.40	1.63±0.69	1.55±0.44
D	*B. subtilis*	5.00±0.71	2.06±0.11	1.93±0.07
E	*P. suwonensis*	2.38±0.48	2.44±0.49	2.12±0.54
F	Control	3.03±0.16	3.00±0.12	3.30±0.87
White blood cell (10^3/mm^3)				
A	*L.sphaericus*	16.33±0.59	26.45±1.03	28.24±0.75
B	*P. aeruginosa*	16.27±0.83	15.38±1.04	18.52±0.82
C	*S. marcescens*	15.83±1.30	15.82±0.42	19.36±1.00
D	*B. subtilis*	15.27±0.81	19.75±1.03	27.44±0.71
E	*P. suwonensis*	15.43±0.99	21.33±1.10	25.37±1.12
F	Control	16.33±1.03	16.33±1.22	16.11±1.14
Lymphocyte (%)				
A	*L.sphaericus*	90.17±9.95	81.15±0.91	94.33±1.43
B	*P. aeruginosa*	86.33±1.33	67.29±0.79	88.33±1.33
C	*S. marcescens*	80.33±1.43	77.37±0.79	90.33±1.23
D	*B. subtilis*	82.03±1.05	73.25±0.98	91.33±1.14
E	*P. suwonensis*	80.12±1.02	83.41±1.16	90.07±1.09
F	Control	80.18±1.04	80.11±0.88	80.13±1.06
Monocyte (%)				
A	*L.sphaericus*	9.34±0.72	11.35±0.69	13.33±1.43
B	*P. aeruginosa*	8.40±0.79	11.33±0.88	11.38±0.94
C	*S. marcescens*	8.63±0.86	10.48±0.84	10.41±0.97
D	*B. subtilis*	8.37±1.00	13.39±0.94	13.39±0.92
E	*P. suwonensis*	8.37±1.09	13.31±0.94	13.15±1.09
F	Control	8.37±1.19	8.30±0.73	8.33±1.06

4.7.2. Effects of isolates on blood chemistry of fishes

The effects of the bacterial isolates on blood chemistry profile of the fishes are presented in Table 11. Exposure of the fishes to the bacterial isolates resulted in significant increase in the aspartate transaminase (AST) enzyme levels all through the experimental period except in the group inoculated with *L.sphaericus*, which showed slight decrease after the first week of inoculation. Statistically, all the isolates had significant effect (p value < 0.05) on the AST value of the fishes [Appendix xiv].

Varying levels of effect were observed in the alanine transaminase (ALT) enzyme level of the fishes exposed to the bacterial isolates. The ALT increased in the 1st and 2nd weeks except slight decrease in *S. marcescens*. However, the effects of the bacterial isolates on the alanine transaminase enzyme level of the fishes were significantly different (p value < 0.05) [Appendix xv].

4.7.3. Effects of isolates on weight of fishes

Table 12 shows the effects of the bacterial isolates on the weights of the fishes. The initial weight of the 10 week old fishes ranged from 125.97±1.05g to 137.13±0.19. At the end of the 2 weeks exposure to the bacterial isolates, the fishes attained weight ranging from 132.00±2.65g to 218.67±7.09g. Percentage weight gain ranged from 4.8% to 59.5%. With regard to the bacterial isolates and experimental periods, a significant difference in weight of the fishes was observed (p value < 0.05) [Appendix xvii]. As observed, the percentage weight gains for the inoculated fishes are less than that of a control.

Table 11: Effects of the bacterial isolates on blood chemistry profile of the fish

Group	Bacterial isolate	Before inoculation	1 week	2 weeks
	Mean Aspartate Transaminase Values			
A	*L.sphaericus*	31.33±1.20	80.32±1.09	79.87±0.61
B	*P. aeruginosa*	31.33±0.86	71.94±0.06	76.00±0.77
C	*S. marcescens*	32.33±1.15	79.26±0.89	82.03±0.88
D	*B. subtilis*	31.33±1.09	69.99±0.51	72.00±0.89
E	*P. suwonensis*	30.33±1.06	80.00±0.76	91.00±0.78
F	Control	30.33±1.07	33.17±0.91	33.94±0.86
	Mean Alanine Transaminase Values			
A	*L.sphaericus*	22.33±1.02	24.00±0.75	29.03±0.80
B	*P. aeruginosa*	23.33±1.05	25.16±0.79	27.01±0.68
C	*S. marcescens*	28.90±0.69	29.03±0.83	28.97±0.62
D	*B. subtilis*	23.19±0.64	26.02±0.74	27.93±0.49
E	*P. suwonensis*	29.22±0.46	29.80±0.58	30.00±2.03
F	Control	21.15±1.05	21.84±0.64	22.02±0.80

Table 12: Effects of the bacterial isolates on weight of the fish

		Weight (g)			
Group	**Isolate**	**Before inoculation**	**1 week**	**2 weeks**	%weight gain
A	*L.sphaericus*	135.31±0.91	164.67±13.05	174.33±4.72	28.8
B	*P. aeruginosa*	136.33±1.04	142.67±2.52	144.00±4.00	5.6
C	*S. marcescens*	125.97±1.05	125.67±5.13	132.00±2.65	4.8
D	*B. subtilis*	137.13 ±0.19	177.33±1.89	218.67±7.09	59.5
E	*P. suwonensis*	136.36±0.63	136.94±1.19	163.00±3.61	19.5
F	Control	131.33 ±0.67	185.00±2.00	218.33±7.64	66.2

4.7.4. Effects of administration of the isolates on the bacterial load of the fish internal organs

The mean bacterial count in gill and liver of fishes during experiment are presented in Fig.4 and 5. There was significant difference in the bacterial count in the gills of the fishes infected with the bacterial isolates (p value < 0.05) [Appendix xvii]. The mean bacterial count 2 weeks after inoculation ranged from $26.93\times10^4\pm1.43$cfu/g to $49.02\times10^4\pm2.79$cfu/g (Fig.4). There was significant increase (p value < 0.05) in the bacterial counts obtained after the experimental period. Highest bacterial count was observed in the group inoculated with *L.sphaericus* while the least was observed in *B. subtilis* (Appendix xvii).

Oneway ANOVA of the bacterial counts obtained in the livers of the fishes upon inoculation of the bacterial isolates (Appendix xviii) shows that there was significant difference (p value < 0.05) in the bacterial counts obtained in all the groups during the experimental period. After 2 weeks inoculation, the bacterial counts obtained ranged from $5.48\times10^4\pm0.07$cfu/g to $20.67\times10^4\pm1.53$cfu/ml (fig.5).

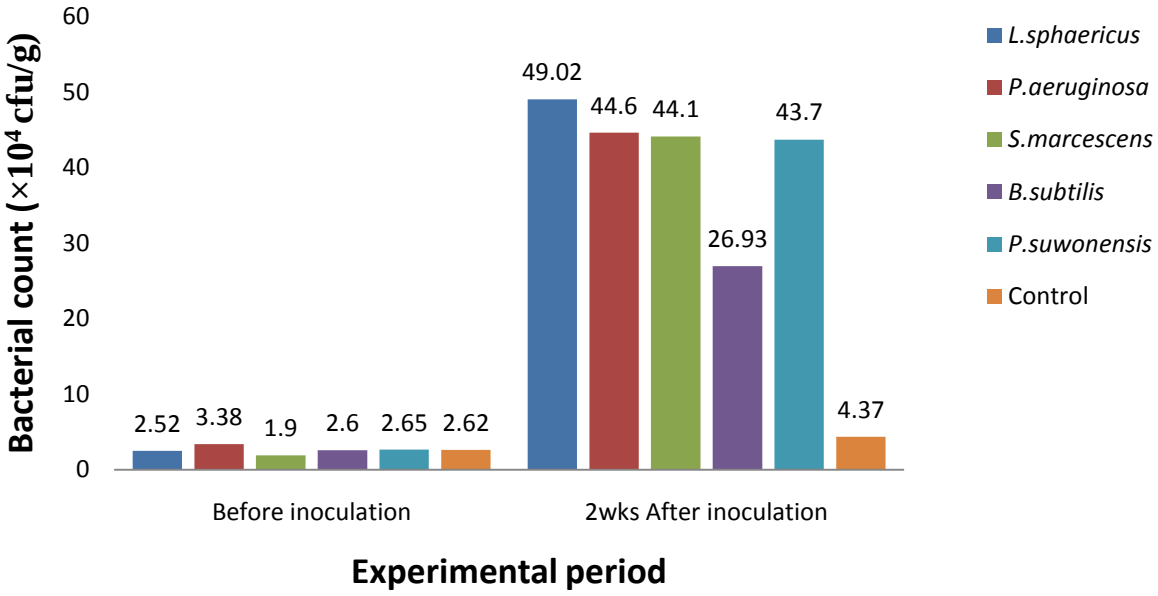

Fig.4:Bacterial count in gills of fishes before and after inoculation

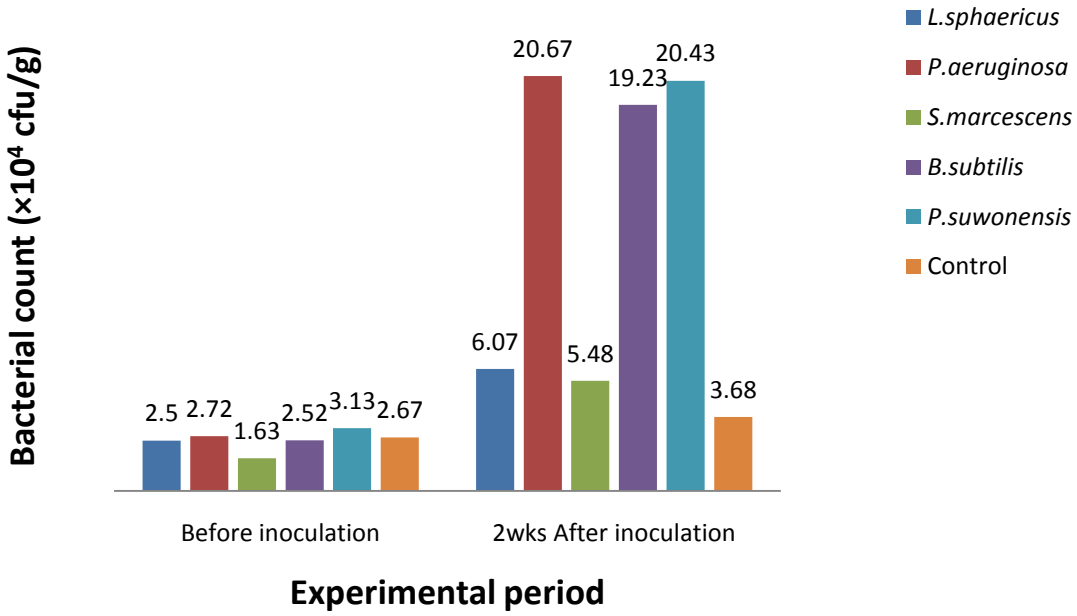

Fig.5: Bacterial count in the livers of fishes before and after inoculation

4.7.5. Histological Examination of Vital Organs of fishes exposed to the bacterial isolates

Plates 1 - 6 show photomicrographs of gill tissues of fish in groups A – F. Plate 1 shows normal gill structure with striated abductormuscle and gill lamellae. Plates 2 and 3 show distorted gill structure of fishes inoculated with *Pseudomonas aeruginosa* and *Serratia marcescens.* Plates 4, 5 and 6, show gill structure, with little or no distortion of the gill architecture.

Plates 7 – 12 show the photomicrographs of the liver tissues of fish in group A – F. Plate 7, 10 and 12, showed normal liver architecture, while Plates 8, 9 and 11, showed visible signs of slight liver impairments.

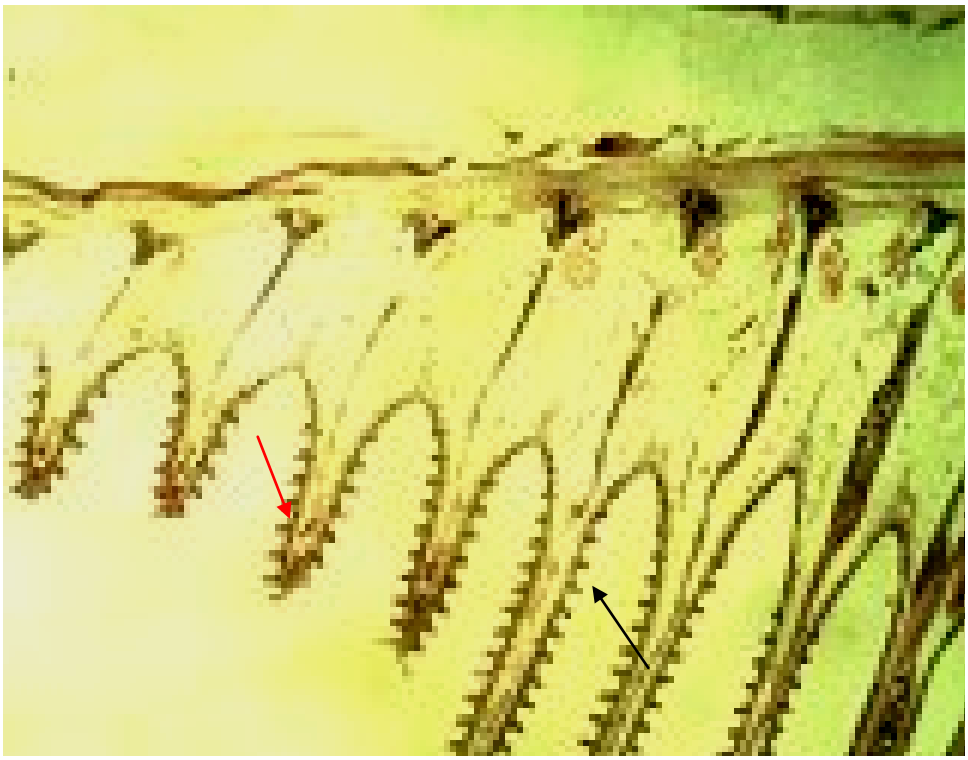

Plate 1: Photomicrograph of fish gills inoculated with *L. sphaericus* showing normal gill structure, striated abductor muscle (black arrow), and the gill lamellae (red arrow)

Plate 2: Photomicrograph of fish gills inoculated with *P.aeruginosa* showing curled lamella tip (black circle)

Plate 3: Photomicrograph of fish gills inoculated with *S. marcescens* showing curled lamellae (black circle).

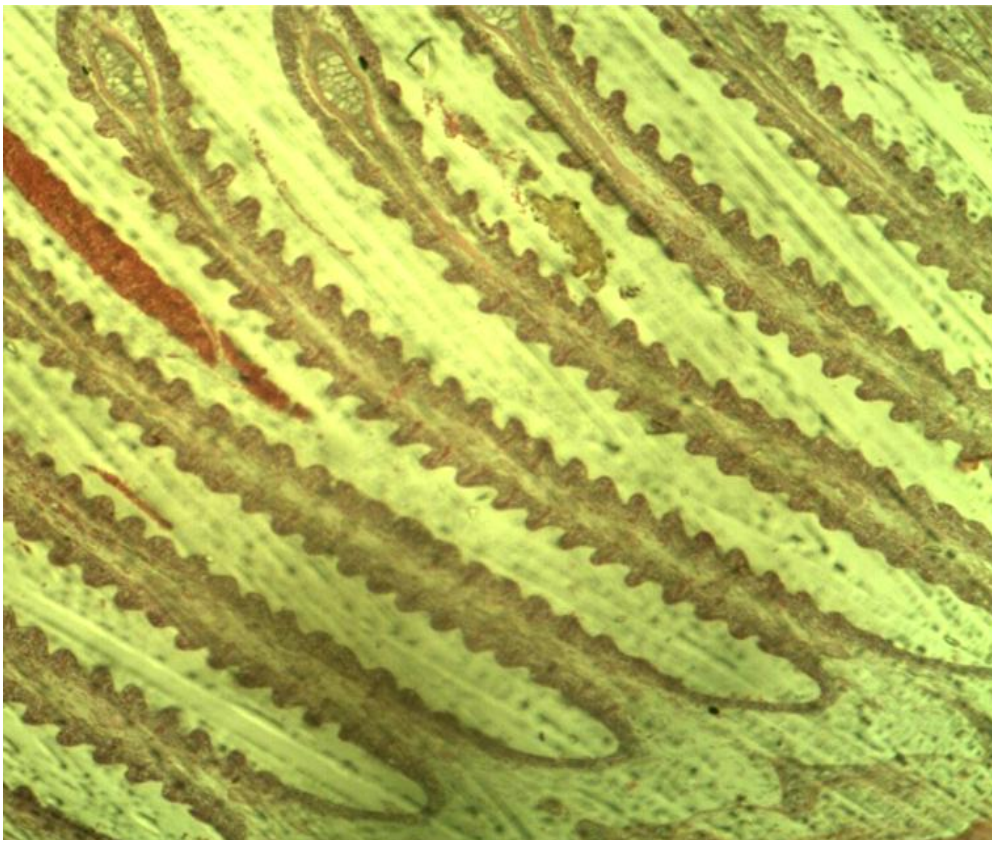

Plate 4: Photomicrograph of fish gills inoculated with *B. subtilis* showing no structural damage

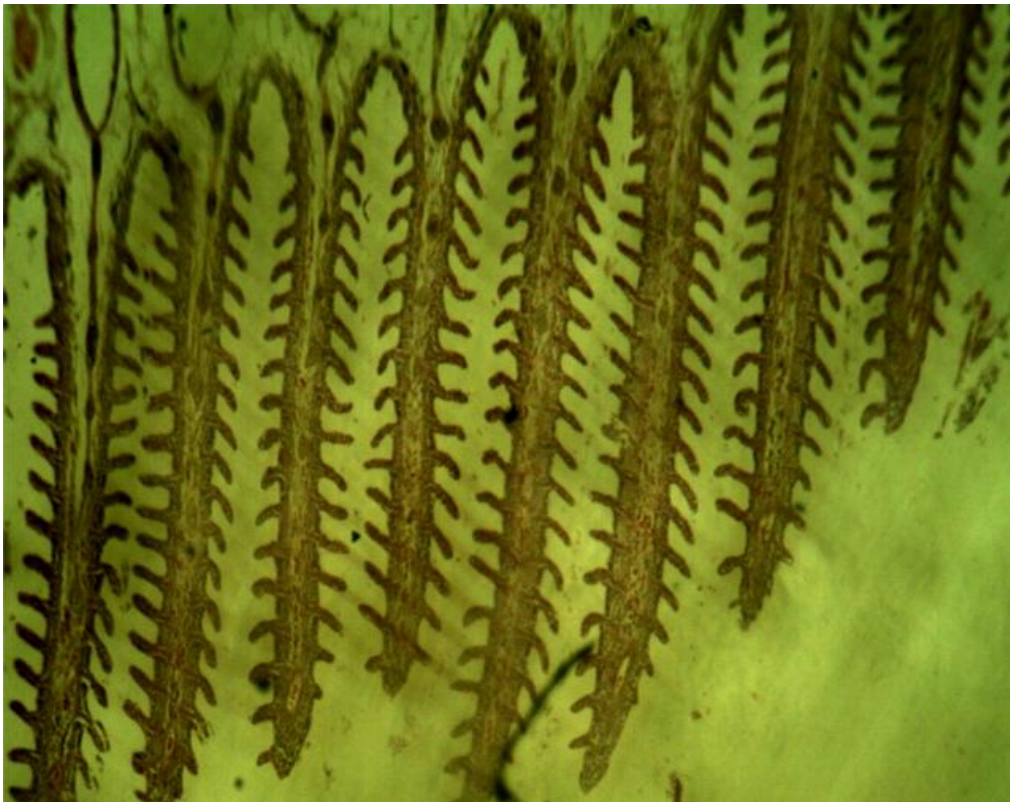

Plate 5: Photomicrograph of fish gills inoculated with *P. suwonensis* showing intact gill architecture

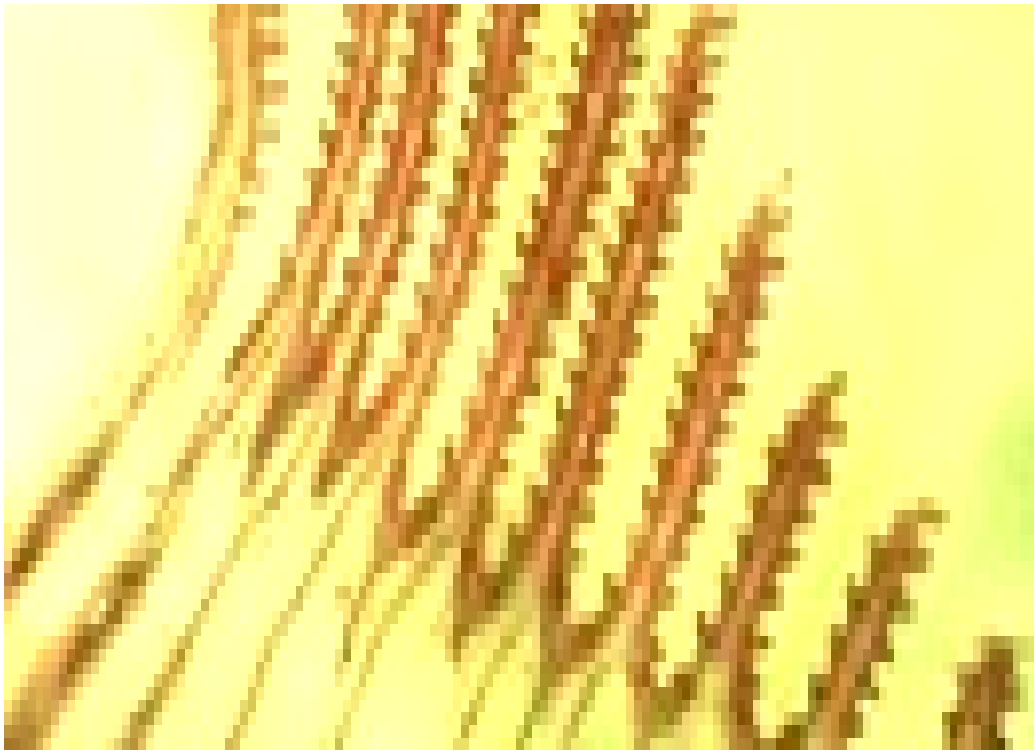

Plate 6: Photomicrograph of control fish gills showing intact gill architecture

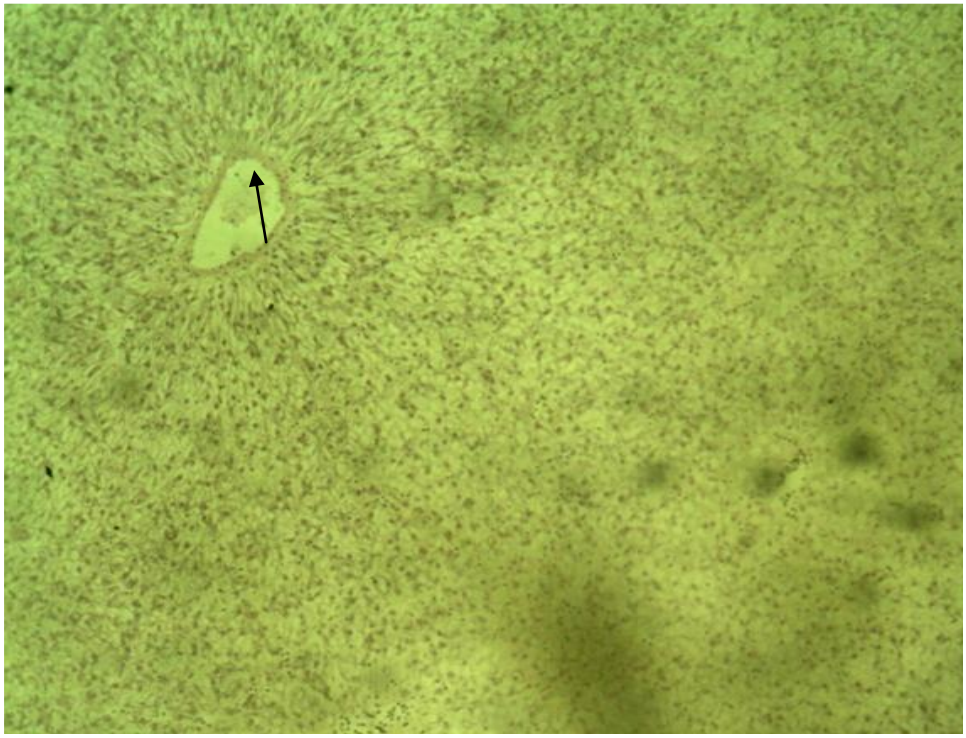

Plate 7: Photomicrograph of fish liver tissue inoculated with *L. sphaericus* showing normal liver architecture, central vein (black arrow)

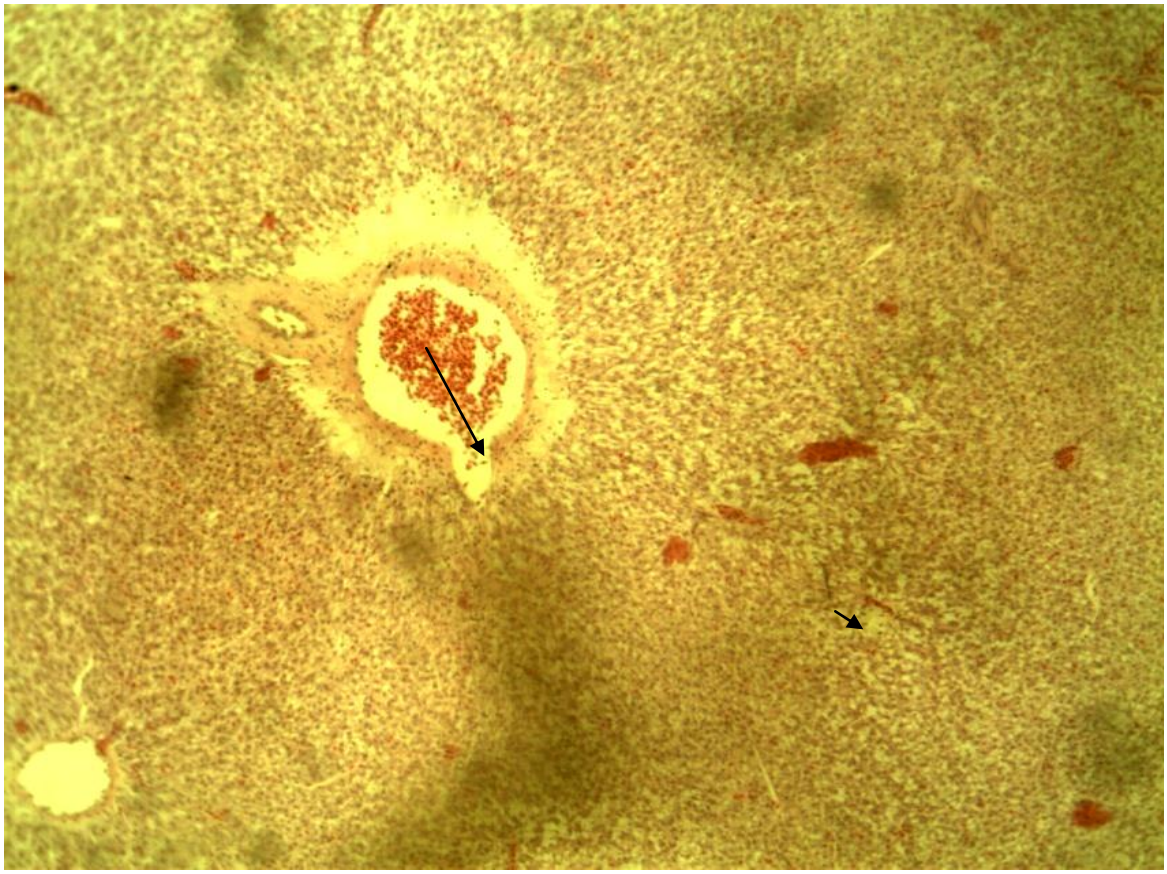

Plate 8: Photomicrograph of fish liver inoculated with *P. aeruginosa* showing periportal inflammatory cell infiltrates (Black arrow).

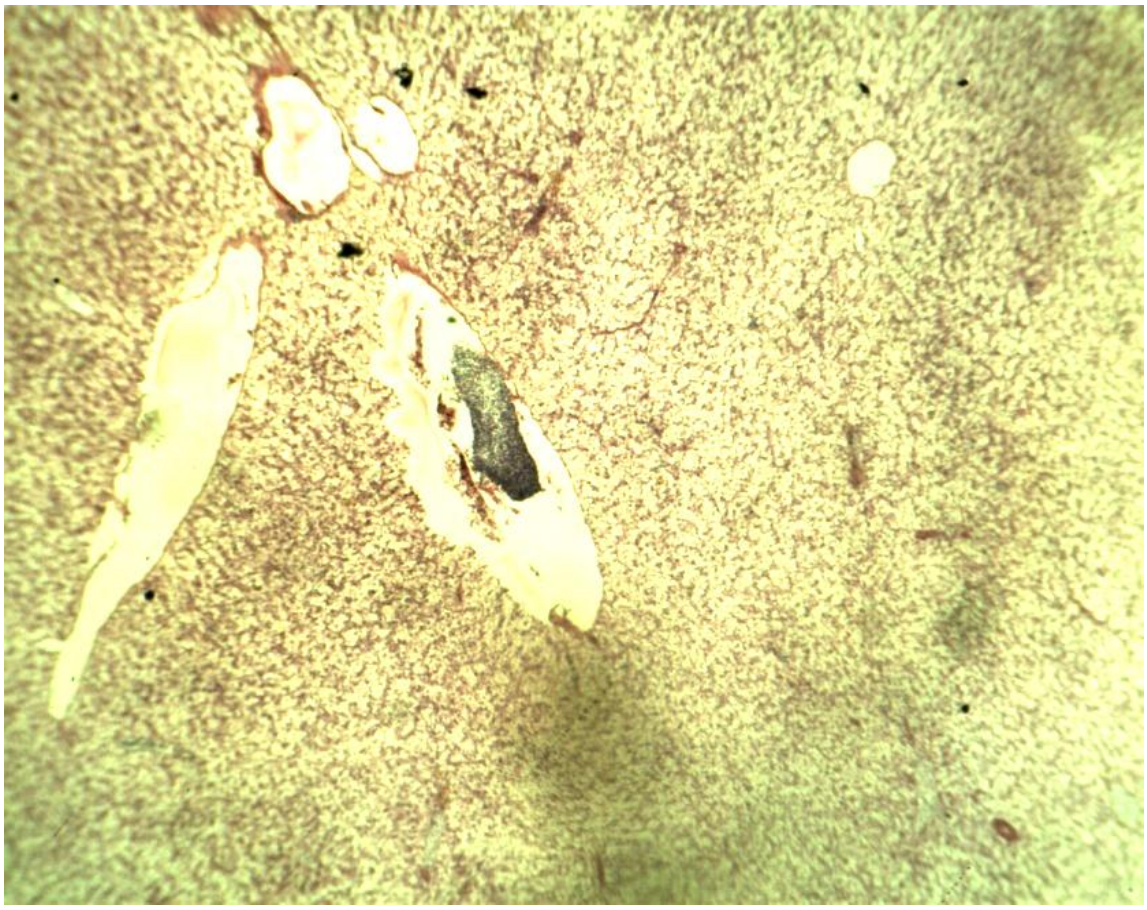

Plate 9: Photomicrograph of fish liver inoculated with *S. marcescens* showing abnormally widened intercalated duct

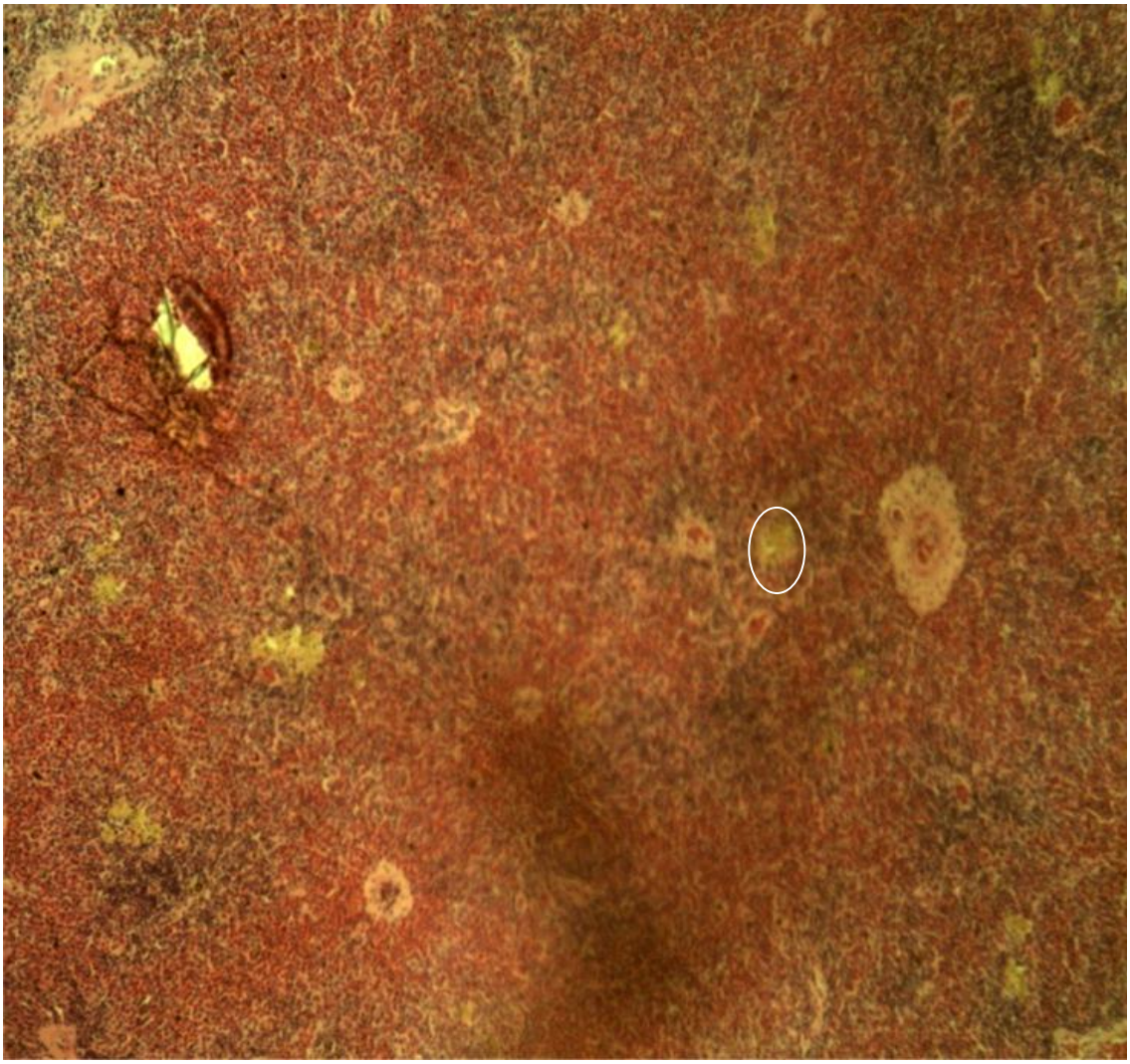

Plate 10: Photomicrograph of fish liver inoculated with *B. subtilis* showing prominent acini (white circle).

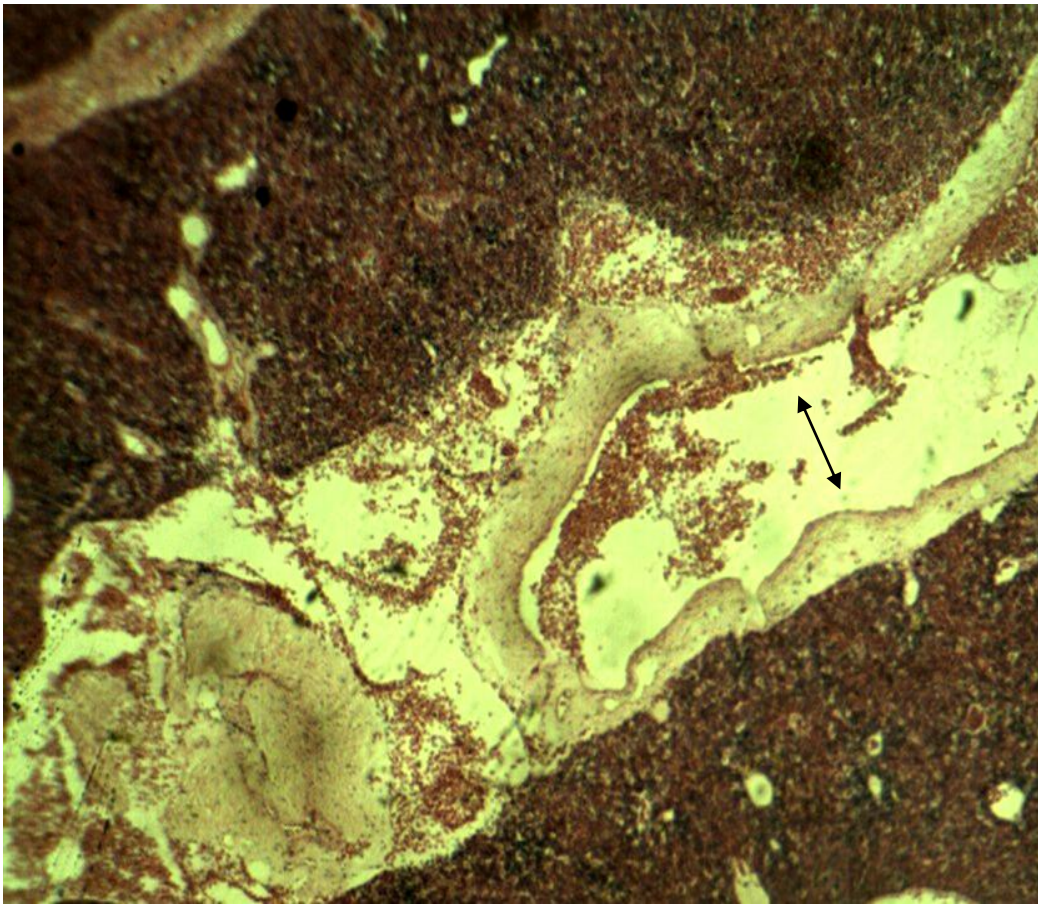

Plate 11: Photomicrograph of fish liver inoculated with *P. suwonensis*, showing widening of liver sinusoids (Black arrow heads)

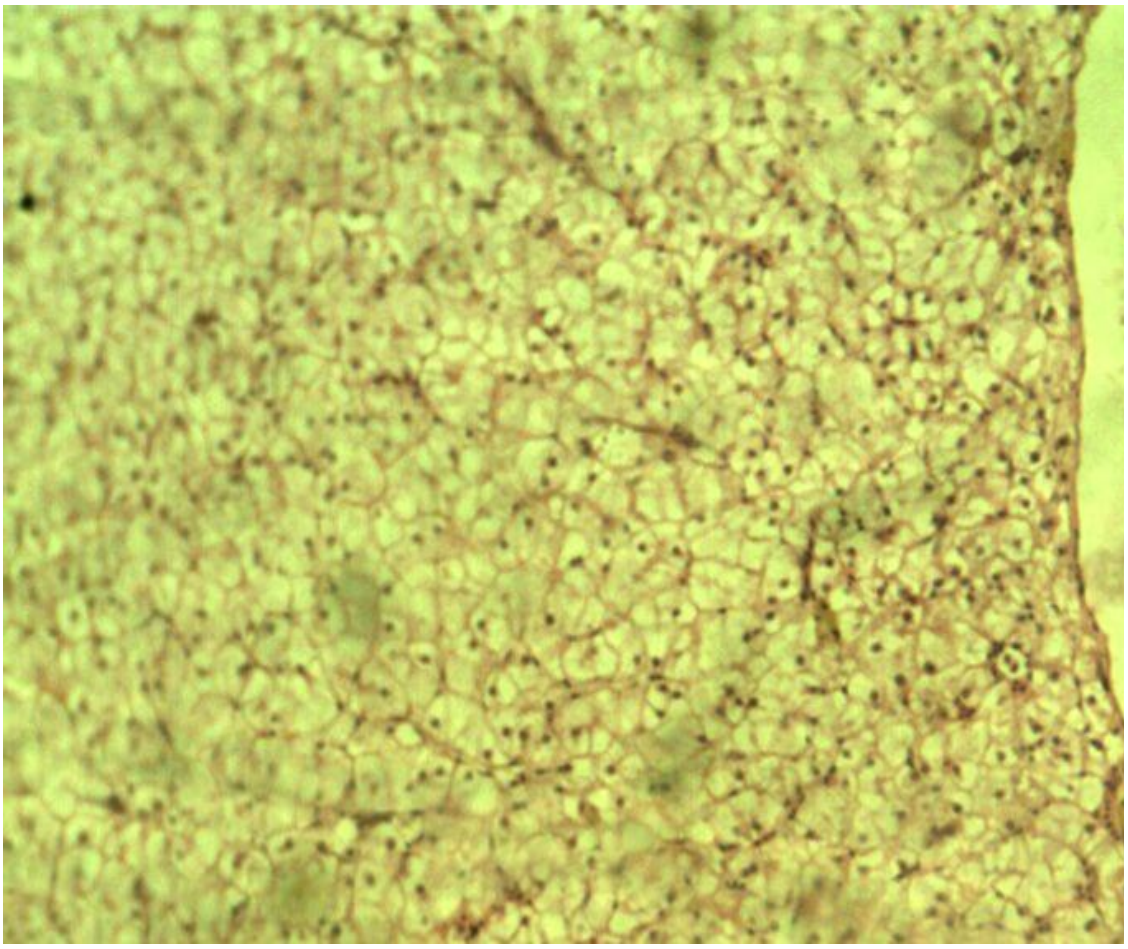

Plate 12: Photomicrograph of control fish liver showing normal liver architecture with no impairment.

4.7.6. Effects of Oral and Subcutaneous Administration of Bacterial Isolates on Healthy Mice

4.7.6.1. Effects of oral administration of bacterial isolates on hematology of mice

Effects of the bacterial isolates administered orally to mice on the hematological profile are shown in table 13. Variable results were observed in all groups after one week of inoculation but decreased after two weeks except in *P. suwonensis* where an increase was maintained. There was significant difference (p value < 0.05) in the effects on the hemoglobin values by the bacterial isolates [Appendix xix]. *Bacillus subtilis* had the most significant effect while *Paenalcaligenes suwonensis* had the least significant effect on the hemoglobin value of the mice.

Varying effects of the bacterial isolates on the packed cell volume (PCV) level of mice were observed after one week of inoculation and a decrease after two weeks inoculation except in *S. marcescens* and *P. suwonensis* where an increase was observed (Table 13). Statistically, the effects of the isolates on the PCV level of the infected mice were significant (p value < 0.05) [Appendix xx].

All the isolates showed significant effect (p value<0.05) on the red blood cells of the mice. Level of the red blood cells of the mice decreased after the experimental period in the groups inoculated with *P. aeruginosa, S. marcescens* and *P. Suwonensis* but not in *B. subtilis*.

The effects of the bacterial isolates on the white blood cell count (WBC) of the mice (Table 13) shows that WBC counts increased after one week of inoculation in all the groups except group A. At the end of the experiment, there was an increase in the white blood cells except in *P. aeruginosa.* However, there was no significant difference (p value > 0.05) in the effects on WBC of the mice upon oral administration of the isolates [Appendix xxii].

The exposure of the bacterial isolates to the mice caused varying changes in the lymphocyte values of the mice. Resultant increase in lymphocyte values were observed in all the groups at the end of the experiment as against the control group (Table 13).

With regard to the effects of the bacterial isolates on the monocyte values of the challenged mice, a decrease was observed in all the groups except in groups B and C, in which there was an increase at the end of experiment. However, there was no significant difference (p value = 0.942) in the effect of the isolates on the monocytes [Appendix xxiv].

Table 13: Effects of orally administered bacterial isolates on hematology profile of the mice

Group	Bacterial isolate	Before inoculation	1week	2weeks
Haemoglobin level (g/dl)				
A	*L.sphaericus*	9.60±0.30	9.97±0.42	8.10±0.32
B	*P. aeruginosa*	8.79±0.11	8.20±0.59	6.50±0.56
C	*S. marcescens*	10.4±0.36	8.57±0.35	8.53±0.06
D	*B. subtilis*	10.2±0.26	11.45±0.39	8.50±0.25
E	*P. suwonensis*	6.27±0.25	7.40±0.36	8.37±0.40
F	Control	7.33±0.35	7.17±0.29	7.20±0.26
Packed cell volume (%)				
A	*L.sphaericus*	22.33±2.52	32.33±1.15	24.40±0.36
B	*P. aeruginosa*	24.35±0.31	24.55±0.48	20.34±0.29
C	*S. marcescens*	31.63±0.15	24.45±0.15	25.41±0.28
D	*B. subtilis*	30.30±0.44	33.57±0.21	25.20±0.26
E	*P. suwonensis*	19.40±0.35	22.69±0.19	25.47±0.45
F	Control	21.63±0.12	21.43±0.16	21.25±0.48
Red blood cell (10^6/mm^3)				
A	*L.sphaericus*	10.5±0.30	9.40±0.26	9.43±0.33
B	*P. aeruginosa*	12.35±0.33	11.47±0.22	10.68±0.19
C	*S. marcescens*	12.63±0.22	12.23±0.38	10.43±0.50
D	*B. subtilis*	12.07±0.45	12.07±0.38	13.33±0.21
E	*P. suwonensis*	12.63±0.06	11.73±0.21	10.53±0.15
F	Control	10.50±0.20	10.70±0.20	10.60±0.26
White blood cell (10^3/mm^3)				
A	*L.sphaericus*	21.38±0.34	19.73±0.25	37.43±0.21
B	*P. aeruginosa*	14.43±0.32	18.83±0.15	11.63±0.47
C	*S. marcescens*	10.70±0.20	15.33±0.47	21.72±0.68
D	*B. subtilis*	14.57±0.35	17.97±0.96	15.07±0.15
E	*P. suwonensis*	11.73±0.75	13.50±0.40	19.20±0.82
F	Control	14.60±0.36	14.77±0.25	14.20±0.26
Lymphocyte (%)				
A	*L.sphaericus*	46.60±0.66	44.53±0.49	49.87±0.78
B	*P. aeruginosa*	48.80±0.26	48.33±0.21	60.54±0.12
C	*S. marcescens*	35.40±0.17	37.83±0.32	57.47±0.45
D	*B. subtilis*	37.43±0.45	37.40±0.36	38.57±0.60
E	*P. suwonensis*	22.53±0.47	29.80±0.70	41.07±1.97
F	Control	34.53±0.47	34.43±1.00	34.10±0.10
Monocyte (%)				
A	*L.sphaericus*	2.70±0.82	2.93±0.31	1.23±0.25
B	*P. aeruginosa*	1.43±0.21	1.73±0.42	3.10±0.36
C	*S. marcescens*	1.73±0.68	1.49±0.27	2.29±0.28
D	*B. subtilis*	1.79±0.41	1.63±0.57	1.66±0.61
E	*P. suwonensis*	2.62±0.11	2.40±0.36	1.23±0.25
F	Control	1.35±0.27	1.20±0.26	1.21±0.35

4.7.6.2. Effects of oral administration of bacterial isolates on blood chemistry profile of mice

The effects of oral inoculation of the bacterial isolates on the blood chemistry profile of the mice are shown in Table 14. The aspartate transaminase (AST) values of the mice increased at the end of the experiment when compared with the initial AST values in all the groups, except in group D and E infected with *B.subtilis* and *P.suwonensis* respectively. The effect of the isolates on the aspartate transaminase values of the mice was significantly different (p value < 0.05) [Appendix xxv].

An increase in alanine transaminase enzyme value was observed in the mice infected by the bacterial isolates at the end of the experiment (Table 14). Statistically, the effects of the isolates on the ALT of the mice were significant (p value < 0.05).

Table 14: Effects of orally administered bacterial isolates on blood chemistry profile of the mice

Group	Bacterial isolate	Before inoculation	1 week	2 weeks
Mean Aspartate Transaminase Values				
A	*L.sphaericus*	47.22±0.33	49.32±0.36	51.43±0.49
B	*P. aeruginosa*	48.29±0.27	51.31±0.29	54.64±0.34
C	*S. marcescens*	43.27±0.17	55.27±0.29	55.62±0.93
D	*B. subtilis*	48.18±0.28	44.40±0.44	40.27±0.37
E	*P. suwonensis*	47.21±0.25	45.33±0.18	43.24±0.28
F	Control	48.50±0.36	48.29±0.19	48.22±0.33
Mean Alanine Transaminase Values				
A	*L.sphaericus*	11.13±0.09	13.74±0.65	14.58±0.80
B	*P. aeruginosa*	11.53±1.16	14.27±0.24	19.52±0.85
C	*S. marcescens*	11.28±0.19	17.35±0.41	19.07±0.98
D	*B. subtilis*	11.20±0.23	15.72±0.68	15.53±0.84
E	*P. suwonensis*	11.86±0.73	15.35±0.48	14.19±0.47
F	Control	13.23±0.24	13.57±0.38	13.39±0.18

*** values represent mean ± SD**

4.7.6.3. Effects of Subcutaneous Inoculation of Bacterial Isolates on the Hematology Profile of Mice

The effects of the subcutaneous inoculation of the bacterial isolates in mice are as shown in Table 15.The results show that the hemoglobin level of the mice inoculated with the bacterial isolates were slightly affected. While an increase was observed in group D and E, a decrease was observed in group A, B and C at the end of the experiment. With regard to the experimental result, no significant difference (p value 0.858) was observed (Appendix xxvii).

The effect of the bacterial isolates on the packed cell volume of the mice (Table 15) showed variations in PCV values of the mice in all the groups during the experimental period. The initial PCV value ranged from 19.15±0.22% to 30.24±0.26%, while the PCV value after the experimental period ranged from 20.17±0.24% to 28.37±0.16%. There was significant difference (p value < 0.05) in the effects on the PCV by the isolates during the experimental periods.

Changes on the red blood cells were observed upon exposure of the bacterial isolates to the mice. However, there was no significant difference (p value > 0.05) in effects of the bacterial isolates on the red blood cells [Appendix xxix]. While an increase in red blood cells was observed in groups A, B and D, and decrease in the red blood cells were observed in groups C and E (Table 15).

Exposure of the mice to the bacterial isolates resulted in an increase in the white blood cell (WBC) count of the group inoculated with *P. aeruginosa* (group B) after the first and second week of infection. A decrease was observed in the group inoculated with *B. subtilis* and *P. suwonensis* (group D and E), and a mixed trend (increase and decrease) in group A and C.

There was increase in lymphocyte value of the mice at the end of the experiment in all the groups except group D upon exposure to the bacterial isolates. While the highest level of effect was observed in groupA, the least effect occurred in group D. There was significant difference (p value < 005) in the effects of the isolates on the lymphocyte values of the mice (Appendix xxxi).

A decrease in monocyte values of the mice at the end of the experiment was observed in groups A,B,C and E, an increase occurred in group D (Table 15). Statistically, there was

significant difference (p value < 0.05) in the effects by the bacterial isolates on the monocyte value of the mice.

Table 15: Effects of the bacterial isolates administered subcutaneously on the hematology profile of the mice

Group	Bacterial isolate	Before inoculation	1week	2weeks
Haemoglobin level (g/dl)				
A	*L.sphaericus*	6.59±0.28	6.59±0.53	6.15±0.18
B	*P. aeruginosa*	10.32±0.28	10.15±0.13	9.19±0.13
C	*S. marcescens*	7.14±0.15	7.24±0.32	7.10±0.10
D	*B. subtilis*	8.00±0.50	7.65±0.18	8.40±0.36
E	*P. suwonensis*	7.32±0.35	7.42±0.52	8.13±0.59
F	Control	9.52±0.17	9.55±0/30	9.55±0.30
Packed cell volume (%)				
A	*L.sphaericus*	19.15±0.22	18.29±0.22	20.17±0.24
B	*P. aeruginosa*	30.24±0.26	31.24±0.26	28.37±0.16
C	*S. marcescens*	22.42±0.39	21.76±0.25	21.47±0.41
D	*B. subtilis*	23.47±1.47	21.44±0.41	25.60±0.21
E	*P. suwonensis*	20.91±0.80	20.14±0.33	21.24±0.26
F	Control	28.20±0.26	28.24±0.40	28.17±0.29
Red blood cell (10^6/mm^3)				
A	*L.sphaericus*	12.49±0.49	13.49±0.44	13.43±0.21
B	*P. aeruginosa*	12.6±0.18	12.61±0.19	13.4±0.18
C	*S. marcescens*	10.5±0.29	10.10±0.10	9.27±0.21
D	*B. subtilis*	10.6±0.26	11.34±0.12	12.07±1.25
E	*P. suwonensis*	11.57±0.12	10.60±0.26	10.5±0.30
F	Control	12.36±0.15	12.33±0.21	12.56±0.21
White blood cell (10^3/mm^3)				
A	*L.sphaericus*	11.36±0.35	13.53±0.41	13.36±0.15
B	*P. aeruginosa*	10.53±0.38	11.54±0.48	17.50±0.30
C	*S. marcescens*	13.62±0.53	10.50±0.44	16.95±0.05
D	*B. subtilis*	18.34±0.42	15.52±0.43	15.30±0.44
E	*P. suwonensis*	15.37±0.48	13.47±0.42	12.87±0.16
F	Control	14.49±0.45	14.16±0.20	14.50±0.45
Lymphocyte (%)				
A	*L.sphaericus*	40.17±0.18	36.19±0.13	44.39±0.24
B	*P. aeruginosa*	26.27±0.26	39.27±0.26	41.27±0.26
C	*S. marcescens*	33.04±0.07	25.10±0.10	38.21±0.27
D	*B. subtilis*	43.3±0.30	37.33±0.25	39.38±0.33
E	*P. suwonensis*	37.40±0.30	34.60±0.66	40.38±0.54
F	Control	58.23±0.25	58.53±0.35	58.27±1.53
Monocyte (%)				
A	*L.sphaericus*	2.77±0.25	1.79±0.25	1.59±0.45
B	*P. aeruginosa*	2.15±0.22	1.42±0.43	1.43±0.18
C	*S. marcescens*	3.47±0.15	3.76±0.51	0.26±0.23
D	*B. subtilis*	0.40±0.46	0.58±0.19	0.50±0.35
E	*P. suwonensis*	1.20±0.26	1.35±0.61	0.32±0.33
F	Control	0.53±0.31	0.50±0.23	0.51±0.10

4.7.6.4. Effects of subcutaneous inoculation of bacterial isolates on the blood chemistry profile of mice

As shown on Table 16, the subcutaneous inoculation of bacterial isolates on the mice had varying and significant effects on the blood chemistry profile of the mice (Appendix xxxiii and xxxiv).

The aspartate transaminase (AST) enzyme values of the mice varied during the experimental period. However, the overall decrease in AST level was observed in all the groups except group A and D at the end of the two weeks experiment.

As in AST, varied results were obtained in the alanine transaminase (ALT) enzyme values of the mice in the groups. While ALT levels of groups A, C and E increased, a decrease was observed in groups B and D. Statistically, there was no significant difference (P value > 0.999) in the effects of the bacterial isolates on the mice. However, *Paenalcaligenes suwonensis* and *Serratia marcescens* had the highest level of effects on the ALT values of the mice (Table 16) [Appendix xxxiv].

Table 16: Effects of the bacterial isolates administered subcutaneously on blood chemistry profile of the mice

Group	Bacterial isolate	Before inoculation	1 week	2 weeks
	Aspartate Transaminase Values			
A	*L.sphaericus*	54.02±0.02	55.36±0.28	57.34±0.43
B	*P. aeruginosa*	45.06±0.08	45.28±0.24	44.08±0.11
C	*S. marcescens*	48.18±0.22	45.55±0.64	41.32±0.28
D	*B. subtilis*	42.95±0.14	47.61±0.51	45.06±0.03
E	*P. suwonensis*	48.45±0.48	44.22±0.26	42.50±0.48
F	Control	43.10±0.06	43.34±0.28	43.23±0.25
	Alanine Transaminase Values			
A	*L.sphaericus*	15.02±0.02	15.24±0.41	15.10±0.08
B	*P. aeruginosa*	17.18±0.17	16.43±0.49	16.38±0.40
C	*S. marcescens*	17.20±0.23	17.22±0.16	17.73±0.68
D	*B. subtilis*	14.33±0.31	14.34±0.29	14.06±0.08
E	*P. suwonensis*	17.13±0.08	17.56±0.56	17.79±0.46
F	Control	16.21±0.25	16.29±0.19	16.25±0.05

4.7.7. Histological Examination of Vital Organs of Mice Exposed to the Bacterial Isolates

4.7.7.1. Examination of the intestinal walls of the mice

The results of the histological examination of the intestinal walls of mice subcutaneously infected with bacterial isolates are presented in Plate 14 – 19. The result showed that *L. sphaericus* inoculated group had minor erosion of the intestinal submucosa (Plate 14). Signs of haemorrhage at the intestinal lumen were observed in the intestinal wall of mice inoculated with *P. aerug*inosa (Plate 15). While the group inoculated with *Serratia marcescens* showed intact villi with minor debris in the lumen (Plate 16), the group inoculated with *B. subtilis and Paenalcaligenes suwonensis* did not present any observable distortion in the intestinal walls (Plate 17 and 18). The control group also had intact submucosa and mucosal glands on the mice's intestinal lumen as shown on Plate19.

4.7.7.2. Examination of the liver cells of the mice

The results of the histological examination of the liver cells of mice subcutaneously inoculated with bacterial isolates are presented in Plate 20 – 25. It showed that all the infected mice experienced some distortion in the liver architecture. The mice inoculated with *L sphaericus* showed fibrotic appearances (Plate 20), macrovesicular fatty changes was observed in group inoculated with *P. aerug*inosa (Plate 21), sinusoid congestion in the liver cells of the mice inoculated with *S. marcescens* (Plate 22), enlarged sinusoids in the mice inoculated with *B. subtilis* (Plate 23) and minor vacuolations in the liver cells of mice inoculated with *Paenalcaligenes suwonensis* (Plate 24). The control group showed no visible signs of tissue damage from the central vein of the liver cells as shown in Plate 25.

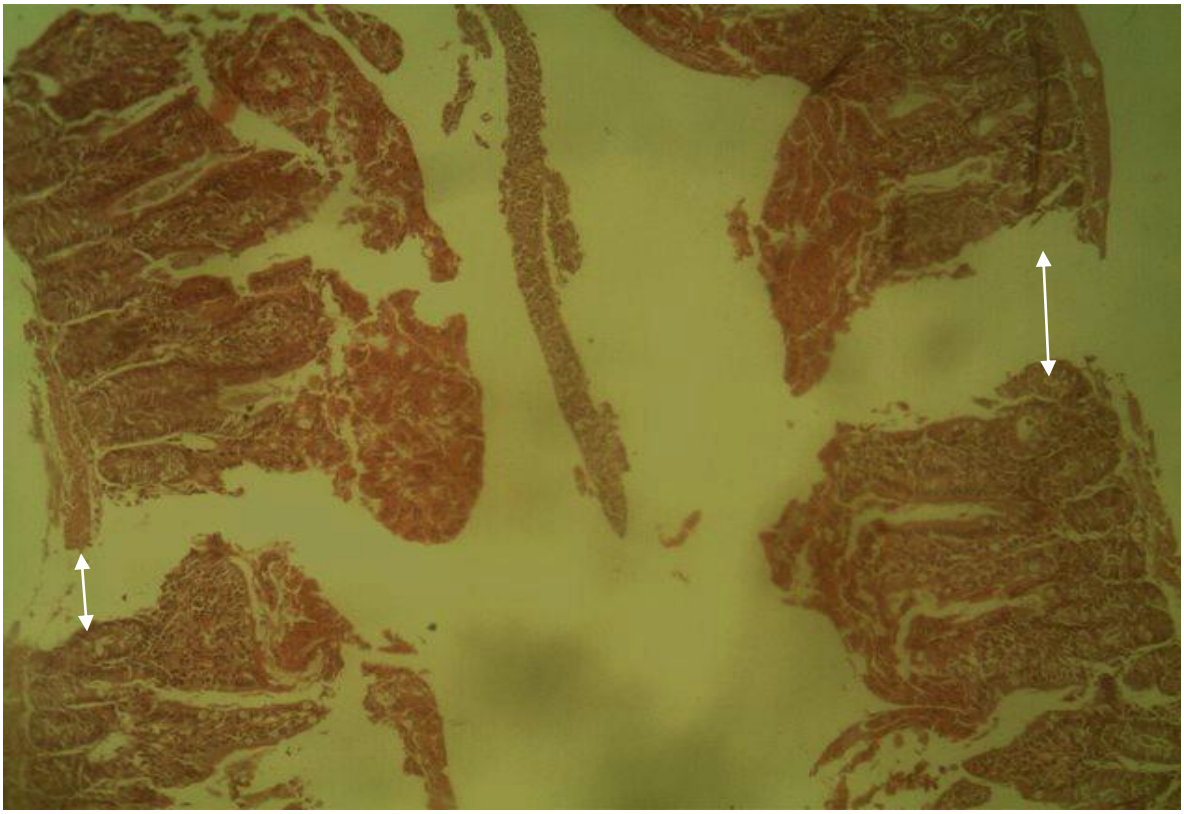

Plate 14: Photomicrograph of mice intestine inoculated with *L. sphaericus* showing minor erosion of intestinal mucosa (white arrow heads).

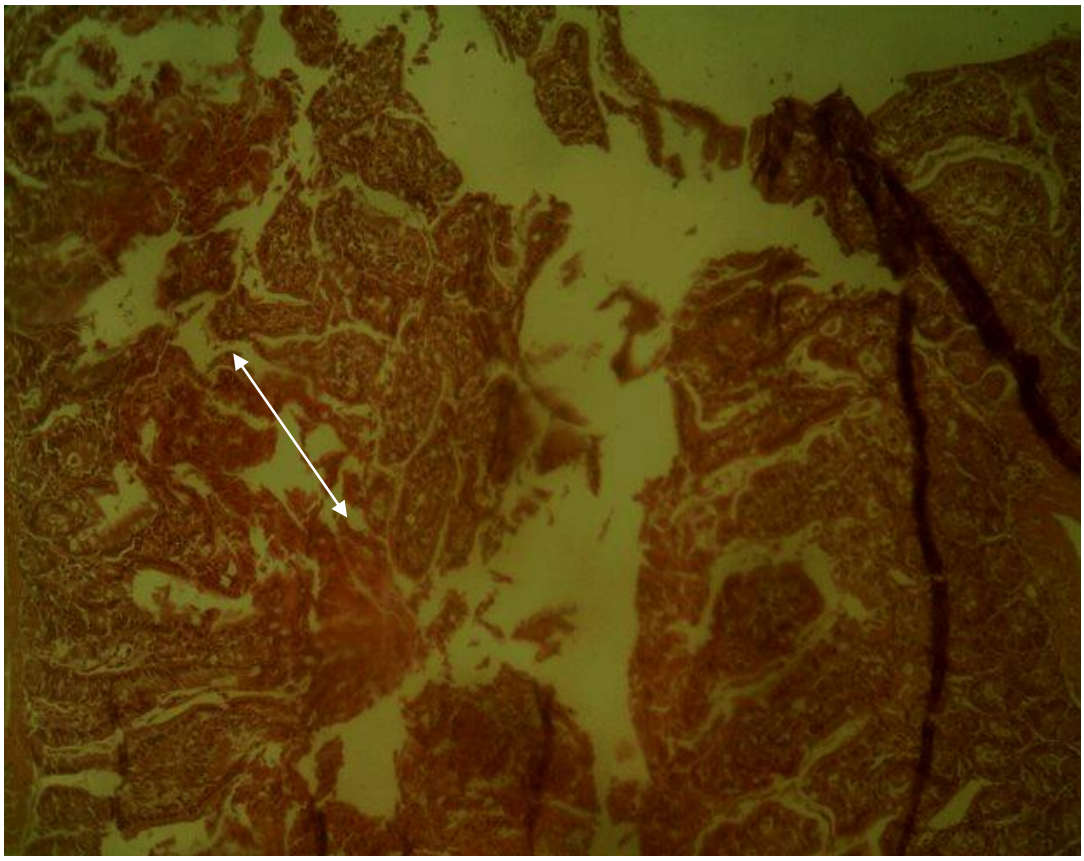

Plate 15: Photomicrograph of mice intestine inoculated with *P. aeruginosa* showing haemorrhage at the intestinal lumen (white arrow).

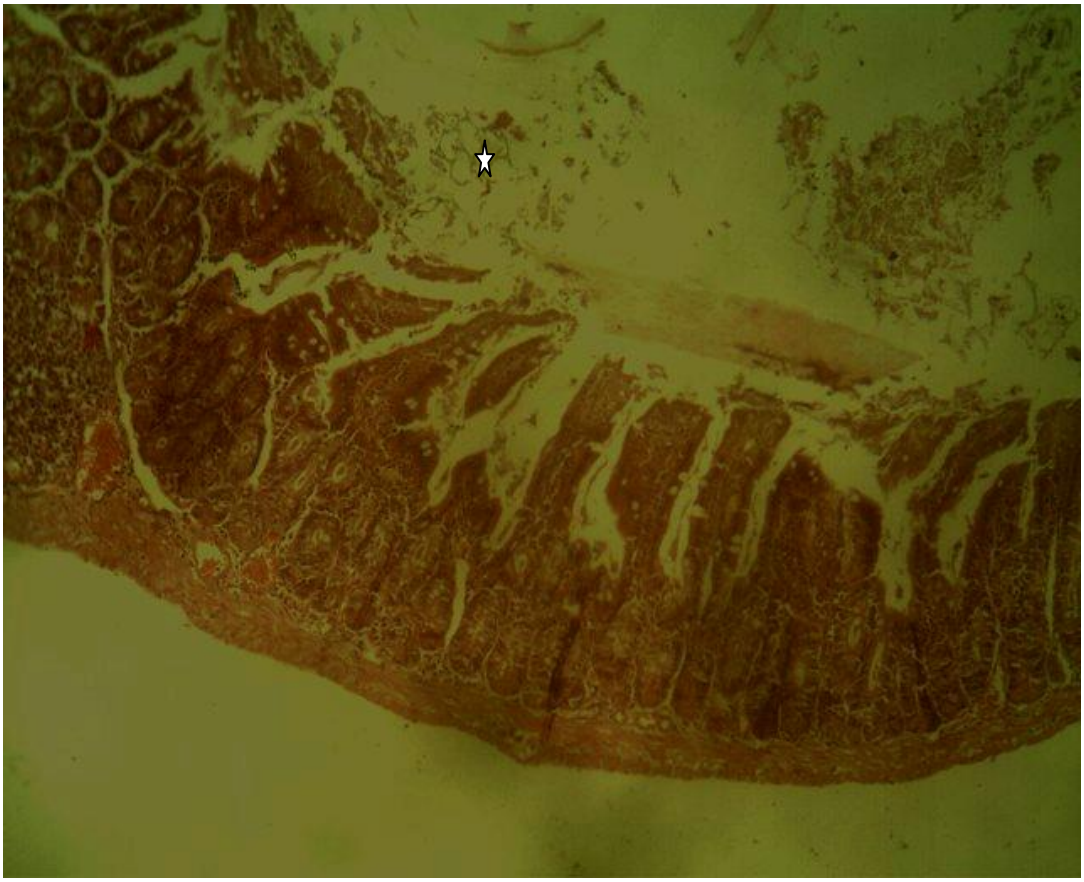

Plate 16: Photomicrograph of mice intestine inoculated with *S. marcescens* showing intact villi with minor debris observed in the lumen (star).

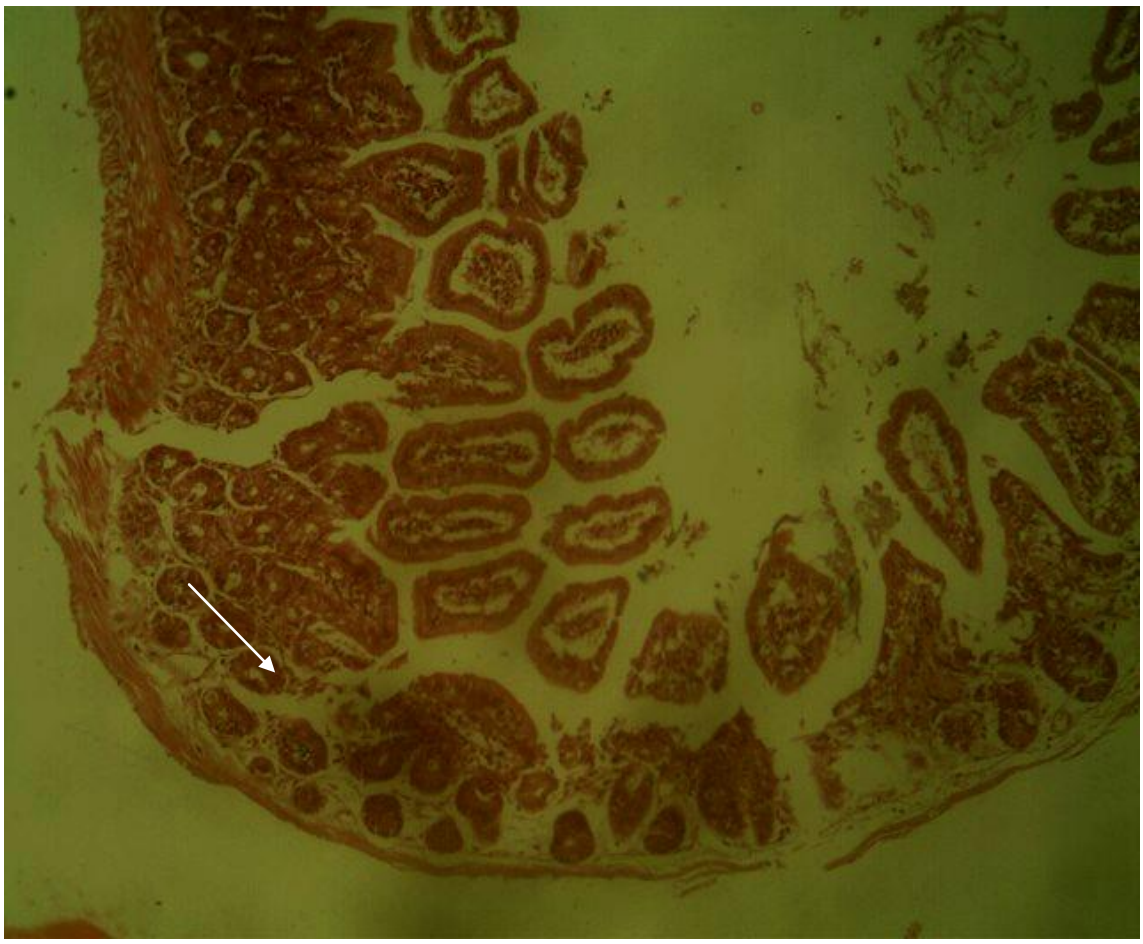

Plate 17: Photomicrograph of mice intestine inoculated with *B. subtilis* showing intact wall architecture with goblet cells (white arrow).

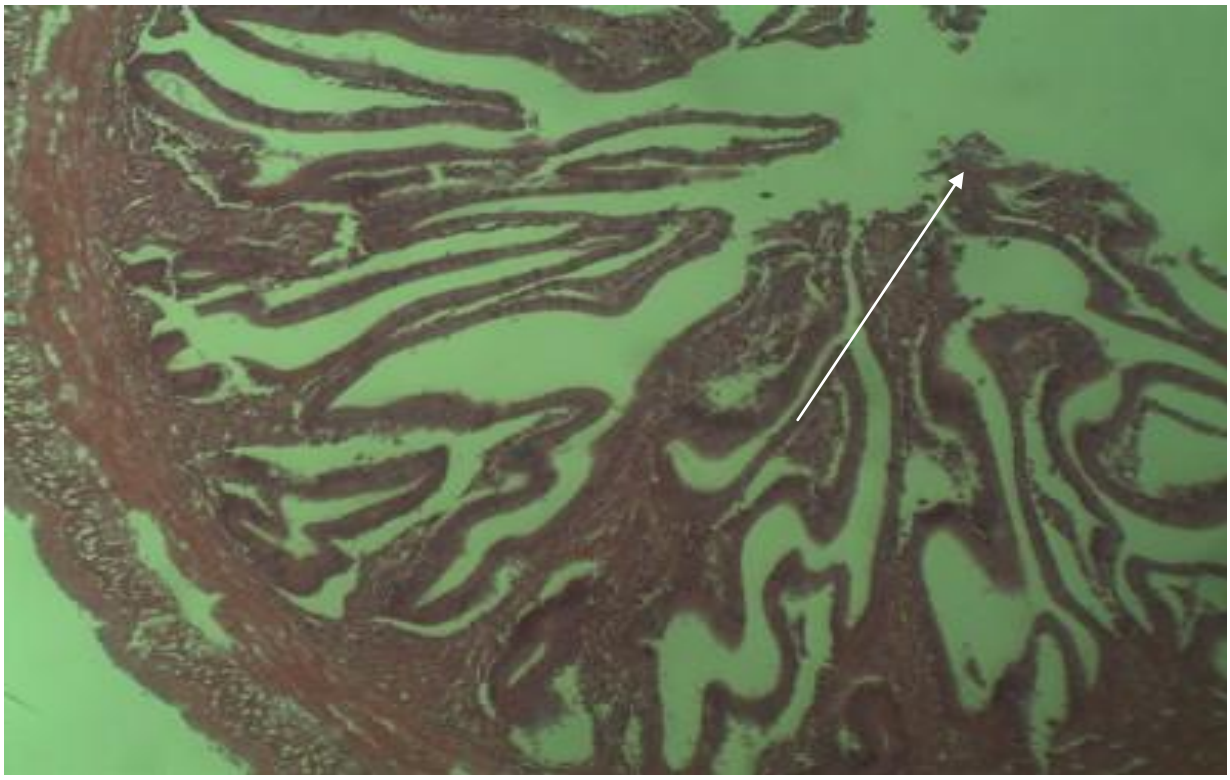

Plate18: Photomicrograph of mice intestine inoculated with *P. suwonensis* showing intact villi architecture (white arrow).

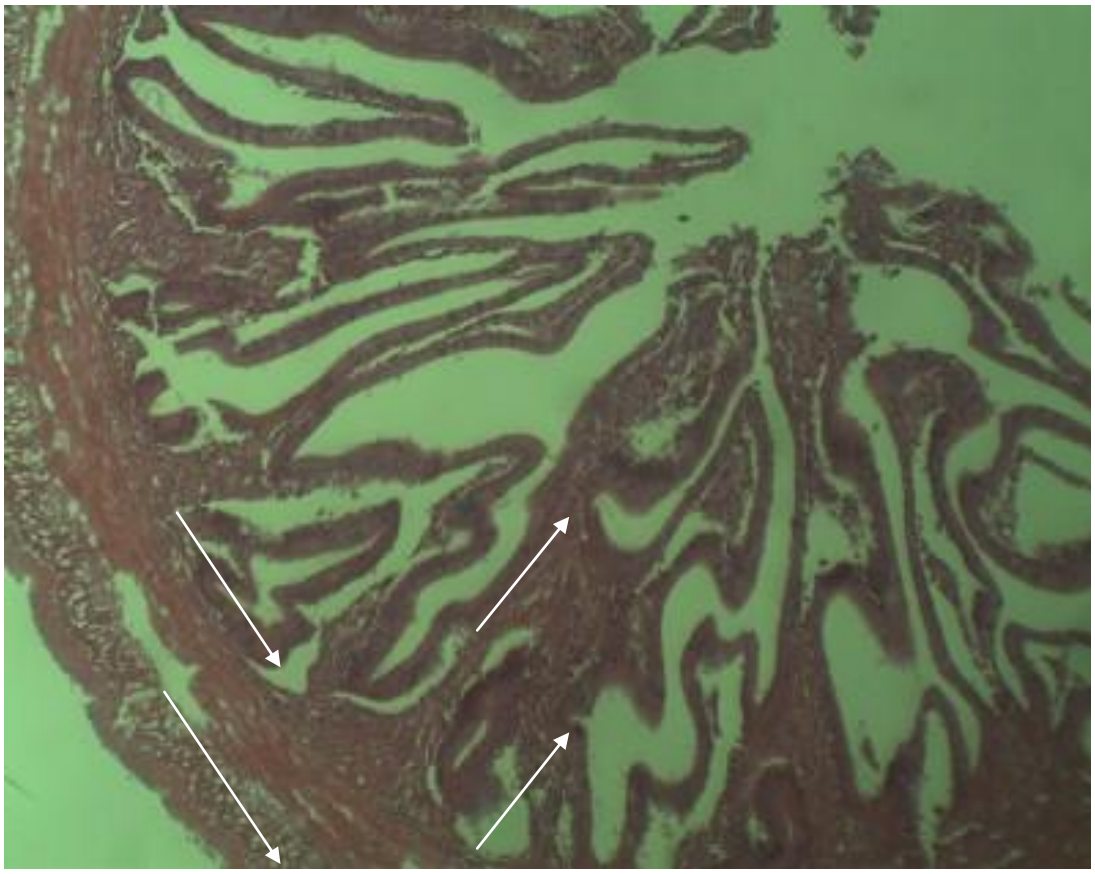

Plate 19: Photomicrograph of control mice intestine showing intact submucosa and mucosal glands (white arrows)

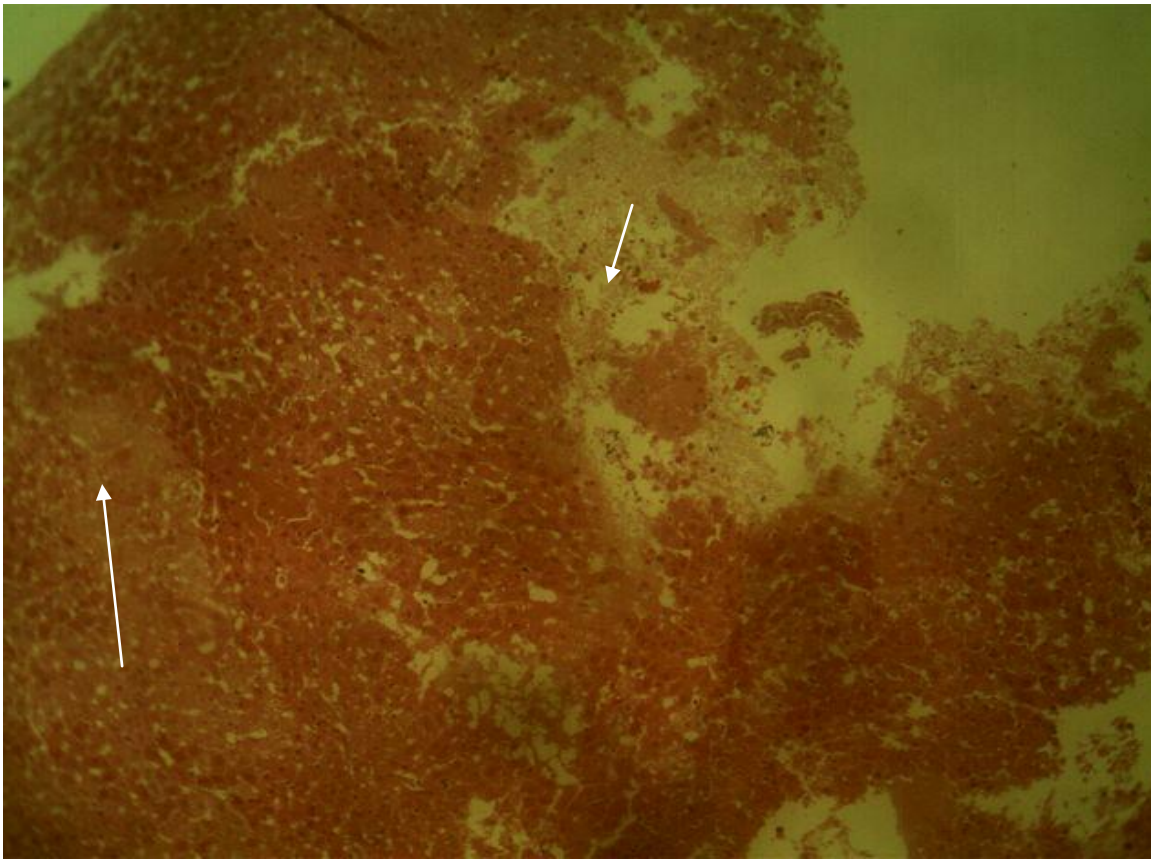

Plate 20: Photomicrograph of mice liver tissue inoculated with *L. sphaericus* showing fibrotic appearances (white arrows).

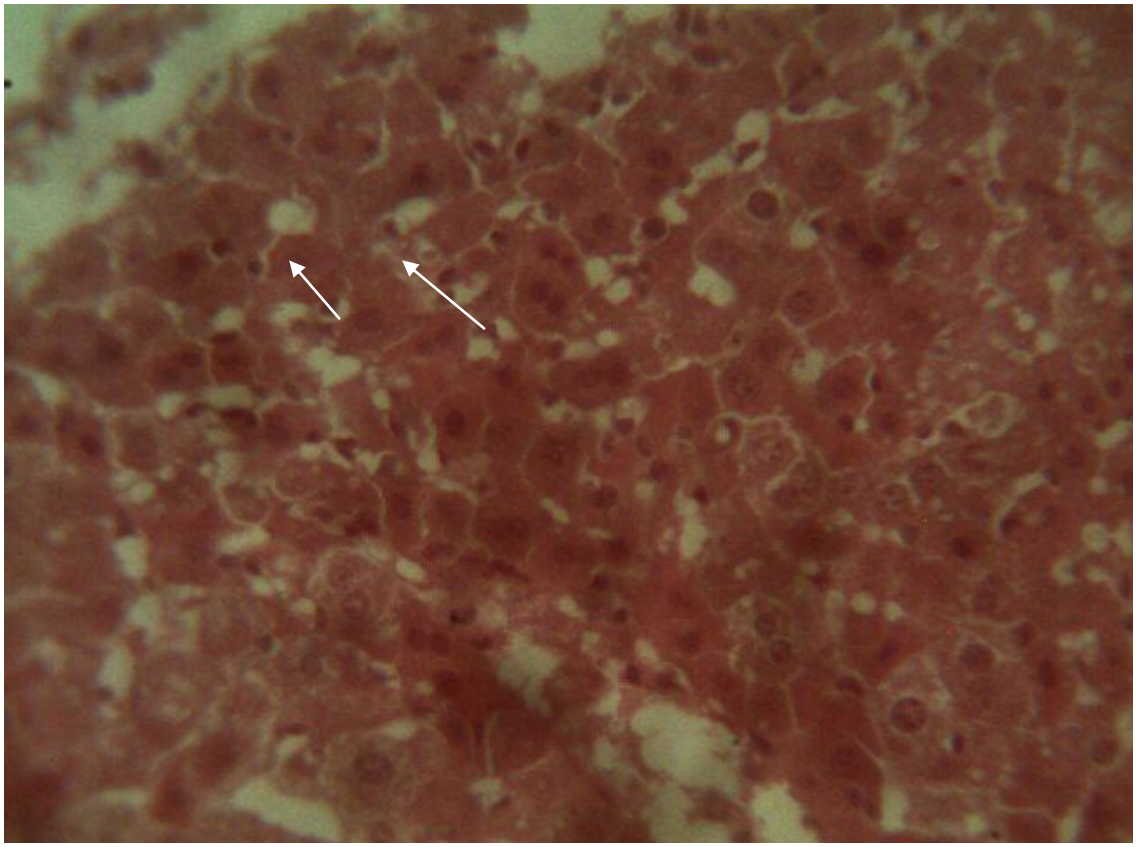

Plate 21: Photomicrograph of mice liver tissue inoculated with *P. aeruginosa* showing macrovascular fatty changes (white arrows).

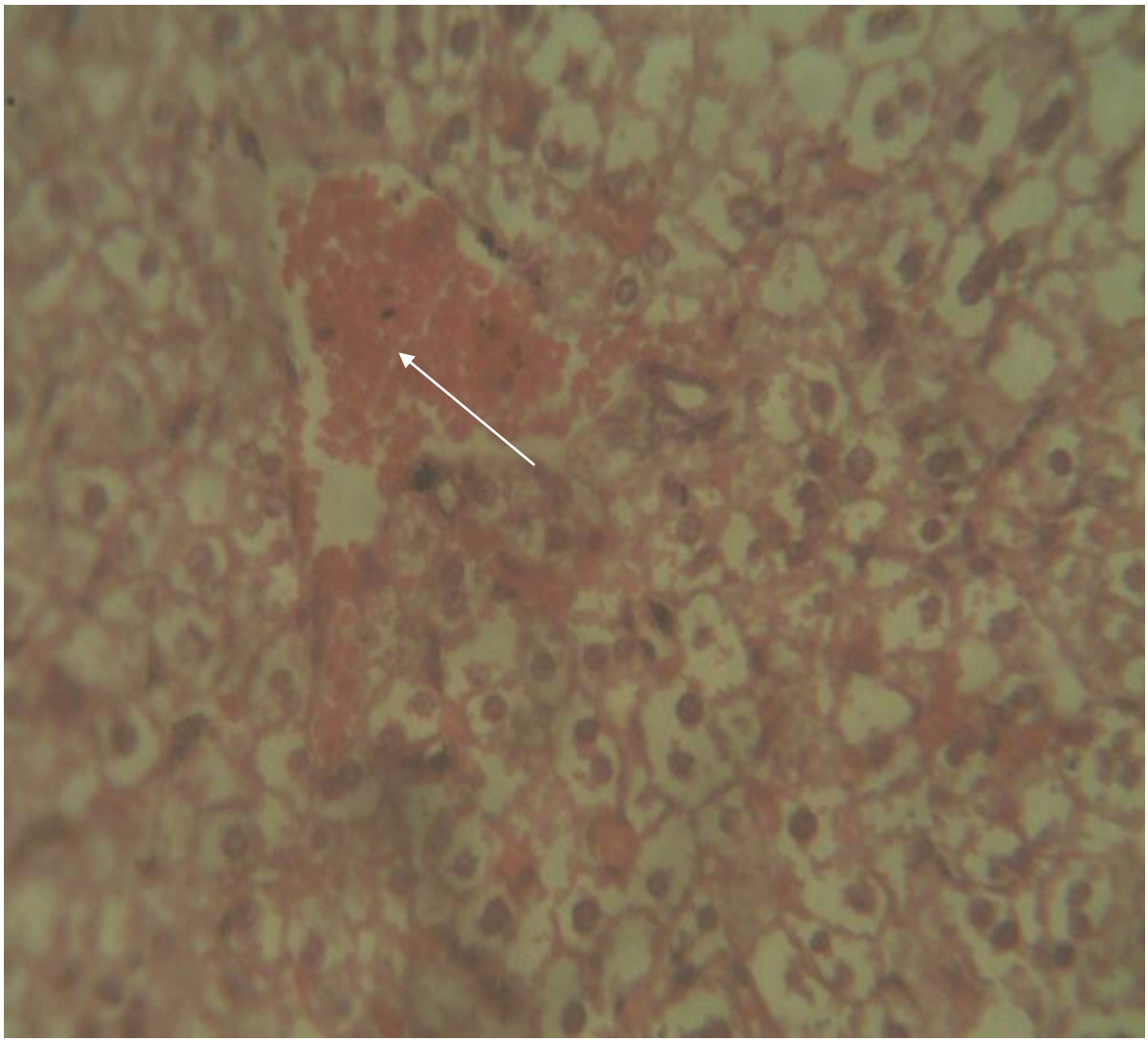

Plate 22: Photomicrograph of mice liver tissue inoculated with *S. marcescens* showing sinusoid congestion (white arrow)

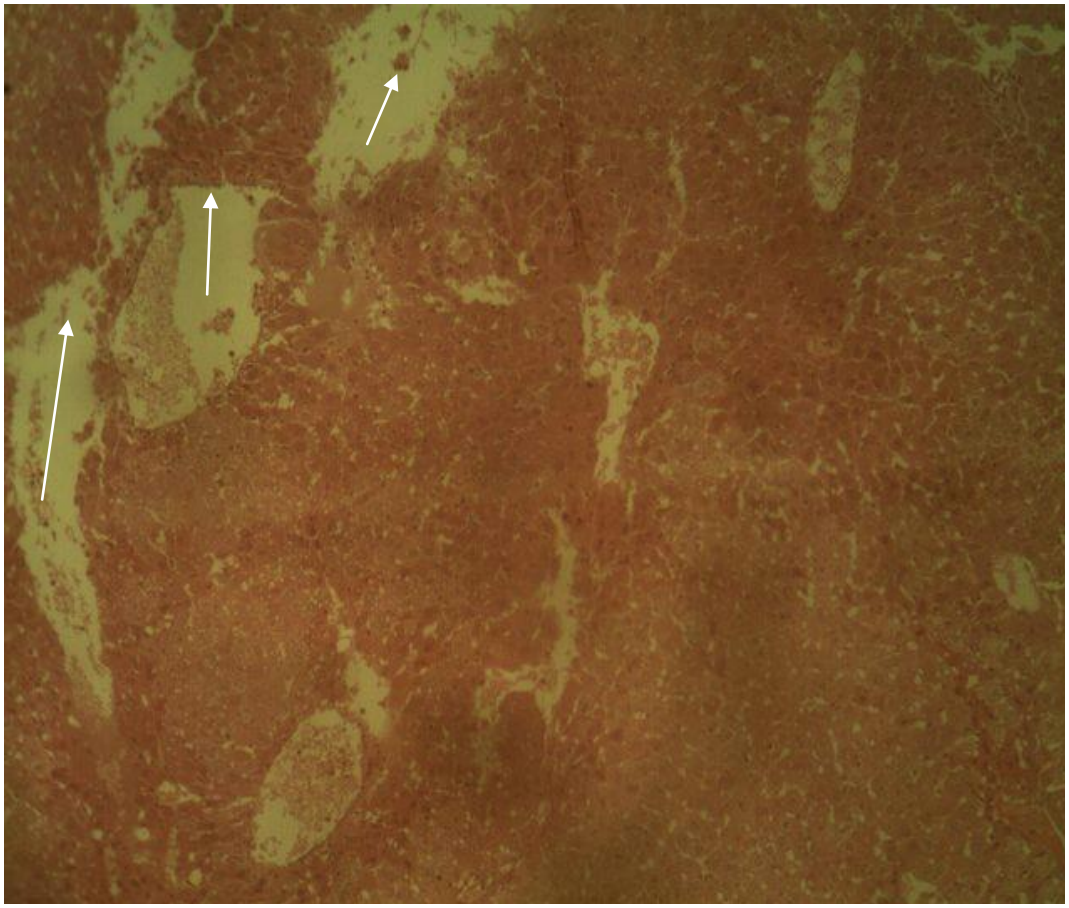

Plate 23: Photomicrograph of mice liver tissue inoculated with *B. subtilis* showing regions of enlarged sinusoids (white arrows).

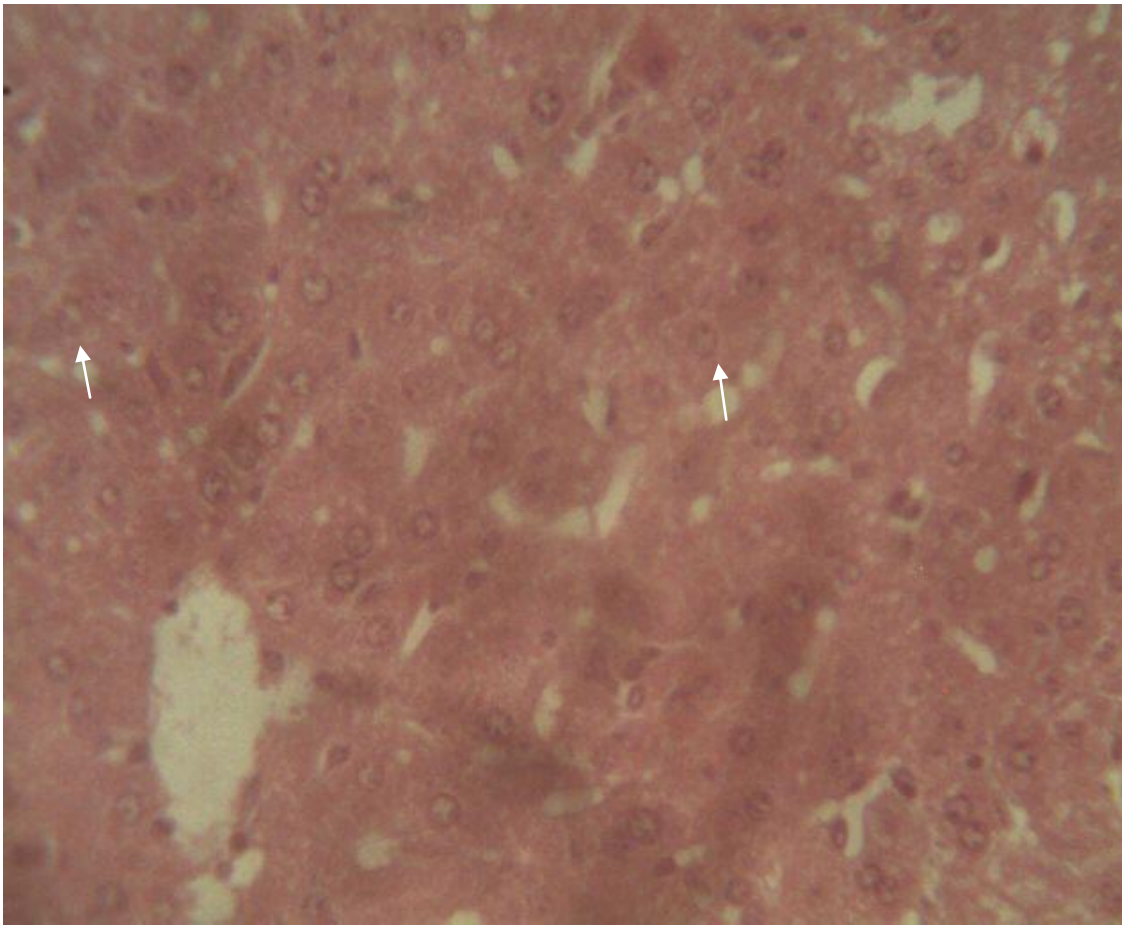

Plate 24: Photomicrograph of mice liver tissue inoculated with *P. suwonensis* showing minor vacuolations (white arrows).

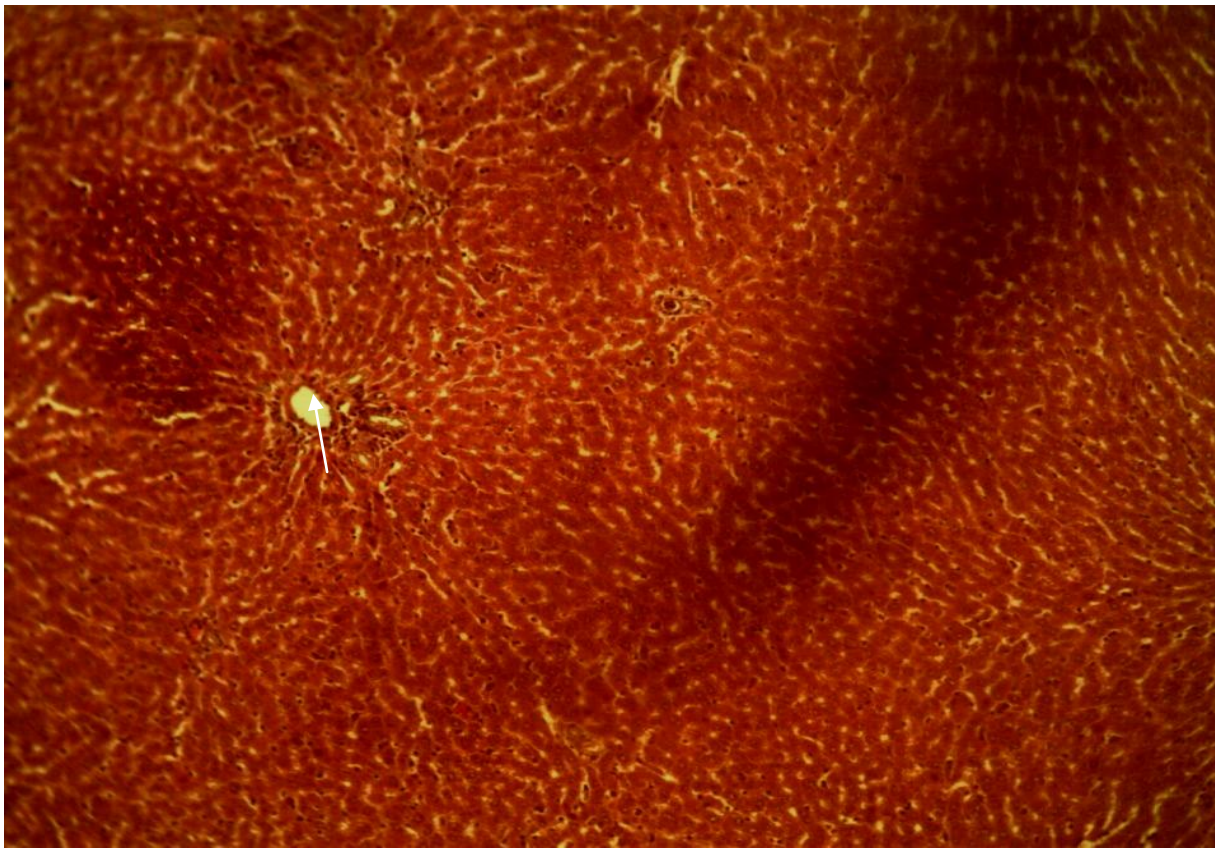

Plate 25: Photomicrograph of control mice liver tissue showing central vein with no visible signs of tissue damage (white arrow).

4.7.7.3. Examination of the Subcutaneous Tissues of the Mice

Plate 26 – 30, are the results of the histological examination of the subcutaneous tissues of mice inoculated with bacterial isolates. Examination of traumatized skin portions from which subcutaneous inoculation was performed showed different distortions of the subcutaneous tissues.

Plate 26 showed an epidermal atrophy in the subcutaneous tissues of mice inoculated with *L. sphaericus*. Loss of stratum corneum, the superficial epidermal layers of the mice inoculated with *P. aerug*inosa was observed (Plate 27). In Plate 28, loss of superficial cell layer of the subcutaneous tissue was seen in the mice inoculated with *S. marcescens*. Presence of adiposites with no visible damage was observed in the mice inoculated with *Bacillus subtilis* (Plate 29). Region of hyperkeratosis was observed in subcutaneous tissues of the mice inoculated with *Paenalcaligenes suwonensis* (Plate 30), while the control group showed presence of pacinian corpuscle with no observable sign of tissue damage (plate 31).

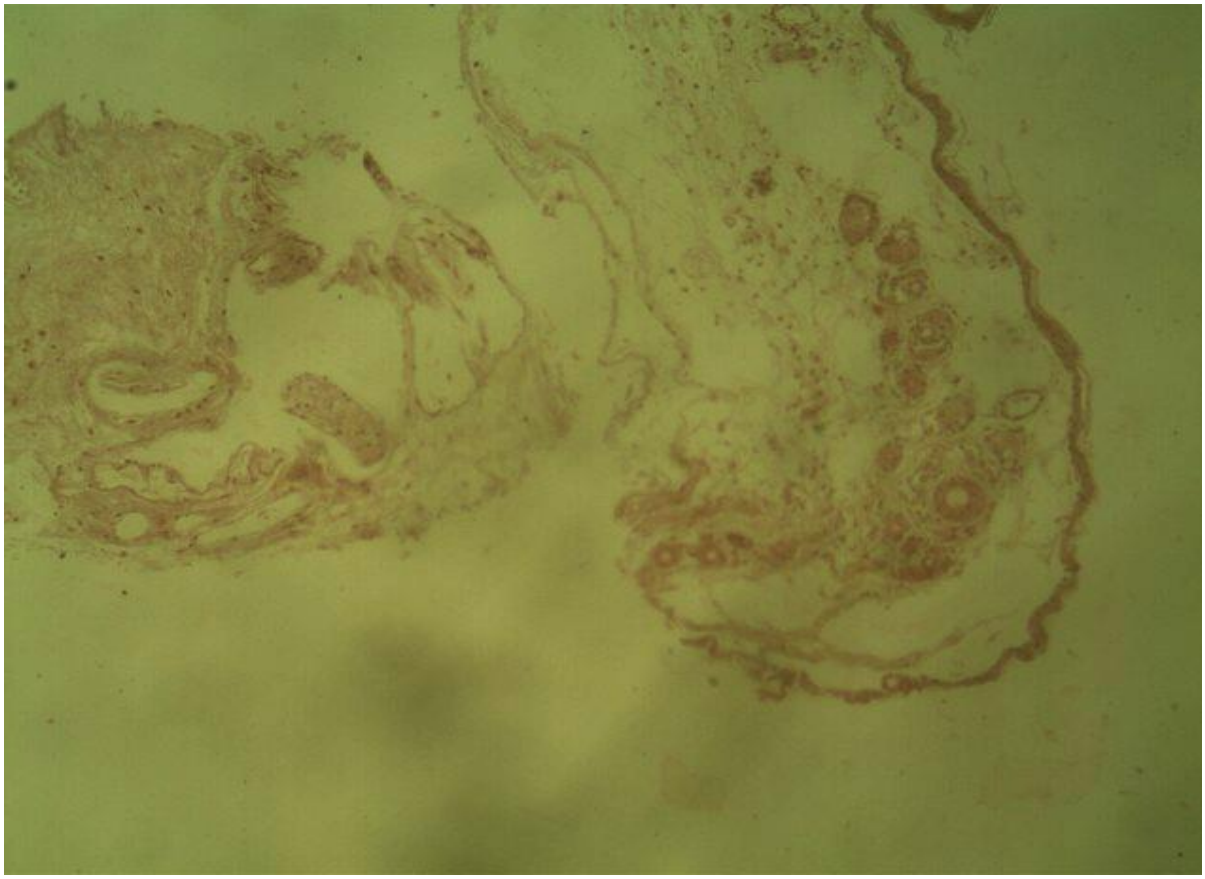

Plate 26: Photomicrograph of subcutaneous tissue of mice inoculated with *L. sphaericus* showing epidermal atrophy

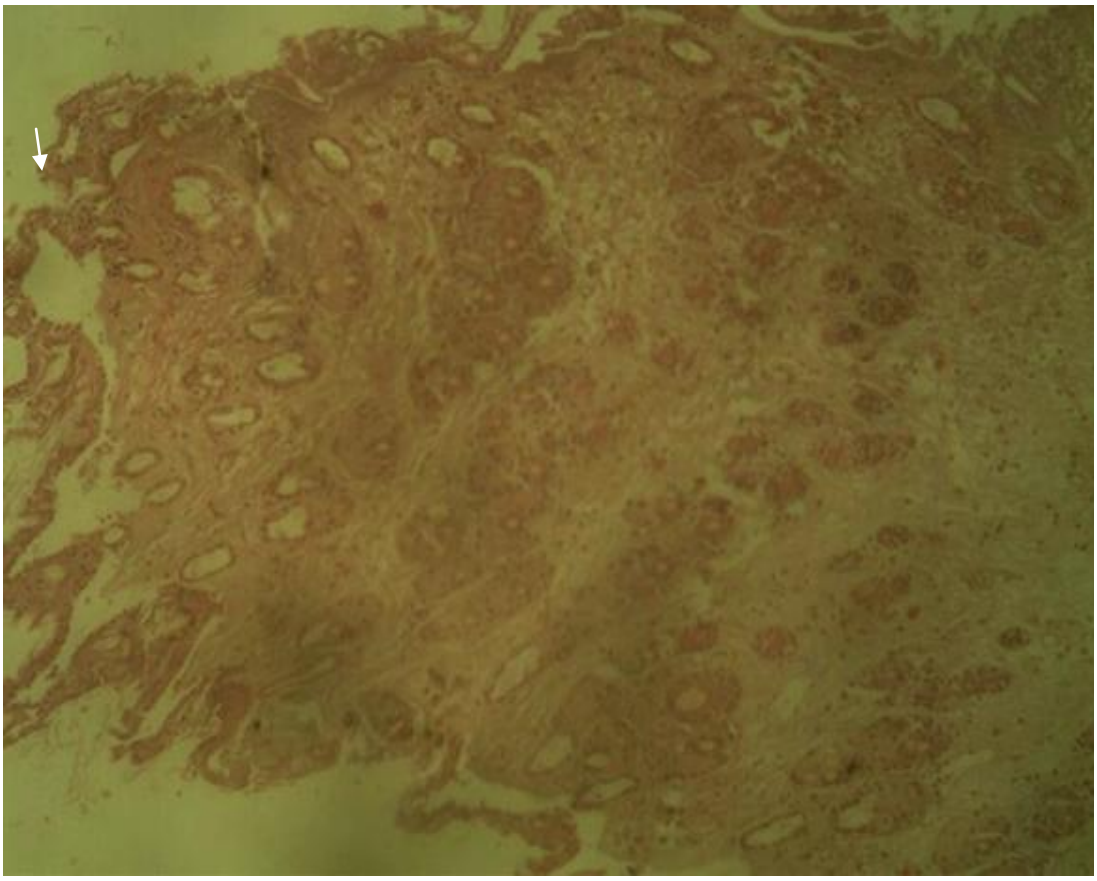

Plate 27: Photomicrograph of subcutaneous tissues of mice inoculated with *P. aeruginosa* showing loss of the superficial epidermal layers, stratum corneum (white arrow)

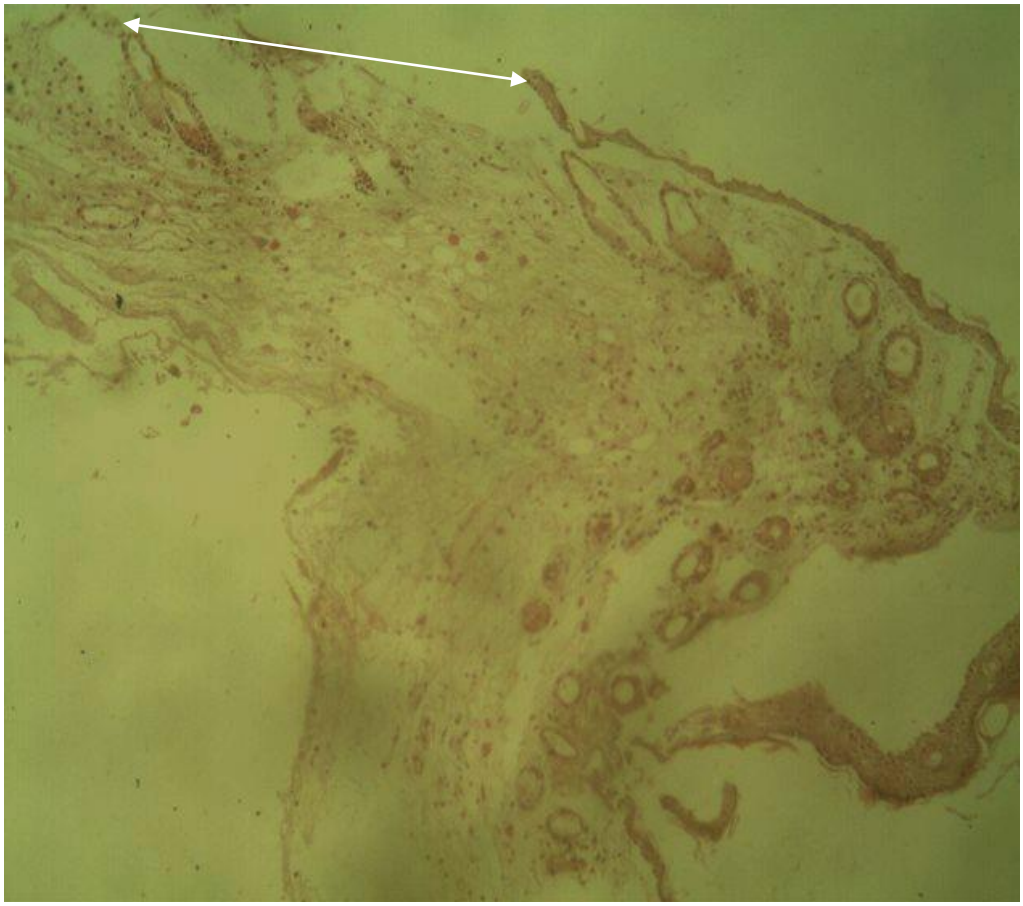

Plate 28: Photomicrograph of mice subcutaneous tissue inoculated with *S. marcescens* showing loss of superficial cell layer (erosion)[white arrow].

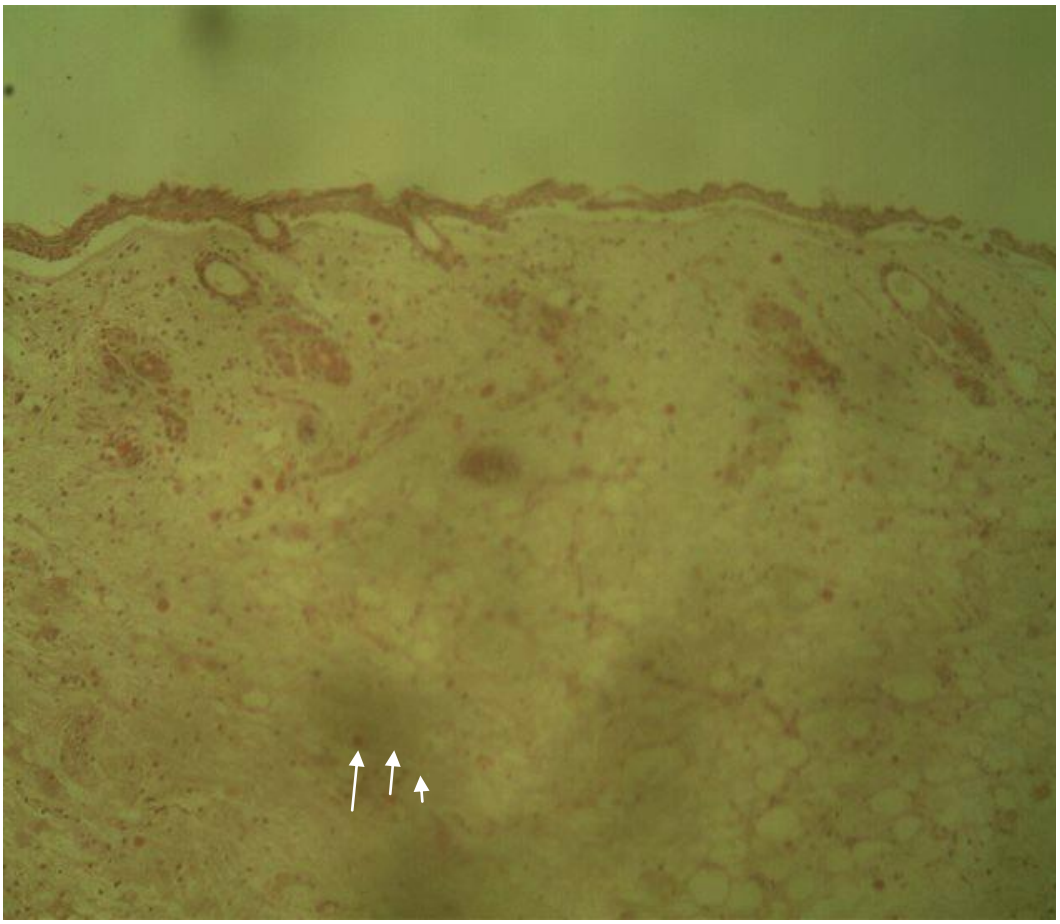

Plate 29: Photomicrograph of mice subcutaneous tissue inoculated with *B. subtilis* showing presence of adiposites with no visible damage signs (white arrows).

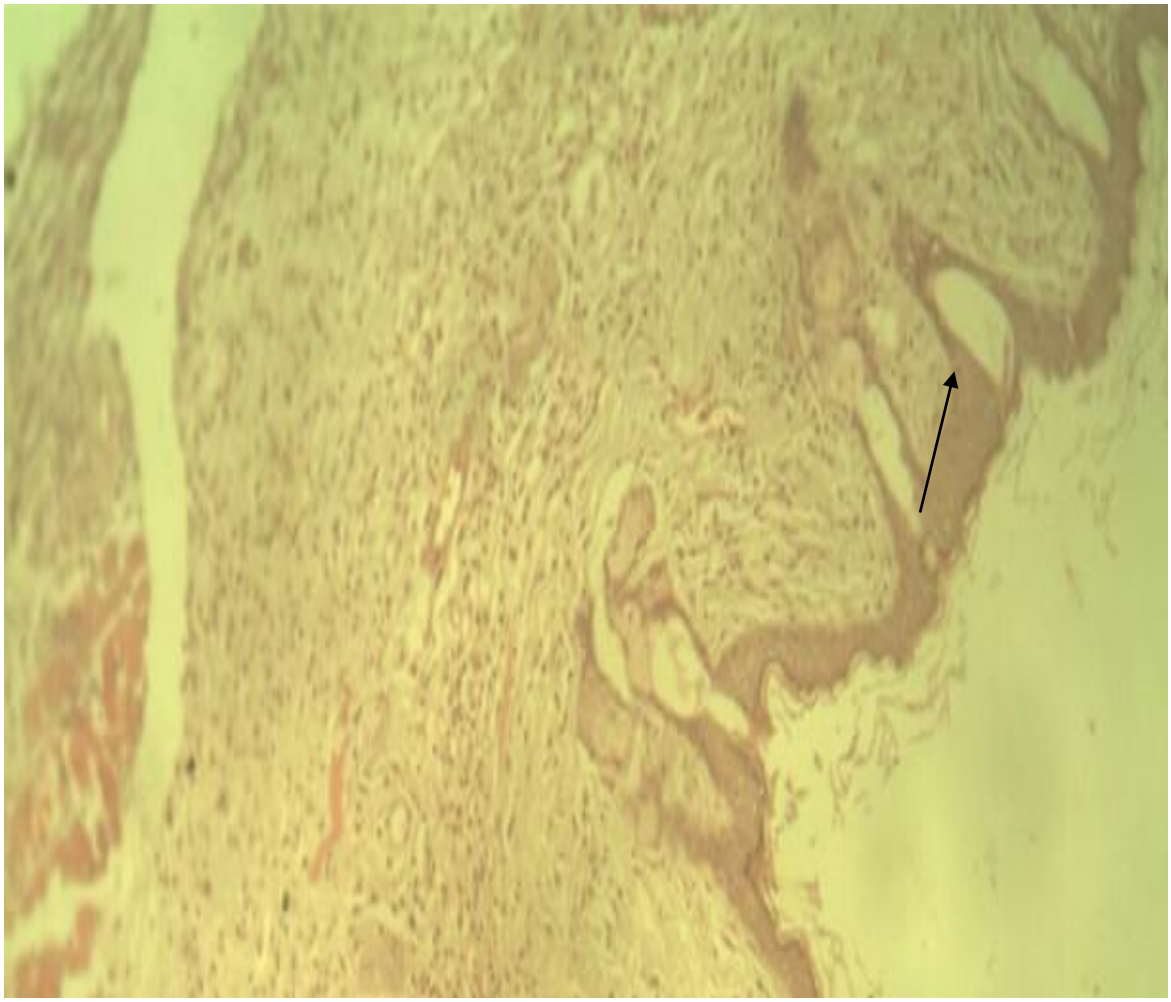

Plate 30: Photomicrograph of mice subcutaneous tissue inoculated with *P. suwonensis* showing region of hyperkeratosis (black arrow).

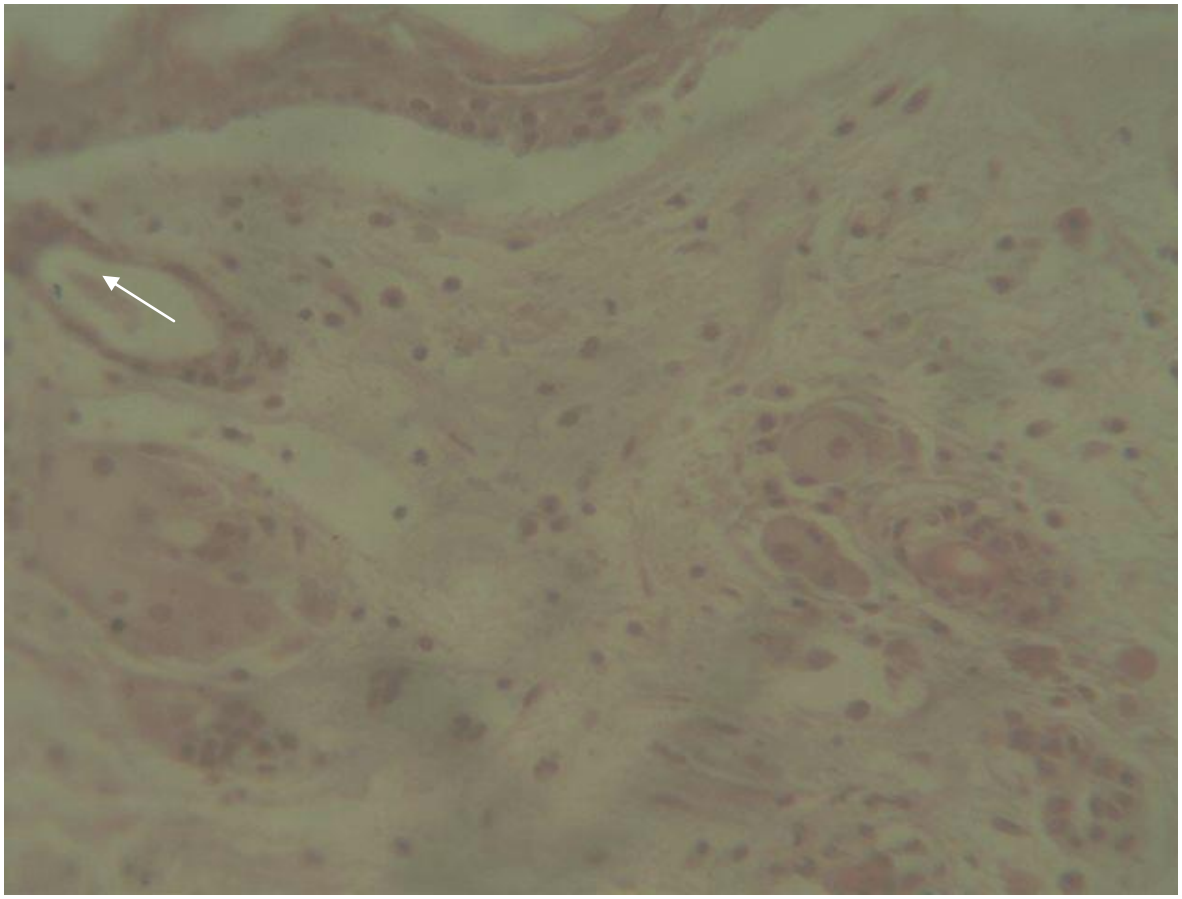

Plate 31: Photomicrograph of control mice subcutaneous tissue showing Pacinian corpuscle with no observable sign of tissue damage (white arrow).

4.8. Mortality of the experimental animals

Fig.6 shows the percentage mortality of the experimental animals due to exposure to the different bacterial isolates. Highest mortality in fish (44.4%) was observed in group C inoculated with *Serratia marcescens* while the highest mortality in mice (66.7%) was observed in group B inoculated with *Pseudomonas aeruginosa.* Zero mortality in mice and fish was observed in group A inoculated with *Lysinibacillus sphaericus,* and in mice alone in groups D and E inoculated with *Bacillus subtilis* and *Paenalcaligenes suwonensis* respectively.

4.9. Plating of the internal organs of the infected fish on selective media

The mean bacterial count of isolates on the internal organs of fish grown on selective media is presented in Fig.7. Significant growth of the isolates was recovered from the internal organs of the fishes. The mean bacterial counts of the bacterial isolates ranged from 10.00±5.00cfu/g to 85.67±13.41cfu/g. There was significant difference (p value < 0.05) in the bacterial growths recovered on the selective media upon inoculation of the fish organs [Appendix xxxvi].

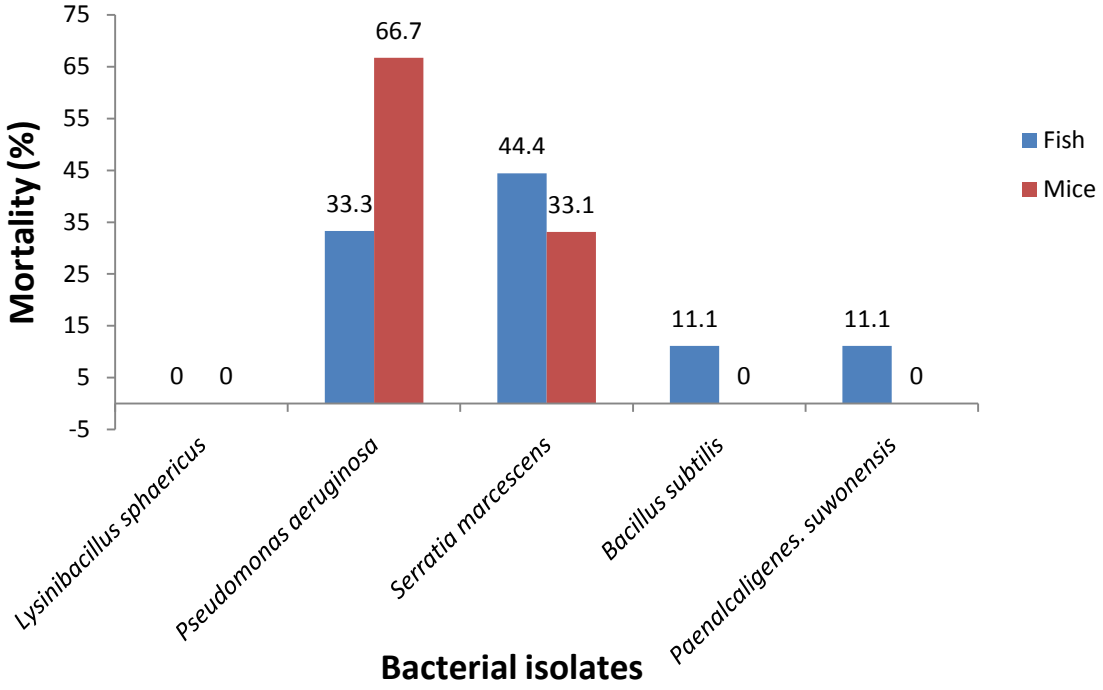

Fig.6: Percentage mortality of experimental animals due to exposure to different bacterial isolates

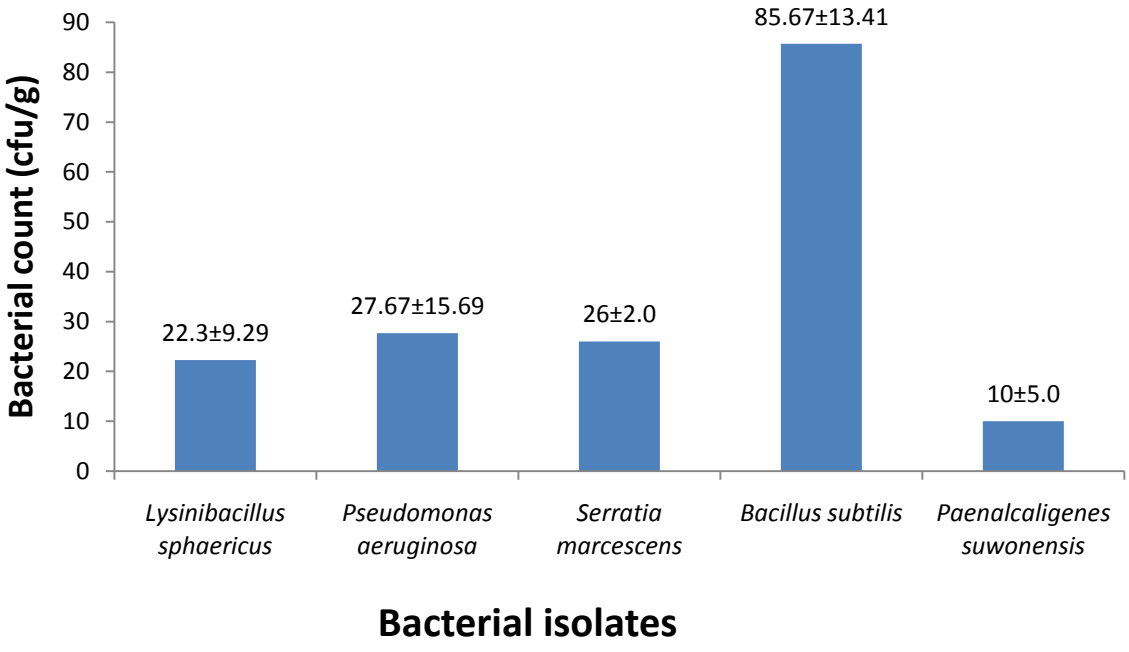

Fig.7: Growth of the bacteria on the internal organs of the infected fishes on selective media

4.10. Treatment of the Infected Fishes with Probiotic and a Conventional Antibiotic

4.10.1. Effects of probiotics on hematology profile of infected fishes

The effects of probiotic use on the hematology profile of fishes infected with the bacterial isolates are shown on Table 17.While change was observed in group A at the end of the experiment, a decrease in hemoglobin values were observed in groups A, B and D and an increase in groups C and E. Statistically, the effects of probiotics on the infected fishes were significant (p value < 0.05) [Appendix xxxvi].

An increase in packed cell volume (PCV) of the infected fishes exposed to probiotic was observed in all the groups at the end of the treatment (Table 17). However, the treatment with probiotic had highest effect on the group infected with *Serratia marcescens* and least in the group infected with *Pseudomonas aeruginosa* (Appendix xxxvii). It was also observed that the highest effect of the probiotic on the infected fishes was on the first week of treatment (Appendix xxxvii).

An increase in red blood cell count was observed in the fishes infected with *Pseudomonas aeruginosa* all through the experimental period while a decrease was observed in groups A and D infected with *Lysinibacillus sphaericus* and *Bacillus subtilis* respectively. Although there was significant effect (p value < 0.05) of the probiotic treatment on all the groups, the highest level of effect at the end of the experiment was observed in the group infected with *Serratia marcescens* (Appendix xxxviii).

A decrease in white blood cell count was observed in groups A, B and C at the end of the 14 days treatment period. The values of fishes in groups D and E increased after first week of treatment, while those in group D decreased, those in group E increased at the end of the 14 days treatment period. Statistically, there was significant difference (p value < 0.05) in the effects of the probiotic on the fishes during the experimental period [Appendix xxxix].

As shown in Table 17, lymphocyte values increased in all the groups at the end of the treatment. The highest effects on lymphocyte values in the treatment course with probiotic were observed in the group inoculated with *P. suwonensis* and least in the *Serratia marcescens* (Appendix xl).

A slight increase in monocyte value was observed in group C infected with *Serratia marcescens* after 14 days treatment, but the values of the other groups decreased. There was no significant difference (p value = 0.113) in the effect observed in groups infected with *Lysinibacillus sphaericus* and *Bacillus subtilis* [Appendix xli].

4.10.2. Effect of probiotics on blood chemistry profile of the infected fishes

Table 18 shows the effects of probiotic on the blood chemistry profile of the infected fishes.

The aspartate transaminase values (AST) before and after treatment with probiotic ranged from 59.80±0.57 to 91.57± 0.38 and 58.00±0.82 to 80.00±0.73 respectively. The effects of the treatment with probiotic on the AST values of the infected fishes were significantly different (p value<0.05)**.** Overall decrease in AST value was observed in all the groups

A decrease in alanine transaminase enzyme values was observed in all the groups at the end of the treatment. The treatment on the fishes infected with *L. sphaericus* and *P. aeruginosa* showed least effect and were not significantly different (p value > 0.05) [Appendix xlii].

4.10.3. Effect of probiotic on weight of the infected fishes

Table 19 shows the weights of the infected fishes during treatment with probiotic (*Saccharomyces cerevisiae*). Increase in weights was observed during the treatment period. The initial weights ranged from 134.33±1.17g to225.32±0.33g, while the final weights after treatment ranged from 156.52±0.50g to 263.33±2.52g. The highest percentage weight gain of 17.5% was observed in group E and the least in the group A. However, these were less than the weight achieved by the control group (43.9%) which was not pre-infected with any organism.

Table17: Effect of Probiotic on hematology profile of the infected fish

Group	Bacterial isolate	Before treatment	1week	2weeks
Haemoglobin level (g/dl)				
A	*L.sphaericus*	6.00±0.66	8.00±0.90	6.00±0.80
B	*P. aeruginosa*	5.33±0.49	5.00±0.78	5.00±0.70
C	*S. marcescens*	7.40±0.82	9.90±0.64	10.00±0.35
D	*B. subtilis*	7.00±0.35	8.00±0.70	6.75±0.64
E	*P. suwonensis*	8.00±0.44	9.00±0.90	9.00±0.92
F	Control	9.07±0.21	9.10±0.10	9.11±0.46
Packed cell volume (%)				
A	*L.sphaericus*	23.97±0.95	30.00±0.90	24.00±0.10
B	*P. aeruginosa*	17.00±0.10	18.00±3.36	17.27±0.50
C	*S. marcescens*	23.97±0.95	31.00±0.61	30.00±0.72
D	*B. subtilis*	24.07±1.00	31.00±0.82	25.00±0.69
E	*P. suwonensis*	25.00±0.80	30.10±1.05	27.00±0.80
F	Control	28.33±0.53	28.30±0.61	28.90±0.95
Red blood cell (10^6/mm^3)				
A	*L.sphaericus*	1.17±0.23	0.93±0.12	0.73±0.12
B	*P. aeruginosa*	1.37±0.31	1.53±1.13	1.90±0.04
C	*S. marcescens*	1.33±0.07	5.33±0.28	2.15±0.09
D	*B. subtilis*	1.58±0.40	1.44±0.11	0.83±0.12
E	*P. suwonensis*	2.44±0.09	2.20±0.87	2.33±0.28
F	Control	3.13±0.15	2.96±0.55	3.03±0.07
White blood cell (10^3/mm^3)				
A	*L.sphaericus*	28.00±0.95	25.03±0.94	24.00±0.70
B	*P. aeruginosa*	18.00±0.75	17.70±0.58	15.00±0.56
C	*S. marcescens*	19.00±0.70	23.83±0.65	17.00±0.89
D	*B. subtilis*	26.93±0.64	30.03±0.78	27.00±0.64
E	*P. suwonensis*	25.03±0.83	25.93±0.74	26.00±0.78
F	Control	19.43±4.67	19.42±2.65	19.40±0.61
Lymphocyte (%)				
A	*L.sphaericus*	94.00± 0.80	95.93± 0.77	96.00±0.90
B	*P. aeruginosa*	88.00±0.90	94.03± 0.79	93.00±0.10
C	*S. marcescens*	89.97±0.80	91.00± 0.73	90.67±0.49
D	*B. subtilis*	90.73±0.46	94.00± 0.67	94.00±0.80
E	*P. suwonensis*	90.00±0.80	105.00 ±0.46	101.00±0.90
F	Control	81.00±1.00	81.00±2.65	81.33±4.12
Monocyte (%)				
A	*L.sphaericus*	13.00± 0.10	11.00± 0.72	11.00±0.78
B	*P. aeruginosa*	11.40± 0.35	06.00±0.30	07.00±0.97
C	*S. marcescens*	10.33± 0.99	10.00±0.67	10.43±0.60
D	*B. subtilis*	13.10± 0.10	10.03±0.30	09.87±0.65
E	*P. suwonensis*	13.00± 0.10	04.00±0.70	08.67±0.44
F	Control	07.67±1.53	07.07±2.53	07.00±1.00

Table18: Effects of Probiotic on blood chemistry profile of the infected fish

Group	Bacterial isolate	Before inoculation	1 week	2 weeks
	Mean Aspartate Transaminase Values			
A	*L.sphaericus*	59.80± 0.57	60.00± 0.77	58.00±0.82
B	*P. aeruginosa*	75.77± 0.52	74.80± 0.59	74.80±0.54
C	*S. marcescens*	82.10± 0.97	81.70± 0.60	80.00±0.56
D	*B. subtilis*	72.33± 1.06	71.00± 0.46	59.67±0.58
E	*P. suwonensis*	91.57± 0.38	80.00± 1.00	80.00±0.73
F	Control	65.14±5.09	65.40±7.91	65.30±4.81
	Mean Alanine Transaminase Values			
A	*L.sphaericus*	28.83± 0.61	21.97±0.77	19.77±0.53
B	*P. aeruginosa*	27.03± 0.84	23.77± 0.52	20.16±0.93
C	*S. marcescens*	28.90± 0.66	25.76± 0.58	25.73±0.57
D	*B. subtilis*	27.83± 0.66	22.00± 0.78	25.87±0.63
E	*P. suwonensis*	28.87± 0.60	29.00± 0.72	26.47±0.69
F	Control	25.39± 0.91	25.15± 1.17	25.85±1.03

Table19: Effect of Probiotic on weight of the infected fish

Grp	Isolate	Before inoculation	1 week	2 weeks	%weight gain
A	*L.sphaericus*	178.57±0.23	181.37±0.46	204.33±6.03	14.4
B	*P. aeruginosa*	148.17±0.38	155.42±0.90	172.33±2.52	16.3
C	*S. marcescens*	134.33±1.17	146.53±2.16	156.52±0.50	16.5
D	*B. subtilis*	225.32±0.33	234.00±4.00	263.33±2.52	16.9
E	*P. suwonensis*	167.41±0.36	185.07±3.22	196.67±1.15	17.5
F	Control	225.16±0.18	245.00±1.00	324.00±1.73	43.9

4.10.4. Effect of Chloramphenicol on hematology profile of the infected fish

Table 20 shows the effects of chloramphenicol on hemoglobin value of the infected fishes. An increase in hemoglobin values was observed in all the groups throughout the treatment period. The hemoglobin level of the infected fishes before and after 2weeks treatment ranged from 5.00±0.36to 8.00±0.72 g/dl and 9.33±0.99 to 12.33±0.61g/dl. The effects of the chloramphenicol on the infected fishes were significantly different (p value< 0.05) during the treatment periods. The treatment effect was highest in the second week of treatment (Appendix xlv).

Treatment of the infected fishes with chloramphenicol for 2 weeks caused significant increase (p value < 0.05) in the packed cell volume values of the infected fishes (Table 20). While the highest treatment effect was observed in the group infected with *L. sphaericus*, the least was observed in the group infected with *P. aeruginosa* (Appendix xlvi).

An increase in red blood cell values of the fishes was observed in all the groups after the 2 weeks treatment period. However, a reduction in RBC value was observed after first week of treatment in group B infected with *P. aeruginosa.* The effects of the treatment with chloramphenicol on the fishes were significantly different (p value < 0.05) among the bacterial isolates [Appendix xlvii].

Effects of the treatment of the infected fishes with chloramphenicol on the white blood cell count (Table 20), shows a decrease in WBC count of the fishes at the end of the treatment period. The initial WBC count ranged from 18.33±0.55 to 28.03±0.40×10^3/mm^3 while the final WBC count ranged from 10.33±0.96 to 27.13±0.92×10^3/mm^3.The highest and least treatment effects were observed in the groups infected with *B. subtilis* and *P. aeruginosa* respectively [Appendix xlviii].

With regard to the effects of the antibiotic on lymphocyte values of the infected fishes, a decrease was observed in all the groups except in groups C and E, infected with *S. marcescens* and *P. suwonensis* respectively. However, there was no significant difference (p value = 0.658) in the effects of the treatment in all the groups except in group B [Appendix xlix].

A significant difference p value < 0.05 was observed in the effects of the treatment with chloramphenicol on the monocyte of infected fishes during the treatment periods at. A decrease in monocyte count of the infected fishes was observed in groups A, B, D and E and an increase occurred only in group C (Table 20).

4.10.5. Effects of Chloramphenicol on blood chemistry profile of the infected fish

The effects of chloramphenicol on the blood chemistry profile of the fishes infected with the bacterial isolates are shown in Table 21. A decrease in aspartate transaminase enzyme value was observed in all the groups at the end of the treatment period except in group C infected with *Serratia marcescens.* Statistically, the effects of the treatment were significant (p value < 0.05). The highest effects were observed in groups infected with *S. marcescens* and *P.suwonensis* (Appendix li).

A decrease in alanine transaminase enzyme (ALT) value was observed in all the groups at the end of the experiment. The range of the ALT value of the infected fishes before and after treatment were 27.10±0.93 to 29.21± 0.86 and 19.33±1.18 to 25.17±0.88 respectively

4.10.6. Effect of Chloramphenicol on weight of the infected fishes

The effects of treatment with chloramphenicol on weight (g) of the fishes infected with the bacterial isolates are presented in Table 22. A continual increase in the weight of the fishes in all the groups was observed throughout the treatment period. However, the highest treatment effect on the weight of the fishes was observed in group D and the least in group C (Appendix liii). The weights of the fishes before and after treatment were in the range of 130.67±0.58 to 226.93±1.02g and 187.33±2.52 to 365.00±2.65g respectively. The highest percentage weight gain (50.4%) was observed in group A infected with *L.sphaericus* while the least percentage weight gain (43.4%) was observed in group C infected with *S. marcescens.* However, the weight gain was significantly higher (p value < 0.05) in the control group.

Table 20: Effects of chloramphenicol on hematology profile of the infected fish

Group	Bacterial isolate	Before treatment	1week	2weeks
Haemoglobin level (g/dl)				
A	*L.sphaericus*	6.00±0.75	9.00±0.53	9.33±0.99
B	*P. aeruginosa*	5.00±0.36	7.00±0.78	9.33±1.07
C	*S. marcescens*	7.00±0.60	9.00±0.61	11.33±0.61
D	*B. subtilis*	7.00±0.35	8.33±0.98	10.33±0.76
E	*P. suwonensis*	8.00±0.72	9.00±0.90	12.33±0.61
F	Control	9.13±0.97	9.17±0.67	9.13±0.31
Packed cell volume (%)				
A	*L.sphaericus*	24.00± 0.90	33.00± 0.10	34.33±0.67
B	*P. aeruginosa*	17.00± 0.80	28.30± 0.70	30.33±0.91
C	*S. marcescens*	24.00± 0.70	30.40± 0.61	30.33±0.83
D	*B. subtilis*	24.00± 0.60	26.20± 0.75	28.33±0.90
E	*P. suwonensis*	25.00± 0.50	30.00± 0.30	35.00±1.00
F	Control	29.17±3.82	29.17±2.05	29.17±0.67
Red blood cell (10^6/mm^3)				
A	*L.sphaericus*	1.33±0.12	1.63±0.39	2.83±0.31
B	*P. aeruginosa*	1.33±0.26	1.20±0.13	1.73±0.32
C	*S. marcescens*	1.33±0.09	1.50±0.09	2.50±0.57
D	*B. subtilis*	1.43±0.42	1.82±0.22	2.07±0.03
E	*P. suwonensis*	2.47±0.06	2.70±0.19	2.50±0.48
F	Control	2.93±0.15	2.92±0.11	2.96±0.12
White blood cell (10^3/mm^3)				
A	*L.sphaericus*	28.03± 0.40	20.37± 0.52	22.33±0.91
B	*P. aeruginosa*	18.33±0.55	8.06±1.03	10.33±0.96
C	*S. marcescens*	19.37± 0.53	15.33± 1.66	17.33±1.06
D	*B. subtilis*	27.33± 0.40	25.20± 0.93	27.13±0.92
E	*P. suwonensis*	25.00± 0.82	22.17± 1.02	18.93±0.64
F	Control	16.66± 1.16	16.33± 0.76	16.45±0.88
Lymphocyte (%)				
A	*L.sphaericus*	94.00± 1.00	90.33±0.88	88.00±1.00
B	*P. aeruginosa*	88.33± 1.43	86.33± 0.88	83.33±0.76
C	*S. marcescens*	90.33± 1.33	90.30± 0.94	91.33±0.94
D	*B. subtilis*	91.33± 1.23	90.33± 0.88	88.33±1.14
E	*P. suwonensis*	90.00± 0.60	91.33± 0.66	90.33±1.33
F	Control	82.43± 0.55	82.02± 1.74	82.80±4.17
Monocyte (%)				
A	*L.sphaericus*	13.00± 0.40	12.23± 0.82	12.33±1.07
B	*P. aeruginosa*	11.50± 0.74	10.33± 0.65	8.33±1.13
C	*S. marcescens*	10.33± 1.08	10.13± 0.86	11.33±1.18
D	*B. subtilis*	13.00± 0.75	11.33± 0.59	11.33±0.83
E	*P. suwonensis*	13.00± 0.70	6.30± 0.76	10.33±1.01
F	Control	8.58± 1.36	8.80± 1.90	8.33±1.14

Table 21: Effects of Chloramphenicol on blood chemistry profile of the infected fish

Group	Bacterial isolate	Before treatment	1 week	2 weeks
	Mean Aspartate Transaminase Values			
A	*L.sphaericus*	60.33± 1.22	66.36±1.10	54.33±1.09
B	*P. aeruginosa*	76.33± 1.18	70.33± 1.07	73.33±1.07
C	*S. marcescens*	82.00± 0.64	86.00± 1.00	88.33±1.43
D	*B. subtilis*	72.33± 1.06	70.33± 0.97	62.33±0.89
E	*P. suwonensis*	91.33±1.09	81.00± 1.33	85.40±0.88
F	Control	63.12± 6.11	63.50± 1.56	63.35±8.44
	Mean Alanine Transaminase Values			
A	*L.sphaericus*	29.17± 0.94	25.33± 1.04	19.33±1.18
B	*P. aeruginosa*	27.10± 0.93	25.33± 1.18	21.33±1.97
C	*S. marcescens*	29.17± 0.94	25.33± 1.29	23.33±1.43
D	*B. subtilis*	28.17± 0.96	27.33± 1.07	24.33±0.89
E	*P. suwonensis*	29.21± 0.86	30.00± 0.63	25.17±0.88
F	Control	22.56± 2.81	22.54± 1.40	22.43±0.51

Table 22: Effects of Chloramphenicol on weight of the infected fish

Grp	Isolate	Weight (g)			%weight gain
		Before treatment	1 week	2weeks	
A	*L.sphaericus*	173.33±2.31	249.33±7.02	260.67±0.58	50.4
B	*P. aeruginosa*	145.67±2.08	218.83±1.04	213.67±1.15	46.7
C	*S. marcescens*	130.67±0.58	134.47±0.42	187.33±2.52	43.4
D	*B. subtilis*	221.67 ±2.89	279.67±2.52	330.67±1.15	49.0
E	*P. suwonensis*	163.00±1.73	230.83±1.04	237.00±2.65	45.4
F	Control	226.93±2.69	253.67±1.53	365.00 ±2.65	60.8

4.11. Effect of treatment on total bacterial load of internal organs of the fishes

The mean bacterial count in gill and liver of fishes after treatment with probiotic and chloramphenicol are presented in Fig.8 and 9 respectively.

The bacterial loads of the gill and liver of the infected fishes were significantly higher (p value < 0.05) in the infected fishes than the control group. The effect of the probiotic on the infected fishes was significantly different (p value < 005) among the groups [Appendix liv]. At the end of the treatment, the bacterial count in the gill ranged from $2.65\times10^4\pm0.01$cfu/g – $10.57\times10^4\pm0.15$cfu/g. The highest bacterial load of the gill was observed in the group infected with *S. marcescens* and the least in the group infected with *B. subtilis.* In the liver, highest bacterial load was seen in the group infected with *P.aeruginosa* and least in the group infected with *P. suwonensis*. The bacterial count in the liver after treatment with probiotic ranged from $2.74\times10^4\pm0.09$cfu/g –$5.63\times10^4\pm0.06$cfu/g (Fig.8).

Treatment with chloramphenicol affected the bacterial load of the gill and liver of the infected fishes. There was significantly higher bacterial count (p value < 005) in the infected fishes than in the control group. The bacterial counts obtained were significantly different (p value < 0.05) among the groups. The counts obtained after 2 weeks treatment ranged from $1.48\times10^4\pm0.21$cfu/g – $3.9\times10^4\pm0.10$cfu/g and $1.45\times10^4\pm0.05$cfu/g – $5.37\times10^4\pm0.15$cfu/g in the gill and liver respectively (Fig.9).

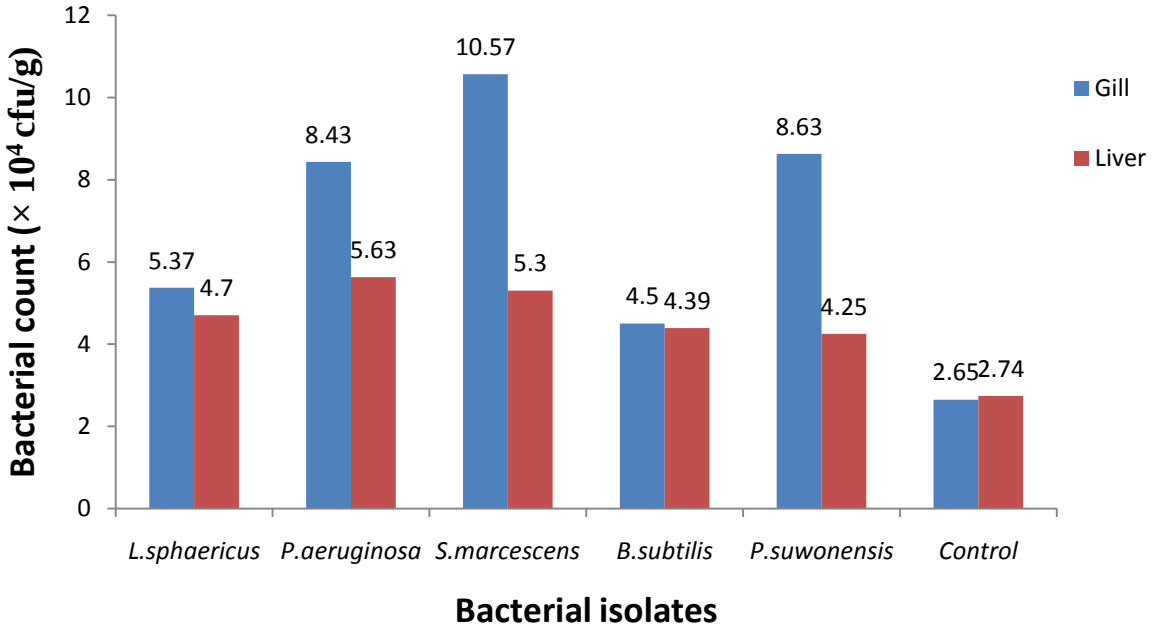

Fig.8. Effect of probiotic on bacterial load of the gill and liver of infected fishes

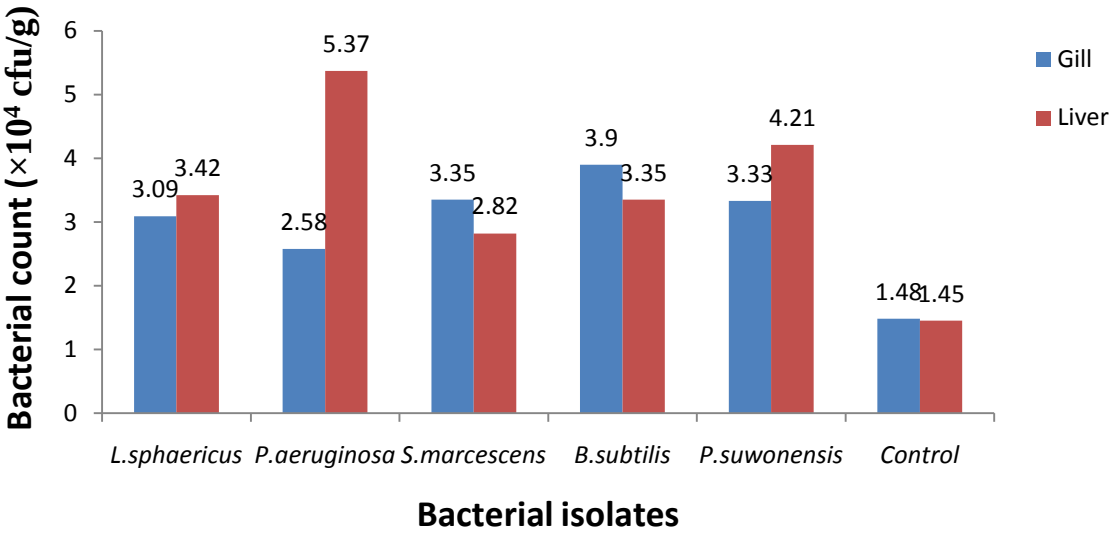

Fig.9. Effect of antibiotic on bacterial load of the gill and liver of infected fishes

DISCUSSION

Fish has become increasingly important source of protein and other elements necessary for the nourishing of the body. Fish aquaculture is a practice done in some parts of Anambra state as a means of ensuring all year supply of fishes.

The seasonal variation of the bacterial load in the internal organs of fish samples obtained from different fish farms in the three senatorial zones in Anambra state visited was carried out. The bacterial loads obtained in the gills of the fish samples were higher than the counts obtained in the liver in both dry and rainy seasons. There was significant difference (p value < 0.05) in the bacterial counts obtained in gill and liver in the different farms, during dry and rainy season and among the three senatorial zones. The high bacterial load in the gills than the liver may be as suggested by Bekele *et al*. (2019), due to the fact that gills play an important role in filtering microscopic organisms while in water, and smaller particles are entrapped by the gill filaments in a mucous leading to higher level of bacterial population. The bacterial count obtained from the gills during the dry season and rainy season ranged from 1.3×10^3 -9.6×10^3 cfu/g. This is in line with the work of Abu and Uwadiriroha (2016), who reported highest bacterial count in the gills than in other organs sampled. They recorded counts ranging from $0.73 \pm 0.0030 \times 10^3$ - $2.55 \pm 0.0128 \times 10^3$ cfu/g. Similar range of bacteria were recorded by Adegunloye and Sanusi (2019), during their work on microbiota of catfish (*Clarias gariepinus*) tissues, and opined that the higher microbial load in gills than liver and intestine is due to the gill's constant interaction with the water environment. On the contrary, very high bacterial load of $1.2 \pm 0.42 \times 10^{12}$ CFU/g and $6.48 \pm 1.06 \times 10^6$ CFU/g was reported by Bekele *et al*. (2019) in the gill of Tilapia, during their work on prevalence and antimicrobial susceptibility of pathogenic bacteria in Nile tilapia, *Oreochromis niloticus L*. They also noted that poor sanitary condition could be the cause of such high load.

The higher bacterial load in fish sample observed during the rainy season than in the dry season in this study may be due to the fact that rain water washes down microorganism in the air into the ponds during rainy season. This view agrees with the work of Mgwede *et al*. (2018), who reported higher microbial load of fresh *Engraulicypris sardella* (Usipa) during the rainy season than the dry season**.** However, the bacterial load of fishes depends on the microbial flora and sanitary conditions of the fish pond water, whether dry season or rainy season.

The bacteriological analysis of the fish samples also revealed the presence of several bacterial genera. The isolation of the bacterial species shown in Table 3 is in line with the work of other researchers who have reported isolation of one or several of the isolates obtained in this study (Akaniro *et al*., 2020; Bekele *et al*., 2019; Wamala *et al*., 2018; Abdel-salam *et al*., 2016). The highest prevalence of *Bacillus* sp obtained in this study (Table 4) supports the work of Akaniro *et al*. (2020), who reported *Bacillus* spp. as the most occurring bacteria during their work on isolation, characterization and antibiotic resistance profile of bacteria from the gut of African catfish. On the contrary, Abdel-salam *et al*. (2016) reported that *Aeromonas sorbia* was the most occurring bacteria during their work on isolation and identification of bacteria flora from catfish, which is contrary to our findings.

The seasonal variation of the bacterial load in the water samples of fish pond obtained from different fish farms in the three senatorial zones in Anambra State showed that bacterial load was higher during rainy season than during the dry season. The bacterial counts obtained in all the pond waters during rainy and dry seasons were significantly different (p value < 0.05).

The high bacterial load of the pond waters observed during the rainy season in this study could be due to conducive temperature for microbial growth in the pond water during rainy season as against the dry season. Although the mean bacterial counts obtained in Anambra south were higher than the mean bacterial counts of other zones in both the rainy and dry seasons, it would be observed that the mean bacterial counts of the three zones during the dry season is similar to the count recorded by Ogeneogaga and Solomon (2017). They reported bacterial counts ranging from 7.983×10^6 cfu/ml to 15.483×10^6 cfu/ml while working on the physico-chemical and bacteriological investigation of selected fish pond in Kuje Area Council, Nigeria, and stated that the temperature of fish pond water ranging from 25°C to 35°C supports the growth of microorganisms in the pond. On the contrary, Umeh *et al*. (2020), recorded quite low bacterial count ($3.981 \times 10^3 - 1.32 \times 10^4$cfu/ml) during their work on physico-chemical, bacteriological and parasitological examination of selected fish pond water samples in Awka and its environment in Anambra state, Nigeria. The unhygienic condition of the fish pond is likely to be contributory to the microbial load of pond water irrespective of the season.

The bacteria isolated from the fish pond water are similar to the organisms isolated by many researchers (Umeh *et al*., 2020; Okafor *et al*., 2020; Njoku *et al*., 2015a; Douglas and

Isor, 2015). *Staphylococcus* spp was observed as the most prevalent bacteria in the pond water while *E. coli* was the least (Table 7). This is contrary to the reports of Douglas and Isor (2015) and Okafor *et al.* (2020), who reported *E. coli* as the most occurring bacteria in fish pond water.

Most of the organisms isolated from the fish samples (Table 3) except *Vibrio* and *Aeromonas* were also recovered from the pond water, while *Enterococcus, Enterobacter, Paenalcaligenes, Lysinibacillus, Acinetobacter, E.coli* only were recovered from the pond water and not in the fish samples. The sources of these organisms in fish and pond include the microbial flora of the fishes, water source, environment and human activities in the pond.

The occurrence of antibiotic resistance among bacteria from livestock has raised considerable concern due to the potential for transfer of resistant pathogens to the human consumers (Gufe *et al*., 2019). The bacterial isolates showed varying susceptibility and resistance to the antibiotics tested. The susceptibility of the isolated bacteria to conventional antibiotics revealed that the organisms had highest percentage susceptibility and least resistance (94.4%) to chloramphenicol, vancomycin, ciprofloxacin and trimethoprim and least susceptibility and highest resistance (72.2%) to erythromycin. Highest resistance and least susceptibility to the antibiotics tested were observed with *Serratia*. While *Staphylococcus* sp., *Enterobacter* sp., *Micrococcus* sp, *E. coli* and *Microbacterium* sp. showed 100% susceptibility, *Pseudomonas* sp., *Bacillus* sp., *Paenalcaligenes* sp, *Lysinibacillus* sp. and *Serratia* sp. were resistant to more than one antibiotic (Table 8). The resistance of the isolates to antibiotics could be attributed to indiscriminate use of these antibiotics in aquaculture. The high susceptibility of the bacterial isolates to chloramphenicol is similar to the work of Kasing (2013). They observed a high susceptibility of the bacterial isolates to chloramphenicol. However, Njoku *et al.* (2015b) noted that bacterial isolates from fish ponds were most susceptible to erythromycin. Highest resistance of 100% to lincomycin and least resistance rate of 13% to sulphamethoxazole by bacterial isolates recovered from fish were reported by Gufe *et al*. (2019). Agoba *et al*. (2017) reported that bacteria isolated from fish farm showed highest resistance to penicillin and least (1.7 – 5.6%) to gentamicin.

Out of the organisms isolated from the fish and pond water sample, five bacterial isolates based on their resistance to antibiotic susceptibility tests were selected and identified following 16S rRNA sequencing technique as *Lysinibacillus sphaericus* G39, *Bacillus subtilis* BB1, *Serratia marcescens* Db11, *Pseudomonas aeruginosa* PA01 and

Paenalcaligenes suwonensis UN24 (fig. 3). The evolutionary relatedness of the isolates is shown in Fig. 3.

The water physico-chemical characteristics are based on climatic conditions and soil factors and these contribute to quality of the water. The physico-chemical characteristics of fish pond water affect the activities and well-being of the fishes. Table 9a and 9b show the results for the physico-chemical analysis of the different pond water samples.

The optimum water temperature for fish survival ranged fom 20-30^{0}C (Ntengwe & Edema, 2008; Sawere & Oghenekowhoyan, 2019). The temperature recorded in this study ranged from 27.1-32.1^{0}C. The values were well within permissible limit for aquaculture. This result corroborates with the report of Fafioye (2011), who also observed a temperature range of 27-28^{0}C in the preliminary studies and water characteristics of bacterial population in Kojalo fish pond. Njoku *et al*. (2015a), also reported a temperature range of 26-29^{0}C, and opined that such temperature range supports fish productivity. Contrary to our findings, Wang *et al*. (2018) reported much lower temperature below 25^{0}C in their study.

Optimum pH directly stabilizes the physico-chemical parameters of pond water, enhancing fish health and productivity, and the maintenance of a proper balance of the microbial ecology in pond water (Makori *et al*., 2017). Fluctuations in pH have been reported to cause extreme stress in fish (Fakorede *et al*., 2019). Fishes are known to have an average blood pH of 7.4 (Kumar *et al*., 2017). It has been confirmed that the pH between 6.5 and 9 is most appropriate for maintenance and increased fish production (WHO, 2020; Bhatnagar & Devi, 2013). The pH recorded in this study ranged from 6.4 – 8.0. These values are within the range required for aquaculture and are similar to the work of Olaniyi (2013), who reported a pH of 6.7- 7.4 in the assessment of water quality characteristics for aquaculture uses in Abeokuta, Nigeria . Contrary to our findings, pH values of 7 -8.3 were reported by Kamal *et al*. (2007), Kumar *et al*. (2017) and Wairimu *et al*. (2019). Kumar *et al*. (2017), recorded pH range from 7.1 – 8.0, While Kamal *et al*. (2007) and Kumar *et al*. (2017), reported pH ranges of 7.3 – 8.3 and 7.1 – 8.0 respectively, Wairimu *et al*. (2019), recorded a pH range of 7.3 -7.9 in their study on impact of aquaculture development on water quality of fish ponds in Gatundu North and South sub-countries, Kenya.

Conductivity is an indicator of the freshness of a waterbody, and high values of conductivity are indicative of pollution (Kumar *et al*., 2017). High level of nutrient content of fish feed is known to contribute to the high conductivity values observed in most pond

water.The FAO acceptable limit for conductivity in aquaculture is 20 -1500 µs/cm (FAO, 2016). The electrical conductivities of the pond water samples varied from 40.0 – 61.0µs/cm throughout the period of study (Table 9a). This is within the permissible limit stated by FAO and suitable for fish production (FAO, 2016). A similar study in Busia County recorded electrical conductivity (EC) levels of 34.67 – 86.67µs/cm (Makori *et al*., 2017). South East Brazil ponds had a wider range of 24 – 610 µs/cm, while analysis carried out in a fish pond in Ghana recorded higher EC levels of 102.2 – 132.30 µs/cm (Ansa *et al*., 2013).

Turbidity in water is caused by presence of suspended particles. At increased turbidity of pond water, the penetration of light is greatly impeded and absorption of nutrient by fishes is slowed down.The WHO standard for turbidity of pond water is 5NTU (WHO, 2020). Contrary to WHO's standard, the turbidity values 5.5 -10.9 NTU obtained from the ponds investigated were not within the range that supports aquatic life (Table 9a). The high turbidity value obtained could be attributed to the types of feeds introduced into the ponds.

Alkalinity is the measure of the quantity of base in water. Optimum alkalinity for increased fish production is 20 – 300mg/L (Njoku *et al*., 2015a). The values (92.0 – 137.3mg/L) obtained in this study were within the limit for fish farming. Similar result was reported by Ehiagbonare and Ogunrinde (2010), who worked on physico-chemical analysis of fish pond water in Okada and its environs, and obtained the alkalinity value ranging from 35 to 135mg/L. Higher alkalinity values of 148.33 – 210.5 mg/L was however, reported by Manjare *et al*. (2010).

Nitrite is termed the invisible fish killer, as it is deadly to the smallest fish even at a concentration as low as 0.25ppm. Excess nitrite concentration in fish pond water has been reported to prevent blood cells from absorbing oxygen from water, thus making fish blood to appear dull brown in colour which is significant of a nitrite poisoning popularly called "brown blood disease" (Njoku *et al*., 2015a). Nitrite concentrations (1.1 – 2.9mg/L) obtained in this study exceeded the maximum permissible limit of 0.1mg/L. This may be as a result of the action of algae through nitrogen fixation.

Phosphate levels in this study were within WHO limits for aquaculture (0.03-3mg/L) [WHO, 2020]. Use of fertilizers and phosphorous–rich fish feeds impact phosphate concentrations in fish ponds significantly and increased phosphate concentration of the pond encourages algal growth (WHO, 2020; Wairimu *et al*., 2019). Phosphate levels (0.8 – 2.3mg/L) in pond water in this study supports the range of 0.69 – 2.41mg/L reported by

Wairimu *et al*., 2019. Kiran (2010), reported a range of 0.51- 1.28 in fish ponds at Karnataka while Raj and Sevarkodiyone (2018), recorded phosphate level ranging from 0.36 – 2.86mg/L. However, Ehiagbonare and Ogunrinde, (2010), recorded a higher range of 1.40 – 4.51mg/L.

Sulphate is known as one of the least toxic anions (Umeh *et al*., 2020). Fishes can survive wide range of sulphate in water. The usual sources of sulphate in fish pond are the borehole water used for rearing the fishes and the sulphate-containing organic feeds. The concentration of sulphate in the ponds as shown in Table 9b, varied from 6.1 – 12.1mg/L. Torimiro *et al*. (2014), recorded a sulphate concentration of 10mg/l for fresh water fish pond while Umeh *et al*. (2020) reported sulphate values ranging from 0.39 mg/l to 4.37 mg/l in the fish pond water samples.

Biochemical Oxygen Demand is the measurement of total dissolved oxygen consumed by microorganism for biodegradation of organic matter (Stevens, 2007). High BOD affects the oxygen cycle and oxygen equilibrium in the water. Water quality criterion for BOD for the protection of aquatic life is 4 mg O_2 l^{-1} (FEPA, 1991). Prasanna and Ranjan (2010) stated that a BOD level above 5mg/L is an indication of water pollution. In this study, values obtained ranged from 1.1 – 3.0mg/L (Table 9b), which is within the range given by Federal Environmental Protection Agency of Nigeria (FEPA, 1991). Sawere and Oghenekowhoyan (2019), noted a higher value of BOD in their study of cat fish ponds in Ozoro town, Nigeria, and reported that the fish ponds were grossly polluted.

Dissolved Oxygen (DO) is known to affect attributes such as growth, survival distribution, behavior and physiology of aquatic organism (Nduka *et al*., 2008). WHO standard for DO concentration which supports aquatic life ranges from 5 – 9.5mg/L (WHO, 2020). At concentration below 5mg/L, the functions and survival of bacteria may be adversely affected, while DO concentration below 3mg/L leads to death of most fish (Swann, 2006). The DO obtained from this study ranged from 6.2 to 12.0mg/L. Ehiagbonare and Ogunrinde (2010), observed DO range of 9.3 – 16.2mg/L during their work on physico-chemical analysis of fish pond water in Okada and its environs, Nigeria, while Wairimu *et al*. (2019), reported a DO range of 4.72 - 5.02mg/L during their study on impact of aquaculture development on water quality of fish ponds in Gatundu North and South sub-countries, Kenya.

The effects of the oral administration of the bacterial isolates on hematological profile of healthy fish are shown in Table 10. The haematological profile of a fish population explains the physiological and health status of the fish.

According to Bolliger and Everds (2012), Haemoglobin (Hb) concentration is defined as the amount of total Hb per volume of whole blood. Bacterial infection of fishes causes rupture of the red blood cell and release of hemoglobin which becomes nutrient for the proliferation of the pathogen (Pishchany & Skaar, 2012). The oral administration of the bacterial isolates on the fishes (Table10) had observable changes in the hemoglobin levels of the fishes. The bacterial isolates showed varying effects on the hemoglobin during the experimental period. Decrease in hemoglobin level was generally observed in all the fishes inoculated with the bacterial isolates. However, highest reduction in hemoglobin was observed in the group inoculated with *Pseudomonas aeruginosa.* This result agrees with the work of Oghenebrorhie *et al*. (2014), who reported a decrease in Hb value of *Clarias gariepinus* infected with *Pseudomonas aeruginosa.* Haque *et al*. (2020) also reported a significant decrease in hemoglobin level of fishes infected with *Pseudomonas aeruginosa*. Karuthapardi and Xavier (2010) reported that there was sharp decrease in the mean value of Hb in Tilapia when infected with *Vibrio anguillarum*. Similar decrease was recorded in Hb value of *Cyprinus carpio* infected with *Flavobacterium columnare* (Tripathi *et al*., 2005). However, *Vibrio anguillarum* and *Flavobacterium columnare* were not used in this study but they did show that Hb value during infection of fish is usually decreased.

The infection of the fishes with the bacterial isolates had significant effects (p value < 0.05) on the packed cell volume (PCV) of the fishes (Table 10). There was significant decrease on the PCV of the fishes. Decrease in the PCV in these fishes indicates that haematopoiesis (process of red blood cell formation in the blood forming tissues of the body) may have been severely affected as a result of bacterial infection. The increase in PCV of the control group is apparently on account of the uninfection of the group. As observed in Table 10, *Pseudomonas aeruginosa* was responsible for the lowest PCV, and this finding is in agreement with the works of Sivagurunathan *et al*. (2011) and Amrevuawho *et al*. (2014), who reported a marked reduction in the PCV of *Cirrhinus mrigala* and *Clarias gariepinus* respectively upon exposure to *P. aeruginosa.* In another study, no significant difference in PCV of Nile tilapia (*Oreochromis niloticus*) naturally infected with *Flavobacterium columnare* and uninfected Nile tilapia, was reported, although, there was a slight decrease in other blood parameters (Sebastiao *et al*., 2011).

Infection of fishes by bacteria causes depletion of the red blood cells. In this study, a decrease in the mean Red blood cell (RBC) count of *C. gariepinus* exposed to the bacterial isolates was observed (Table 10). The difference in the effects caused by the isolates was significant (p value< 005). This result corroborates the findings of Bektas and Ayik (2009), who reported a significant decrease in RBC count of *Pseudomonas putida*- infected rainbow trout (*Oncorhynchus mykiss*). Oghenebrorhie *et al.* (2014), in their pathological study of *Clarias gariepinus* sub-adult artificially infected with *Pseudomonas aeruginosa*, noted a signficiant difference in the total RBC count of healthy and infected fishes. Sebastiao *et al.* (2011), also recorded significant decrease in RBC upon exposure of fish to *F. columnare.* On the contrary, Manoj *et al*. (2010), reported an increase in RBC of infected fish, while Zorriehzahar *et al*. (2010), noted that there was no significant difference in RBC count of infected and uninfected rainbow trout (*O. mykiss*) fry.

An increase in white blood cell count of the fishes exposed to the bacterial isolates was demonstrated in this study (Table 10). The body system synthesizes white blood cells as the frontline defence of the immune system. High WBC count is usually associated with microbial infection or the presence of antigens in the blood (Tiamiyu *et al*., 2019). In this study, there were variations in the level of effects of the bacterial organisms on the fish samples. A significantly (p value < 0.05) higher WBC counts were obtained in the fishes infected with the bacterial isolates than in the control fishes. The WBC count of healthy fish (control group) was observed to be within the range of normal limits indicated for cat fish (Okorie-Kanu & Unakalamba, 2014). The elevated WBC count of the infected fishes observed in this study, is in line with the work of Yildiz and Aydin (2006), who noted significantly higher WBC count in the fish infected with A*rcobacter cryaerophilus* during their work on pathological effects of *Arcobacter cryaerophilus* infection in rainbow trout (*Oncorhynchus mykiss* Walbaum). Oghenebrorhie *et al*. (2014) also worked on the pathological effect on *Clarias gariepinus* sub-adult artificially infected with *Pseudomonas aeruginosa* and reported an increased WBC count. On the contrary, reduction in WBC count was reported by Omoya and Akharaiyi (2015), in fish inoculated with *Bacillus* species.

Effects of the bacterial isolates on the differential WBC counts (monocytes and lymphocytes) of the fishes are presented in Table 10. Increased presence of lymphocyte and monocyte in the blood indicates active immune response to an antigen. As presented in Table 10, the monocyte count of fishes in all the groups increased significantly (p value < 0.05), from initial range of 8.37±1.00 - 9.34±0.72% to 10.41±0.97 - 13.39±0.92% after the 2 weeks

experiment. This result is in line with the work of Adeyemi *et al*. (2013), who reported higher monocyte count in infected fishes than the uninfected ones. Martins *et al*. (2008), on the contrary, reported decreased number of monocytes in tilapia fish injected with $1x10^6$ *Enterococcus*/mL. Rafiq *et al*. (2001) did not observe any alteration in the differential counts of WBCs in tilapia challenged with *Aeromonas hydrophila.*

Significant increase in lymphocyte count of the fish was observed in this study (Table10) upon exposure of the fishes to bacterial isolate, thus, the bacterial isolates ilicited significant lymphocyte activity. Contrary to our findings, Adeyemi *et al*. (2013), reported a reduction in lymphocyte count in fish samples challenged with *Vibrio fischeri* and *E.coli* in their work on hematological assessment of health status of African catfish *Clarias gariepinus* (Burchell 1822) experimentally challenged with *Escherichia coli* and *Vibrio fischeri.*

Biochemical profiling is essential for the evaluation of fish health status. Measurement of the activity of various enzymes in fishes can be used for confirming maturity and monitoring any changes in the quality of water (Shahsavani *et al*., 2010). There was an observable increase in the mean aspartate transaminase (AST) and mean alanine transaminase (ALT) values in all the fishes challenged (Table11). All the bacterial isolates showed significant effect (p value < 0.05) on the AST and ALT level of the fishes. These results corroborate the findings of Rozas-Serri *et al*. (2017), on the increase in AST and ALT concentrations of fish samples challenged with *Piscirickettsia salmonis*-like isolates. They opined that the increase in serum levels of ALT and AST in diseased fish indicates increased permeability of cellular membranes and various levels of damage to hepatocytes, and is consistent with the presence of microscopic liver lesions. ALT concentration of fishes experimentally challenged with *Arcobacter cryaerophilus* increased from 73.0 ± 23.5 to 80.7 ± 28.3 (Aydin *et al*., 2009).

The weight of the infected fishes was significantly lower than the uninfected group. Aside the control group which had the highest percentage weight gain of 66.2%, the fishes infected with *Bacillus subtilis* showed relatively high percentage weight gain while the least weight gain was observed in the fishes infected with *Serratia marcescens* (Table 12). The relatively high weight gain observed in the group infected by *Bacillus subtilis* may be as a result of the probiotic nature of the isolate.

Infection of fish by pathogens can lead to dysbiosis and disease (Hess *et al*., 2015). Usually a diverse microbiome is associated with healthy phenotypes, but disruptions to this

equilibrium can lead to an increase in abundance of opportunistic pathogens and disease susceptibility (Reid *et al*., 2017). The inoculation of the bacterial isolates into the fishes resulted in observable variations in the mean bacterial populations of the fish internal organs (Fig.4 and 5). There was significant increase (p value < 0.05) in the mean bacterial counts in the gills of the infected fishes. After 2weeks inoculation, the increase in bacterial count in the gills was highest in the fishes infected with *Lysinibacillus sphaericus* (Fig.4).

In the liver of the infected fishes, the bacterial count 2 weeks post infection was significantly different (p value < 0.05) among the groups (Fig 5). Highest count was observed in fish infected with *Pseudomonas aeruginosa*. It can be observed that the bacterial count 2 weeks post infection was higher in the gills than the liver. While the count obtained in the liver ranged from 2.68×10^4 - 20.67×10^4cfu/g, the count in the gill ranged from 4.37×10^4 - 49.02×10^4cfu/g. Rosado *et al*. (2019), reported that infection of fishes with pathogens led to a decrease in microbial load which translates into a decrease in host immunity.

Histopathological changes on the organs of the fishes infected with the bacterial isolates as presented in Plates 1-6, revealed an observable distortion of the gill tip and lamella in the fishes infected with *P aeruginosa* (Plate 2) and *S. marcescens* (Plate 3). No observable change was observed in the gill architecture of fishes infected with the other organisms (Plates 1,4,5 and 6). The distortion in gill structures is an indication of pathogenic invasion of the isolates into the gill tissues of the fishes. The gills are the primary initial target of toxicity, and the cytological changes in gills morphology in fish usually occur as a result of contaminant exposure (Alaa *et al*., 2012). Our finding supports the work of Hanna *et al*. (2014), who reported the curling of the lamellae, hypertrophy and hyperplasia of the epithelial cells lining the secondary lamellae, resulting in lamellar fusion in the gills of fishes infected with *Pseudomonas aeruginosa*. Abdelhamed *et al*. (2017) reported thickening of the primary and secondary lamellae in approximately 75 – 80% of gill lamellar surface, with a moderate number of lymphocyte invasion in their work on characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent *Aeromonas hydrophila*. El-Barbary and Hal (2017), also had a similar report. They observed that the gills of fishes infected with *S.marcescens* exhibited epithelia lifting and hyperplasia, aneurism, congestion and collapsed secondary lamellae in the fish.The observation of no histological changes in the fishes infected with *L.sphaericus, B.subtilis, P.suwonensis* is in line with the findings of Sahoo *et al*. (2000), who reported no remarkable changes in the gill of *Anabas testudineus* exposed to *Edwardsiella tarda.*

Amrevuawho *et al*. (2014), opined that histopathological changes observed in diseased fish show that microbial invasion affects the fish internal organs such as liver, gills and intestine. Visible signs of liver impairments of periportal inflammation, accumulated cell infiltrates, abnormal widening of liver sinusoids and cytoplasmic vacuolations were observed in the fish groups infected with *Pseudomonas aeruginosa* (Plate 8), *Serratia marcescens* (Plate 9) and *Paenalcaligenes suwonensis* (Plate 12). These observations corroborate with the result of Abdel-Salam *et al*. (2017), who reported coagulative necrosis, leukocytic infiltration and congestion of hepatic blood vessels in the liver of *Clarias gariepinus* infected with *Aeromonas sobria*. Amerevuawho *et al*. (2014) also observed marked degeneration of the hepatocytes which is usually the primary or secondary target. Omoya and Akharaiyi (2015), reported presence of slightly vacuolated cells in the liver of catfish treated with *Bacillus* spp, showing evidence of fatty degeneration.

The bacterial isolates were inoculated into adult albino mice. This was carried out to elucidate possible effects of the isolates in humans upon possible entrance.The commonly used route of inoculation employed by most researchers in mice is the intraperitoneal route (Abdullah *et al*., 2013; Jesse *et al*., 2013; Raetz & Whitfield, 2002). Other routes that are used, less commonly, include subcutaneous and intramuscular. The routes of inoculation used in this study were oral and subcutaneous.

Haematological changes caused by bacterial infections are first detected during routine blood analysis. However, an animal's defensive mechanisms can react quite differently to different bacteria; therefore, there is no singular pattern in complete blood analysis that indicates a bacterial infection. The effects of the oral administration of the bacterial isolates on hematological profile of healthy mice are shown in Table 13.

There was significant difference (p value < 005) in the effects of the bacterial isolates on the hemoglobin of the mice. An overall decrease in hemoglobin level was observed in all the mice groups except the group infected with *P. suwonensis* (Table 13) after 2 weeks experimental period. However, a marked increase in hemoglobin level was observed in the mice infected with *B.subtilis* after first week of inoculation, but decreased by the second week. A significant reduction in the level of Hb in rat orally inoculated with *Bacillus* sp., and *Citrobacter* sp was reported by Odiete *et al*. (2019). Ali *et al*. (2015) also reported that there was a decrease in hemoglobin level of mice orally infected with *Pastuerella multocida*, and at increasing doses of the organism, there was significant decrease in Hb of the infected mice.

Adetunji and Anyanwu (2011), recorded similar effect on hemoglobin of mice orally infected with *B. thuringiensis.*

The oral administration of the bacterial isolates in mice caused an increase in packed cell volume (PCV) of the mice infected with *L.sphaericus* and *P. suwonensis,* and a decrease in the group infected with *P.aeruginosa, S .marcescens* and *B.subtilis* (Table 13). The effects of oral administration of the isolates on the PCV of the mice were significantly different (p value < 0.05) [Appendix xx]. Odiete *et al*. (2019), recorded slight reduction in PCV of mice infected with *Bacillus cereus*, *Bacillus* sp., *Staphylococcus* sp. and *Salmonella* in their work on hematological and pathological studies of bacteria associated with mobile phones from handlers of diverse lifestyles in the rural community. They opined that reduction of PCV may be attributed to more than one factor, one of which is the failure to supply the blood circulation with cells from haem hepatic tissues, since liver has an important role in the regeneration of erythrocyte or possible destruction effect on erythrocyte pathogenesis of the bacteria inoculated. However, Boshuizen *et al*. (2019) recorded a stable hemoglobin level in rat infected with *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* during a 14 day disease duration.

A slight decrease in the mean RBC counts was observed in the mice inoculated with *L. sphaericus, P.aeruginosa, S. marcescens* and *P.suwonensis*, while an increase was noted with *B. subtilis* (Table 13). Abdullah *et al*. (2013), recorded a decrease in red blood cell count of mice infected with *Pasteurella multocida* and indicated that there was significant difference in the RBC of the infected and uninfected mice. Similar reduction in RBC was also reported by Ali *et al*. (2015), in their work on haematological and histopathological vicissitudes following oral inoculation of graded doses of *Pasteurella multocida* Type B: 2 and its lipopolysaccharide in mice.

Univariate analysis of the data obtained on the effects of the bacterial isolates on the white blood cells counts of mice infected through oral administration, shows that there was significant difference (p value < 0.05) in the WBC obtained in the various groups, indicating observable variations in the effects by the different isolates on the WBC count of the mice (Table 13). Increase in WBC count was observed in all the groups at the end of the experiment except the group infected with *P. aeruginosa* which had reduction in WBC (Table 13). However, a significant increase (p value < 0.05) in WBC was observed in the group infected with *P. aeruginosa* in the firstweek of the experiment before a decrease in the second

week. This observation is similar to the work of Odiete *et al.* (2019), who reported a decrease in WBC count of mice inoculated orally with bacteria. Increase in WBC observed with most of the isolates as suggested by Lockhart (2017), may have resulted from tissue necrosis, inflammation disorder, crush injuries, stress, uremia, collagen vascular disorder. Ajay *et al*. (1991) had reported a significant increase in leucocyte (white blood cell) count of Japanese quail from 3-7days post infection. They noted that it was due to an increase in the percentage of heterophils and decrease in lymphocytes**.**

An increase in lymphocyte count known as lymphocytosis, was observed in the mice upon oral inoculation with the isolates (Table 13). Lymphocytes are involved in adaptive immunity and when the cells encounter antigen, they multiply in order to amass sufficient numbers of cells to mount an effective response (Nester *et al*., 2004). As demonstrated in this study, there was a significant difference (p values < 0.05) in the effects of the isolates on the lymphocyte counts of the mice, during the experimental period. The increase in lymphocyte counts observed in this study could be as a result of inflammation in response to the presence of these bacterial isolates, as suggested by Nester *et al*. (2004). Contrary to this result in which *B. subtilis* showed an increase in lymphocyte cells, Odiete *et al*. (2019) and Adetunji and Anyanwu (2011), observed a reduction in lymphocyte counts of mice inoculated orally with *Bacillus cereus* and *Bacillus spp* respectively. In a study by Zhang *et al*. (2020), using piglet as the experimental animal, they noted an increase in lymphocyte count of the piglets after oral administration of *Bacillus subtilis.*

In the effects of the bacterial isolates on the monocyte count of mice (Table 13), it shows that the group orally infected with *L. sphaericus, B.subtilis* and *P. suwonensis* had reduced monocyte while an increase was observed in the group infected with *P.aeruginosa* and *S. marcescens*. The difference in the effects of the isolates on the mice monocyte count was significant (p value < 0.05), however, with respect to the experimental periods, there was no significant difference in the monocyte counts obtained (p value > 0.05). The variations in monocyte count observed in this study agrees with the report of Gihan *et al*. (2016), who stated that monocytes and neutrophils act as host defences against infections by chemotactically migrating to the site of infection. Abdullah *et al*. (2013) also observed an increase in monocyte count after intraperitoneal inoculation of mice with *Pasteurella multocida*. Chao and Eric (2011) reported that studies of different diseases revealed the multipotency of monocytes in different inflammatory environments. In contrast with our result on the effect of *B. subtilis* on monocyte reduction, Odiete *et al*. (2019), observed a

slight increase in monocyte count after oral inoculation of *Bacillus cereus*. Similarly, Toapanta *et al*. (2015), observed reductions in the percentages of monocytes as measured by flow cytometry after oral inoculation of wild-type *Salmonella typhi* in individuals who developed typhoid disease.

The effects of oral inoculation of the bacterial isolates on the blood chemistry profile indicate that there were changes in the AST and ALT of all the mice. Increased activity of AST and ALT is a sensitive sign of impaired organs membrane and indicates disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane, and membrane transport system (Al-Jubury*et al*., 2010). The effects of the oral administration of the isolates on the AST and ALT value of the mice during the experimental period were significantly different (p value < 0.05). There was an increase in the AST value of the mice groups infected with *L.sphaericus, P. aeruginosa* and *S. marcescens,* while the groups infected with *B. subtilis* and *P. suwonensis* had a decreased AST value. A significant increase (p value < 0.05) in ALT value was observed in all the groups. The result obtained is in line with the work of Al-Jubury *et al*. (2010), who reported increase in AST and ALT level of mice infected with *Pseudomonas aeruginosa*. Abdullah *et al*. (2013), reported that there was no significant difference (p>0.05) in the levels of ALT and AST of mice infected with *Pasteurella multocida.*

Subcutaneous administration is one of the most efficient methods to obtain a reproductible sublethal infection as it mimics to a certain extent the natural route of most infection (Motameni *et al*., 2005). Chung *et al.* (2015) observed that subcutaneous inoculation results in rapid onset and produced more consistent results compared to intranasal or oral route. Subcutaneous administration of the bacterial isolates to the mice caused changes in the hematology and biochemistry profile of the mice. Results obtained (Table 15), showed a decrease in the hemoglobin level of mice infected subcutaneously with *L. sphaericus, P. aeruginosa* and *S. marcescens* and an increase in mice infected with *B.subtilis* and *P suwonensis.* A significant difference (p value > 0.05) was observed in the effects by the different isolates.

There was significant variation (p value < 0.05) in the effects of the bacterial isolates on the PCV value of the mice, as presented in Table 15. A decrease in PCV was observed in mice infected with *P.aeruginosa* and *S.marcescens*, while an increase was observed in the other groups. Similar to our findings, Abdullah *et al*. (2013) observed that among the

challenged groups, the animals that received the inoculums containing live *Pasteurella multocida* showed significant difference in PCV.

The effects of subcutaneous administration of the bacterial isolates on the mice red blood cell counts (RBC) [Table 15] show a rise in red blood cell count of all the mice groups except the groups infected with *S.marcescens* and *P. suwonensis.* The effects caused by the bacterial isolates were significant (p value < 0.05). Rise in red blood cell count observed in the mice infected with *S.marcescens* and *P. suwonensis* could be associated with inflammation and this observation supports the findings of Praveena *et al.* (2010), who concluded that inflammation is able to reduce red blood cell count, leading to anaemia. This they observed in their work on cytokines profiles, apoptosis and pathology of experimental *Pasteurella multocida* serotype A1infection in mice. Chung *et al*. (2015), observed a significant increase (p < 0.05) in red blood cell count in buffaloes inoculated subcutaneously with *Pasteurella multocida* Type B. Brown *et al*. (2013), reported a decrease in RBC counts.They however, noted that decreased RBC count occur secondary to conditions that include blood loss, immune-mediated hemolysis, inflammatory disease, renal disease, iron deficiency, myelodysplastic disease, genetic disorders, and neoplasia.

White blood cell is the body's first immune response to any infection. An increased white blood count above the normal range is indicative of severe infection. In this study, an increase in white blood cell count was observed in all the groups except the group subcutaneously infected with *B.subtilis* and *P.suwonensis*, which showed reduction in white blood cell (Table 15). Statistically, the effects of the bacterial isolates on the white blood cell count of the mice during the experimental periods were significant (p value < 0.05) [Appendix xxx]. Mölne and Tarkowski (2000), reported significant increase in number of white blood cells of mice within two days after inoculation of *Staphylococcus aureus* and decreased 2 weeks later to levels below the original values.

An increase in lymphocyte count was observed in all the groups except the mice group infected with *B.subtilis* which showed decrease in lymphocyte count (Table 15). The result of this study agrees with the work of Dengler *et al*. (2014), who recorded an increase of absolute numbers of lymphocytes and total white blood cells (WBC) on days 6 to 8 of mice infection with Influenza A. Lee *et al*. (2011), found out that cats inoculated subcutaneously had significantly higher WBC values than those inoculated through other routes.

As presented in Table 15, a reduction in the monocyte count of the mice was observed in all the groups except the group infected with *B.subtilis.* The observed effects on the monocyte count of the mice by the bacterial isolates statistically significant ($p < 0.05$). Contrary to our observation, Brown *et al*. (2013) reported an increase in monocyte count of mice infected with *Salmonella enterica.*

In this study (Table 16), the effects of the bacterial isolates administered via subcutaneous route on the AST of the mice were statistically significant (p value < 0.05) [Appendix xxxiii]**.** The decrease in mean aspartate transaminase values observed in mice infected with *P. aeruginosa, S. marcescens* and *P.suwonensis* agrees with the work of Munukka *et al*. (2017), who reported a decrease in hepatic AST values in *Faecalibacterium prausnitzii*- treated mice. Increase in AST values observed in mice infected with *L.sphaericus and B. subtilis* also supports the work of Keskitalo *et al*. (2018), who reported that *Enterobacter cloaca* inoculation of mice significantly increased (p value < 0.05) AST values and consequently AST/ALT ratio to over 2:1. They also noted that such increased AST level is an indication of liver fibrosis or cirrhosis.

A significant difference (p value < 0.05) was demonstrated in the effects of the bacterial isolates in the ALT values of mice upon subcutaneous administration (Table 16). The isolates presented varying levels of effect on the ALT of the mice**.** The increase in ALT values of mice infected with *L.sphaericus, S. marcescens* and *P. suwonensis* demonstrated in this study is in line with the report of Zhang *et al*. (2019b), who recorded an increase in ALT and AST of mice experimentally infected with influenza A virus. They opined that the high ALT and AST values probably reflect the liver damage suffered by the infected mice.

Histological examination of the vital organs of the mice exposed to the bacterial isolates revealed that changes occurred in the structure of the intestinal wall, liver and subcutaneous tissues (Plates 14-31).

The intestinal wall histology of the mice infected with the bacterial isolates showed the presence of distortions ranging from minor erosion of the intestinal submucosa, haemorrhage at the intestinal lumen and presence of minor debris in the lumen of the intestine (Plate 14 -16). Similar histolological results to our findings were reported by Azevedo *et al*. (2014), who observed induced hypertrophy of several layers of the intestinal wall of mice. Ali *et al*. (2015), noted inflammation, congestion, degeneration and necrosis of the intestine of mice exposed to *Pasteurella multocida.* Murine haemorrhagic pneumonia was reported by Gonzalez-Juarbe *et al*. (2015), in mice inoculated with *S. marcescens*

intranasally. However, Schook *et al*. (1976) observed that histological sections of the intestines were void of any focal areas of inflammation or necrosis. They observed gross enlargement of the mesenteric lymph nodes, which, under microscopic examination, did not show any evidence of bacteria.

Several researchers have reported the use of *Bacillus subtilis* as a probiotic **(**Rhayat *et al*., 2019; Madani *et al*., 2018; El shaghabee *et al*., 2017). Probiotics ameliorates or prevents gut inflammation and other intestinal or systemic disease phenotype (Hemarajata and Versalovic, 2013). *Bacillus subtilis* and *P.suwonensis* did not cause any observable distortion to the intestinal walls (Plate 17 – 18). *B. subtilis* could have acted as a probiotic, preventing functional damage to the intestinal mucosal barrier. Our observation is in agreement with the work of Sun *et al*. (2018), who noted no inflammatory injury or histopathological changes in the intestines of mice after *B. subtilis* inoculation.

Histological examinations of the liver cells of the mice inoculated with the isolates are shown in Plate 19 – 24. All the isolates caused an observable distortion in the liver structure such as fibrotic appearances, macrovesicular fatty changes, sinusoid congestion, enlarged sinusoids and presence of minor vacuolations.This observation is in line with the report of Lemos *et al*. (2003), who observed a septal fibrosis of the liver in *Capillaria hepatica*-infected rats. Bayo *et al*. (1980), reported hyperplasia and hypertrophy of lymhiod, myeloid, megacaryocytic and mononuclear phagocytic cells in the liver of infected mice. Essan *et al*. (2013), observed congestion of central vein, enlargement and vacuolation of hepatocyte, stenosis of sinusoid and infilteration of inflammatory cells. Normal architecture with occasional dilated central vein with feathery degeneration of hepatocytes was seen in the work of Shivshetty *et al*. (2014). However, contrary to our observations, Chauhan and Prabha (2019), observed no histological change in the liver of mice infected with *Serratia marcescens*, in their work on evaluation of sperm imparing factor from *Serratia marcescens* as male contraceptive in mouse model. Also Yu *et al*. (2015) and Tang *et al*. (2016), observed no signs of toxicity or damage in the liver function of the rats after inoculation of *B subtilis*. Moon *et al*. (2014), reported isolation of *P. suwonensis* from spent mushroom compost, a waste product of button mushroom cultivation, and no reported case of its use in mouse model or any infection in mice has been recorded.

Animal models of external traumatic skin infections reported by different investigators vary in animal species used, microorganism strains and the number of microorganisms applied (Reizner *et al*., 2014; Dai *et al*., 2011). Examination of traumatized

skin portions from which subcutaneous inoculation was performed showed different distortions of the subcutaneous tissues (Plate 25 -30).

Inoculation with *Lysinibacillus sphaericus* showed an epidermal atrophy in the subcutaneous tissues of the mice (Plate 25). Subcutaneous administration of *P. aeruginosa* showed an attack on the stratum corneum of the skin by the organism, which is one of its typical signs of skin infections. Inoculation with *Bacillus subtilis*, showed presence of adiposites with no visible damage (Plate 28).These findings are similar to the report of Wang *et al*. (2016), who observed notable adipocyte hypertrophy in the mice. Keskitalo *et al*. (2018) noted that *Enterobacter cloacae* administration increased adipose tissue hypertrophy and hepatic damage in the high-fat diet fed mice. Pletzer *et al*. (2017) indicated that an inflammatory infiltrate penetrated into the deep skeletal muscle tissue within the first 24h, thereby causing local tissue swelling, increased tension, induration and accumulation of cellular debris. Seo *et al*. (2016) observed painful nodules and erosions in patients with cutaneous *Serratia marcescens* infections in Korea, while the histopathological findings by Park and Seo (2013), showed a non-specific inflammatory reaction in an immunocompetent patient after filler injection. Rivera-Rodriguez and Dussan-Garzon (2008), observed no skin lesions, irritation or any apparent changes on the skin of mice inoculated with *Bacillus sphaericus.*

The mortality rate of the experimental animals (Fig.6), shows mortality rate ranging from 0 - 44.4% in fish and 0 – 66.7% in mice.

The highest mortality was observed in the fish group infected with *Serratia marcescens* and no mortality was recorded in the group infected with *Lysinibacillus sphaericus*. Mortality is the peak of pathogencity and resulting from severe infection. The result *Serratia marcescens* agrees with work of El-Barbary and Hal (2017), who reported mortality rate ranging form 40 - 60% in *Oreochromis niloticus* and *Serratia marcescens* as one the isolates that caused the highest mortality. The mortality of the fish by *Pseudomonas aeruginosa* observed in this study is in line with the work of Magdy *et al*. (2014). They recorded 40% mortality in *Clarias gariepinus* infected with *Pseudomonas aeruginosa*. Pękala-Safińska (2018), reported that *Pseudomonas fluorescens* can cause sudden mortality, reaching even 100% of fish population. Oh *et al*. (2019), observed onset of mortality at 7days post infection and recorded mortality rates of 100% when the organism titer was higher than 6×10^{7}CFU/fish, and less mortality at lower titer. The mortality observed in the group infected

with *Bacillus subtilis* demonstrated in this study supports the work of Gu *et al*. (2019), who reported mortality of fish upon exposure to *Bacillus subtilis.* On the contrary, several studies have reported the probiotic effect of *B.subtilis* on growth and immune performance of fish (Kuebutornye *et al*., 2020; Addo, 2017; Zokaeifar *et al*., 2012). *Lysinibacillus sphaericus* did not cause any death in the fishes in this study (Fig.6), and no possible reason can adduced. However, antagonistic effect of *Lysinibacillus sphaericus* against common fish pathogens in *Clarias batrachus* have been reported (Ganguly *et al*., 2018).

The mortality rate of mice inoculated with the bacterial isolates is presented in Fig.6. The highest mortality (66.7%) was observed in the mice group infected with *Pseudomonas aeruginosa* (Fig.6), while no mortality was observed in mice infected with *L.sphaericus, B.subtilis* and *P.suwonensis.*The mortality of mice group infected with *P. aeruginosa* supports the work of Kamal *et al*. (2016), who studied the virulence of pigmented and non-pigmented *Pseudomonas aeruginosa* in mice and reported 100% mice mortality with pyocyanin and fluorescein producing *P.aeruginosa* within 24 and 48h post inoculation respectively. Furuya *et al*. (1993), observed 80% mortality rate within 11-13 days post infection amongst leucopaenic mice infected with *P. aeruginosa* D4. The mortality of the mice by *Serratia marcescens* in this study supports the work of González-Juarbe *et al*. (2015). While we observed a mortality of 33.1%, González-Juarbe *et al*. (2015) had 100% mortality. The difference in percentage mortality may have been as a result of route of infection and the dose of bacteria used in our study. Contrary to our finding of no mortality by *B. subtilis*, Gu *et al*. (2019) reported the > 60% mortality of mice inoculated with *B. subtilis* G7 in their study of the virulence potential of a *Bacillus subtilis* isolate from deep-sea hydrothermal vent. Super infection of influenza A virus-infected mice by *Bacillus thringiensis* H34 or 3a3 resulted in 40% mortality of the mice, and at increasing concentration of *B. thuringiensis* inoculums, the mortality rate increased upto 100% (Hernandez *et al*., 2000).

Koch's postulate was carried out to establish the etiology of the disease conditions in the fish. Fig.7 demonstrated that significant growth of the isolates was recovered from the internal organs of the fishes. The mean bacterial count on selective media plate ranged from10.00±5.00cfu/g to 85.67±13.41cfu/g. Although the bacterial counts of the isolates on the selective media were minimal, the re-isolation of the bacterial isolates from the organs of the infected fishes indicates that they were present in the fish organs and are likely to be associated with the histological, hematological and biochemical changes observed in the

fishes. The identification and isolation of an organism from clinically diseased fish, reproduction of clinical symptoms and mortality in healthy fish, and re-isolation of the same pathogen fulfils Koch's postulates (Tattiyapong *et al*., 2017). Anshary *et al*. (2014) experimentally infected a healthy tilapia with *Streptococcus* spp. which caused 70% morbidity and 100% mortality 6 days post infection. They re-isolated the bacteria from the morbid and dead fishes and noted that the isolate was the causative agent of streptoccoccosis in the fish.

Numerous studies have demonstrated the significant effects of probiotics in protecting aquatic animals against infection by pathogens (Yu *et al*., 2019; Cha *et al*., 2013; Korkea-aho *et al*., 2012). The use of *Saccharomyces cerevisiae* (2.5×10^6 cfu/g) as probiotic for the treatment of the infected fishes demonstrated in this study is in line with the report of other researchers (Opiyo *et al.*, 2019; Boonanuntanasarn *et al*., 2019; Hassaan *et al*., 2014). In the past few years, the trend of environment friendly aquaculture has been evolving globally to overcome the shortage of fish vaccines and drawbacks of chemotherapeutics (Abu-Elala *et al*., 2013). Numerous biotic forms have been widely used to boost both growth performance as well as immune response in different aquatic species. On the top list of these agents, the use of *Saccharomyces cerevisiae* as a whole or extracts is globally documented (Abu-Elala *et al*., 2013). The hematology profile of the treated fishes (Table 17) shows that the effects of the treatment with *S. cerevisiae* vary among the infected fishes.

There was significant difference (p value < 0.05) in the effects of the treatment with *S. cerevisiaie* on the hemoglobin level of the infected fishes. An increase in hemoglobin level was observed in all the groups by first week post treatment, but by 2 weeks post treatment, hemoglobin level decreased in fish infected with *P. aeruginosa* and *B.subtilis.* An increase was observed in group infected with *S. marcescens* and *P. suwonensis* and this result is in line with the work of Goran *et al*. (2017), who reported significant increase in hemogblobin level of fish, in their work on assessment of yeast as a dietary additive on haematology and water quality of common carp in a recirculating aquaculture system. Also, Hassaan *et al*. (2014) reported an increase in hemoglobin of fish samples synbiotically fed with *Bacillus licheniformis* and *Saccharomyces cerevisiae*. As the diet was supplemented with higher concentration of *B. licheniformis*, the hemoglobin value decreased. On the contrary, freeze-dried microencapsulated probiotic *S. cerevisiae* used as supplementary fish feed had no detectable effects on hematological indices, although the fish growth performance was improved (Boonanuntanasarn *et al*., 2018).

The packed cell volume of all the fishes increased upon treatment with *S.cerevisiae* at the end of the experiment (Table 17). The levels of effects of the treatment on the fishes differ significantly and highest on the first week post-treatment (Table 17) [Appendix xxxvii]**.** This result is in agreement with the report of Abu-Elala *et al*. (2013), who observed increase in PCV value of fishes challenged with some fish pathogens upon treatment with *Saccharomyces cerevisiae.*

There was significant difference (p value < 0.05) in the treatment effects on the red blood cell count of the infected fishes, with highest level of effect observed in the group infected with *S.marcescens* (Appendix xxxviii)**.** At the end of the treatment period, increase in RBC was observed in fishes infected with *P.aeruginosa* and *S.marcescens.* Studies have shown that incorporation of probiotics stimulates hemopoesis and also induces the non specific immunity in fish (Marzouk *et al*., 2008). Opiyo *et al.* (2019) reported that blood samples from fish fed on probotic-treated diets contained a significantly higher number of red blood cells. Increase in RBC has been reported in Nile tilapia fed on diet supplemented with *S cereisiae* and *Bacillus* spp (Addo *et al*., 2017; Elsabagh *et al*., 2018 and Selim & Reda, 2015).

A decrease in white blood cell count observed in the fishes infected with *L. sphaericus, P. aeruginosa and S. marcescens* in this study (Table 17) is similar to the work of Yazici *et al*. (2015), who reported a reduction in WBC of fish fed with *Lactobacillus plantarum*-supplemented feed. Their study was on effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. Mohapatra *et al*. (2014), recorded a decrease in WBC count of *Labeo rohita* fingerlings fed with probiotic (*B. subtilis, L. lactis* and *S. cerevisiae*) supplemented feed. They opined that the reduction of WBC count might also be associated with anti-stress substances released/produced by probiotic organisms. The increase in WBC of fishes infected with *B. subtilis* and *P. suwonensis* and treated with *S. cerevisiae* in this study (Table 17) is in line with work of Abu-Elala *et al*. (2013), who reported increase in WBC of fishes fed with *S.cerevisiae* in their work on use of different *Saccharomyces cerevisiae* biotic forms as immune-modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens.

The treatment of the infected fishes with *S.cerevisiae* caused an increase in the lymphocyte value of all the fishes (Table 17). The highest treatment effect was observed in

the fish infected with *Paenalcaligenes suwonensis* (Appendix xl). There was significant difference (p value < 0.05) in the effects observed in all the groups. Increase in lymphocyte value is one of the non-specific immune response that is triggered by probiotic; probiotics activates immune response by producing effector molecules, cytokines and chemokines (Hoseinifar *et al*., 2018; Remus *et al*., 2012). Kim and Austin (2006), Nayak *et al*. (2007) and Kumar *et al*. (2008), reported increase in lymphocytes in various fishes following treatment with probiotics.

There were variations in the effects observed on the monocyte values of the infected fishes upon treatment with probiotic (Table 17). The increase in monocyte value of fish infected with *S.marcescens* upon treatment with probiotic is in line with the work of Yazici *et al*. (2015), who reported an increase in monocyte value of fish fed with *Bacillus subtilis*-supplemented feed. Reda *et al*. (2018) did not observe any significant difference in the monocyte value of *Clarias gariepinus* treated with three different strains of *Bacillus.*

Treatment of the infected fishes with probiotic initiated varying changes in the biochemistry profile of the infected fishes (Table 18).

The effects of the treatment with probiotic on the AST value of the infected fishes were significantly different (p value<0.05) [Table 18]. The observed decrease in AST value is in line with the work of Dawood *et al*. (2020), who reported reduction in AST of Nile tilapia fed with *Saccharomyces cerevisiae*-supplemented diet.They opined that the probiotic improved digestibility of date palm seed meal. Contary to our findings, El-feky *et al*. (2017), reported an increase in AST value in *Clarias gariepinus* treated with *Saccaromyces cerevisiae* at 0.2% concentration of the probiotic, and noted that at increasing concentration of the probiotic, the AST values decreased.

The ALT values of all the infected fishes decreased after 2 weeks treatment with *S. cerevisiae* (Table 18). There was significant difference (p value < 0.05) in treatment effects among the fishes infected with the isolates. Similar decrease in ALT value was recorded by Adorian *et al*. (2019), in fish fed with diet supplemented with 1×10^6CFU/g probiotic *Bacillus.* Hassaan *et al*. (2014), also recorded significant decrease in ALT value of *Oreochromis niloticus* with an increase in *B. licheniformis* level up to 0.48×10^6 CFU g^{-1}

In Table 19, an increase in the weight of the fishes was observed upon treatment with *S.cerevisiae.* The highest percentage weight gain (17.5%) was observed in the group infected

with *P. suwonensis* and least (14.4%) in the fish infected with *L.sphaericus.* Lara-Flores *et al*. (2003) reported that live yeast improves protein digestibility, and this probably explains the enhanced growth rate of the fishes observed in this study. Mohammadi *et al*. (2015) reported that growth performance significantly increased with probiotic, *S. cerevisiae,* especially at 2% probiotic concentration. El-feky *et al*. (2017) reported very high percentage growth rate (82.5 – 114.43%) in *Clarias garipinus* treated with *S. cerevisiae.*

Antibiotics are largely used for prophylaxis and treatment to eliminate or reduce bacterial contamination to a degree that enhances host defense mechanism (Shalaby *et al*., 2006). Chloramphenicol is one of the commonly used antibiotics in aquaculture.

Several studies have investigated the effects of antibiotics on the microbiome of fish organs (Pindling *et al*., 2018; Carlson et *al*., 2017; Carlson *et al*., 2015). In this study, the administration of chloramphenicol to the infected fishes caused an increase in the hemoglobin level of the fishes (Table 20). The treatment effect was highest 2 weeks post treatment, and showed no significant difference (p value > 0.05) among the fishes [Appendix xlv]. Similar increase in haemoglobin level was reported in *Aeromonas hydrophilia*–infected fish treated with chloramphenicol (Salah *et al*., 2008). Shalaby *et al*. (2006) also reported significant haemoglobin increase in fish fed with chloramphenicol-supplemented diet. In contrast, Amrevuawho *et al*. (2016) reported that there were no changes in the haemoglobin of *Pseudomonas aeruginosa*-infected catfishes treated with chloramphenicol. Kasagala and Pathiratne (2008) also reported no significant effect in hemoglobin level of healthy fish exposed to different concentrations (2 - 10 mg/L) of chloramphenicol for 10 days.

Treatment with chloramphenicol for 2 weeks caused significant increase ($p<0.05$) in the packed cell volume (PCV) value of the infected fishes (Table 20). There was significant difference ($p<0.05$) in the effects of the treatment in the PCV of the infected fishes. This is line with the report of Amrevuawho *et al*. (2016), who in their study on the effects of *Allium cepa* and chloramphenicol on haematological parameters, histopathology and survival of catfish *Clarias gariepinus* sub-adult infected with *Pseudomonas aeruginosa* recorded improvement in PCV of *P.aeruginosa*-infected fishes. Nwani *et al*. (2013), reported insignificant effect on PCV of *Clarias gariepinus* (Burchell 1822) on day 1 post- exposure to chloramphenicol, but observed that it however induced both concentration- and time dependent significant decrease in PCV from day 5 onward.

An increase in red blood cell (RBC) count was observed in all the fishes after two weeks treatment with chloramphenicol (Table 20). The effects of the treatment with chloramphenicol on the fishes were significantly different (p value < 0.05) among the bacterial isolates. Increase in RBC implies improvement in the hemopoietic tissues of the infected fishes. Shalaby *et al*. (2006) revealed that treatment with chloramphenicol induced significant increase in RBC of treated fish, and opined that decrease or increase of blood indices may be attributed to a defense reaction against the antibiotic, which occurs by stimulation of erthropoiesis. Faisal (2003) also reported significantly increased values of RBC count in catfish, *Clarias gariepinus*, at the 1st and 3rd days after administration of antibiotics.

The effects of treatment of infected fishes with chloramphenicol on the white blood cell count (WBC) [Table 20], demonstrated a decrease in WBC count of all the fishes at the end of the treatment period. WBCs are involved in the regulation of immunological function in many organisms and changes in WBC count are indicative of protective response (Saravanan *et al*., 2011). There was significant difference (p value < 0.05) in the effects of the antibiotic in all the groups in this study and the decrease observed is similar to the report of Amrevuawho *et al*. (2016), and Ezeri (2001), who reported reduction in WBC of infected fishes after treatment with antibiotics. On the contrary, Saravanan *et al*. (2011) reported a significant increase in WBCs in *Clarias carpio* exposed to clofibric acid and diclofenac. Significant increase in WBCs has also been reported in *Cirrhinus mrigala* after exposure to different concentrations of ibuprofen (Saravanan *et al*., 2012).

The lymphocyte values of the infected fishes decreased except the fish infected with *S. marcescens* and *P. suwonensis* (Table 20). The increase in lymphocyte of *Serratia marcescens* and *P. suwonensis* -infected fishes treated with chloramphenicol in this study is contrary to the work of Amrevuawho *et al*. (2016), who recorded an increase in lymphocyte value of *P.aeruginosa* infected-*Clarias gariepinus* treated with chloramphenicol. In our study, lymphocyte value with *P. aeruginosa* infected fish showed a decrease. Nwani *et al*. (2013) also recorded an increase in lymphocyte value of *Clarias gariepinus* exposed to sublethal concentration of chloramphenicol. A similar increase was observed in *O. mykiss* treated with sulphadiazine and trimethoprin mixture (Lunden and Bylund, 2002).

An increase in monocyte was observed only in the fishes infected with *S.marcescens*, while a decrease was observed in the other groups. A decrease in monocyte value of

Aeromonas hydrophilia-infected *Pangasius hypophthalmus* treated with natural antibacterial agent was recorded by Riauwaty *et al*. (2019). An increase in monocyte value of *P.aeruginosa*-infected *Clarias gariepinus* treated with chloramphenicol was reported by Amrevuawho *et al*. (2016). This is contrary to our findings in which *P. aeruginosa*-infected fish treated with chloramphenicol has a decrease in monocyte. Nwani *et al*. (2013) recorded no significant effect on monocytes of fish treated with chloramphenicol.

Aspartate transaminase enzyme (AST) is considered to be important in assessing the state of the liver and some other organs (Shalaby *et al*. 2006). In this study, AST values decreased significantly (p value < 0.05) in all the fishes except that infected with *Serratia marcescens.* This finding agrees with the report of Faisal (2003), who noted reduced AST value in serum of catfish after ampicillin administration. The increase in AST value observed in *S. marcescens*-infected fish in this study corroborates the report of Soltan *et al*. (2013), who recorded an increase in AST value of *Oreochromis niloticus* fed with oxytetracycline supplemented feed. At increasing dose of the antibiotic, the AST value of *O.niloticus* increased.

A significant decrease (p value < 0.05) in alanine transaminase enzyme (ALT) value was observed at the end of 2 weeks treatment (Table 21). El-Sayed *et al*. (2013), observed a decrease in AST of *Aeromonas hydrophilia*-infected *Clarias lazera* treated with florfenicol. Reda *et al*. (2013) also reported significant decrease in ALT value of *Oreochromis niloticus* fed with oxytetracycline supplemented diet, however with florfenicol, no significant ALT activity was observed. In contrast to our observation, Soltan *et al*. (2013) reported a significant increase in ALT of fish exposed to florfenicol.

The effect of chloramphenicol on weight (g) of the fishes infected with the bacterial isolates is presented in Table 22. The highest percentage weight again was observed in fish infected with *Lysinibacillus sphaericus* and least with *Serratia marcescens*. Shalaby *et al*. (2006), reported increase in weight of *O.niloticus* fed with chloramphenicol supplemented feed. Reda *et al*. (2013) reported significant increase in weight of *O.niloticus* fed with oxytetracycline and florfenicol. Pandit and Jaiswal (2020), in their study on impairments induced due to chloramphenicol and ginger on growth parameters of *Channa punctatus* (Bloch), reported that 1.33kg chloramphenicol- supplemented feed increased 1kg body weight of the test fish.

The treatment of the infected fishes with probiotic and antibiotic in this study caused significant changes in the bacterial count of fish internal organs (Figs 8 and 9).

The effect of probiotic on the gill and liver of the infected fishes was significantly different (p value < 0.05). The bacterial counts were higher in the gills than in the liver in all the groups (Fig.8). No explanation to the reason for this can be adduced. The bacterial count was highest in the group infected with *S.marcescens* and least in the group infected with *B. subtilis* (Appendix liv)**.** Although, a reduction in bacterial count was observed in the infected fishes treated with probiotic when compared to the bacterial count before treatment, it modulated the microbial population in the gill, hence the relatively lower reduction in bacterial load observed in this study. Verschuere *et al*. (2000) stated that the interaction between the microbiota and probiotics is not limited to the intestinal tract, rather, the activities of the probiotic bacteria could also be observed on the gills or the skin.

The effect of antibiotic on the bacterial load of the internal organs of the infected fishes was significantly higher (p value < 0.05) in the infected fishes than the control group. The bacterial load was higher in fishes infected with *P.aeruginosa* and *P.suwonensis*, and lesser in fishes infected with *S.marcescens* (Appendix lv).

With reference to the initial bacterial load of the infected fishes before treatment (Fig.4 and 5), probiotic and antibiotic caused a decrease in bacterial count in the gill and liver of the fishes. The probiotic and antibiotic inhibited the growth of microbial populations in the fish organs. This corroborates the report of Kashem *et al*. (2014) and Islam *et al*. (2015). While Kashem *et al*. (2014) noted a reduction of overall bacterial load below 1 log in the gills of *Labeo rohita* (Rohu) fish upon exposure to oxytetracycline, Islam *et al*. (2015), reported significant reduction in gill bacterial load after 21 day treatment of *Barbonymus gonionotus* with oxytetracycline.

However, the mean estimate of the treatment methods (Appendix lvi) revealed that the bacterial load was higher after treatment with probiotic than antibiotic. This indicates that the antibiotics reduced the bacterial populations in the internal organs more than the probiotic. Our findings support the decrease in microbial diversity detected by Zhou *et al.* (2018) in fish organs which include the liver of fishes when treated with antibiotics. Rosado *et al*. (2019) reported a clear reduction in taxonomic diversity in gut microbiome of the Atlantic salmon treated with oxytetracycline, and opined that alongside decreased microbial population effect of antibiotic on fish, there is usually an increased susceptibility to secondary

infection. The use of broad spectrum rifampicin led to a decrease of diversity in the skin microbiome of *Gambusia affinis* after 2.6 days of antibiotic administration (Carlson *et al*., 2017). On the contrary, Ran *et al*. (2016) reported that yeast supplementation had no significant influence on the diversity of allochthonous microbiota of Nile Tilapia. Wanguyun *et al*. (2019) reported that probiotic caused an increase in the gut microbial population of tilapia reared in copper-tainted water and opined that the improving of bacterial population in fish by probiotic can help improve nutrient absorption and metabolism which increase immune system in fish.

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CONCLUSIONS AND RECOMMENDATIONS

Rearing of fishes in artificial ponds has been observed to contain large population of bacteria which are opportunistic pathogens to fish and man. The bacterial load in the pond water varied with season, among the senatorial zones and in the fish tissues.

Serratia sp showed the highest resistance to tested antibiotics, while the highest antibiotic resistance by the bacterial isolates was to erythromycin. The most antibiotic resistant bacterial organisms obtained in this study demonstrated diverse effects on the weight, blood hematology and biochemistry of fishes and mice upon oral and subcutaneous administration. The highest mortality in fish and mice was observed with *Serratia marcescens* and *Pseudomonas aeruginosa* respectively.

The comparative treatment of the infected fishes with chloramphenicol and *Saccharomyces cerevisiae* showed that antibiotic and probiotic use were effective at reducing the bacterial infection and restoring the blood parameters. However, probiotic use is recommended to avoid the severity of antibiotics.

It is, therefore, necessary that all the health problems caused by bacteria in commercial fish farming settings be addressed through improvement in the working environment. Training of fish farmers plays a vital role in reducing the occurrence of these problems. They should be educated on how to adopt good fish farming practices and use of probiotics in order to reduce the microbial load, as well as economic losses in the industry.

REFERENCES

Abdel-Hamed, H., Ibrahim, I., Baumgartner, W., Lawrence, M.L. and Karsi, A. (2017). Characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent *Aeromonas hydrophila*. *Frontiers in Microbiology,* **8**, 15-19

Abdel-Salam, S. S., Ghaly, M.F., Baraka, D.M., Mahmoud, S.H., El-Makhzangy, A.A. (2017). Histopathological changes in diseased catfish (*Clarias gariepinus*) and treated by ciprofloxacin and clove extracts. *Journal of Veterinary Medicine and Allied Science*, **1**(1), 41-48

Abdel-Salam, S.S., Ghaly, F.M., Baraka, D. M., Mahmoud, S. H. and El-Makhzangy, A.A. (2016). Isolation and identification of bacterial flora from catfish (*Clarias gariepinus*) with antimicrobial susceptibility and herbal sensitivity. *Journal of Pure and Applied Microbiology*, **10**(3), 1835-1846

Abdullah, F.F.J, Adamu, L., Osman, A.Y., Haron, A.W., Saharee, A.A., Abdullah, R., Saad, M.Z. and Zakaria, Z. (2013). Biochemical and hematological alterations in mice inoculated with outer membrane protein, lipopolysaccharides and whole cells of *Pasteurella multocida* Type B: 2 *American Journal of Animal and Veterinary Sciences,* **8**(3), 152-158

Abdurrahman, D., Zargar, H., Asif, M. and Ramola, S. (2017). A survey on fish marketing system in Dehradun, India. *Archive of Life Science and Environment***, 1**(2), 1-6

Abowei, J.F.N., Briyai, O.F. and Bassey, S.E. (2011). A review of some viral, neoplastic,environmental and nutritional diseases of African Fish. *British Journal of Pharmacology and Toxicology*, **2**(5), 227 – 235.

Abu, O. M. G. and Uwadirioha, U. (2016). Comparative study on bacterial load in intestine, gills and skin of cultured African Catfish (*Clarias gariepinus*) from different locations in Rivers State, Nigeria. *International Journal of Innovative Studies in Aquatic Biology and Fisheries*, 2(3), 21-29

Abu-Elala, N., Marzouk, M. and Moustafa, M. (2013). Use of different *Saccharomyces cerevisiae* biotic forms as immune-modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. *International Journal of Veterinary Science and Medicine*, **1**, 21-29.

Acha, P.N. and Szyfres, B. (2003). Zoonoses and Communicable Diseases common to man and animals. *In*: *Bacterioses and Mycoses*, 3rd edition. Pan American Health Organisation, Washington. p 384.

Acharya, G.D., Hathi, M.V., Patel, A.D. and Parmar, K.C. (2008). Chemical properties of groundwater in Bhiloda Taluka Region, North Gujarat, India. *E-Journal of Chemistry*, **5**(4), 792 – 796.

Adams, M. R. and Moss, M. O. (2000). Bacterial agents of food borne illness. *In: Food Microbiology*. 2nd edition. The Royal Society of Chemistry Publishers, Cambridge, UK. pp 184-271.

Adebayo–Tayo, B.C., Odu, N.N., Igiwiloh, N.J.P.N and Okonko, I.O. (2012). Microbiological and physicochemical level of fresh catfish (*Ariushendelotic*) from different markets in Akwa Ibom State, Nigeria. *New York Science Journal*, **5**(4), 46-52.

Adegunloye, D. V. and Sanusi, A. I. (2019). Microbiota of catfish (*Clarias gariepinus*) tissues harvested from vials polluted with soil from e-wastes dumpsite. *International Journal of Fisheries and Aquaculture*, **11**(5), 104-111.

Adeshina, I., Umma, S.B., Adesanmi, O. and Adewale, Y.A. (2016). Distribution of bacterial load in fish tissues, pond water and sediment in Oyo State, Nigeria. *Alexandria Journal of Veterinary Sciences*, **51**(1), 1-9.

Adetunji, V.O. and Anyanwu, S.C. (2011). Clinico-Pathological changes in Wistar rats administered *B. thuringiensis* isolate from soft cheese wara. *Advances in Biological Research*, **5**, 206-214.

Adeyemi, J.A., Atere, T.G., Oyedara, O.O., Olabiyi, K. O. and Olaniyan, O.O. (2013). Hematological assessment of health status of African catfish *Clarias gariepinus* (Burchell 1822) experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. *Comparative Clinical Pathology*, **22**(3), 1-8.

Adeyeye, S. A.O. (2016). Traditional fish processing in Nigeria: A critical review. *Nutrition and Food Science*, **46** (3), 321-335.

Addo, S. (2017). Effects of *Bacillus subtilis* strains on growth, immune parameters, and *Streptococcus iniae* susceptibility in Nile Tilapia, *Oreochromis niloticus.Journal of the World Aquaculture Society*, **48**(2), 257 -267

Addo, S., Carrias, A.A. and Williams, M.A. (2017). Effects of *Bacillus subtilis* strains and the prebiotic previda on growth, immune parameters and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia *Oreochromis niloticus*. *Aquaculture Research,* **48**, 4798 – 4810.

Adorian, T.J., Jamali, H., Farsani, H.G., Darvishi, P., Hasapour, S., Bagheri, T. and Roozbehfar, R. (2019). Growth performance, digestive enzyme activity and hematological parameters of Asian sea bass, *Lates calcarifer* (Bloch). *Probiotics and Antimicrobial Proteins*, **11**, 248 – 255

Agoba, E.E., Adu, F., Agyare, C., Boamah, V.E. and Boakye, Y.D. (2017). Antibiotic resistance patterns of bacterial isolates from hatcheries and selected fish farms in the Ashanti region of Ghana. *Journal of Microbiology and Antimicrobials*, **9**(4), 35 – 46.

Ajay, P., Sadana, J. R. and Asrani, R. k. (1991). Studies on clinical signs and haematological alterations in pneumonic aspergillosis due to *Aspergillus flavus* in Japanese quail *MycopathoIogia*, **116**, 119-123

Akaniro, I.R., Anumudu, O.H., Oweredaba, C.I., Koledowo, A.K., Egboka, K.C., Ofonegbu, M.N. (2020). Isolation, characterization and antibiotic resistance profile of bacteria from the gut of African catfish, *Clarias gariepinus*. *Journal of Scientific and Engineering Research*, **7**(3), 188-195

Akanmu, O.A. (2018). Probiotics, an alternative measure to chemotherapy in fish production. https://www.intechopen.com/books/probiotics-current-knowledge-and-future-prospects/probiotics-an-alternative-measure-to-chemotherapy-in-fish-production. Accessed on 14/5/2018

Akter, M.N., Parvez, I. and Patwary, Z.P. (2016). Beneficial effects of probiotics in aquaculture. *International Journal of Fisheries and Aquatic Studies*, **4**(5), 494-499.

Alaa EL-Din, H.S., Imam, A.M. and Usama, M.M. (2012). Histopathological alterations in some body organs of adult *Clarias gariepinus* (Burchell, 1822) exposed to 4-nonylphenol. http://www.intechopen.com/books/zoology/histopathological-alterations-in-some-body-organs-of-adult-clariasgariepinus-burchell-1822-exposed- Accessed on 15/4/2018.

Ali, D.A., Tadesse, B. and Ebabu, A. (2020). Prevalence and antibiotic resistance pattern of *Salmonella* isolated from caecal contents of exotic chicken in Debre Zeit and Modjo, Ethiopia. *Hindawi International Journal of Microbiology*, **2020**(3), 1-7

Ali, Y., Hossen, S., Azad, S.M, Alom, S., Mondal, A. H.,Ali, M.M. and Hossain, M.B. (2017).Fish availability and marketing system at local markets of a Coastal District Southern Bangladesh. *Asian Journal of Animal Sciences*, **11**(5), 221-229

Ali, O.S., Adamu, L., Abdullah, F.F.J., Abba, Y., Hamzah, H.B., Mohd-Azmi, M.L., Abdul, W. H. and Mohd, Z. (2015). Haematological and histopathological vicissitudes following oral inoculation of graded doses of *Pasteurella multocida* Type B: 2 and its lipopolysaccharide in mice. *Journal of Veterinary Science and Technology*, **6**, 220

Aliyu, A., Ibrahim, Y.K. and Oyi, R.A. (2018). Bacteriological and elemental quality of *Clarias gariepinus* (cat fish) samples from River Lavun, Bida, Niger State, Nigeria. *Nigerian Journal of Pharmaceutical Resources*, **12**, 139 – 147.

Al-Jubury, N.O., Sahood, A.S. and Hussein, O.R. (2010). Effect of *P. aeruginosa* metabolic products on some hematological, biochemical and histological parameters of male rats. *Al-Kufa University Journal for Biology*, **2**(2), 1-10

Al Mamum, M.A., Nasren, S. and Rathore, S.S. (2019). Assessment of probiotics in aquaculture: Functional changes and impact on fish gut. *Microbiology Research Journal International*, **29** (1), 1-10.

Alyahya, S.A., Ameen, F., Al-Niaeem, K.S., Al-Saadi, B.A., Hadi, S. and Mostafa, A.A. (2018). Histopathological studies of experimental *Aeromonas hydrophilia* infection in blue tilapia, *Oreochromis aureus*. *Saudi Journal of Biological Sciences*, **25**, 183–185.

American Public Health AssociationAPHA (2012). Standard methods for the Examination of Water, 22nd edn. American Public Health Association, Washington.

Amrevuawho, M. O., Akinyemi, A. A., Oyewusi, A. J., Bankole, O. M. and Ezeri, G. N. O. (2016). Effects of onion (*Allium cepa*) and chloramphenicol on haematological parameters, histopathology and survival of catfish *Clarias gariepinus* (burchell, 1822) sub-adult infected with *Pseudomonas aeruginosa*. *Vom Journal of Veterinary Science*, **11**, 1 – 12

Amrevuawho, M.O., Akinyemi, A.A., Ezeri, G.N.O., Bankole, O.M. and Takeet, O.V.A. (2014). Pathological study of *Clarias gariepinus* (Burchell, 1822) Sub-adult artificially infected with *Pseudomonas aeruginosa*. *Brazilian Journal of Aquatic Science and Technology*, **18**(2), 65-70

Angulo, F.J. (2000). Antimicrobial agents in aquaculture: potential impact on health. *APUA Newsletter*, **18**, 1–6

Ansa, Y.B., Frimpong, E.A. and Amisah, S. (2013). Characterization of potential aquaculture pond effluents and physicochemical and microbial assessment of effluent receiving waters in Central Ghana. *African Journal ofAquatic Science*, **38**(2), 185–192.

Anshary, H., Kumiawan, R.A., Sriwulan, S., Ramli, R. and Baxa, D.V. (2014). Isolation and molecular identification of the etiological agents of streptococcosis in Nile tilapia (*Oreochromis niloticus*) cultured in net cages in Lake Sentani, Papua, Indonesia. *Springer Plus*, **3**, 627 – 638

Antao, E-M., Vincze, S., Hanke, R., Klimmek, L., Suchecka, K., Lubke-Becker, A. and Wieler, L.H. (2018). Antibiotic resistance, the 3As and the road ahead. *Gut Pathogens*, **10**, 52

Anya, C. (2017). Economic analysis of fish marketing in the rural communities of Ebonyi state. Project Writers Nigeria.https://www.projectwriters.ng/. Assessed on 12/5/2019

Aquatic Life Support Systems (ALSS) (2013). Sanatoga Station Road, Pottstown, Pennsylvania, USA. EAI, Emperor Aquatics, Inc. http://www.info@emperoraquatics.com. Accessed on 26th December 2018.

Arıg, N., Suzer, C., Gokvardar, A., Basaran, F., Coban, D., Yldırım, S., Okan Kamac, H., Fırat, K. and Saka, S. (2013) Effects of probiotic (*Bacillus* sp.) supplementation during larval development of Gilthead sea bream (*Sparus aurata*, L.). *Turkish Journal of Fish Aquatic Science*, **13**, 407–414

Ashraf, M., Mustafa, B-E., Rehman, S., Basir, M.K. and Ashraf, M. A. (2019). Emergence of antimicrobial resistance, causes, molecular mechanisms, and prevention strategies: A bovine perspective. https://www.intechopen.com/books/bovine-science-a-key-to-sustainable-development/emergence-of-antimicrobial-resistance-causes-molecular-mechanisms-and-prevention-strategies-a-bovine Accessed on 12/5/2019.

Assefa, A. and Abunna, F. (2018). Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Hindawi Veterinary Medicine International*, **2018**, 1-11

Austin, B. and Austin, D.A. (2016). Bacterial fish pathogens. Diseases of farmed and wild fish, 6th ed., Springer International Publishing Switzerland.

Austin, B. (2002). The bacterial microflora of fish. *The Scientific World Journal*, **2**, 558-572

Aydin, S., Gure, H. Cakici, H., Colakoglu, S. and Bircan, R. (2009). Gross pathology, blood chemistry, lipid and peroxide contents in rainbow trout (*Oncorhynchus Mykiss* Walbaum) affected by experimental *Arcobacter Cryaerophilus* infection at low water temperature. *Acta Veterinaria Hungarica*, 57(2), 305–317.

Azad, A. K., Sarker, M. and Wan, D. (2018). Immunomodulatory effects of probiotics on cytokine profiles. *Hindawi BioMed Research International*, **2018**, 1-11

Azevedo, J.F., Hermes-Uliana, C., Lima, D.P., Alves, G. and Araujo, E.J.A. (2014). Probiotics protect the intestinal wall of morphological changes caused by malnutrition. *Anais da Academia Brasileira de Ciencias*, **86** (3),1303-1314.

Balouiri, M., Sadiki, M. and Ibnsouda, S. K (2016). Methods for In vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, **6**(2), 71-79

Bauer, A.W., Kirby, W.M., Sheris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *American Journal of Chemical Pathology*, **45**(4), 493 – 496

Bayo, S., Petit, J.C. and Sicard, D. (1980). Histopathological observations of experimental hamatogenous infection with *Pseudomonas aeruginosa* in mice. *Pathological Biology*, **28** (4), 241-246

Bekele, B., Workagegn, K.B. and Natarajan, P. (2019). Prevalence and antimicrobial susceptibility of pathogenic bacteria in Nile Tilapia, *Orechromia niloticus* L. *International Journal of Aquaculture and Fishery Sciences*, **5**(4), 22 – 26.

Bektas, S. and Ayik, O. (2009). Haematological parameters and erythrocytes osmotic fragility in Rainbow trout (*Oncorhynchus mykiss*) experimentally infected with *Pseudomonas putida. Journal of Fisheries andAquatic Science*, **4**, 246-253.

Bene, C., Arthur, R., Norbury, H., Allison, E.H., Beveridge, M. and Bush, S. (2016). Contribution of fisheries and aquaculture to food security and poverty reduction: Assessing the current evidence. *World Development*, **79**, 177 – 196.

Bernardi, C., Ripamonti, B., Stella, S., Tirloni, E., Bersani, C. and Cattaneo, P. (2011). Effect of the lactic acid bacteria on the control of listerial activity and shelf life of smoked salmon scraps. *International Journal of Food Science and Technology*, **46**, 2042–2051.

Bhatnagar, A. and Devi, P. (2013). Water quality guidance for the management of pond fish culture. *International Journal of Environmental Sciences*, **3**(6), 1980-1993.

Biswas, P.C., Sultana, S., Kabiraj, M. and Hossain, S.M.S. (2019). Role of probiotics in aquaculture practice of Satkhira region of Bangladesh. *International Journal of Fisheries and Aquatic Studies*, **7**(5), 174-181.

Bolliger, A.P. and Everds, N. (2012). Haematology of the mouse. *In: The Laboratory mouse*, Second edition. Elsevier Limited, Netherland. pp 331-347.

Boonanuntanasarn, S., Ditthab, K., Jangprai, A. and Nakharutha, C. (2019). Effects of microencapsulated *Saccharomyces cerevisiae* on growth,hematological indices, blood chemical, and immune parameters and intestinal morphology in striped Catfish, *Pangasianodon hypophthalmus*. *Probiotics and Antimicrobial Proteins*, **11**(2), 427-437.

Boshuizen, M., van Bruggen, R., Zaat, S. A., Schultz, M. J., Aguilera, E., Motos, A., Senussi, T., Idone, F. A., Pelosi, P., Torres, A., Bassi, G. L. and Juffermans, N. P. (2019).

Development of a model for anemia of inflammation that is relevant to critical care. *Intensive Care Medicine Experimental*, **7**(1), 47

Bourre, J. M. (2005). Where to find omega-3 fatty acids. *Journal of Nutrition Health Aging*, **9**(1), 31-38.

Breijyeh, Z., Jubeh, B. and Karaman, R. (2020). Resistance of gram negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, **25**(6), 1340

Brincat, J. P, Carosati, E., Sabatini, S., Manfroni, G., Fravolini, A., Raygada, J. L. (2011). Discovery of novel inhibitors of the Nor A multidrug transporter of *Staphylococcus aureus. Journal of Medicinal Chemistry*, **54**, 354-365

Brown, D.E., Libby, S.J., Moreland, S.M., McCoy, M.W., Brabb, T., Stepanek, A., Fang, F.C. and Detweiler, C.S. (2013). *Salmonella enterica* causes more severe inflammatory disease in C57/BL6 *Nramp1G169* mice than Sv129S6 mice. *Veterinary Pathology*, **50**, 867–876

Bruun, M.S., Madsen, L. and Dalsgaard, I. (2003). Efficiency of oxytetracycline treatment in rainbow trout experimentally infected with *Flavobacterium psychrophilum* strains having different invitro antibiotic susceptibilities. *Aquaculture*, **215**, 11–20.

Burnett-Boothroyd, S.C. and McCarthy, B.J. (2011). Antimicrobial treatments of textiles for hygiene and infection control applications: an industrial perspective. *In*: *Textiles for Hygiene and Infection Control.* Woodhead Publishing Limited, UK. pp 196-209

Caballero-Franco, C., Keller, K., De Simone, C. and Chadee, K. (2007). The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *AmericanJournal of Physiology and Gastrointestinal Liver Physiology*, **292**, 315-322.

Carlson, J. M., Leonard, A. B., Hyde, E. R., Petrosino, J. F. and Primm, T. P. (2017). Microbiome disruption and recovery in the fish *Gambusia affinis* following exposure to broad-spectrum antibiotic. *Infection and Drug Resistance,* **10**, 143

Carlson, J. M., Hyde, E. R., Petrosino, J. F., Manage, A. B. and Primm, T. P. (2015). The host effects of *Gambusia affinis* with an antibiotic-disrupted microbiome. *Comparative Biochemistry and Physiology - Part C*, **178**, 163–168.

Cerrato, R.M. (2000). What fish biologists should know about bivalve growth? *Fish Research*, **46**, 39–49

Cha, J.H., Rahimnejad, S., Yang, S.Y., Kim, K.W. and Lee, K.J. (2013). Evaluations of *Bacillus* spp. as dietary additives on growth performance, innate immunity and disease resistance of olive flounder (*Paralichthys olivaceus*) against *Streptococcus iniae* and as water additives. *Aquaculture*, **402**, 50–57.

Chae-Woo M, Cho Y-S, Oh K-H (2009) Removal of pathogenic bacteria and nitrogens by *Lactobacillus* sp. JK-8 and JK-11. *Aquaculture*, **287**, 266–270

Chao, S. and Eric G. P. (2011). Monocyte recruitment during infection and inflammation *Nature Review Immunology*. **11**(11), 762–774.

Chauhan, A. and Prabha, V. (2019). Evaluation of sperm impairing factor from *Serratia marcescens* as male contraceptive in Mouse Model. *HindawiBioMed Research International,* **2019**, 1-10

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries-Part2. Cambridge University Press, New York. pp: 47-55.

Chi, T.T.K., Clausen, J.H., Van, P.T., Tersbol, B. and Dalsgaard, A. (2017). Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. *Aquaculture Reports*, **7**, 40-47

Chung, E. L. T., Abdullah, J.F.F., Adamu, L., Marza, A.D., Ibrahim, H. H., Zamri-Saad, M., Haron, A.W., Saharee, A.A., Lila, M.A.M., Omar, A.R., Bakar, M. Z.A. and Norsidin, M.J. (2015). Clinico-pathology, hematology and biochemistry responses toward *Pasteurella multocida* Type B: 2 via oral and subcutaneous route of infections. *Veterinary World*, **8**(6), 783-792.

Claudious, G., Tinashe, C.H., Bernar, M., Shuvai, M., Gilbert, J., Pious, V.M. and Jairus, M. (2019). Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. *International Journal of Microbiology,* **201**9, 1-7.

Clinical and Laboratory Standards Institute (CLSI). (2017). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, 27th edition, Wayne, USA.

Croci, L. and Suffredini, E. (2003). Microbiological risk associated with seafood consumption. *Annali dell'Istituto Superiore di Sanità*, **39**(1), 35-45

Cruz,P.M., Ibanez,A.L., Hermosillo, O.A.M. and Saad, H.C.R. (2012). Use of probiotics in aquaculture. *International Scholarly Research Network Microbiology*, **2012**, 1-14

Dai, T., Kharkwal, G. B., Tanaka, M., Huang, Y. Y., Bil de Arce, V. J. and Hamblin, M. R. (2011). Animal models of external traumatic wound infections. *Virulence*, *2*(4), 296–315.

Dalgaard, P. (1995). Qualitative quantitative characterisation of spoilage bacteria from packed fish. *International journal of food microbiology,* **26**, 319- 333

Danba, E.P., Bichi, A.H., Ishaku, S., Ahmad, M.K., Buba, U., Bingari, M.S., Barau, B.W. and Fidelis, U.F. (2014). Occurrence of pathogenic bacteria associated with *Clarias gariepinus* selected fish farms of Kumbotso Local Government Area of Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences,* **7**(2), 145-149.

Dat, T.T.H., Tam, V.T.T., Dung, T.T.K., Bui, L.M., Anh, H.L.T. and Oanh, P.T.T. (2019). Isolation and screening of cellulose and organic matter degrading bacteria from aquaculture ponds for improving water quality in aquaculture. IOP Conference Series: *Earth and Environmental Science,* 266012002.

Dawood, M.A.O., Eweedah, N.M., Khalafalla, M.M., Khalid, A., El Asely, A., Fadl, S.E., Amin, A.A., Paray, B.A. and Ahmed, H.A. (2020). *Saccharomyces cerevisiae*

increases the acceptability of Nile tilapia (*Oreochromis niloticus*) to date palm seed meal. *Aquaculture Reports*, **17**, 100314.

Delince, G. (1992). The ecology of the fish pond ecosystem. *In*: *Developments in Hydrobiology*. 2ND edition. Kluwer Academic Publishers, Netherlands. p 230

Dengler, L., Kühn, N., Shin, D.L., Hatesuer, B., Schughart, K. and Wilk, E. (2014). Cellular changes in blood indicate severe respiratory disease during influenza infections in mice. *Plos One,* **9**(7), 103-149.

DENR Administrative Order (DAO) (2016). Water Quality Guidelines and General Effluent Standards.

Divya, K.R., Akbarali, I., Ramasubramanian, V., Sureshkumar, S.and Thundiparambi-Sathrajith, A. (2012). Colonization of probiotic bacteria and its impact on ornamental fish *Puntius conchonius. Journal of Environmental Biology*, **33**(5), 551-555

Douglas, S. I. and Isor, F.N. (2015). Bacteriological investigation of pond water quality from Ogoniland, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology,* **9**(2), 36-41.

Efuntoye, M.O., Olurin, K.B. and Jegede, G.C. (2012). Bacterial flora from healthy *Clarias gariepinus* and their antimicrobial resistance pattern. *Advance Journal of food Science and Technology*, **4**(3), 121-125.

Ehiagbonare, J.E. and Ogunrinde, Y.O. (2010). Physicochemical analysis of fish pond water in Okada and its environs, Nigeria. *African Journal of Biotechnology*, **9**(36), 5922-5928.

Elayaraj, B., Selvaraju, M. and Ajayan, K.V. (2016). Assay on water quality variations of pasupatheswarar temple pond, Annamalai Nagar, Tamil Nadu, India. *Journal of International Academic Research for Multidisciplinary*, **3**(12), 97 – 108.

El-Barbary, M.I. and Hal, A.M. (2017). Molecular identification and pathogenicity of *Citrobacter* and *Serratia* species isolated from cultured *Oreochromis niloticus*. *Egyptian Journal of Aquatic Research,* **43**, 255–263

El-Dakar, A.Y., Shalaby, S.M. and Saoud, I.P. (2007) Assessing the use of dietary probiotic/prebiotic as an enhancer of spine foot rabbit fish (Singanus rivulatus), survival and growth. *Aquaculture Nutrition,* **13**, 407–412.

El-feky, M., Essa, A., Osman, A., Shalaby, M. and Moustafa, M. (2017). Growth performance of African catfish *Clarias gariepinus* (Burchell, 1822) treated with live bakers yeast (*Saccharomyces cerevisiae*) in Egypt. *International Journal of Biotechnology and Bioengineering,* **3**, 171-182

Elsabagh, M., Mohammed, R. and Moustafa, E.M. (2018). Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia. *Oreochromis niloticus*. *Aquaculture Nutrition*, **26** (6), 1-10.

El-Sayed, M. G., El-Komy, A.A., Shaheen, A.A. and Ibrahim, M. H. (2013). Effects of florfenicol on hepatic and renal functions in Nile catfish (*Clarias lazera*). *Benha Veterinary Medical Journal*, 24(2), 19-26

El-Shafai, S., Gijzen, H. J., Nasr, F. A. and El-Gohary, F. A. (2004). Microbial quality of tilapia reared in faecal- contaminated ponds. *Environmental Resources,* **95**, 231-238.

El-shaghabee, F., Rokana, N., Gulhane, R., Sharma, C. and Panwar, H. (2017). *Bacillus* as potential probiotics: status, concerns, and future perspectives. *Frontiers in Microbiology*, **8**, 1-15

Emborg, J., Laursen, B.G., Rathjen, T., Dalgaard, P. (2002). Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2 degrees C. *Journal of Applied Microbiology*, **92**, 790-799

Endo, H. and Wu, H. (2019). Biosensors for the assessment of fish health: A Review: *Fisheries Science*, **85**, 641-654

Essan, F., Saleh, B.H. and Hussain, S.M. (2013). Determination of lethal dose (LD50) of Exotoxin A from *Pseudomonas aeruginosa* 26A in mice histopathology. *Journal of Pharmacy and Biological Sciences*, **7**(**6**), 49-54

Ezeri, G.N.O. (2001). Haematological response of *Clarias gariepinus* to bacterial infection and prophylactic treatment with antibiotic. *Journal of Aquatic Sciences,* **16**, 22-24

Fafioye, O.O. (2011). Preliminary studies on water characteristics and bacterial population in high yield kajola fish ponds. *Journal of Agricultural Extension and Rural Development*, **3**, 68-71.

Faisal, A.S.R. (2003). Adverse effects of some antimicrobial agents used in fish. Ph.D Thesis presented to the Faculty of Veterinary Medicine, Cairo University, Cairo. pp: 238.

Fakorede, C.N., Fatokun, E. N., Philip-kantiok, B., Iwu, C.J. and Jaja, I.F. (2019). Bacteriological assessment and antibiotics susceptibility profile of bacteria recovered from pond water, fish skin and gut in Ile-Ife, Osun State, Nigeria. *Preprints*, https://www.preprints.org/manuscipt/201904.0108/v1. Assessed on April 18th 2019

Fazio, F. (2018). Fish Hematology analysis as an important tool of aquaculture: *A Review Article in Aquaculture*, **500**, 237-242

Federal Environmental Protection Agency (FEPA). (1991). Guidelines and standards for environmental pollution control. Federal Environmental Protection Agency, 27, 20.

Ferreira, N.F., Cavasin-Oliveria, G.M., Alves, C.B., Nascimento, M.N. and Oliveira, E.M. (2006). Effect of using probiotics based on *Lactobacillus sp* on weight gain and development of the intestinal mucosa of rats (*Rattus norvegicus*). *Brazilian Journal of Animal Science*, **7**, 217-222.

Floyd, F.R. (2005) Introduction to fish health management. IFAS Extension Fisheries and Aquatic Science Department University of Florida United States CIR, **921**, 1-4.

Food and Agricultural Organization (FAO). (2020). The state of world fisheries and Aquaculture. Sustainability in action. Food and Agriculture Organization of the United Nations. Rome.

Food and Agricultural Organization (FAO). (2018). The state of world fisheries and Aquaculture-meeting the sustainable development goals. Food and Agriculture Organization of the United Nations. Rome. Pp 86-120

Food and Agriculture Organization (FAO) (2017). Towards gender-equitable small-scale fisheries governance and development – a handbook. Food and Agriculture Organization of the United Nation, Italy. p174.

Food and Agricultural Organization (FAO) (2016). Aquaculture production. *In: The state of world fisheries and aquaculture.* Food and Agriculture Organization of the United Nations: Rome. p 18

Food and Agriculture Organization (FAO) (2007). Aquaculture. *In The state of the world Fisheries and Aquaculture.* FAO Viale delle Terme di Caracalla,00153 Rome. p 16

Food and Agriculture Organization/World Health Organization (FAO/WHO) (2001). Health and nutritional properties of probiotics in food including powder milk with Live Lactic Acid Bacteria. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with Live Lactic Acid Bacteria. Food and Agriculture Organization of the United Nations, World Health Organization.

Food and Agriculture Organization (FAO). (1997). Aquaculture development. *In: FAO technical guidelines for responsible fisheries.* FAO of the United Nations, Rome. pp 40

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, **66**, 365-378.

Furuya, N., Hirakata, Y., Tomono, K., Matsumoto, T., Tateda, K., Kaku, M. and Yamaguchi, K. (1993). Mortality rates amongst mice with endogenous septicaemia caused by *Pseudomonas aeruginosa* isolates from various clinical sources. *Journal of Medical Microbiology*, **39**, 141-146

Galdeano, C.M. and Perdigón, G. (2006). The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. *Clinical, Vaccine and Immunology,* **13**, 219-226.

Ganguly, S. and Prasad, A. (2011). Microflora in fish digestive tract plays significant role in digestion and metabolism. *Reviews in Fish Biology and Fisheries*, **22**(1), 11-16

Ganguly, A., Banerjee, A., Mandal, A., Khan, M. and Das-Mohapatra, P. (2018). Isolation and characterization of bacteria from the intestine of *Clarias batrachus* for probiotic organism. *Proceedings of the Zoological Society*, **72**, 1-6.

Gaulin, C., Vincent, C., Alain, L. and Ismail, J. (2002). Outbreak of Salmonella *paratyphi* B Linked to aquariums in the province of Quebe, 2000. *Canada Communicable Diseases Report*, **28**(11), 89-93, 96.

Gihan, M. M, Zeinab, M. K., Moustafa, A. N. and Abd El Razak A. A.(2016). Effect of dermatophytes on neutrophils and monocytes chemotaxis. http://medcraveonline.com/MOJI/MOJI-03-00089.php. Accessed on 8/3/ 2019

González-Juarbe, N., Mares, C.A., Hinojosa, C.A., Medina, J.L., Cantwell, A., Dube, P.H., Orihuela, C.J. and Bergman, M.A. (2015). Requirement for *Serratia marcescens* cytolysin in a murine model of hemorrhagic pneumonia. *Infection and Immunity,* **83**(2), 614-624

Goran, S.M.A., Omar, S.S. and Youns, A. (2017). Assessment of yeast as a dietary additive on haematology and water quality of common carp in a recirculating aquaculture system. *AIP Conference Proceedings of 6th International Conference and Workshops on Basic and Applied Sciences,* **1888** (1), 1-7

Govind, P., Madhuri, S., Shirvastav, A.B. and Sahni, Y.P. (2012). Overview of the treatment and control of common fish disease. *International Research Journal of Pharmacy*, **3**(7), 123 – 127.

Gram, L., Ravn, L., Rasch, M., Bruhn, J., Christensen, A., Givskov, M. (2002). Food spoilage - Interactions between food spoilage bacteria. *International Journal of Food Microbiology,* **78**, 79- 97

Gram, L. and Dalgaard, P. (2002). Fish spoilage bacteria--problems and solutions. *Current Opinion Biotechnology*, **13**, 262-266

Gufe, C., Hodobo,T.C., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P.V. and Machakwa, J. (2019). Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. *Hindawi International Journal of Microbiology*, **2019**, 1 – 7.

Gu, H., Sun, Q., Luo, J., Zhang, J.and Sun, L. (2019). A first study of the virulence potential of a *Bacillus subtilis* isolate from deep-sea hydrothermal vent. *Frontiers in Cellular and Infection Microbiology,* **9**,183

Gupta, M.V. and Acosta, B.O. (2004). A review of global tilapia farming practices. *Aquaculture Asia Magazine,* **10**(1), 7-16.

Hai, N.V. (2015). The use of probiotics in aquaculture. *Journal of Applied Microbiology*, **119** (4), 917-935

Hanna, M.I., El-Hady, M.A., Ahmed, H.A., Elmeadawy, S.A. and Kenwy, A.M. (2014). A contribution on *Pseudomonas aeruginosa* infection in African catfish (*Clarias gariepinus*). *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **5**(5), 575 – 588

Harikrishnan, R., Kim, M.C., Kim, J.S., Balasundaram, C. and Heo, M.S.(2011). Immunomodulatory effect of probiotics enriched diets on Uronema marinum infected olive flounder. *Fish Shellfish Immunology*, **30**(3), 964-971.

Hassaan, M.S., Soltan, M.A. and Ghonemy, M.M.R. (2014). Effect of symbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical

indices of the Nile tilapia (*Oreochromis niloticus*). *The Egyptian Journal of Aquatic Research*, **40**(2), 199-208

Haque, S., Bandyopadhyay, P.K. and Mondal, K.(2020). Studies on growth, behavior and blood profile in *Anabas testudineus* infected with *Pseudomonas aeruginosa*. *Proceedings of the Zoological Society*. https://doi.org/10.1007/s12595-020-00330-w. Accesssed on 18/6/2020

Harwood, V. J., Gandhi, J. P. and Wright, A. C. (2004). Methods for isolation and confirmation of *Vibrio vulnificus* from oysters and environmental sources. *Journal ofMicrobiological Methods*, **59**, 301-316

Hemarajata, P. and Versalovic, J. (2013). Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therapeutic Advances in Gastroenterology*, **6**(1), 39–51

Hentschel, D.M., Park, K.M., Cilenti, L., Zervos, A.S., Drummond, I. and Bonventre, J.V. (2005). Acute renal failure in zebrafish: a novel system to study a complex disease. *American Journal of Physiology and Renal Physiology*, **288**(5), 923-929

Hernandez, G.C., Ulloa, P.J., Vergara, O.J.A., Espejo, T.R. and Cabello, C.F. (2005).*Vibrio parahaemolyticus* infections and algal intoxications as emergent public health problems in Chile. *Review Medicine Chile*, **133**, 1081–1088.

Hernandez, E., Ramisse, F., Gros, P. and Cavallo, J. (2000). Super-infection by *Bacillus thuringiensis* H34 or 3a3b can lead to death in mice infected with the influenza A virus. *FEMS Immunology and Medical Microbiology*, **29**, 177 -181

Hess, S., Wenger, A. S., Ainsworth, T. D. and Rummer, J. L. (2015). Exposure of clownfish larvae to suspended sediment levels found on the Great Barrier Reef: impacts on gill structure and microbiome. *Scientific Report*, **5**, 10561

Hooper, L. and Gordon, J. (2001). Commensal host–bacterial relationship. *Science*, **292**(5519), 1115-1118

Hoseinifar, S. H., Sun, Y. Z., Wang, A., and Zhou, Z. (2018). Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. *Frontiers in microbiology*, **9**, 2429

Hummel, S., Veltman, K., Cichon, C., Sonnenborn, U., and Schmidt, M.A. (2012). Differential targeting of the E-Cadherin/beta-catenin complex by gram-positive probiotic lactobacilli improves epithelial barrier function. *Applied Environmental Microbiology*, **78**, 1140-1147.

Huss, H. H., Ababouch, L. and Gram, L. (2004). Assessment and Management of seafood quality and safety. *Fisheries Technical Paper*, **444**, 223.

Ibrahim, A., Ali, H.A. and El-Deen, N.A. (2014). Molecular, biochemical and histological effects of tea seed cake on different organs of *Oreochromis niloticus*. *Global Veterinaria*, **13**(5), 711-719

Idowu, T.A., Adedeji, H.A. and Sogbesan, O.A. (2017). Fish disease and health management in aquaculture production. *International Journal of Environment and Agricultural Science*, **1**, 1-7.

Idowu, T.A., Onyia, L.U. and Kefas, M. (2016). Fish diseases and health management. *In: Contextual Aquaculture and Fisheries Digest*, Maiden Edition. Paraclete Publisher pp: 155-171.

Ifeka, A.C. and Akinbobola, A. (2015). Land use/land cover change detection in some selected stations in Anambra State. *Journal of Geography and Regional Planning*, **18**(1), 1-11.

Ikape, S. I. and Cheikyula, J. O. (2017). Food spoilage in the Tropics: A review. *Octa Journal of Biosciences***, 5** (2), 34-37

Ikeogu, F.C., Nsofor, C.I. and Ikpeze, O.O. (2010). A Review of risk factors for fish diseases in Aquatic Environments. *Proceedings of the 6th National Conference of the Society for Occupational Safety and Environmental Health (SOSEH).* November 3-6, 2010. Princess Alexandra Auditorium, University of Nigeria, Nsukka, Enugu State, Nigeria

Ikpi, G. and Offem, B. (2011). Fish fauna of Agbokum waterfalls in South Eastern Nigeria. *Journal of Asian Scientific Research*, **1**(6), 299-311

Islam, M. J., Rasul, M.G., Kashem, M.A., Hossain, M. M., Liza, A.A., Sayeed, M.A. and Hossain, M.M. (2015). Effect of Oxytetracycline on Thai Silver Barb (*Barbonymus gonionotus*) and on its culture environment. *Journal of Fisheries and Aquatic Science*, **10**, 323-336.

Jahangiri, L. and Esteban, M.A. (2018). Administration of probiotics in the water in fin fish aquaculture systems: A Review. *Fishes*, **3**(3), 1-11

Jamal, M.T., Abdulrahman, I.A., Al Harbi, M. and Chithambaran, S. (2019). Probiotics as an alternative control measure in shrimp aquaculture: A review. *Journal of Applied Biology and Biotechnology*, **7**(3), 69-77.

Jammal, A., Bariche, M., Dohna, H. and Kambris, Z. (2017). Characterization of the cultivable gut microflora in wild-caught Mediterranean fish species. *Current Nutrition and Food Science*, **13**,147-154

Jay J. M. (2000). Modern Food Microbiology. 6th edition. Aspen Publishers Inc., Maryland. pp 35-56

Jesse, F.F.A., Lawan, A., Abdinasir, Y.O., Zamri-Saad, M. and Zakaria, Z. (2013). Acute phase protein profiles and clinico-pathological changes in mice associated with the infection of *Pasteurella multocida* type B and the bacterial lipopolysaccharide and outer membrane protein immunogens. *Journal of Animal Veterinary Advances*, **12**: 186-193

Jonasson, E., Matuschek, E. and Kahlmeter, G. (2020). The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. *Journal of Antimicrobial Chemotherapy*, **75**, 968-978

Johnson-Henry, K.C., Hagen, K.E., Gordonpou,r M., Tompkins, T.A. and Sherman, P.M. (2007). Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157:H7 adhesion to epithelial cells. *Cell Microbiology*, **9**, 356-367.

Kamal, M.A., Pambuk, C.I.A. and Husein, A.S. (2016). The virulence of pigmented and non- pigmented *Pseudomonas aeruginosa* in mice with antibiotics susceptibility. *International Journal of Vaccines and Vaccination*, **3**(1), 56

Kamal, D., Khan, A.N., Raham, M.A. and Ahamed, A. (2007). Study on physicochemical properties of water of Mouri River, Khulna, Bangladesh. *Pakistan Journal of Biological Sciences*, **10**(5), 710-717.

Karunasagar, I., Kumar, V., Kennedy, B., Vijay, A., Deepanjali, A., Umesh, K. and Karunasagar, I. (2005). Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy. *In*: *Diseases in Asian Aquaculture.*Walker, P., Lester, R. and Bondad-Reantaso, M.G. edition. Fish Health Section, Asian Fisheries Society, Manila. pp. 535-542.

Karuthapardi, M. and Xavier, I. B. (2010). Immune response of *Tilapia mosambicus* administered with rat serum vaccine for *Vibrio anguillarum*. *Current Biotica,* **4**(1), 63-73.

Kasagala, K.H.D.T. and Pathiratne, A. (2008). Effects of waterborne chloramphenicol and oxytetracycline exposure on hematological parameters and phagocytic activity in the blood of koi carp, *Cyprinus carpio*. In: Diseases in Asian Aquaculture VI. Fish Health Section. Bondad-Reantaso, M.G, Mohan, .C.V., Crumlish, M. and Subasinghe, R.P, eds. Malina, Philippines: Asian Fisheries Society pp 283-296.

Kashem, M.A., Uddin, M.N., Hossain, M.M., Hasan, M.T., Haque, S.A., Khan, M.N.A, Hassan, M.M and Hossain, F.M.A. (2014). Effect of oxytetracycline on bacterial load of *Labeo rohita* (Rohu) fish in culture pond. *Global Advanced Research Journal of Microbiology*, **3**(2), 018-024.

Kerala Agricultural University (KAU) (2013). Pond Preparation.Kerala Agricultural University Infotech Portal Centre for E –Learning Kerala Agricultural University; Kerala Agricultural University. Accessed online on 18/5/2019

Kesarcodi- Watson, A., Kaspar, H., Lategan, M.J. and Gibson, L. (2008). Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture* **274**,1–14

Keskitalo,A., Munukka, E., Toivonen, R., Hollme'n, M., Kainulainen, H., Huovinen, P., Jalkanen, S. and Pekkala, S. (2018). *Enterobacter cloacae* administration induces hepatic damage and subcutaneous fat accumulation in high-fat diet fed mice. *PLoS ONE*, **13**(5), e0198262.

Khalafalla, M.M. (2013). Nutritive value of diets containing digeston-1 as a feed additive for Nile tilapia (*Oreochromisniloticus*) fingerlings. *Journal of Aquaculture Research and Development*, **4**(5), 2-5.

Khalighi, A., Behdani, R. and Kouhestani, S. (2016). Probiotics: A comprehensive review of their classification, mode of action and role in human nutrition. https://www.intechopen.com/books/probiotics-and-prebiotics-in-human-nutrition-and-health/probiotics-a-comprehensive-review-of-their-classification-mode-of-action-and-role-in-human-nutrition. Accessed on 18/5/2019.

Khan, Z. A., Siddiqui, M. F. and Park, S. (2019). Current and emerging methods of antibiotics susceptibility testing. *Diagnostics*, **9**(2), 49

Kim, D. H. and Austin, B. (2006). Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. *Fish and Shellfish Immunology*, **21**, 513–524.

Kiran, B.R. (2010). Physicochemical characteristics of fish ponds of Bhadra project at Karnataka. *Rasayar Journal*, **3**(4), 671- 676.

Kohanski, M., Dwyer, D. and Collins, J. (2010). How antibiotics kill bacteria: From targets to networks. *Nature Reviews Microbiology*, **8**(6), 423-435

Kolenda, C., Dupieux, C., Decousser, J-W., Larsen, A, R., Pichon, B., Holmes, M., Skov, R., Kearns, A. and Laurent, F. (2017). Comparison of automated antimicrobial susceptibility testing systems to detect mecC-positive methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, **55**(12), 3554-3356

Korkea-aho, T.L., Papadopoulou, A., Heikkinen, J., Avon, W., Adams, A., Austin, B. and Thompson, K.D. (2012). *Pseudomonas* M162 confers protection against rainbow trout fry syndrome by stimulating immunity. *Journal of Applied Microbiology*, **113**, 24–35.

Kousar, R., Shafi, N., Andleeb, S., Ali, N.M., Akhtar, T. and Khalids, S. (2020). Assessment and incidence of fish associated bacterial pathogens at hatcheries of Azad Kashmir, Pakistan. *Brazilian Journal of Biology*, **80** (3), 607-614.

Kuebutornye, F.K.A., Abarike, E.D., Lu, Y., Hlordzi, V., Sakyi, M.E., Afriyie, G., Wang, Z., Li, Y. and Xie, C.X. (2020). Mechanisms and the role of probiotic *Bacillus* in mitigating fish pathogens in aquaculture. *Fish Physiology and Biochemistry*, **46**(3), 819-841

Kumar, D.G., Karthik, M. and Rajakumar, R. (2017). Study of seasonal water quality assessment and fish pond conservation in Thanjavur, Tamil Nadu, India. *Journal of Entomology and Zoology Studies*, **5**(4), 1232 – 1238.

Kumar, V. and Roy, S. (2017). Aquaculture drugs: sources, active ingredients, pharmaceutics preparations and methods of administration. *Journal of Aquaculture Research and Development*, **8**(9), 1-13

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and evolution*, **33**(7), 1870-1887

Kumar, R., Mukherjee, S., Ranjan, R. and Nayak, S. (2008). Enhanced innate immune parameters in *Labeo rohita* (Ham.) following oral administration of *Bacillus subtilis*. *Fish and Shellfish Immunology*, **24**, 168–172.

Laaksonen T. (2008). Elintarviketekniikka 3, lesson material. Turku University of Applied Sciences.

L'Abee-Lund, T.M. and Sorum, H. (2001). Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. *Microbial Drug Resistance*, **7**, 263–272

Lara-Flores, M., Olvera-Novoa, M. A., Guzman-Mendez, B. E. and Lopez- Madrid, W. (2003). Use of the Bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the Yeast *Saccharomyces cerevisiae* as Growth Promoters in Nile Tilapia (*Oreochromis niloticus*). *Aquaculture*, **216**, 193–201.

Lee, J.J., Kim, D.H., Lim, J.J., Kim, D.G., Chang, H.H., Lee, H.J., Kim, S.H., Rhee, M. H., Endale, M., Imada, Y., Kim, O.J. and Kim, S. (2011). Characterization and identification of *Erysipelothrix rhusiopathiae* isolated from unnatural host, a cat, with a clinical manifestation of depression. *Journal of Veterinary Medical Science*, **73**(2), 149-154.

Lem, A., Tietze, U., Ruckes, E. and van Anrooy, R. (2004). Fish marketing and credit in VietNam. Food and Agricultural Organization Fisheries Technical Paper. No 468. Food and Agricultural Organization, Rome. Pp 174.

Lemos, Q.T., Magalhaes-Fernandes, I. and Andrade, Z.A. (2003). Immunological basis of septal fibrosis of the liver in *Capillaria hepatica*-infected rats. *Brazilian Journal of Medical Biological Research*, **36**, 1201-1207.

Lewis, K. (2020). The science of antibiotics discovery. *Cell*, **181**(1), 29-45.

Li, Y., Pei, X., Yan, J., Liu, D., Zhang, H., Yu, B., Li, N. and Yang, D. (2019). Prevalence of food borne pathogens isolated from retail fresh water fish and shell fish in China. *Food Control*, **99**, 131 – 136.

Li, Y., Yang, X. and Zhao, W. (2017). Emerging microtechnologies and automated systems for rapid bacterial identification and antibiotics susceptibility testing. *SLAS Technology*, **22**(6), 585-608.

Li, X.Z. and Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria: an update. *Drugs*, **69**(12), 1555-1623

Lim, M.H. and Kasing, A. (2013). Antimicrobial susceptibilities of *Vibrio parahaemolyticus* isolate from tiger shrimps (*Panaeus monodon*) aquaculture in Kuching Sarawak. *Research Journal of Microbiology*, **8**(1), 55-62.

Liu, Q., Yu, Z., Tian, F., Zhao, J., Zhang, H., Zhai, Q. and Chen, W. (2020). Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. *Microbial Cell Factories*, **19**(23), 1-11

Lockhart, T. (2017). High white blood cell count: causes and home remedies *General Health* http://www.doctorshealthpress.com/general-health-articles/high-white-blood-cell-count- causes-home-remedies/. Accessed on 20/9/ 2018.

Lulijwa, R., Rupia, E.J. and Alfaro, A.C. (2019). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Review in Aquaculture*, **12**(2), 640-663.

Lunden, T. and Bylund, G. (2002). Effect of sulphadiazine and trimethoprim on the immune response of rainbow trout (*Oncorhynchus mykiss*). *Veterinary Immunology and Immunopathology*, **85**, 99–108.

Madani, N., Adorian, T., Farsani, G.H. and Hossein, S.H. (2018). The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) post larvae. *Aquaculture Research*, **49**(5), 1926-1933

Magdy, I.H., El-Hady, M., Ahmed, H., Saher, A. and Amany, M. (2014). Contribution on *Pseudomonas aeruginosa* infection in African Catfish (*Clarias gariepinus*). *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **5**(5), 575

Mahdhi, A., Amoun, F. and Bakhrouf, A. (2011) Inhibitory activity and adhesive ability of potential probiotics Bacillus sp. to confer protection for Artemia gnotobiotic culture against pathogenic Vibrio sp. *Turkish Journal of Veterinary and Animal Science,* **35**(4), 227–233

Mahmud, Z.H., Wright, A.C., Mandal, S.C., Dai, J., Jones, M.K., Hasan, M., Rashid, M.H., Islam, M.S., Johnson, J.A., Gulig, P.A., Morris, J.G. Jr. and Ali, A. (2018). Genetic characterization of *Vibrio vulnificus* strains from tilapia aquaculture in Bangladesh. *Applied Environmental Microbiology*, **76**, 4890–4895

Makori, A.J., Abuom, P.O., Kapiyo, R., Anyona, D.N. and Dida, G.O. (2017). Effects of water physicochemical parameters on tilapia (*Oreochromis niloticus*) growth in Teso North sub County, Busia County. *Fisheries and Aquatic Sciences*, **20**(30), 1-10

Manjare, S.A., Vhanalakar, S.A. and Muley, D.V. (2010). Water quality assessment of Vadgaon tank of Kolhapur (Maharashtra) with special reference to zooplankton. *International Journal of Advanced Biotechnology and Research*, **1**, 91-95.

Manoj, C.K., Mohanakumaran, C., Patel, M.R. and Salim, K.R. (2010). Haematobiochemical and histopathological changes in *Labeo rohita* infected with *Aeromonas hydrophila* by immersion challenge. *Fisheries Technology*, **47**(2), 151-160

Martins, M.L., Mourino, J.L.P., Amaral, G.V., Vieira, F.N., Dotta, G., Jatoba, A.M.B., Pedrotti, F.S., Jeronimo, G.T., Buglione-Neto, C.C. and Pereira-Jr. G. (2008). Haematological changes in Nile tilapia experimentally infected with *Enterococcus sp*. *Brazilian Journal of Biology*, **68**(3), 657-661.

Marzouk, M.S., Moustafa, M.M. and Mohamed, N.M. (2008). Evaluation of immunomodulatory effects of some probiotics on cultured O*reochromis niloticus*. *8th International Symposium on Tilapia in Aquaculture*, 1043–1058

Meck, N. (1996). Pond water chemistry, San Diego, Koi Club. Http://users.vcnet.com/rrenshaw/h20quality.html. Asssessed on 19th June, 2019

Medellin-Pena, M.J., Wang, H., Johnson, R. and Griffiths, M.W. (2007). Probiotic effects virulence related gene expression in *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, **73**, 4259– 4267

Mgwede, C.W., Msiska, O. and Kapute, F. (2018).Comparative assessment of microbiological safety of fresh and parboiled *Engraulicypris sardella* (Usipa) from selected selling points in the city of Mzuzu, Malawi. *MOJ Food Processing and Technology*, **6**(4), 355–360.

Michigan State University Board of Trustees (2011). Antimicrobials. Antimicrobial Resistance Learning Site, Pharmacology Module Outline, pp. 1-23.

Miller, M.B. and Bassler, B.L. (2001). Quorum sensing in bacteria. *Annual Review of Microbiology*, **55**, 165-199.

Miranda,C. D., Gody, F.A. and Lee, M.R. (2018). Current status of the use of antibiotics and the antimicrobial resistance in the Chilean Salmon Farms. *Frontiers in Microbiology*, **18**, 1284

Mirzoyan, N., Tal, Y. and Gross, A.(2010). Anaerobic digestion of sludge from intensive recirculating aquaculture systems. *Review in Aquaculture*, **306**, 1–6.

Mohammadi, F., Mousavi, S. M., Ahmadmoradi, E., Zakeri, M., and Jahedi, A. (2015). Effects of *Saccharomyces cerevisiae* on survival rate and growth performance of Convict Cichlid (*Amatitlania nigrofasciata*). *Iranian journal of veterinary research*, **16**(1), 59–62.

Mohapatra, S., Chakraborty, T., Prusty, A. K., PaniPrasad, K., and Mohanta, K. N. (2014). Beneficial effects of dietary probiotics mixture on hemato-immunology and cell apoptosis of *Labeo rohita* fingerlings reared at higher water temperatures. *PloS ONE*, **9**(6), e100929.

Mölne, L. and Tarkowski, A. (2000). An experimental model of cutaneous infection induced by super antigen-producing *Staphylococcus aureus*. *Journal of Investigative Dermatology*, **114**(6), 1120-1125

Moon, J.Y., Lim, J.M., Ahn, J.H., Weon, H.Y., Kwon, S. W. and Kim, S.J. (2014). *Paenalcaligenes suwonensis* sp. Nov., isolated from spent mushroom compost. *International Journal of Systematic and EvolutionaryMicrobiology*, **64**, 882-886.

Motameni, A.T., Bates, T.C., Juncadella, I.J., Petty, C., Hedrick, M.N. and Anguita, J. (2005). Distinct bacterial dissemination and disease outcome in mice subcutaneously infected with *Borrelia burgdorferi* in the midline of the back and the footpad, *FEMS Immunology and Medical Microbiology*, **45**(2), 279–284.

Mukwabi, D. and Okemo, K.P. (2019). Temperature and pH influence on bacterial pathogens infecting farmed Nile tilapia in aquaculture systems in Bungoma County Kenya. *International Journal of Fish Aquatic Studies*, **7**, 190-197.

Munoz-Atienza, E., Beatriz, G.S., Carlos, A., Cristina, C., Rosa, D.C., Pablo, E.H., Carmen, H. and Luis, M.C. (2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology*, **13**, 15

Munukka, E., Rintala, A., Toivonen, R., Nylund, M, Yang, B., Takanen, A., Hanninen, A., Vuopio, J., Huovinen, P., Jalkanen, S. and Pekkala, S. (2017). *Faecalibacterium prausnitzii* treatment improves hepatic health and reduces adipose tissue inflammation in high-fat fed mice. *Multidisciplinary Journal of Microbial Ecology*, **11**, 1667-1679.

Murdoch, D. R., Werno, A. M. and Jennings, L. C. (2019). Microbiological Diagnosis of Respiratory Illness: Recent Advances. In: *Kendig's Disorders of the Respiratory Tract in Children*. Elsevier publishers, UK. pp 396–405.

Nair, G., Ramamurthy, T., Bhattacharya, S. K., Dutta, B., Takeda, Y. and Sack, D. A. (2007). Global dissemination of *Vibrio parahaemolyticus* serotype O3: K6 andits serovariants. *Clinical Microbiology Reviews*, **20**, 39-40.

National Bureau of statistics (NBS) (2011). Annual Abstract of Statistics. https://www.nigeriastat.gov.ng. Accessed on 18/4/2019

Nawar, F. (2016). Determination of phosphorus: Ascorbic acid procedure protocols.io. https://www.protocols.io/view/determination-of-phosphorus-ascorbic-acid-procedure-ggcbtsw. Accessed on 18th April, 2019

Nayak, S.K. (2010). Probiotics and Immunity: A fish perspective. *Fish and Shell fish Immunology*, **29**, 2-14.

Nayak, S., Swain, P.and Mukherjee, S. (2007). Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita* (Ham.). *Fish and Shellfish Immunology*, **23**, 892–896.

Nduka, J.K., Orisakwe, O.E. and Ezenweke, L.O. (2008). Some physicochemical parameter of portable water supply in Warri, Niger Delta Area of Nigeria. *Scientific Research and Essay*, **3**(11), 547 – 551.

Nester, E. W., Anderson, D. G., Roberts, E. C., Pearsall, N. N. and Nester, M. T. (2004). Fungi *In: Microbiology A human Perspective* 4th Ed Mc Graw Hill New York. pp 307-314

Ng, S., Hart, A., Kamm, M.A., Stagg, A.J. and Knight, S. (2009). Mechanisms of Action of Probiotics: Recent Advances. *Inflammatory Bowel Diseases*, **15**(2), 300-310

Nickelson, R. I. McCarthy, I. S. and Finne, G. (2001). Fish, crustaceans and precooked seafoods. *In: Compendium of methods for the microbiological examination of foods*. Downes, F.P. and Ito, K., (eds.). *American Public Health Association*, pp: 497-505.

Njoku, O.E., Agwa, O.K. and Ibiene, A.A. (2015). An Investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. *European Journal of Food Science and Technology*, **3**(4), 20 – 31.

Njoku, O. E., Agwa, O. K. and Ibiene, A. A. (2015a). An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. *African Journal of Food Science*, **9**(3), 1-12.

Njoku, O. E., Agwa, O. K. and Ibiene, A. A. (2015b). Antibiotic susceptibility profile of bacteria isolates from some fishponds in Niger Delta Region of Nigeria. *British Microbiology Research Journal*, **7**(4), 167-173.

Nordmann, P. and Poirel, L. (2005). Emergence of plasmid mediated resistance to quinolones in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, **56**, 463–469

Noel, K.R. and John, H.G. (1984). Bergey's Manual of Systematic Bacteriology, Barbara T. edition. William and Wilkins publishers, Baltimore, USA. Pp 85-115.

Novotny, L., Dvorska, L., Lorencova, A., Beran, V. and Pavlik, I. (2004). Fish: a potential source of bacterial pathogens for human beings. *Veterinary Research Institute*, **9**, 343-358.

Ntengwe, F.W. and Edema, M. O. (2008). Physico-chemical and microbiological characteristics of water for fish production using small ponds. *Physics and Chemistry of the Earth*, **33(**8), 701-707.

Ntsama, I.S.B., Tambe, B.A., Takadong, J.J.T., Nama G.M. and Kansci, G. (2018).Characteristics of fish farming practices and agrochemicals usage therein in four regions of Cameroon. *Egyptian Journal of Aquatic Research,* **44**, 145–153.

Nwani, C.D., Mkpadobi, B.N., Onyishi, G., Echi, P.C., Chukwuka, C.O., Oluah, S.N. and Ivoke, N. (2013). Changes in behavior and hematological parameters of fresh water African catfish, *Clarias gariepinus* (Burchell 1822) following sub lethal exposure to chloramphenicol. *Drug and Chemical Toxicology*, **37**(1), 107– 113O

Odiete, J.A., Olayiwola, J.O. and Abiala, M.A. (2019). Hematological and pathological studies of bacteria associated with mobile phones from handlers of diverse lifestyles in the rural community. *American Journal of Pharmacology and Therapeutics*, **3**(1), 1-7.

Ogawa, M., Shimizu, K., Nomoto, K., Takahashi, M. and Watanuki, M. (2001). Protective effect of *Lactobacillus casei* strain Shirota on Shiga toxin-producing *Escherichia coli* O157:H7 infection in infant rabbits. *Infection and Immunity*, **69**, 1101- 1108

Ogeneogaga, O.I. and Solomon, R. J. (2017). Physico-chemical and bacteriological investigation of selected fish pond in Kuje Area Council, Nigeria. *Researcher*, **9**(4), 31- 45

Oghenebrorhie, O., Adeolu, A., Onyenoro, E., Mobolaji, B. and Vivian, T. (2014). Pathological study of *Clarias gariepinus* (Burchell, 1822) sub-adult artificially

infected with *Pseudomonas aeruginosa*. *Brazilian Journal of Aquatic Science and Technology*, **18**(2), 65-69.

Oh, W.T., Kim, J.H., Jun,J.W., Giri, S.S., Yun, S., Kim, H.J., Kim,S.G., Kim, S.W. Han, S.J., Kwon,J. and Park, S.C. (2019). Genetic characterization and pathological analysis of a novel bacterial pathogen, *Pseudomonas tructae*, in Rainbow Trout (*Oncorhynchus mykiss*). *Microorganisms*, **7**, 432 – 444

Ojiako, J. C., Okafor, C.M. and Igbokwe, E.C. (2015). Geospatial mapping of fish farms in animal state using GIS Approach. *International Journal of Scientific and Engineering Research*, **6**(11), 1-12.

Okafor, U.C., Ezeanochie, P. E. and Obubu, M. (2020). Microbial assessment of some selected fish ponds in Awka, Anambra State: Comparative study and modelling. *Agricultural and Biological Sciences Journal*, **6**(2), 91-99

Okonko, I. O., Ogunjobi, A.A., Fajobi, E.A., Onoja, B.A., Babalola, E.T. and Adedji, A.O. (2008). Comparative studies and microbial risk assessment of different ready-to-eat (RTE) frozen sea-foods processed in Ijora-Olopa, Lagos state, Nigeria. *Journal of Biotechnology***, 7**, 2898-2901

Okorie-Kanu, C.O. and Unakalamba, N. J. (2014). Haematological and blood biochemistry values of cultured *Heterobranchus longifilis* in Umudike, Abia State, Nigeria. *Animal Research International*, **11**(2), 1987 – 1993

Olaniyi, O. (2013). Assessment of water quality characteristics for aquaculture uses in Abeokuta Nigeria. *Lakes, Reservoirs and Ponds*, **7**(1), 9–19.

Omojowo, F.S., and Sogbesan, O.A. (2003). Fish losses due to bacterial flora and infections of fishes in Kainji lake fish Disease, Nigeria. A Review: *Nigeria Veterinary Journal*, **24**, 41-47.

Omoya, F.O. and Akharaiyi, F.C. (2015). Physiological changes in *Clarias gariepinus* induced with *Bacillus* species used as biological agent in aquatic environment. *Journal of Scientific Research and Reports*, **7**(2), 117-128.

Opiyo, M.A., Jumbe, J., Ngugi, C.C. and Charo-Karisa, H. (2019).Dietary administration of probiotics modulates non-specific immunity and gut microbiota of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *International Journal of Veterinary Science and Medicine*, **7**(1), 1-9

Pages, J. M., Janes, C. E. and Winterhalter, M. (2008). The porin and the permeating antibiotic: A selective diffusion barrier in gram negative bacteria. Nature Reviews. *Microbiology*, **6**, 893-903

Pal, M., Ketema, A., Anberber, M., Mulu, S. and Dutta, Y. (2016). Microbial quality of fish and fish products. *Beverage and Food World*, **43**(2), 46-49.

Pandit, D. and Jaiswal, A. (2020). Impairment induced due to chloramphenicol and ginger on growth parameters of *Channa punctatus* (Bloch). *International Journal on Advances in Life Sciences*, **8**(1), 102-108

Park, K. Y. and Seo, S. J. (2013). Cutaneous *Serratia marcescens* infection in an immunocompetent patient after filler injection. *Acta Dermato- Venereologica,* **93**, 191-192.

Pascual, L., Ruiz, F., Giordano, W. and Barberis, L. (2009). Vaginal colonization and activity of the probiotic bacterium *Lactobacillus fermentum* L23 in a murine model of vaginal tract infection. *Journal of Medical Microbiology,* **59(**3), 360-364.

Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research,* **62(**3), 261–267.

Pereckaite, L., Tatarunas, V. and Giedraitiene, A. (2018). Current antimicrobial susceptibility testing for beta-lactamase-producing Enterobacteriaceae in clinical settings. *Journal of Microbiology Methods*, **152,** 1-12

Phu, T.M., Phuong, N.T., Scippo, M.L. and Dalsgaard, A. (2015). Quality of antimicrobial products used in striped catfish (*Pangasianodon hypophthalmus*) in Vietnam. *PLoS ONE*, **10**(4), e0124267

Pindling, S., Azulai, D., Zheng, B., Dahan, D. and Perron, G. G. (2018). Dysbiosis and early mortality in zebrafish larvae exposed to subclinical concentrations of streptomycin. *FEMS Microbiology Letters,* **365**, 188

Pishchany, G. and Skaar, E.P. (2012). Taste for blood: hemoglobin as a nutrient source for pathogens. *PLoS Pathogen,* **8**(3), 1 -10

Pletzer, D., Mansour, S.C., Wuerth, K., Rahanjam, N. and Hancock, R.E.W. (2017). New mouse model for chronic infections by gram-negative bacteria enabling the study of anti-infective efficacy and host-microbe interactions. *mBio*, **8**(1), 1-17

Poirel, L., Rodriquez-Martinez, J.M., Mammeri, H., Liard, A. and Nordmann, P. (2005). Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrobial Agents and Chemotherapy*, **49**, 3523–3525

Porex Barrier Technology (PBT) (2017). Antimicrobial Porous Media. https://www.porex.com/wp-content/uploads/2020/01/Porex-Whitepaper-Antimicrobial-Wound-Care-Foams.pdf . Accessed on 23/5/2019.

Pramanick, P., Biswas, P., Zaman, S. and Mitra, A. (2019). Probiotics: A pathway for upgrading aquaculture sector. *International Journal of Research and Review*, **6**(1), 95 – 99.

Prameena, J.L. and Sheeja (2016). Physico-chemical characterization of pond water from Vaduvur, Mannargudi (TK), Thiruvarur Disctrict (India). *Der pharma Chemica*, **8**(10), 6-11.

Prasanna, M. and Ranjan, P.C. (2010) Physicochemical properties of water collected from Dhamra estuary. *International Journal of Environmental science*, **1**(3), 334 – 342.

Praveena, P. E., Periasamy, S., Kumar, A.A. and Singh, N. (2010). Cytokines profiles, apoptosis and pathology of experimental *Pasteurella multocida* serotype A1infection in mice. *Research Veterinary Science,* **89**, 332-339.

Pridgeon, J.W. and Klesius, P.H. (2012). Major bacterial diseases in Aquaculture and their vaccine development. *CAB Review*, **7**, 16.

Prieur, G., Nicolas, J.L., Plusquellec, A. and Vigneulle, M. (1990). Interactions between bivalves mollusks and bacteria in the marine environment. Oceanography and marine Biology. *An Annual Review*, **28**, 227-352.

Priyanka, Y., Yadav, V.K., Yadav, A.K. and Khare, P.K. (2013). Physico-Chemical characteristics of a fresh water pond of Orai, U.P., Central India. *Octa Journal of Biosciences,* **1**(2), 177 – 184.

Purwandari, A.R. and Chen, H.Y. (2013) Effects of probiotic *Bacillus subtilis* on intestinal microbial diversity and immunity of orange spotted grouper *Epinephelus coioides*. *Journal of Applied Biotechnology,* **1**(1), 25–36

Raetz, C.R.H. and Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry*, **71**, 635-700.

Rafiq, M., Sarder, I., Thompson, K., Penman, D. and Mcandrew, B.J. (2001). Immune responses of Nile tilapia (*Oreochromis niloticus* L.) clones: I. Non-specific responses. *Developmental and Comparative Immunology*, **25**(1), 37-46

Raj, J. A. and Sevarkodiyone, S.P. (2018). A Study on physico-chemical parameters of Urinjikulam pond, Thiruthangal (Virudhungar District, Tamil Nadu). *International Journal of Aquaculture and Fishery Sciences,* **4**(1), 010-012

Ramos, C.L., Thorsen, L. and Schwan, R.F. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiology,* **36**(1), 22–29.

Ran, C., Huang, L., Liu, Z., Xu, L., Yang, Y, Tacon, P., Auclair, E. and Zhou, Z. (2016). A comparison of the beneficial effects of live and heat-inactivated baker's yeast on Nile Tilapia: Suggestions on the role and function of the secretory metabolites released from the yeast. *PLOS ONE*, **11**(3), e0151207

Reid, K. M., Patel, S., Robinson, A.J., Bu, L., Jarungsriapisit, J., Moore, l.J. and Salinas, I. (2017). Salmonid alphavirus infection causes skin dysbiosis in Atlantic salmon (*Salmo salar* L.) post-smolts. *PloS ONE,* **12**(3), e0172856

Reda, R.M., El-Hady, M.A., Selim, K.M. and El-Sayed, H.M. (2018). Comparative study of three predominant guts *Bacillus* strain and a commercial *B. amyloliquefaciens* as probiotics on the performance of *Clarias gariepinus*. *Fish and Shellfish Immunology*, **80**, 416 – 425

Reda, R.M., Ibrahim, R.E., Ahmed, E.G. and El-Bouhy, Z.M. (2013). Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured *Oreochromis niloticus. The Egyptian Journal of Aquatic Research,* **39(**4), 241-248

Reitman, S. and Frankel, S. (1957). Colorimetric methods for the determination of serum transaminases. *American Journal of Clinical Pathology,* **28**, 56-61.

Reizer, W., Hunter, J. G., O'Malley, N.T., Southgate, R. D., Schwarz, E. M. and Kates, S.I. (2014). A systemic review of animal models for *Staphylococcus aureus* Osteomylitis. *European Cells and Materials*, **27**, 196-212

Reller, L. B., Weinstein, M., Jorgensen, J.H. and Ferraro, M. J. (2009). Antimicrobial susceptibility testing. A review of general principles and contemporary practices. *Clinical Infectious Diseases*, **49**(11), 1749-1755.

Remus, D. M., van Kranenburg, R., van Swam, I. I., Taverne, N., Bongers, R. S., Wels, M., Wels, J.M., Bron, P. A. and Kleerebezem, M. (2012). Impact of *Lactobacillus plantarum* capsular polysaccharide clusters on surface glycan composition and host cell signaling. *Microbial Cell Factories*, **11**, 149.

Rhayat, L., Maresca, M., Nicoletti, C., Perrier, J., Brinch, K. S., Christian, S., Devillard, E. and Eckhardt, E. (2019). Effect of *Bacillus subtilis* strains on intestinal barrier function and inflammatory response. *Frontiers in immunology*, **10**, 564

Riauwaty, M., Windarti, W., Yulika Sari, T. E. and Adelina, A. (2019). Blood condition of *Pangasius hypophthalmus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila*. IOP Conf. Series: *Earth and Environmental Science,* **348** (1**)**, 012036

Ringo, E. (2020). Probiotics in shellfish aquaculture: A Review article. *Aquaculture and Fisheries*, **5**, 1-27

Ripabelli, G., Sammarco, M. L., Fanelli, I. and Grasso. G. M. (2004). Detection of *Salmonella*, *Listeria* spp, *Vibrio* spp and *Yesinia entrocolitica* in frozen seafood and comparison with enumeration for faecal indicators: implication for public health. *AppliedMicrobiology*, **16**(4), 531-539

Rivera-Rodriguez, S. C. and Dussan-Garzan, J. (2008). Trial evaluation of toxicity and pathology in murine tissues using *Bacillus sphaericus* Colombian strain. *Actual Biology*, **30**(89), 125-134

Robert, T. and Stadler, K.M. (2000). Safe and nutritious seafood in Virgina. http://www.ext.vt.edu/pubs/foods/348-961/htm. Accessed in 28/8/2018.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P. and Pickup, R.W. (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant TetA. *Applied and Environmental Microbiology*, **66**, 3883–3890

Romero, J., Ringoe, E. and Merrifield, D. (2014). The gut microbiota of fish. *In*: *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*. Merrifield, D. and Ringø, E. edition. John Wiley and Sons, Ltd, Chichester, UK. pp 75-100

Rosado, D., Xavier, R., Severino, R., Tavares, F., Cable, J. and Perez-Losada, M. (2019). Effects of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed seabass (*Dicentrarchus labrax*). *Scientific Report*, **9**, 18946

Rozas-Serri, M., Ildefonso, R., Pena, A. Enrlıquez, R., Barrientos, S. and Maldonado, L. (2017). Comparative pathogenesis of piscirickettsiosis in Atlantic salmon (*Salmosalar* L.) post- smolt experimentally challenged with LF-89-like and EM-90-like *Piscirickettsia salmonis* isolates. *Journal of Fish Diseases*, **2017**, 1–22.

Safaeian, S. and Khanzadi, S. (2018). Microbiology of fish and sea food. The first National Conference on Recent Advances in Engineering and Modern Science.

Safinska, A.P. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research*, **62**, 261–267

Saglam, N. and Yonar, M. E. (2009). Effects of sulfamerazine on selected haematological and immunological parameters in rainbow trout (*Onchorhynchus mykiss*, Walbaum, 1792).*Aquaculture Research*, **40**(4), 395-404.

Sahoo, P.K., Swaim, P., Sahoo, S.K., Makherjee, S.C. and Sahu, A.K. (2000). Pathology caused by the bacterium *Edwardsiella tarda* in *Anabas testudineus* (Blouch). *Asian Fishery Science*, **13**, 357-362.

Sajitha, V. and Vijayamma, S.A. (2016). Study of physico-chemical parameters and pond water quality assessment by using water quality index at Athiyannoor Panchayath, Kerala, India. *Emergence Life Science Research*, **2**(1), 46 – 51.

Sakata, T. (1990). Microflora in the digestive tract of fish and shellfish. *In*: *Microbiology of Poekilotherms*. Lesel, R. edition. Elsevier, Amsterdam. pp 217–223

Salah, M. A, Nashwa, M. A. A and Mohamed, F. M. (2008). Effect of garlic feeding on the survival, growth, resistance and quality of *Oreochromis niloticus*. *8th Int. Symposium on Tilapia in Aquacultur*e. pp 277-296

Salyers, A.A., Gupta, A. and Wang, Y. (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in Microbiology*, **12**, 412- 416.

Sander-Miranda, L., Vinuesa, P., Cravioto, A. and Morales- Espinosa, R. (2018). The genomic basis of intrinsic and acquired antibiotic resistance in the genus Serratia. *Frontiers in Microbiology*, **9**, 828

Sandoval, K., Labana, R., Cada, K. J. and Dungca, J. (2017). Physico-chemical analysis of fish pond water in Candaba, Pampanga, Philippines. *Philippine Journal of Systematic Biology*, **11**(1), 67-70.

Saravanan, M., Usha, D. K., Malarvizhi, A. and Ramesh, M. (2012). Effects of Ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp *Cirrhinus mrigala. Environmental Toxicology Pharmacology*, **34**,14–22

Saravanan, M., Karthikas, S., Malarvizhi, A. and Ramesh, M. (2011). Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical ion regulatory and enzymological responses. *Journal of Hazardous Materials*, **195**, 188–194.

Sawere, B. T. and Oghenekowhoyan, O.C. (2019). Determination of some physicochemical parameters and selected heavy metals from water collected from concrete fish ponds in Ozoro town. *International Journal of Advances in Scientific Research and Engineering*, **5**(5), 1-7

Sawyer, C. N., Mc Carty, P.L., and Parkin, G.F. (2000). Chemistry for environmental engineering. 4th Edn. Tata Mc Graw-Hill Publishing Company Limited.

Schook, L.B., Carrick Jr, L. and Berk. R.S. (1976). Murine gastro intestinal tract as a portal of entry in experimental *Pseudomonas aeruginosa* infections. *Infection and Immunity*, **14**(2), 564-570

Schumacher,A., Vranken, T., Malhotra, A., Arts, J.J.C. and Habibovic, P. (2018). *In vitro* antimicrobial susceptibility testing methods: agar dilution to 3D tissue- engineered models. *European Journal of Clinical Microbiology and Infectious Diseases*, **37,** 187-208

Sebastiao, F.A., Nomura, D., Sakabe, R. and Pilarski, F. (2011). Haematology and productive performance of Nile Tilapia (*O. niloticus*) naturally infected with *Flavobacterium columnare*. *Brazilian Journal ofMicrobiology*, **42**, 282-289.

Selim, K.M. and Reda, R.M. (2015). Improvement of immunity and disease resistance in the Nile tilapia, *Oreochromis niloticus*, by dietary supplementation with *Bacillus amyloliquefaciens*. *Fish Shellfish Immunology*, **44**, 496-503.

Selvamuthukumaran,M. and Khanum, F. (2014). Effect of modified atmosphere packaging on physicochemical, sensory and microbiological properties of spray-dried sea Buckthorn fruit juice powder. *Journal of Food Quality*, **37**, 149-156.

Senok, A.C., Ismaeel, A.Y. and Botta, G.A. (2005). Probiotics: facts and myths. *Clinical Microbiology and Infect*ion, **11**(12), 958-966

Seo, J., Shin, D., Oh, S.H., Lee, J.H., Chung, K.Y., Lee, M. G. and Kim, D.Y. (2016). Cutaneous *Serratia marcescens* infections in Korea: A retrospective analysis of 13 patients. *The Journal of Dermatology*, **43**(2), 149-155.

Seo, J.S., Jeon, E.J., Jung, S.H., Park, M.A. and Kim, N.Y. (2015). Pharmacokinetics of Amoxicillin trihydrate in cultured olive flounder (*Paralichthys olivaceus*). *Journal of Veterinary Pharmacology and Therapeutics*, **38**, 86 – 92.

Shahsavani, D., Mohri, M. and Gholipour, H. (2010). Determination of normal values of some blood serum enzymes in *Acipenser stellatus* (Pallas). *Fish physiology and Biochemistry*, **36**(1), 39-43

Shalaby, A. M., Khattab, Y. A. and Abdel Rahman, A. M. (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia *(Oreochromis niloticus*). *Journal of Venomous Animals and Toxins including Tropical Diseases*, **12**(2), 172-201

Shivshetty, N., Hosamani, R., Ahmed, L., Oli, A.k., Sannaquallah, S., Sharanbassappa, S., Patil, S.A. and Kelmani, C.R. (2014). Experimental protection of diabetic mice

against lethal *Pseudomonas aeruginosa* infection by Bacteriophage. *HindawiBioMed Research International*, **2014**, 1-12.

Shobana, R., Suriyakala, K., Arockia, S.P., Dharmalingam, V. and Soruba, R. (2014). Evaluation of water quality of Sriperumpudur in Kancheepuram District in Tamilnadu. *Der Chemica Sinica*, **5**(5), 91 – 97.

Sikoki, F.D. and Veen, F. (2004). Aspects of water quality and the potential for fish production of Shinro Reservour. *Nigeria Living System Sustainable Development*, **2**, 1-7.

Sivagurunathan, A., Amila Meera, K. and Xavier, B.I. (2011). Investigation of immunostimulant potential of *Zingiber officinale* and *Curcuma longa* in *Cirrhinus mrigalla* exposed to *Pseudomonas aeruginosa* – Haematological assessment. *International Journal of Research Ayurveda and Pharmacolog*, **2**(2), 899-904

Snoussi, M., Ghaieb, K., Manmoud, R. and Bakhrouf, A. (2006). Quantitative study, identification and antibiotics sensitivity of some *Vibrionaceae* associated to marine fish hatchery. *Annuals of Microbiology*, **56**, 289-293.

Soltan, M. A., Hassaan, M. S., El-Nagaar, G. O. and Wahead, E. (2013). Effect of rearing temperature and hormone treatment on sex ratio, survival and body weight of *Oreochromis niloticus* fry. *Egyptian Journal of Aquatic Biology and Fisheries*, **17**(4), 13-23.

Sorum, H. (2006). Antimicrobial drug resistance in fish pathogens. *In*: *Antimicrobial resistance in bacteria of animal origin*, Aerestrup, F.M. edition.ASM Press, Washington DC. pp 213- 238.

Statistical Year Book (2011). Population density of local Government Area. pp 12.

Stevens, C. (2007). Dissolved Oxygen Science and Technology. *Aquaculture Networks*, **1**, 14-16.

Stokes, H.W., Holmes, A.J., Nield, B.S., Holley, M.P., Nevalainen, K.M.H., Mabbutt, B.C. and Gillings, M.R. (2001). Gene cassette PCR: sequence- independent recovery of entire genes from environmental DNA. *Applied and Environmental Microbiology*, **67**, 5240–5246

Strzyzewska, E., Szarek, J. and Babinska, I. (2016). Morphologic evaluation of the gills as a tool in the diagnostic of pathological conditions in fish and pollution in the aquatic environment: a review. *Veterinarni Medicina*, **61** (3), 123-132.

Subbiah, M., Caudell, M.A. and Mair, C. (2020). Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nature Communications*, **11**, 228.

Sun, H., Lin, Z., Zhao, L., Chen, T., Shang, M., Jiang, H., Tang, Z., Zhou, X., Shi, M., Zhou, L.,Ren, P., Qu, H., Lin, J., Li, X., Xu, J., Huang, Y. and Yu, X. (2018). *Bacillus subtilis* spore with surface display of paramyosin from *Clonorchis sinensis* potentializes a promising oral vaccine candidate. *Parasites Vectors*, 11(156), 1-10

Swann, L. (2006). A fish farmer's guide to understanding water quality. http://aquatic.org/publicat/il-in/as-530htlm. Assessed on 19/6/2019

Swarnendu, C., Urmimala, C. and Rajarshi, B. (2010). Development and assessment of a pO fish feed to assist in aquaculture nutrition management. *Research*, **2**(5), 63 – 75.

Tafalla, C., Beatriz, N., Alvarez, J.M. and Antonio, F. (2002). *In vivo* and *in vitro* effect of oxytetracycline treatment on the immune response of turbot, *Scophthalmus maximus*(L.). *Journal of Fish Disease*, **22**(4), 271-276

Tang, Z., Shang, M., Chen, T. Ren, P., Sun, H., Qu, H., Lin, Z., Zhou, L., Yu, J., Jiang, H., Zhou X., Li, X., Huang, Y. and Yu, X. (2016). The immunological characteristics and probiotic function of recombinant *Bacillus subtilis* spore expressing *Clonorchis sinensis* cysteine protease. *Parasites Vectors*, **9**, 648

Tankeshwar, A. (2016). Preparation of McFarland turbidity standards. https://microbeonline.com/preparation-mcfarland-turbidity-standards/ Accessed on 14/5/2019.

Tara, J.S., Kour, R. and Sharma, S. (2011). Studies on the occurrence and seasonal abundance of aquatic coleopteran in relation to some physico chemical parameters of water of Gharana Wetland Reserve Jammu. *The Bioscan*, **6**(2), 257-261.

Tattiyapong, P., Dachavichitle, W. and Surachetpong, W. (2017). Experimental infection of Tilapia Lake Virus (TiLV) in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis* spp.). *Veterinary Microbiology*, **207**, 170 – 177.

Teh, L.C.,Teh, L., Starkhouse,B., and Sumaila, R. U. (2009).An overview of socio-economic and ecological perspectives of Fiji's inshore reef fisheries. *Marine Policy*, **33**(5), 807-817

Tenover, F.C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*, **119**(6), 3-10

Tendencia, D. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture*, **195**, 193- 204.

Thornber, K., Verner-Jeffreys, D. Hinchliffe, S., Rahman, M.M., Bass, D. and Tyler, C.R. (2019). Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture*, **1**, 21.

Tiamuyu, A.M., Olatoye, I.O. and Adedji, O.B (2019). Blood indices of African catfish (*Clarias gariepinus*) following dietary administration of *Talinum triangulare*. *International Journal of Research- Granthaalayah*, **7**(4), 1-14.

Toapanta, F. R., Bernal, P. J., Fiesnay, S., Darton, T. C., Jones, C., Waddington, C.S., Blohmke, C. J., Dougan, G., Angus, B., Levine, M.M. and Pollard, A.J. (2015).Oral wild-type *Salmonella typhi* challenge induces activation of circulating monocytes and dendritic cells in individuals who develop typhoid disease. *PLoS Neglected Tropical Diseases*, **9**(6), e0003837.

Torimiro, N., Bebe, P.T., Ogundipe, F.E., Esan, D.M.and Aduwo, A.I.(2014). The bacteriology and physicochemical analysis of freshwater fish ponds. *International Research Journal of Microbiology*, **5**(3), 28-32

Tripathi, N.K., Latimer, K.S., Gregory, C.R. Ritchie, B.W., Wooley, R.E. and Walker, R.L. (2005). Development and evaluation of an experimental novel of cutaneous columnaris disease in koi (*Cyprinus carpio*).*Journal of Veterinary Diagnosis Investigation*, **17**, 45-54

Umeh, O. R., Chukwura, E. I., and Ibo, E. M. (2020). Physicochemical, bacteriological and parasitological examination of selected fish pond water samples in Awka and its environment Anambra State, Nigeria. *Journal of Advances in Microbiology*, **20**(3), 27-48

Van Belkum, A., Burnham, C-A.D., Rossen, J.W.A., Mallard, F., Rochas, O. and Dunne, W.M. (2020). Innovativeand rapid antimicrobial susceptibility testing systems. *Nature Reviews Microbiology*, 1-12

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, **64**(4), 655-671

Vignesha, R., Karthikeyanb, B.S., Periyasamya, N. and Devanathana, K. (2011). Antibiotics in aquaculture: An overview. *South Asian Journal of Experimental Biology*, **1**(3), 1-7

Vincent, R. (2010), Koch's Postulate in the 21st century. Virology blog http://www.virology.ws/2010/01/22/kochs-postulates-in-the-21st-century/. Accessed 20/11/2018.

Vlab. (2012). Biological Oxygen Demand. Virtual Amrita Laboratories Universalizing Education. www.Vlab. amrita.edu. Accessed on 25/9/2018

Wairimu, T.O., Kitur, E., Ngugi, C.C., Mwangi, B.M. and Nzere, J.K. (2019). Impact of aquaculture development on water quality of fish ponds in Gatundu North and South sub-countries, Kenya. *International Journal of Fisheries and Aquatic Studies*, **4**, 01-06.

Wamala, S.P., Mugimba, K. K., Mutoloki, S., Evensen, Q., Mdegela, R., Byarugaba, D. K. and Sørum, H. (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. *Fisheries and Aquatic Sciences*, **21**, 6-16.

Wang, S., Payne, G. F. and Bentley, W E.(2020). Quorum sensing communication: Molecularly connecting cells, their neighbors, and even devices. *Annual Review of Chemical and Biomolecular Engineering*, **11**(11), 1-11.

Wang, W., Gu, X., Zhou, L., Chen, H., Zeng, Q. and Mao, Z.(2018). Antibiotics in crab ponds of Lake Guchenghu Basin, China. Occurrence, temporal variations, and ecological risks. *International Journal of Environmental Research and Public Health*, **15**(3), 548

Wang, A., Luo, J., Moore, W., Alkhalidy, H., Wu, L., Zhang, J., Zhen, W., Wang, Y., Clegg, D.J., Xu, B., Cheng, Z., McMillan, R.P., Hulver, M.W. and Liu, D. (2016). GPR30 regulates diet-induced adiposity in female mice and adipogenesis *in vitro*. *Scientific Report*, **6,** 34302

Wanguyun, A. P., Hayati, A. and Utomo, B. (2019). The effects of probiotic feed supplementation on tilapia (*Oreochromis niloticus*) in copper-tainted water. *Ecology, Environment and Conservation Paper*, **25**, 13-17.

Wollenberger, L., HallingSorensen, B. and Kusk, K.O. (2000). Acute and chronic toxicity of veterinary antibiotics of *Daphnia magna*. *Chemosphere*, **40**, 723- 730.

WorldFish (2018). WorldFish Nigeria Strategy: 2018-2022. http://pubs.iclarm.net/resource_centre/2018-09.pdf. Assessed on 11/6/2020

World Health Organization (WHO) (2020). Water Quality Requirements. https://www.who.int/water_sanitation_health/resourcesquality/wpcchap2.pdf. Assessed on 11/6/2020.

World Health Organization (WHO) (2018). Antimicrobial resistance. https://www.who.int/news- room/fact-sheets/detail/antimicrobial-resistance . Accessed on 25/4/2019.

World Health Organization (WHO) (2012). The evolving threat of antimicrobial resistance: options for action. http://whqlibdoc.who.int/publications/2012/9789241503181_eng.pdf Accessed on 23/8/2018

Wintrobe, P.K. (1967). Clinical Haematology. Williams and Wilkins publisher, Lippincott. p 230

Yan, F. and Polk, D.B. (2006) Probiotics as functional food in the treatment of diarrhea. *Current Opinion in Clinical Nutrition Metabolic Care*, **9**, 717-721.

Yang, C., Song, G. and Lim, W. (2020). A review of the toxicity in fish exposed to antibiotics. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, **237**(108840)

Yasin, R., Jabeen, F. Ali, M., Samiullah, K. and Makhdoom, S. (2018). Effects of yeast (*Sacchaoromyces cerevisiae*) on the intestinal microbiota of GIFT Tilapia (*Oreochromis mossambicus*). *International Journal of Biosciences*, 12(4), 283–291.

Yassir, A.L., Adel, M.E. and Azze, A. (2002). Use of probiotics bacteria as growth promoters antibacterial and the effect on physiological parameters of *Oreochromis niloticus*. *Journal of Fish Diseases*, **25**, 633-642.

Yazici, I. S., Hisar, O., Yilmaz, S. and Yigit, M. (2015). Effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. *Marine Science and Technology Bulletin*, **4**(2), 21-28

Yildiz, H. and Aydin, S. (2006). Pathological effects of *Arcobacter cryaerophilus* infection in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Acta veterinaria Hungarica*, **54**, 191 - 199

Yu, L., Qiao, N., Li, T., Yu, R., Zhai, Q., Tian, F., Zhao, J., Zhang, H., and Chen, W. (2019). Dietary supplementation with probiotics regulates gut microbiota structure and function in Nile tilapia exposed to aluminum. *PeerJ*, **7**, e6963

Yu, J., Chen, T., Xie, Z., Liang, P., Qu, H., Shang, M., Mao, Q., Ning, D., Tang, Z., Shi, M., Zhou, L., Huang, Y. and Yu, X. (2015). Oral delivery of *Bacillus subtilis* spore expressing enolase of *Clonorchis sinensis* in rat model: induce systemic and local mucosal immune responses and has no side effect on liver function. *Parasitological Research*, **114**(7), 2499-2505.

Zarate, N., Perez-Olmos, R., dos Reis, B. F. (2011). Turbidimetric determination of sulfate in rainwater employing a LED based photometer and multicommuted flow analysis system with in-line preconcentration. *Journal of the Brazilian Chemical Society,* **22**(6), 1009-1014

Zhang, S., Mou, C., Cao, Y., Zhang, E. and Yang, Q. (2020). Immune response in piglets orally immunized with recombinant *Bacillus subtilis* expressing the capsid protein of porcine circovirus type 2. *Cell Community Signal*, **18**(1), 23

Zhang, Z., Li, D., Xu, W., Tang, R. and Li, L. (2019a). Microbiome of co-cultured fish exhibits host selection and niche differentiation at the organ scale. *Frontiers in Microbiology*, **10**, 2576.

Zhang, S., Hu, B., Xu, J., Ren, Q., Wang, L. and Wang, S. (2019b). Influenza A virus infection induces liver injury in mice. *Microbial Pathogenesis*, **137**, 103-136.

Zhou, L., Limbu, S.M., Shen, M., Zhai, W., Qiao, F., He, A., Du, Z. and Zhang, M. (2018). Environmental concentrations of antibiotics impair Zebrafish gut health. *Environmental Pollution*, **235**, 245–254

Zokaeifar, H., Balcázar, J.L., Saad, C.R., Kamarudin, M.S., Sijam, K., Arshad, A. and Nejat, N. (2012). Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology*, **33**, 683 - 689

Zorriehzahra, M.J., Hassan, M.D., Gholizadeh, M. and Saidi, A.A. (2009). Study of some hematological and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. *Iranian Journal of Fisheries Sciences*, **9**(1), 185-198

APPENDICES

Appendix i: Questionnaire for fish handlers

1. Name of pond……
2. Type of pond (concrete or earthen) ……..
3. Location of pond……...
4. Breed of fish …….
5. Age of fishes ……
6. Size of pond ……..
7. Population of fish ……
8. Do you use organic feed (a) Yes (b) No
9. Do you use commercial feed? (a) Yes (b) No
10. What water source is used? (a) Bore-hole (b) Stream (c) Tap (d) Well
11. How often is the pond washed? (a) Daily (b) Weekly (c) Fortnightly (d) Monthly
12. How often is the water changed? (a) Daily (b) Weekly (c) Fortnightly (d) Monthly
13. What season is mortality and morbidity high? (a) 1st quarter (Jan – March) (b) 2nd quarter (April – June) (c) 3rd quarter (July – Sept.) (d) 4th quarter (Oct. – Dec.)
14. Which of these clinical signs do you usually observe in infected fish? (a) White fluffy appearance (b) Bloody spot at the site of infection (c) Cottony mycelium over the body (d) Descaliny of the body (e) Fungoid patches on the body (f) Lesions and ulcerations (g) Complete loss of tail.

Appendix ii: Selective media composition

a. Selective medium for isolation of *Lysinibacillus sphaericus* - Polymyxin Pyruvate Egg Yolk MannitolBromothymol Blue Agar (PEMBA)

Composition (g /100 ml)
Peptic digest of animal tissue - 0.1
Mannitol- 1
Sodium pyruvate - 1
Disodium phosphate- 0.25
Sodium chloride - 0.2
Potassium dihydrogen phosphate - 0.025
Magnesium sulphate. heptahydrate- 0.01
Bromothymol blue - 0.01
Agar 1.8
pH- 7.4±0.2
Temperature - 25°C

b. Selective medium for isolation of *Pseudomonas aeruginosa* – Cetrimide agar

Composition (g/L)
Peptone from Gelatin- 20
$MgCl_2$ - 1.4
K_2SO_4 - 10
N-Cetyl-N,N,N-trimethylammoniumbromide (Cetrimide) - 0.3
Agar-Agar - 13.6
Glycerol - 10ml/L

c. Selective medium for isolation of *Serratia marcescens* - Caprylate-thallous (CT5) agar

Composition (%)
Yeast extract - 0.01
Caprylic (n-octanoic) acid- 0.1
Thallous sulfate - 0.025

d. Selective medium for isolation of *Bacillus subtilis*- Mannitol Egg Yolk Polymyxin(MYP) Agar

Composition
Beef Extract - 1 g/L
Enzymatic Digest of Casein - 10 g/L
D-Mannitol - 10 g/L
NaCl- 10 g/L
Phenol Red - 0.025 g/L
Agar 12-18 g/L
Polymyxin B Sulfate 10^5 IU
20% Egg Yolk Emulsion - 100 ml/L
Water - 900 ml/L
pH- 7.2 ± 0.2
Temperature - 25 °C

e. Selective medium for isolation of *Paenalcaligenes suwonensis*–Blood agar

Composition
Agar - 15g/L
Beef extract - 10 g/L
Peptone - 10 g/L
Sodium Chloride (NaCl) - 5g/L
Sheep blood, defibrinated = 50mL
pH 7.3 ± 0.2
Temperature - 25°C

Appendix iii: Univariate Analysis of Variance of bacterial count on fish samples

Tests of Between-Subjects Effects

Dependent Variable: Bacterial count

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1509613689.583[a]	79	19109034.045	299.900	.000
Intercept	1387464309.112	1	1387464309.112	21775.105	.000
Farms	120978687.847	17	7116393.403	111.686	.000
Season	2699125.312	1	2699125.312	42.361	.000
Zones	.000	0	.	.	.
ORGAN	950730309.112	1	950730309.112	14920.926	.000
Farms * Season	38655251.736	17	2273838.337	35.686	.000
Farms * Zones	.000	0	.	.	.
Farms * ORGAN	122131354.514	17	7184197.324	112.750	.000
Season * Zones	.000	0	.	.	.
Season * ORGAN	1819150.234	1	1819150.234	28.550	.000
Zones * ORGAN	.000	0	.	.	.
Farms * Season * Zones	.000	0	.	.	.
Farms * Season * ORGAN	38381696.181	17	2257746.834	35.433	.000
Farms * Zones * ORGAN	.000	0	.	.	.
Season * Zones * ORGAN	.000	0	.	.	.
Farms * Season * Zones * ORGAN	.000	0	.	.	.
Error	10194866.667	160	63717.917		
Total	2852974900.000	240			
Corrected Total	1519808556.250	239			

a. R Squared = .993 (Adjusted R Squared = .990)

1. Fish farm

Estimates

Dependent Variable: Bacterial count

Fish farm	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
A	1452.500[a]	72.869	1308.592	1596.408
B	895.833[a]	72.869	751.925	1039.742

C	1283.333[a]	72.869	1139.425	1427.242
D	2813.333[a]	72.869	2669.425	2957.242
E	1109.167[a]	72.869	965.258	1253.075
F	1482.500[a]	72.869	1338.592	1626.408
G	2217.500[a]	72.869	2073.592	2361.408
H	1978.333[a]	72.869	1834.425	2122.242
I	3175.833[a]	72.869	3031.925	3319.742
J	3495.000[a]	72.869	3351.092	3638.908
K	3617.500[a]	72.869	3473.592	3761.408
L	3645.833[a]	72.869	3501.925	3789.742
M	4557.500[a]	72.869	4413.592	4701.408
N	2247.500[a]	72.869	2103.592	2391.408
O	2896.667[a]	72.869	2752.758	3040.575
P	1015.000[a]	72.869	871.092	1158.908
Q	3202.500[a]	72.869	3058.592	3346.408
R	2750.000[a]	72.869	2606.092	2893.908
S	2207.500[a]	72.869	2063.592	2351.408
T	1094.167[a]	72.869	950.258	1238.075

a. Based on modified population marginal mean.

Univariate Tests

Dependent Variable: Bacterial count

	Sum of Squares	Df	Mean Square	F	Sig.
Contrast	257365331.250	19	13545543.750	212.586	.000
Error	10194866.667	160	63717.917		

The F tests the effect of Fish farm. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

2. Season

Estimates

Dependent Variable: Bacterial count

Season	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound

DRY	2234.250[a]	23.043	2188.742	2279.758
RAINY	2479.500[a]	23.043	2433.992	2525.008

a. Based on modified population marginal mean.

Univariate Tests

Dependent Variable: Bacterial count

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	3608853.750	1	3608853.750	56.638	.000
Error	10194866.667	160	63717.917		

The F tests the effect of Season. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

3. Senatorial zones

Estimates

Dependent Variable: Bacterial count

Senatorial zones	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Anambra south	1654.062[a]	25.763	1603.183	1704.942
Anambra North	3456.528[a]	29.748	3397.777	3515.278
Anambra central	2194.306[a]	29.748	2135.555	2253.056

a. Based on modified population marginal mean.

Univariate Tests

Dependent Variable: Bacterial count

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	136386643.403	2	68193321.701	1070.238	.000
Error	10194866.667	160	63717.917		

The F tests the effect of Senatorial zones. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

4. Organ

Estimates

Dependent Variable: Bacterial count

Organ	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
GILL	4303.333[a]	23.043	4257.826	4348.841
LIVER	410.417[a]	23.043	364.909	455.924

a. Based on modified population marginal mean.

Univariate Tests

Dependent Variable: Bacterial count

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	909288010.417	1	909288010.417	14270.523	.000
Error	10194866.667	160	63717.917		

The F tests the effect of Organ. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Post Hoc Tests

Fish farm

Homogeneous Subsets

Bacterial count

Student-Newman-Keuls

Fish farm	N	Subset								
		1	2	3	4	5	6	7	8	9
B	12	895.8333								
P	12	1015.0000								
T	12	1094.1667	1094.1667							
E	12	1109.1667	1109.1667							
C	12		1283.3333	1283.3333						
A	12			1452.5000						
F	12			1482.5000						

H	12				1978.3333					
S	12				2207.5000	2207.5000				
G	12				2217.5000	2217.5000				
N	12					2247.5000				
R	12						2750.0000			
D	12						2813.3333			
O	12						2896.6667			
I	12							3175.8333		
Q	12							3202.5000		
J	12								3495.0000	
K	12								3617.5000	
L	12								3645.8333	
M	12									4557.5000
Sig.		.167	.161	.133	.056	.920	.331	.796	.311	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means. The error term is Mean Square(Error) = 63717.917.

a. Uses Harmonic Mean Sample Size = 12.000. b. Alpha = .05

Senatorial zones

Homogeneous Subsets

Bacterial count

Student-Newman-Keuls

Senatorial zones	N	Subset		
		1	2	3
Anambra south	96	1654.0625		
Anambra central	72		2194.3056	

Anambra North	72			3456.5278
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 63717.917.

a. Uses Harmonic Mean Sample Size = 78.545.

b. The group sizes are unequal. The harmonic mean of the group sizes is used.
Type I error levels are not guaranteed.

c. Alpha = .05.

Appendix iv: **Univariate Analysis of Variance of bacterial count on fish pond water samples**

Tests of Between-Subjects Effects

Dependent Variable: Bacterial count in the fish pond water samples

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	12099.418[a]	39	310.241	389.587	.000
Intercept	61592.037	1	61592.037	77344.543	.000
Zone	.000	0	.	.	.
Farm	1936.457	17	113.909	143.042	.000
Season	7181.080	1	7181.080	9017.681	.000
Zone * Farm	.000	0	.	.	.
Zone * Season	.000	0	.	.	.
Farm * Season	1536.190	17	90.364	113.475	.000
Zone * Farm * Season	.000	0	.	.	.
Error	63.707	80	.796		
Total	76057.800	120			
Corrected Total	12163.125	119			

a. R Squared = .995 (Adjusted R Squared = .992)

Estimated mean for Season

Dependent Variable: Bacterial count in the fish pond water samples

Season	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Dry season	15.263[a]	.115	15.034	15.493
Rainy season	30.887[a]	.115	30.657	31.116

a. Based on modified population marginal mean.

Post Hoc Tests

Senatorial zone

Homogeneous Subsets

Bacterial count in the fish pond water samples

Student-Newman-Keuls[a,b,c]

Senatorial zone	N	Subset		
		1	2	3
Anambra north	36	19.7611		
Anambra central	36		21.9194	
Anambra south	48			26.4271
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .796.

a. Uses Harmonic Mean Sample Size = 39.273.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Fish farm

Homogeneous Subsets

Bacterial count in the fish pond water samples

Student-Newman-Keuls[a,b]

Fish farm	N	Subset										
		1	2	3	4	5	6	7	8	9	10	11
D	6	11.3333										
G	6		16.6667									
F	6		17.5167	17.5167								
Q	6			18.5167								
R	6			18.6667								
O	6				20.9167							
B	6				21.7500	21.7500						
J	6					22.3667	22.3667					
A	6					22.5667	22.5667					
C	6					22.6000	22.6000					
E	6					22.8000	22.8000					
S	6						23.5333	23.5333				
T	6							24.3333				
P	6								25.5500			
L	6									26.7333		
I	6									26.9167		
N	6										28.3333	
K	6										28.4500	
H	6										28.5667	
M	6											33.3833
Sig.		1.000	.103	.072	.110	.258	.167	.124	1.000	.723	.893	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .796.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Appendix v: Respondent's Answer to the pond structure they operate in the fish farm

S/No	Questions	Number (%)
A	Is the pond made of concrete	100
B	Is the pond made of earth	0
C	Do you breed *Clarias gariepinus*	80
D	Do you breed other fish species	20
E	Is your fish population 100 – 500 per pond	35
F	Is your fish population 500 – 1000 per pond	50
G	Is your fish population >1000 fishes	15

Appendix vi: Respondent's Answer to feeding and sanitary practices employed in fish farm

S/No	Questions	Number(%)
A	Do you use organic feed	28
B	Do you use commercial feed	100
C	Is your water sourced from bore-hole	100
D	Is your water sourced from stream	0
E	Do you use any other water source apart from C and D above	0
F	Is the pond washed and water changed daily	0
G	Is the pond washed and water changed weekly	10
H	Is the pond washed and water changed fortnightly	88
I	Is the pond washed and water changed monthly	2

Appendix vii: Respondent's Answer to infection morbidity and mortality of fishes in the farm

S/No	Questions	Number(%)
A	Do you have more fish infection and death in rainy season	85
B	Do you experience more fish infection and death in dry season	15
C	Do you find white fluffy patched on the fish when they are infected	86
D	Do you experience blood spots on the fish skin when they are infected	64
E	Do you notice descaling of the fishes when infected	12
F	Do you notice complete tail loss when fishes are infected	54

Appendix viii: Univariate Analysis of Variance of Pathogenicity effects on Hemoglobin of fish

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effects on Hemoglobin of fish

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	89.014[a]	17	5.236	9.108	.000
Intercept	3817.804	1	3817.804	6641.263	.000
Organism	31.357	5	6.271	10.909	.000
Time	31.592	2	15.796	27.478	.000
Organism * Time	26.065	10	2.606	4.534	.000
Error	20.695	36	.575		
Total	3927.513	54			
Corrected Total	109.709	53			

a. R Squared = .811 (Adjusted R Squared = .722)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effects on Hemoglobin of fish

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset	
		1	2
Pseudomonas aeruginosa	9	7.3778	
Lysinibacillus sphaericus	9	7.4944	
Serratia marcescens	9		8.4333
Bacillus subtilis	9		8.5667
Control	9		9.2167
Paenalcaligens suwonensis	9		9.3611
Sig.		.746	.062

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .575.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effects on Hemoglobin of fish

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
2 weeksafter inoculation	18	7.3278	
1 week after inoculation	18		8.9056
Before inoculation	18		8.9917
Sig.		1.000	.735

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .575.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix ix: Univariate Analysis of Variance of Pathogenicity effect on Packed cell volume of fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on Packed cell volumeof fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	734.559[a]	14	52.469	93.568	.000
Intercept	32714.882	1	32714.882	58340.950	.000
Organism	392.889	4	98.222	175.161	.000
Time	93.718	2	46.859	83.564	.000
Organism * Time	247.952	8	30.994	55.272	.000
Error	16.823	30	.561		
Total	33466.264	45			

Corrected Total	751.382	44			

a. R Squared = .978 (Adjusted R Squared = .967)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on Packed cell volume of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Pseudomonas aeruginosa	9	23.1867		
Lysinibacillus sphaericus	9		26.0289	
Bacillus subtilis	9		26.4333	
Serratia marcescens	9		26.8889	
Paenalcaligenes suwonensis	9			32.2767
Sig.		1.000	.053	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .561.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on Packed cell volumeof fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
2 weeks after inoculation	15	24.9253	

1 week after inoculation	15		27.8807
Before inoculation	15		28.0827
Sig.		1.000	.466

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .561.

a. Uses Harmonic Mean Sample Size = 15.000.

b. Alpha = .05.

Appendix x: Univariate Analysis of Variance of Pathogenicity effect of Red blood cells on fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of Red blood cells on fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33.374[a]	17	1.963	7.480	.000
Intercept	303.455	1	303.455	1156.214	.000
Organism	8.724	5	1.745	6.648	.000
Time	11.941	2	5.971	22.749	.000
Organism * Time	12.709	10	1.271	4.842	.000
Error	9.448	36	.262		
Total	346.277	54			
Corrected Total	42.822	53			

a. R Squared = .779 (Adjusted R Squared = .675)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of Red blood cells on fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Serratia marcescens	9	1.8389		
Lysinibacillus sphaericus	9	2.0822		
Pseudomonas aeruginosa	9	2.1978	2.1978	
Paenalcaligenes suwonensis	9	2.3167	2.3167	
Control	9		2.7867	2.7867
Bacillus subtiis	9			3.0011
Sig.		.215	.051	.380

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .262.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of Red blood cells on fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
2 weeks after inoculation	18	1.8111		
1 week after inoculation	18		2.3389	
Before inoculation	18			2.9617
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .262.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xi: Univariate Analysis of Variance of pathogenicity effect on white blood cell

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on White blood cells of fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1086.042[a]	17	63.885	68.803	.000
Intercept	19788.081	1	19788.081	21311.370	.000
Organism	419.299	5	83.860	90.315	.000
Time	411.448	2	205.724	221.561	.000
Organism * Time	255.295	10	25.530	27.495	.000
Error	33.427	36	.929		
Total	20907.549	54			
Corrected Total	1119.468	53			

a. R Squared = .970 (Adjusted R Squared = .956)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on White blood cells of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	16.2566		
Pseudomonas aeruginosa	9	16.7222		
Serratia marcescens	9	17.0033		
Paenalcaligenes suwonensis	9		20.7133	
Bacillus subtilis	9		20.8189	
Lysinibacillus sphaericus	9			23.6733

Sig.		.059	.818	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .929.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on White blood cells of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before inoculation	18	15.7444		
1 week after inoculation	18		19.1783	
2 weeks after inoculation	18			22.5056
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .929.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xii: Univariate Analysis of Variance of Pathogenicity effect on Lymphocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on Lymphocyte of fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2415.989[a]	17	142.117	21.283	.000

Intercept	373639.569	1	373639.569	55955.986	.000
Organism	410.142	5	82.028	12.284	.000
Time	1330.916	2	665.458	99.659	.000
Organism * Time	674.932	10	67.493	10.108	.000
Error	240.386	36	6.677		
Total	376295.945	54			
Corrected Total	2656.375	53			

a. R Squared = .910 (Adjusted R Squared = .867)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on Lymphocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	80.1400		
Pseudomonas aeruginosa	9	80.6511		
Bacillus subtilis	9	82.2067	82.2067	
Serratia marcescens	9	82.6778	82.6778	
Paenalcaligenes suwonensis	9		84.5311	
Lysinibacillus sphaericus	9			88.5500
Sig.		.286	.151	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6.677.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subset

Pathogenicity effect on Lymphocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
1 week after inoculation	18	77.0956		
Before inoculation	18		83.1944	
2 weeks after inoculation	18			89.2561
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6.677.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xiii: Univariate Analysis of Variance of Pathogenicity effects on Monocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effects on Monocyte of fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	162.165[a]	14	11.583	12.887	.000
Intercept	4791.989	1	4791.989	5331.448	.000
Organism	64.412	4	16.103	17.916	.000
Time	65.606	2	32.803	36.496	.000
Organism * Time	32.147	8	4.018	4.471	.001
Error	26.964	30	.899		
Total	4981.118	45			
Corrected Total	189.129	44			

a. R Squared = .857 (Adjusted R Squared = .791)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effects on Monocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Paenalcaligenes suwonensis	9	8.3344		
Serratia marcescens	9		9.8389	
Pseudomonas aeruginosa	9		10.3678	
Lysinibacillus sphaericus	9			11.3500
Bacillus subtilis	9			11.7056
Sig.		1.000	.246	.433

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .899.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effects on Monocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
Before inoculation	15	8.6267	
1 week after inoculation	15		10.9707
2 week after inoculation	15		11.3607
Sig.		1.000	.269

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .899.

a. Uses Harmonic Mean Sample Size = 15.000.

b. Alpha = .05.

Appendix xiv: Univariate Analysis of Variance of Pathogenicity effects on AST of fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effects on AST of fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26113.682[a]	17	1536.099	1858.234	.000
Intercept	195308.258	1	195308.258	236266.341	.000
Organism	2153.732	5	430.746	521.078	.000
Time	22836.346	2	11418.173	13812.677	.000
Organism * Time	1123.604	10	112.360	135.924	.000
Error	29.759	36	.827		
Total	221451.699	54			
Corrected Total	26143.441	53			

a. R Squared = .999 (Adjusted R Squared = .998)

Post Hoc Tests

Bacterial isolates

Homogeneous Subset

Pathogenicity effects on AST of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Control	9	32.4800				
Bacillus subtilis	9		57.7733			
Pseudomonas aeruginosa	9			59.7578		

Lysinibacillus sphaericus	9				63.8411	
Serratia marcescens	9				64.5411	
Paenalcaligenes suwonensis	9					67.1111
Sig.		1.000	1.000	1.000	.111	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .827.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effects on AST of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before inoculation	18	31.1667		
1 week after inoculation	18		72.4467	
2 weeks after inoculation	18			76.8067
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .827.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xv: Univariate Analysis of Variance of Pathogenicity effects of ALT on fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effects of ALT on fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	457.660[a]	17	26.921	47.822	.000
Intercept	36635.679	1	36635.679	65079.103	.000
Organism	293.361	5	58.672	104.224	.000
Time	98.979	2	49.489	87.912	.000
Organism * Time	65.321	10	6.532	11.603	.000
Error	20.266	36	.563		
Total	37113.605	54			
Corrected Total	477.926	53			

a. R Squared = .958 (Adjusted R Squared = .938)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effects of ALT on fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	21.6700		
Lysinibacillus sphaericus	9		25.1200	
Pseudomonas aeruginosa	9		25.1678	
Bacillus subtilis	9		25.7144	
Serratia marcescens	9			28.9667
Paenalcaligenes suwonensis	9			28.9744
Sig.		1.000	.226	.983

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .563.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effects of ALT on fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before inoculation	18	24.5206		
1 week after inoculation	18		25.8089	
2 weeks after inoculation	18			27.8111
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .563.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xvi: Univariate Analysis of Variance of Pathogenicity effects on weight of fishes

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effects on weight of fishes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	44367.635[a]	17	2609.861	122.694	.000
Intercept	1292707.261	1	1292707.261	60772.394	.000
Organism	18781.521	5	3756.304	176.590	.000
Time	15379.351	2	7689.675	361.505	.000
Organism * Time	10206.763	10	1020.676	47.984	.000
Error	765.766	36	21.271		
Total	1337840.662	54			

Corrected Total	45133.401	53			

a. R Squared = .983 (Adjusted R Squared = .975)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effects on weight of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Serratia marcescens	9	127.8778				
Pseudomonas aeruginosa	9		141.0000			
Paenalcaligenes suwonensis	9			145.4322		
Lysinibacillus sphaericus	9				158.1033	
Bacillus subtilis	9					177.7000
Control	9					178.2211
Sig.		1.000	1.000	1.000	1.000	.812

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 21.271.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effects on weight of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset

		1	2	3
Before inoculation	18	133.7333		
1 week after inoculation	18		155.3783	
2 week after inoculation	18			175.0556
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 21.271.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xvii: Univariate Analysis of Variance of bacterial count in gill before and after inoculation

Tests of Between-Subjects Effects

Dependent Variable: **Bacterialcount in gill of fishes before and after inoculation**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14069.297[a]	11	1279.027	255.677	.000
Intercept	13048.874	1	13048.874	2608.463	.000
Isolates	2177.251	5	435.450	87.046	.000
Time	9714.402	1	9714.402	1941.904	.000
Isolates * Time	2177.645	5	435.529	87.062	.000
Error	120.060	24	5.003		
Total	27238.231	36			
Corrected Total	14189.358	35			

a. R Squared = .992 (Adjusted R Squared = .988)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Bacterial count in gill of fishes before and after inoculation

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	6	3.4933		
Bacillus subtilis	6		14.7667	
Serratia marcescens	6			23.0167
Paenalcaligens suwonensis	6			23.1767
Pseudomonas aeruginosa	6			24.0050
Lysinibacillus sphaericus	6			25.7733
Sig.		1.000	1.000	.171

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 5.003.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Appendix xviii: Univariate Analysis of Variance of bacterial count in liver before and after inoculation

Tests of Between-Subjects Effects

Dependent Variable: Bacterialcount in liver before and after inoculation

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1971.615[a]	11	179.238	797.833	.000
Intercept	2012.719	1	2012.719	8959.132	.000
Time	881.496	1	881.496	3923.767	.000
Isolates	579.003	5	115.801	515.458	.000
Time * Isolates	511.116	5	102.223	455.022	.000
Error	5.392	24	.225		
Total	3989.725	36			
Corrected Total	1977.007	35			

a. R Squared = .997 (Adjusted R Squared = .996)

Post Hoc Tests

Bacterial isolates

Homogeneous Subset

Bacterialcount in liver of fishes before and after inoculation

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
6.00	6	2.6733				
Serratia marcescens	6		3.5550			
Lysinibacillus sphaericus	6			4.2850		
Bacillus subtilis	6				10.8750	
Pseudomons aeruginosa	6					11.6917
Paenalcaligenes suwonensis	6					11.7833
Sig.		1.000	1.000	1.000	1.000	.741

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .225.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Appendix xix: Univariate Analysis of Variance of Pathogenicity effect on oral administration on hemoglobin of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on hemoglobin of mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	104.019[a]	17	6.119	49.853	.000
Intercept	3898.780	1	3898.780	31765.314	.000
Organism	62.582	5	12.516	101.977	.000
Time	10.357	2	5.179	42.193	.000

Organism * Time	31.080	10	3.108	25.322	.000
Error	4.419	36	.123		
Total	4007.218	54			
Corrected Total	108.437	53			

a. R Squared = .959 (Adjusted R Squared = .940)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on hemoglobin of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset			
		1	2	3	4
Control	9	7.2333			
Paenalcaligenes suwonensis	9	7.3444			
Pseudomonas aeruginosa	9		7.8411		
Serratia marcescens	9			9.1667	
Lysinibacillus sphaericus	9			9.3356	
Bacillus subtilis	9				10.0611
Sig.		.505	1.000	.313	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .123.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on hemoglobin of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
2 weeks after inoculation	18	7.8778	
1 week after inoculation	18		8.7972
Before inoculation	18		8.8161
Sig.		1.000	.872

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .123.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xx: Univariate Analysis of Variance of Pathogencity effect of oral administration on PCV of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogencity effect on PCV of mic

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	896.023[a]	17	52.707	102.360	.000
Intercept	34014.014	1	34014.014	66056.606	.000
Organism	438.314	5	87.663	170.245	.000
Time	71.886	2	35.943	69.802	.000
Organism * Time	385.824	10	38.582	74.929	.000
Error	18.537	36	.515		
Total	34928.574	54			
Corrected Total	914.560	53			

a. R Squared = .980 (Adjusted R Squared = .970)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogencity effect on PCV of mic

Student-Newman-Keuls

Bacterial isolates	N	Subset				
		1	2	3	4	5
Control	9	21.4366				
Paenalcaligenes suwonensis	9		22.5200			
Pseudomonas aeruginosa	9		23.0800			
Lysinibacillus sphaericus	9			26.3556		
Serratia marcescens	9				27.1667	
Bacillus subtilis	9					29.6889
Sig.		1.000	.107	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .515.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogencity effect on PCV of mic

Student-Newman-Keuls

Experimental period	N	Subset		
		1	2	3
2 weeks after inoculation	18	23.6789		
Before inoculation	18		25.1089	
1 week after inoculation	18			26.5050
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .515.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxi: Univariate Analysis of Variance of Pathogenicity effect of oral administration on Red blood cell of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on Red blood cell of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.947[a]	17	4.056	48.327	.000
Intercept	7024.682	1	7024.682	83704.667	.000
Organism	36.293	5	7.259	86.493	.000
Time	3.861	2	1.931	23.006	.000
Organism * Time	28.792	10	2.879	34.308	.000
Error	3.021	36	.084		
Total	7096.650	54			
Corrected Total	71.968	53			

a. R Squared = .958 (Adjusted R Squared = .938)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on Red blood cell of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset			
		1	2	3	4
Lysinibacillus sphaericus	9	9.7778			
Control	9	10.6000	10.6000		
Pseudomonas aeruginosa	9		11.5022	11.5022	

Paenalcaligenes suwonensis	9			11.6333	
Serratia marcescens	9			11.7644	
Bacillus subtilis	9				12.4889
Sig.		1.000	.093	.148	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .084.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on Red blood cell of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
2 weeks after inoculation	18	11.1694	
1 week after inoculation	18	11.2678	
Before inoculation	18		11.7794
Sig.		.315	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .084.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxii: Univariate Analysis of Variance of Pathogenicity effect of oral administration on White blood cell of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on White blood cell of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1865.400[a]	17	109.729	490.614	.000
Intercept	15689.411	1	15689.411	70149.303	.000
Organism	916.491	5	183.298	819.549	.000
Time	257.037	2	128.518	574.622	.000
Organism * Time	691.872	10	69.187	309.344	.000
Error	8.052	36	.224		
Total	17562.863	54			
Corrected Total	1873.451	53			

a. R Squared = .996 (Adjusted R Squared = .994)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on White blood cell of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset		
		1	2	3
Control	9	14.5222		
Paenalcaligenes suwonensis	9	14.8111		
Pseudomonas aeruginosa	9	14.9667		
Bacillus subtilis	9		15.8667	
Serratia marcescens	9		15.9222	
Lysinibacillus sphaericus	9			26.1833
Sig.		.128	.805	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .224.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on White blood cell of mice

Student-Newman-Keuls

Experimental period	N	Subset		
		1	2	3
1.00	18	14.5694		
2.00	18		16.6889	
3.00	18			19.8778
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .224.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxiii: Univariate Analysis of Variance of Pathogenicity effect of oral administration on lymphocyte of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on lymphocyte of mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4729.991[a]	17	278.235	606.947	.000
Intercept	90830.589	1	90830.589	198139.805	.000
Organism	2991.575	5	598.315	1305.177	.000
Time	906.412	2	453.206	988.633	.000
Organism * Time	832.005	10	83.200	181.495	.000
Error	16.503	36	.458		

Total	95577.083	54			
Corrected Total	4746.494	53			

a. R Squared = .997 (Adjusted R Squared = .995)

Post Hoc Tests

Bacterial isolate

Homogeneous Subsets

Pathogenicity effect on lymphocyte of mice

Student-Newman-Keuls

Bacterial isolate	N	Subset					
		1	2	3	4	5	6
Paenalcaligenes suwonensis	9	31.1333					
Control	9		34.4300				
Bacillus subtilis	9			37.8000			
Serratia marcescens	9				43.5667		
Lysinibacillus sphaericus	9					46.9978	
Pseudomonas aeruginosa	9						52.5567
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .458.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on lymphocyte of mice

Student-Newman-Keuls

Experimental period	N	Subset

		1	2	3
Before inoculation	18	37.5500		
1 week after inoculation	18		38.7211	
2 weeks after inoculation	18			46.7672
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .458.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxiv: Univariate Analysis of Variance of Pathogenicity effect of oral administration on monocyte of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on monocyte of mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	18.947[a]	17	1.115	6.442	.000
Intercept	197.380	1	197.380	1140.910	.000
Organism	4.018	5	.804	4.645	.002
Time	.021	2	.010	.059	.942
Organism * Time	14.909	10	1.491	8.618	.000
Error	6.228	36	.173		
Total	222.555	54			
Corrected Total	25.175	53			

a. R Squared = .753 (Adjusted R Squared = .636)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on monocyte of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset		
		1	2	3
Control	9	1.2533		
Bacillus subtilis	9	1.6967	1.6967	
Serratia marcescens	9	1.8378	1.8378	1.8378
Paenalcaligenes suwonensis	9		2.0844	2.0844
Pseudomonas aeruginosa	9		2.0889	2.0889
Lysinibacillus sphaericus	9			2.2889
Sig.		.167	.207	.117

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .173.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on monocyte of mice

Student-Newman-Keuls

Experimental period	N	Subset
		1
2 weeks after inoculation	18	1.8972
1 week after inoculation	18	1.8989
Before inoculation	18	1.9394
Sig.		.950

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .173.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxv: Univariate Analysis of Variance of Pathogenicity effect of oral administration on Aspartate transaminase of mice

Dependent Variable: Pathogenicity effect on Aspartate transaminase of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	921.446[a]	17	54.203	376.696	.000
Intercept	125581.691	1	125581.691	872762.497	.000
Organism	413.872	5	82.774	575.262	.000
Time	56.385	2	28.192	195.930	.000
Organism * Time	451.189	10	45.119	313.566	.000
Error	5.180	36	.144		
Total	126508.316	54			
Corrected Total	926.626	53			

a. R Squared = .994 (Adjusted R Squared = .992)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on Aspartate transaminase of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset				
		1	2	3	4	5
Bacillus subtilis	9	44.2856				
Paenalcaligenes suwonensis	9		45.2627			
Control	9			48.3367		
Lysinibacillus sphaericus	9				49.3244	
Serratia marcescens	9					51.3878
Pseudomonas aeruginosa	9					51.4144

Sig.		1.000	1.000	1.000	1.000	.882

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .144.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on Aspartate transaminase of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
Before inoculation	18	46.7800	
2 weeks after inoculation	18		48.9058
1 week after inoculation	18		48.9872
Sig.		1.000	.524

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .144.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxvi: Univariate Analysis of Variance of Pathogenicity effect of oral administration on ALT of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on ALT of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	327.232[a]	17	19.249	51.011	.000
Intercept	10967.940	1	10967.940	29065.695	.000
Organism	50.415	5	10.083	26.720	.000
Time	184.729	2	92.364	244.771	.000
Organism * Time	92.089	10	9.209	24.404	.000
Error	13.585	36	.377		
Total	11308.757	54			
Corrected Total	340.817	53			

a. R Squared = .960 (Adjusted R Squared = .941)

Post Hoc Test

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on ALT of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset			
		1	2	3	4
Lysinibacillus sphaericus	9	13.1511			
Control	9	13.3978			
Paenalcaligenes suwonensis	9	13.8033	13.8033		
Bacillus subtilis	9		14.1500		
Pseudomonas aeruginosa	9			15.1078	
Serratia marcescens	9				15.9000
Sig.		.076	.239	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .377.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on ALT of mice

Student-Newman-Keuls

Experimental period	N	Subset		
		1	2	3
Before inoculation	18	11.7067		
1 week after inoculation	18		15.0011	
2 weeks after inoculation	18			16.0472
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .377.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxvii: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on hemoglobin of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of subcutaneous administration on hemoglobin of mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	86.890[a]	17	5.111	46.025	.000
Intercept	3553.153	1	3553.153	31995.444	.000
Organism	82.231	5	16.446	148.095	.000
Time	.034	2	.017	.153	.858
Organism * Time	4.625	10	.462	4.164	.001
Error	3.998	36	.111		
Total	3644.041	54			
Corrected Total	90.888	53			

a. R Squared = .956 (Adjusted R Squared = .935)

Post Hoc Tests

Bacterial organisms

Homogeneous Subsets

Pathogenicity effect subcutaneous on mice

Student-Newman-Keuls

Bacterial organisms	N	Subset					
		1	2	3	4	5	6
Lysinibacillus sphaericus	9	6.4433					
Serratia marcescens	9		7.1589				
Paenalcaligenes suwonensis	9			7.6222			
Bacillus subtilis	9				8.0167		
Control	9					9.5422	
Pseudomonas aeruginosa	9						9.8867
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .111.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subset

Pathogenicity effect of subcutaneous administration on hemoglobin of mice

Student-Newman-Keuls

Experimental period	N	Subset
		1

2 weeks after inoculation	18	8.0889
1 week after inoculation	18	8.0994
Before inoculation	18	8.1467
Sig.		.862

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .111.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxviii: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on PCV of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect subcutaneous on mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	845.897[a]	17	49.759	213.081	.000
Intercept	30747.905	1	30747.905	131671.190	.000
Organism	796.352	5	159.270	682.041	.000
Time	3.236	2	1.618	6.928	.003
Organism * Time	46.309	10	4.631	19.831	.000
Error	8.407	36	.234		
Total	31602.209	54			
Corrected Total	854.304	53			

a. R Squared = .990 (Adjusted R Squared = .986)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on PCV of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset					
		1	2	3	4	5	6
Lysinibacillus sphaericus	9	19.2033					
Paenalcaligenes suwonensis	9		20.7622				
Serratia marcescens	9			21.8844			
Bacillus subtilis	9				23.5033		
Control	9					28.2033	
Pseudomonas aeruginosa	9						29.9511
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .234.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on PCV of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
2.00	18	23.5178	
3.00	18		24.0044
1.00	18		24.0644
Sig.		1.000	.712

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .234.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxix: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on red blood cell of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect on Red blood cell of mice ...subcutaneous

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	80.663[a]	17	4.745	32.300	.000
Intercept	7478.894	1	7478.894	50910.819	.000
Organism	69.746	5	13.949	94.956	.000
Time	.320	2	.160	1.091	.347
Organism * Time	10.597	10	1.060	7.214	.000
Error	5.288	36	.147		
Total	7564.845	54			
Corrected Total	85.952	53			

a. R Squared = .938 (Adjusted R Squared = .909)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on red blood cell of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset				
		1	2	3	4	5
Serratia marcescens	9	9.9589				
Paenalcaligenes suwonensis	9		10.8889			
Bacillus subtilis	9			11.3356		
Control	9				12.4189	
Pseudomonas aeruginosa	9					12.8711
Lysinibacillus sphaericus	9					13.1378
Sig.		1.000	1.000	1.000	1.000	.149

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .147.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on red blood cell of mice

Student-Newman-Keuls

Experimental period	N	Subset
		1
Before inoculation	18	11.6878
1 week after inoculation	18	11.7456
2 weeks after inoculation	18	11.8722
Sig.		.330

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .147.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxx: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on white blood cell of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of subcutaneous administration on white blood cell of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	257.299[a]	17	15.135	101.120	.000
Intercept	10577.001	1	10577.001	70666.015	.000
Organism	72.139	5	14.428	96.393	.000
Time	35.796	2	17.898	119.578	.000

Organism * Time	149.365	10	14.936	99.792	.000
Error	5.388	36	.150		
Total	10839.689	54			
Corrected Total	262.687	53			

a. R Squared = .979 (Adjusted R Squared = .970)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect on white blood cell of mice...subcutaneous

Student-Newman-Keuls

Bacterial isolates	N	Subset			
		1	2	3	4
Lysinibacillus sphaericus	9	12.7500			
Pseudomonas aeruginosa	9		13.1911		
Serratia marcescens	9			13.6900	
Paenalcaligenes suwonensis	9			13.9044	
Control	9			14.3833	
Bacillus subtilis	9				16.3856
Sig.		1.000	1.000	.132	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .150.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect on white blood cell of mice...subcutaneous

Student-Newman-Keuls

Experimental period	N	Subset		
		1	2	3
1 week after inoculation	18	13.1194		
Before inoculation	18		13.7861	
2 weeks after inoculation	18			15.0806
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .150.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxi: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on lymphocyte of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogencity effect on lymphocyte of mice...subcutaneous

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4560.531[a]	17	268.267	1216.731	.000
Intercept	88896.985	1	88896.985	403194.791	.000
Organism	3693.630	5	738.726	3350.513	.000
Time	272.822	2	136.411	618.695	.000
Organism * Time	594.080	10	59.408	269.447	.000
Error	7.937	36	.220		
Total	93465.453	54			
Corrected Total	4568.469	53			

a. R Squared = .998 (Adjusted R Squared = .997)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on lymphocyte of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset				
		1	2	3	4	5
Serratia marcescens	9	32.1178				
Pseudomonas aeruginosa	9		35.6033			
Paenalcaligenes suwonensis	9			37.4589		
Bacillus subtilis	9				40.0033	
Lysinibacillus sphaericus	9				40.2489	
Control	9					58.3433
Sig.		1.000	1.000	1.000	.275	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .220.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogencity effect on lymphocyte of mice...subcutaneous

Student-Newman-Keuls

Experimetal period	N	Subset		
		1	2	3
1 week after inoculation	18	38.3372		
Before inoculation	18		39.7361	
2 weeks after inoculation	18			43.6483
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .220.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxii: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on monocyte of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of subcutaneous administration on monocyte of mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1097.224[a]	17	64.543	637.761	.000
Intercept	116659.222	1	116659.222	1152738.021	.000
Organism	917.204	5	183.441	1812.624	.000
Time	21.514	2	10.757	106.295	.000
Organism * Time	158.505	10	15.851	156.623	.000
Error	3.643	36	.101		
Total	117760.090	54			
Corrected Total	1100.867	53			

a. R Squared = .997 (Adjusted R Squared = .995)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on monocyte of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset		
		1	2	3
Control	9	43.2200		
Pseudomonas aeruginosa	9		44.8078	
Lysinibacillus sphaericus Pseudomonas aeruginosa Serratia marcescens	9		45.0144	
Paenalcaligenes suwonensis	9		45.0533	
Bacillus subtilis	9		45.2089	
Lysinibacillus sphaericus	9			55.5733

Sig.		1.000	.052	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .101.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on monocyte of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
2 weeks after inoculation	18	45.5878	
1 week after inoculation	18		46.8928
Before inoculation	18		46.9583
Sig.		1.000	.540

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .101.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxiii: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on Aspartate transaminase of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of subcutaneous administration on Aspartate transaminase of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1097.224[a]	17	64.543	637.761	.000
Intercept	116659.222	1	116659.222	1152738.021	.000

Organism	917.204	5	183.441	1812.624	.000
Time	21.514	2	10.757	106.295	.000
Organism * Time	158.505	10	15.851	156.623	.000
Error	3.643	36	.101		
Total	117760.090	54			
Corrected Total	1100.867	53			

a. R Squared = .997 (Adjusted R Squared = .995)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on Aspartate transaminase of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset		
		1	2	3
Control	9	43.2200		
Pseudomonas aeruginosa	9		44.8078	
Serratia marcescens	9		45.0144	
Paenalcaligenes suwonensis	9		45.0533	
Bacillus subtilis	9		45.2089	
Lysinibacillus sphaericus	9			55.5733
Sig.		1.000	.052	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .101.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on Aspartate transaminase of mice

Student-Newman-Keuls

Experimental period	N	Subset	
		1	2
2 weeks after inoculation	18	45.5878	
1 week after inoculation	18		46.8928
Before inoculation	18		46.9583
Sig.		1.000	.540

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .101.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxiv: Univariate Analysis of Variance on Pathogenicity effect of subcutaneous administration on alanine transaminase of mice

Tests of Between-Subjects Effects

Dependent Variable: Pathogenicity effect of subcutaneous administration on alanine transaminase of mice

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	77.337[a]	17	4.549	41.577	.000
Intercept	14138.408	1	14138.408	129214.029	.000
Organism	74.609	5	14.922	136.373	.000
Time	.000	2	7.963E-005	.001	.999
Organism * Time	2.728	10	.273	2.493	.022
Error	3.939	36	.109		
Total	14219.684	54			
Corrected Total	81.276	53			

a. R Squared = .952 (Adjusted R Squared = .929)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on alanine transaminase of mice

Student-Newman-Keuls

Bacterial isolates	N	Subset				
		1	2	3	4	5
Bacillus subtilis	9	14.2422				
Lysinibacillus sphaericus	9		15.1200			
Control	9			16.1833		
Pseudomonas aeruginosa	9				16.6600	
Serratia marcescens	9					17.3867
Paenalcaligenes suwonensis	9					17.4933
Sig.		1.000	1.000	1.000	1.000	.498

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .109.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Pathogenicity effect of subcutaneous administration on alanine transaminase of mice

Student-Newman-Keuls

Experimental period	N	Subset
		1
Before inoculation	18	16.1794
1 week after inoculation	18	16.1800
2 weeks after inoculation	18	16.1833

Sig.		.999

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .109.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxv: Oneway Analysis of variance of growth on selective media

ANOVA

Growth on Selective media

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10455.333	4	2613.833	24.113	.000
Within Groups	1084.000	10	108.400		
Total	11539.333	14			

Post Hoc Tests

Homogeneous Subsets

Growth on Selective media

Student-Newman-Keuls[a]

Bacterial isolates	N	Subset for alpha = 0.05	
		1	2
Paenalcaligenes suwonensis	3	10.0000	
Lysinibacillus sphaericus	3	22.3333	
Serratia marcescens	3	26.0000	
Pseudomonas aeruginosa	3	27.6667	
Bacillus subtilis	3		85.6667
Sig.		.225	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix xxxvi: Univariate Analysis of Variance of treatment effect of probiotic on Hemoglobin of fishes

Tests of Between-Subjects Effects

Dependent Variable: Treatment effects on Hb of fishes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	145.570[a]	17	8.563	19.626	.000
Intercept	3195.503	1	3195.503	7324.158	.000
Organism	119.559	5	23.912	54.806	.000
Time	9.885	2	4.942	11.328	.000
Organism * Time	16.126	10	1.613	3.696	.002
Error	15.707	36	.436		
Total	3356.780	54			
Corrected Total	161.277	53			

a. R Squared = .903 (Adjusted R Squared = .857)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on Hemoglobin of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Pseudomonas aeruginosa	9	5.1111			
Lysinibacillus sphaericus	9		6.6667		
Bacillus subtilis	9			7.3333	
Paenalcaligenes suwonensis	9				8.6667
Serratia marcescens	9				9.1111
Control	9				9.2567
Sig.		1.000	1.000	1.000	.146

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .436.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on Hemoglobin of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
Before treatment	18	7.1333	
2 weeks after treatment	18		7.7722
1 week after treatment	18		8.1722
Sig.		1.000	.078

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .436.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxvii: Univariate Analysis of Variance of treatment effect of probiotic on Packed cell volume of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of probiotic on PCV of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1071.613[a]	17	63.036	94.081	.000
Intercept	35723.537	1	35723.537	53317.091	.000
Organism	785.411	5	157.082	234.444	.000
Time	173.100	2	86.550	129.175	.000
Organism * Time	113.101	10	11.310	16.880	.000
Error	24.121	36	.670		
Total	36819.270	54			

Corrected Total	1095.733	53			

a. R Squared = .978 (Adjusted R Squared = .968)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on PCV of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Pseudomonas aeruginosa	9	17.4222			
Lysinibacillus sphaericus	9		25.9889		
Bacillus subtilis	9		26.6889	26.6889	
Paenalcaligenes suwonensis	9			27.3667	
Serratia marcescens	9				28.3233
Control	9				28.5333
Sig.		1.000	.078	.088	.590

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .670.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experiental period

Homogeneous Subsets

Treatment effect of probiotic on PCV of fishes

Student-Newman-Keuls[a,b]

Experiental period	N	Subset		
		1	2	3
Before treatment	18	23.7228		

2 weeks after treatment with S.cerevisiae	18		25.3722	
1 week after treatment with S.cerevisiae	18			28.0667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .670.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxviii: Univariate Analysis of Variance of treatment effect of probiotic on Red blood cell of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of probiotic on red blood cell of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	63.326[a]	17	3.725	49.578	.000
Intercept	220.988	1	220.988	2941.212	.000
Organism	34.718	5	6.944	92.416	.000
Time	3.850	2	1.925	25.620	.000
Organism * Time	24.758	10	2.476	32.952	.000
Error	2.705	36	.075		
Total	287.020	54			
Corrected Total	66.031	53			

a. R Squared = .959 (Adjusted R Squared = .940)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on red blood cell of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset

		1	2	3	4	5
Lysinibacillus sphaericus	9	.9467				
Bacillus subtilis	9		1.2833			
Pseudomonas aeruginosa	9			1.6000		
Paenalcaligenes suwonensis	9				2.3244	
Serratia marcescens	9					2.9389
Control	9					3.090
Sig.		1.000	1.000	1.000	1.000	.419

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .075.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on red blood cell of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
2 weeks after treatment with S. cerevisiae	18	1.8311	
Before treatment	18	1.8372	
1 week after treatment with S. cerevisiae	18		2.4006
Sig.		.947	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .075.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xxxix: Univariate Analysis of Variance of treatment effect of probiotic on white blood cell of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of probiotic on white blood cell of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1011.104[a]	17	59.477	28.423	.000
Intercept	27471.846	1	27471.846	13128.522	.000
Organism	889.970	5	177.994	85.062	.000
Time	35.269	2	17.635	8.427	.001
Organism * Time	85.865	10	8.586	4.103	.001
Error	75.331	36	2.093		
Total	28558.281	54			
Corrected Total	1086.435	53			

a. R Squared = .931 (Adjusted R Squared = .898)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on white blood cell of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Pseudomonas aeruginosa	9	16.9002			
Control	9		19.4833		
Serratia marcescens	9		19.6244		
Paenalcaligenes suwonensis	9			25.6556	
Lysinibacillus sphaericus	9			25.6778	
Bacillus subtilis	9				27.9889
Sig.		1.000	.838	.974	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 2.093.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on white blood cell of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
2 weeks after treatment with S. cerevisiae	18	21.4389	
Before treatment	18		22.9011
1 week after treatment with S. cerevisiae	18		23.3257
Sig.		1.000	.384

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 2.093.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xl: Univariate Analysis of Variance of treatment effect of probiotic on lymphocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of probiotic on lymphocyte of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1720.626[a]	17	101.213	54.862	.000
Intercept	457424.074	1	457424.074	247943.499	.000
Organism	1237.181	5	247.436	134.121	.000
Time	259.505	2	129.752	70.331	.000
Organism * Time	223.940	10	22.394	12.138	.000
Error	66.415	36	1.845		
Total	459211.115	54			
Corrected Total	1787.041	53			

a. R Squared = .963 (Adjusted R Squared = .945)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on lymphocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Control	9	81.1100				
Serratia marcescens	9		90.5444			
Pseudomonas aeruginosa	9		91.6778	91.6778		
Bacillus subtilis	9			92.9111		
Lysinibacillus sphaericus	9				95.3111	
Paenalcaligenes suwonensis	9					98.6667
Sig.		1.000	.085	.062	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.845.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on lymphocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
Before treatment with S. cerevisiae	18	88.9500	
2 weeks after treatment	18		93.3333
1 week after treatment	18		93.8278
Sig.		1.000	.282

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.845.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xli: Univariate Analysis of Variance of treatment effect of probiotic on monocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable **Treatment effect of probiotic on monocyte of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	313.199[a]	17	18.423	22.022	.000
Intercept	5015.114	1	5015.114	5994.704	.000
Organism	104.759	5	20.952	25.044	.000
Time	130.438	2	65.219	77.958	.000
Organism * Time	78.002	10	7.800	9.324	.000
Error	30.117	36	.837		
Total	5358.431	54			
Corrected Total	343.317	53			

a. R Squared = .912 (Adjusted R Squared = .871)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on monocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	7.2467		
Pseudomonas aeruginosa	9	8.1333		
Paenalcaligenes suwonensis	9	8.5556		
Serratia marcescens	9		10.2556	

Bacillus subtilis	9		10.9667	10.9667
Lysinibacillus sphaericus	9			11.6667
Sig.		.595	.108	.113

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .837.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on monocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
1 week after treatment	18	8.0167		
2 weeks after treatment	18		9.1611	
Before Treatment with S. cerevisiae	18			11.7333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .837.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlii: Univariate Analysis of Variance of treatment effect of probiotic on AST of fishes

Tests of Between-Subjects Effects

Dependent Variable: Treatment effects on AST of Mice

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	5785.185[a]	17	340.305	51.330	.000
Intercept	273650.089	1	273650.089	41275.780	.000

Organism	5032.221	5	1006.444	151.806	.000
Time	399.960	2	199.980	30.164	.000
Organism * Time	353.004	10	35.300	5.325	.000
Error	238.673	36	6.630		
Total	279673.947	54			
Corrected Total	6023.858	53			

a. R Squared = .960 (Adjusted R Squared = .942)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effects of probiotic on AST of fish

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Lysinibacillus sphaericus	9	59.2667				
Control	9		65.2800			
Bacillus subtilis	9		67.6667			
Pseudomonas aeruginosa	9			75.1444		
Serratia marcescens	9				81.2667	
Paenalcaligenes suwonensis	9					83.8556
Sig.		.592	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 6.630.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effects of probiotic on AST of fish

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
2 weeks after treatment	18	67.7883		
1 week after treatment	18		71.3222	
Before treatment with S.cerevisiae	18			74.4506
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 6.630.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xliii: Univariate Analysis of Variance of treatment effect of probiotic on ALT of fishes

Dependent Variable: **Treatment effect of probiotic on ALT of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	441.163[a]	17	25.951	45.574	.000
Intercept	35319.842	1	35319.842	62027.106	.000
Organism	146.225	5	29.245	51.359	.000
Time	143.131	2	71.565	125.680	.000
Organism * Time	151.807	10	15.181	26.660	.000
Error	20.499	36	.569		
Total	35781.504	54			
Corrected Total	461.662	53			

a. R Squared = .956 (Adjusted R Squared = .935)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on ALT of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Lysinibacillus sphaericus	9	23.5222			
Pseudomonas aeruginosa	9	23.6556			
Bacillus subtilis	9		25.2333		
Control	9			26.1300	
Serratia marcescens	9			26.7967	
Paenalcaligenes suwonensis	9				28.1111
Sig.		.710	1.000	.069	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .569.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on ALT of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
2 week after treatment	18	23.9756		
1 week after treatment	18		24.9400	
Before treatment with S. cerevisiae	18			27.8089
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .569.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xliv: Univariate Analysis of Variance of treatment effect of probiotic on weight of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of probiotic on weight of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	120862.832[a]	17	7109.578	1446.294	.000
Intercept	2092771.414	1	2092771.414	425730.362	.000
Organism	97912.895	5	19582.579	3983.664	.000
Time	15044.594	2	7522.297	1530.253	.000
Organism * Time	7905.344	10	790.534	160.818	.000
Error	176.966	36	4.916		
Total	2213811.212	54			
Corrected Total	121039.798	53			

a. R Squared = .999 (Adjusted R Squared = .998)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of probiotic on weight of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset					
		1	2	3	4	5	6
Serratia marcescens	9	145.7956					
Pseudomonas aeruginosa	9		158.6389				
Paenalcaligens suwonensis	9			183.0500			
Lysinibacillus sphaericus	9				188.0889		
Bacillus subtilis	9					240.8844	
Control	9						264.7200
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 4.916.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of probiotic on weight of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before treatment	18	179.8261		
1 week after treatment	18		191.2317	
2 weeks after treatment	18			219.5311
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 4.916.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlv: Univariate Analysis of Variance of treatment effect of chloramphenicol on Hemoglobin of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on Hemoglobin of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	167.646[a]	17	9.862	18.260	.000
Intercept	4052.534	1	4052.534	7503.998	.000
Organism	41.293	5	8.259	15.292	.000
Time	107.985	2	53.992	99.977	.000
Organism * Time	18.369	10	1.837	3.401	.003
Error	19.442	36	.540		
Total	4239.622	54			
Corrected Total	187.088	53			

a. R Squared = .896 (Adjusted R Squared = .847)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on Hemoglobin of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Pseudomonas aeruginosa	9	7.1111			
Lysinibacillus sphaericus	9		8.1111		
Bacillus subtilis	9		8.5556	8.5556	
Serratia marcescens	9			9.1111	9.1111
Control	9			9.3111	9.1433
Paenalcaligenes suwonensis	9				9.7778
Sig.		1.000	.208	.088	.146

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .540.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on Hemoglobin of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before treatment with Chloramphenicol	18	6.9722		
1 week after treatment	18		8.5833	
2 weeks after treatment	18			10.4333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .540.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlvi: Univariate Analysis of Variance of treatment effect of chloramphenicol on PCV of fishes

Tests of Between-Subjects Effects

Dependent Variable: Treatment effects on PCV of fishes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	962.348[a]	17	56.609	37.623	.000
Intercept	42779.556	1	42779.556	28431.951	.000
Organism	206.845	5	41.369	27.495	.000
Time	541.584	2	270.792	179.972	.000
Organism * Time	213.919	10	21.392	14.217	.000
Error	54.167	36	1.505		
Total	43796.070	54			
Corrected Total	1016.514	53			

a. R Squared = .947 (Adjusted R Squared = .922)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on PCV of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Pseudomonas aeruginosa	9	25.1111		
Bacillus subtilis	9	26.1111		
Serratia marcescens	9		28.1111	
Control	9		29.1000	29.1700

Paenalcaligenes suwonensis	9			30.0000
Lysinibacillus sphaericus	9			30.4444
Sig.		.092	.096	.065

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.505.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on PCV of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before treatment with chloramphenicol	18	23.8611		
1 week after treatment	18		29.1611	
2 weeks after treatment	18			31.4167
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.505.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlvii: Univariate Analysis of Variance of treatment effect of chloramphenicol on Red blood cell of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on RBC of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	22.418[a]	17	1.319	17.812	.000

Intercept	231.882	1	231.882	3132.125	.000
Organism	14.959	5	2.992	40.411	.000
Time	3.329	2	1.664	22.481	.000
Organism * Time	4.130	10	.413	5.579	.000
Error	2.665	36	.074		
Total	256.965	54			
Corrected Total	25.083	53			

a. R Squared = .894 (Adjusted R Squared = .844)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on RBC of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Pseudomonas aeruginosa	9	1.4222			
Bacillus subtilis	9		1.7722		
Serratia marcescens	9		1.7778		
Lysinibacillus sphaericus	9		1.9333		
Paenalcaligenes suwonensis	9			2.5556	
Control	9				2.937
Sig.		1.000	.429	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .074.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on RBC of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
Before treatment with chloramphenicol	18	1.8389	
1 weeks after treatment	18	1.9617	
2 weeks after treatment	18		2.4161
Sig.		.184	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .074.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlviii: Univariate Analysis of Variance of treatment effect of chloramphenicol on white blood cell of fishes

Tests of Between-Subjects Effects

Dependent Variable: Treatment effects on WBC of fishes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1599.098[a]	17	94.065	129.791	.000
Intercept	20969.470	1	20969.470	28933.827	.000
Organism	1219.521	5	243.904	336.541	.000
Time	206.211	2	103.105	142.266	.000
Organism * Time	173.366	10	17.337	23.921	.000
Error	26.091	36	.725		
Total	22594.658	54			
Corrected Total	1625.189	53			

a. R Squared = .984 (Adjusted R Squared = .976)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on WBC of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Pseudomonas aeruginosa	9	12.2444				
Control	9		16.4800			
Serratia marcescens	9		17.3444			
Paenalcaligenes suwonensis	9			21.7000		
Lysinibacillus sphaericus	9				23.5778	
Bacillus subtilis	9					26.5556
Sig.		1.000	.194	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .725.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on WBC of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
1 week after treatment	18	18.0778	
2 weeks after treatment	18	18.5861	
Before treatment with chloramphenicol	18		22.4539
Sig.		.082	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .725.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix xlix: Univariate Analysis of Variance of treatment effect of chloramphenicol on lymphocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on lymphocyte of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	696.480[a]	17	40.969	20.130	.000
Intercept	421464.841	1	421464.841	207085.228	.000
Organism	572.311	5	114.462	56.241	.000
Time	28.390	2	14.195	6.975	.003
Organism * Time	95.779	10	9.578	4.706	.000
Error	73.268	36	2.035		
Total	422234.589	54			
Corrected Total	769.748	53			

a. R Squared = .905 (Adjusted R Squared = .860)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on lymphocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	82.4167		
Pseudomonas aeruginosa	9		86.0000	
Bacillus subtilis	9			90.0000
Paenalcaligenes suwonensis	9			90.5556
Serratia marcescens	9			90.6556
Lysinibacillus sphaericus	9			90.7778
Sig.		1.000	1.000	.658

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 2.035.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on lymphocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2
2 weeks after treatment	18	87.3556	
1 week after treatment	18		88.6083
Before treatment with chloramphenicol	18		89.0722
Sig.		1.000	.336

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 2.035.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix l: Univariate Analysis of Variance of treatment effect of chloramphenicol on monocyte of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on monocyte of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	198.412[a]	17	11.671	11.697	.000
Intercept	5989.885	1	5989.885	6003.248	.000
Organism	105.443	5	21.089	21.136	.000
Time	32.669	2	16.335	16.371	.000
Organism * Time	60.300	10	6.030	6.043	.000

Error	35.920	36	.998		
Total	6224.218	54			
Corrected Total	234.332	53			

a. R Squared = .847 (Adjusted R Squared = .774)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on monocyte of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset		
		1	2	3
Control	9	8.5700		
Paenalcaligenes suwonensis	9		9.8889	
Pseudomonas aeruginosa	9		10.0556	
Serratia marcescens	9		10.6000	
Bacillus subtilis	9			11.8889
Lysinibacillus sphaericus	9			12.5222
Sig.		1.000	.298	.187

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .998.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on monocyte of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset	
		1	2

1 week after treatment	18	9.6944	
2 weeks after treatment	18	10.3333	
Before treatment with chloramphenicol	18		11.5683
Sig.		.063	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .998.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix li: Univariate Analysis of Variance of treatment effect of chloramphenicol on AST of fishes

Tests of Between-Subjects Effects

Dependent Variable: Treatment effects on AST of fishes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	6158.073[a]	17	362.240	50.591	.000
Intercept	286975.224	1	286975.224	40079.260	.000
Organism	5299.924	5	1059.985	148.039	.000
Time	48.916	2	24.458	3.416	.044
Organism * Time	809.233	10	80.923	11.302	.000
Error	257.767	36	7.160		
Total	293391.064	54			
Corrected Total	6415.840	53			

a. R Squared = .960 (Adjusted R Squared = .941)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effects on AST of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5

Lysinibacillus sphaericus	9	60.3444				
Control	9		63.2500			
Bacillus subtilis	9			68.3333		
Pseudomonas aeruginosa	9				73.3333	
Serratia marcescens	9					85.4444
Paenalcaligenes suwonensis	9					86.0222
Sig.		1.000	1.000	1.000	1.000	.650

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 7.160.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on AST of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset
		1
1 week after treatment	18	72.1456
2 weeks after treatment	18	72.3111
Before treatment with chloramphenicol	18	74.2422
Sig.		.061

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 7.160.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix lii: Univariate Analysis of Variance of treatment effect of chloramphenicol on ALT of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on ALT of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	543.563[a]	17	31.974	22.073	.000
Intercept	34713.277	1	34713.277	23963.420	.000
Organism	183.636	5	36.727	25.354	.000
Time	289.542	2	144.771	99.939	.000
Organism * Time	70.385	10	7.038	4.859	.000
Error	52.149	36	1.449		
Total	35308.989	54			
Corrected Total	595.712	53			

a. R Squared = .912 (Adjusted R Squared = .871)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Treatment effect of chloramphenicol on ALT of fishes

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset			
		1	2	3	4
Control	9	22.5100			
Pseudomonas aeruginosa	9		24.5889		
Lysinibacillus sphaericus	9		24.6111		
Serratia marcescens	9		25.9444	25.9444	
Bacillus subtilis	9			26.6111	
Paenalcaligenes suwonensis	9				28.1244
Sig.		1.000	.057	.248	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.449.

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on ALT of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
2 weeks after treatment	18	22.1556		
1 week after treatment	18		26.3461	
Before treatment with chloramphenicol	18			27.5611
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 1.449.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix liii: Univariate Analysis of Variance of treatment effect of chloramphenicol on weight of fishes

Tests of Between-Subjects Effects

Dependent Variable: **Treatment effect of chloramphenicol on weight of fishes**

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	204708.995[a]	17	12041.706	1946.280	.000
Intercept	2705025.852	1	2705025.852	437208.608	.000
Organism	115371.141	5	23074.228	3729.447	.000
Time	70865.966	2	35432.983	5726.971	.000
Organism * Time	18471.887	10	1847.189	298.558	.000
Error	222.733	36	6.187		
Total	2909957.580	54			
Corrected Total	204931.728	53			

a. R Squared = .999 (Adjusted R Squared = .998)

Post Hoc Tests

Bacterial isolate

Homogeneous Subsets

Treatment effect of chloramphenicol on weight of fishes

Student-Newman-Keuls[a,b]

Bacterial isolate	N	Subset					
		1	2	3	4	5	6
Serratia marcescens	9	150.8222					
Pseudomonas aeruginosa	9		192.7222				
Paenalcaligenes suwonensis	9			210.2778			
Lysinibacillus sphaericus	9				229.2000		
Bacillus subtilis	9					277.3333	
Control	9						282.5333
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

a. Uses Harmonic Mean Sample Size = 9.000.

b. Alpha = .05.

Experimental period

Homogeneous Subsets

Treatment effect of chloramphenicol on weight of fishes

Student-Newman-Keuls[a,b]

Experimental period	N	Subset		
		1	2	3
Before treatment	18	177.5889		
1 week after treatment	18		227.8000	
2 weeks after treatment	18			266.0556
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = 6.187.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Appendix liv: Univariate Analysis of Variance of the effect of probiotic on bacterial load of gill and liver of fish

Tests of Between-Subjects Effects

Dependent Variable: Effect of probiotic

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	192.574[a]	11	17.507	513.017	.000
Intercept	1127.952	1	1127.952	33053.545	.000
Organ	43.143	1	43.143	1264.264	.000
Organism	109.732	5	21.946	643.120	.000
Organ * Organism	39.698	5	7.940	232.664	.000
Error	.819	24	.034		
Total	1321.345	36			
Corrected Total	193.393	35			

a. R Squared = .996 (Adjusted R Squared = .994)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Effect of probiotic

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset					
		1	2	3	4	5	6
Control	6	2.6983					
B.subtilis	6		4.4433				
L. sphaericus	6			5.0333			
P.suwonensis	6				6.4433		
P.aeruginosa	6					7.0333	
S.marcescens	6						7.9333

Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .034.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Appendix lv: Univariate Analysis of Variance of effect of antibiotic on bacterial load of gill and liver of fish

Tests of Between-Subjects Effects

Dependent Variable: Effect of antibiotic

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38.754[a]	11	3.523	79.919	.000
Intercept	373.907	1	373.907	8481.815	.000
Organ	1.630	1	1.630	36.973	.000
Organism	25.058	5	5.012	113.683	.000
Organ * Organism	12.067	5	2.413	54.744	.000
Error	1.058	24	.044		
Total	413.719	36			
Corrected Total	39.812	35			

a. R Squared = .973 (Adjusted R Squared = .961)

Post Hoc Tests

Bacterial isolates

Homogeneous Subsets

Effect of antibiotic

Student-Newman-Keuls[a,b]

Bacterial isolates	N	Subset				
		1	2	3	4	5
Control	6	1.4633				
S.marcescens	6		3.0850			
L. sphaericus	6			3.4217		
B.subtilis	6			3.6250	3.6250	
P.suwonensis	6				3.7700	3.7700
P.aeruginosa	6					3.9717
Sig.		1.000	1.000	.106	.243	.109

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .044.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Appendix lvi: Univariate Analysis of Variance of effect of probiotic and antibiotic on bacterial load of gill and liver of fish

Tests of Between-Subjects Effects

Dependent Variable: Effect of antibiotic

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	332.835[a]	23	14.471	370.065	.000
Intercept	1400.351	1	1400.351	35810.798	.000
Organism	101.840	5	20.368	520.864	.000
Treatment	101.508	1	101.508	2595.823	.000
Organ	14.001	1	14.001	358.040	.000
Organism * Treatment	32.950	5	6.590	168.525	.000
Organism * Organ	21.092	5	4.218	107.878	.000
Treatment * Organ	30.772	1	30.772	786.924	.000
Organism * Treatment * Organ	30.672	5	6.134	156.876	.000
Error	1.877	48	.039		
Total	1735.063	72			

Corrected Total	334.712	71			

a. R Squared = .994 (Adjusted R Squared = .992)

Estimated Marginal Mean

1. Bacterial isolates

Dependent Variable: Effect of antibiotic

Bacterial isolates	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
L. sphaericus	4.228	.057	4.113	4.342
P.aeruginosa	5.503	.057	5.388	5.617
S.marcescens	5.509	.057	5.394	5.624
B.subtilis	4.034	.057	3.919	4.149
P.suwonensis	5.107	.057	4.992	5.221
Control	2.081	.057	1.966	2.196

2. Treatment method

Dependent Variable: Effect of antibiotic

Treatment method	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
antibiotic	3.223	.033	3.157	3.289
probiotic	5.598	.033	5.531	5.664