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Bacteriological Evaluation and Antibacterial Susceptibility Profile of Bacteria obtained from Major Fish Ponds in Akungba-Akoko

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ABSTRACT

Fishes are good source of dietary protein, reared in artificial ponds in most countries; poor sanitary condition predisposes the fishes to infestation by pathogenic microorganisms. The study aimed at evaluating the bacteriological and antimicrobial susceptibility profile of major fish ponds in Akungba-Akoko. All 6 pond water samples collected were subjected to physicochemical analysis (pH and temperature). Isolates were identified on the basis of their cultural, morphological, and biochemical characteristics. Kirby-Bauer disc diffusion method was used to determine antibacterial activity. The results showed pH range of 8.1 to 8.6 while temperature ranges from 29 to 31°C. The bacterial load ranged from 3.7 x 10^3 to 2.5 × 10^4 cfu/ml. This study revealed diversified forms of bacteria that include member of genera; *Proteus, Staphylococcus, Streptococcus, Escherichia, Enterococcus, Klebsiella,* and *Salmonella.* The organisms with the highest percentage of occurrence are the *Staphylococcus* spp. and *Escherichia* spp. Percentage susceptibility of isolates to antibiotics was highest with azithromycin (93.7%) and the least with streptomycin (62.5%). The presence of some pathogenic organisms shows lack of qualitative pond management services which could become harmful to both fishes and humans in the food web system. Therefore, proper sanitary and enlightenment campaign are necessary to prevent disease outbreak among fish consumers.

Keywords: Bacteriological evaluation, antibacterial susceptibility, bacteria, fish ponds

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1 INTRODUCTION

The bacteriological evaluation and antibacterial susceptibility profile of fish ponds play a crucial role in ensuring the health and safety of aquatic organisms, as well as the consumers of fish products. Understanding the microbial composition and antibiotic susceptibility patterns in these environments is essential for effective disease control, sustainable aquaculture practices, and public health considerations.

In many countries, fishes are consumed and are considered to be a good source of human diet. This study showed high bacterial contamination of fish pond waters, physicochemical parameters at variance with the WHO standard and presence of antibiotic resistant organisms. protein (Claudious *et al.,* 2019). 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 fisheries and aquaculture sector significantly expanded in the past decades and total production, trade and consumption reached an all-time record of 179 million tons in 2018. 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. Nigeria is the largest aquaculture fish producer in sub-Saharan Africa, accounting for 52 percent of the total farmed fish production in the region (FAO, 2002). 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 *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). 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 *et al.*, 2019).

The pollutants of fish pond water include; residual food, fecal 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 waters. The 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 raising worldwide (Claudious *et al.*, 2019). Resistant pathogens are capable of undermining effective health outcomes and prolonging hospitalization of patients (Fakorede *et al.,* 2019). The use of antibiotics has accompanied the growth in aquaculture. It is used for prophylaxis and for treatment in fish farming. The intensives 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).

Often the primary driver of such food intoxication is the farming environment and feed given to the fish. Since fish lives in water, the quality of water directly impacts fish productivity, fish products, human and environmental health. Water quality is one of the most overlooked aspects of pond management until it adversely affects the quality of fish production. The factors which influence the use of water for fish culture include dissolved oxygen, pH, hardness, turbidity, alkalinity, ammonia and

temperature. The level of pollution of a given water body is indicated by other parameters such as biological oxygen demand and chemical oxygen demand (Wang *et al.,* 2018; Mukwabi *et al.,* 2019).

Fishes are reared in different culture media or confinement such as concrete, earthen or plastics ponds. Concrete and earthen ponds have been the widely used culture system for fish. Earthen pond system of fish cultivation has been the most established method of fish culture in Nigeria. Fishes reared in these environments are contaminated by both pathogenic and opportunistic microorganisms. 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 (Abdel-Waheed *et al.*, 2018; Mukwabi *et al.,* 2019). Due to the high cost of feeding, farmers use animal manure to supplement feeding. The use of organic manure also leads to the release of high concentration of opportunistic and pathogenic microorganisms into the ponds, which pose a threat not only to fish health but also to the environment (Wang *et al.,* 2018). Also, these microorganisms in fish and fish ponds portend grave consequences for public health (Li *et al.*, 2019; Yanestria *et al.,* 2019). Some of these microorganisms possess resistant genes, which enhances their potential for infecting consumers. Such resistant pathogens are capable of undermining effective health outcomes and prolonging hospitalization of patients. The specific objectives of this study are to isolate and identify bacteria species obtained from major fish pond in Akungba-Akoko and to evaluate the antimicrobial susceptibility profile of all isolates.

2 LITERATURE REVIEW

2.1 Empirical Review of Literature

Several studies have explored the bacteriological evaluation and antibacterial susceptibility profiles of fish ponds, providing valuable insights into the microbial diversity and antibiotic resistance patterns. For example, a study by Smith et al. (2018) investigated the bacteriological quality of fish ponds in Ghana and found high levels of bacterial contamination, including pathogenic species such as Aeromonas spp. and Vibrio spp. This highlights the importance of regular evaluations to prevent disease outbreaks (Smith et al., 2018).

In another study conducted by Li et al. (2020) in China, the antibacterial susceptibility profiles of bacterial isolates from fish ponds were examined. The results revealed varying degrees of resistance to commonly used antibiotics, emphasizing the need for judicious antibiotic use in aquaculture.

Similarly, Chioma et al. (2020) which aimed at evaluating the bacteriological and physicochemical characteristics of fish pond waters in three senatorial zones in Anambra State, Nigeria and the antibiogram of the isolates determined. This study showed high bacterial contamination of fish pond waters, physicochemical parameters at variance with the WHO standard and presence of antibiotic resistant organisms.

In another study conducted by Cecilia et al. (2020) evaluating bacteriological quality and antibiotics' susceptibility profile of small-medium scale commercial fish farms in Nigeria. Results revealed that the concrete pond and earthen pond had the highest load of heterotrophic and coliform bacteria. The water quality parameters (temperature and pH) and the type of bacteria detected in all ponds did not differ significantly. The study also revealed that all the ponds were contaminated with potential pathogenic bacteria that could lower fish yield, cause diseases and economic loss, and equally endanger public health, particularly if the fish harvested from the ponds are not properly cooked before consumption.

Moreover, a study by Odesiri-Euteyen et al. (2022) on bacteriological and physicochemical analysis of fish pond effluents in Warri and its environment, Nigeria. This study revealed that the physicochemical characteristics and bacteriological quality of the earthen and concrete fish ponds were not significantly different, but there were slight differences in the concentration of nutrients which could be attributed to leaching of these substances into the soil of the earthen ponds. This study also revealed that both ponds were wholly soiled with pathogenic bacteria that could affect cultured fishes by causing

diseases thereby lowering fish yield and resulting into economic loss, and jeopardizing Human's health and also fouling the environment.

A study conducted by Okafor et al. (2020), the results showed that there was no fish pond water sample that was free from bacteria and fungi, an indication that the entire fish pond water samples were contaminated by microorganisms. Showing that the contamination could have arisen from different sources which include air, source of water and fish feeds could have been responsible for the introduction of these organisms into the fish pond. Also, it was shown in this study that the gamma distribution provided best fit for the microbial count.

Furthermore, another study by Judith, et al. (2021) on microbial assessment and antibiotic susceptibility profile of bacterial fish isolates in an aquaculture production site in Mefou Afamba division of Cameroon, revealed that the microbial load of samples (fish parts, mud and pond water) generally exceeded the acceptable levels in terms Total Viable Aerobic Bacterial Count, Fungal Count, Staphylococcus aureus count, Total Coliform Count and Feacal Coliform Count. This study has also shown that fish are sources of fish and human pathogenic bacteria. Curiously, all these bacteria exhibited high resistance against the studied drugs except chloramphenicol. This finding is of clinical and epidemiological relevance suggesting the application of strict hygiene measures during handling, processing, and consumption of fish cultured at the Mfou aquaculture site.

Additionally, a study carried out by Nwachukwu et al. (2020) on bacterial isolates and antibiotic susceptibility profile of fish pond water in Owerri, Imo State, Nigeria. This study was carried out to isolate and identify the bacteria present in fish pond water in Owerri, Imo State, Nigeria and to determine their antibiotic susceptibility profile. The study found that the fish pond water samples contained different types of bacteria, some of which were potential pathogens and exhibited multiple antibiotic resistance. The study also observed that the fish pond water samples had high levels of nitrate, phosphate and total dissolved solids, which could affect the quality of the water and the health of the fish.

Another evaluation study by Njoku et al. (2015) on an investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. This study was aimed at determining the bacterial load and antibiotic susceptibility profile of bacteria isolated from fish pond water in Niger Delta, Nigeria. The study detected various bacteria in the fish pond water samples, some of which were opportunistic pathogens and showed resistance to several antibiotics. The study also noted that the fish pond water samples had low dissolved oxygen, high biochemical oxygen demand and high total suspended solids, which could impair the growth and survival of the fish.

Similarly, another study conducted by Ogundipe et al. (2021) on bacteriological quality and antibiotics' susceptibility profile of bacteria isolated from fish pond in Ile-Ife, Nigeria. This study was conducted to determine the bacteriological quality and antibiotics' susceptibility profile of bacteria isolated from fish pond in Ile-Ife, Nigeria. The study found that the fish pond water was polluted with bacteria of public health importance and that the isolated bacteria were resistant to most of the commonly used antibiotics.

In another study by Anas et al. (2022) on the investigation of the bacterial contamination and antibiotic susceptibility profile of bacteria isolated from bottled drinking water. This study analyzed how lifestyle factors affect the overall health of people with bacterial infections from the water. The article describes significance of the research because many people do not have access to clean, safe drinking water where this water is essential to life, and many die of waterborne bacterial infections. So, the aim of the study is to draw attention to the major factors of the most dangerous bacteria transmitted through water marketed in Al Anbar Province of Iraq: Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Furthermore, the specific significant contribution of the study has been to show the most important treatments for treating infections caused by the bacteria diagnosed in the study.

A study by Ibemenuga and Okeke (2014) on bacteriological quality of freshwater fish caught from two natural lakes in the rainforest region of South-Eastern Nigeria. This study aimed to evaluate

the bacteriological quality of freshwater fish caught from Oguta and Agulu lakes in the rainforest region of South-Eastern Nigeria. The authors collected 24 fish samples from each lake over four months and analyzed them using standard methods. They isolated some bacterial genera from the fish samples, namely Escherichia, Salmonella, Listeria, Vibrio, Coliforms, and Staphylococcus. They also determined the antibiotic resistance patterns of the isolates and compared them with the WHO standards. The results showed that the fish from both lakes had high bacterial counts and contained waterborne pathogens of public health significance. The authors concluded that the fish quality was poor and recommended proper treatment of sewage before disposal into aquatic ecosystems, as well as careful handling of fish at post-harvest.

Moreover, a study by Eghomwanre et al. (2019) on bacteriological and physicochemical assessment of fish pond waters collected from idogbo community, Edo State, Nigeria. The study evaluated the bacteriological and physicochemical quality of fish pond waters collected from Idogbo community, Edo State, Nigeria. The authors collected 30 water samples from 10 different fish ponds (15 samples each from concrete and plastic ponds, respectively) and analyzed them using standard methods. The results showed that the mean physicochemical values for pH, temperature, Electrical Conductivity, Total Dissolved Solid, Dissolved Oxygen, Biological Oxygen Demand, nitrate and phosphate were within the Federal Environmental Protection Agency (FEPA) guidelines for water quality. However, the mean heterotrophic bacterial counts and total coliform counts were high and exceeded the permissible limits. The authors identified seven bacterial genera from the water samples, namely Pseudomonas, Klebsiella, Proteus, Bacillus, Staphylococcus, Escherichia, and Serratia. They also performed antibiotic susceptibility tests on the isolates and found that most of them were resistant to commonly used antibiotics such as ampicillin, tetracycline, and erythromycin. The authors concluded that the fish pond waters in Idogbo community contained waterborne pathogenic bacteria of public health significance and recommended proper monitoring of the ponds and education of the farmers on the effect of the use of organic manure as food additives in fish ponds.

2.6. Summary

Bacteriological evaluation and antibacterial susceptibility of fish pond lies in the importance of assessing the bacteriological quality of fish ponds and understanding the potential risks associated with antimicrobial resistance (AMR) in aquaculture settings. Fish is an important source of protein and income for many people in Nigeria, especially in rural areas. However, fish production and consumption are threatened by various factors, such as environmental pollution, overfishing, and bacterial infections. Bacterial infections can cause diseases and mortality in fish, as well as pose health risks to consumers and fish farmers. Therefore, it is essential to monitor the bacteriological quality and antimicrobial susceptibility of fish and water samples from fish ponds.

3 METHODOLOGY

Six water samples were collected from six major fish pond in Akungba-Akoko. The samples were from School Farm fish ponds in Adekunle Ajasin University Akungba-Akoko, Dr. Ajayi's fish ponds along Foursquare Gospel Church Street, Permanent-site, Akungba-Akoko and fish pond at the Department of Mass Communication's Building, Adekunle Ajasin University Akungba-Akoko. The samples were collected directly into six different sterile sample bottles, cork-screwed, labeled and transported immediately to the Microbiology laboratory of Adekunle Ajasin University, Akungba-Akoko where bacteriological evaluation and antimicrobial susceptibility profile were carried out. The samples were transported immediately to the Microbiology laboratory where the pH and Temperature were measured, using pH meter and thermometer respectively while the values gotten was recorded (APHA, 2012).The temperature of the water sample from fish pond was measured at the laboratory using a mercury-in-glass thermometer calibrated in degree Celsius. The temperature probe was gently inserted into the water sample and allowed to stand for 2 minutes. The temperature was recorded (APHA, 2012).

The pH of the water sample was determined using a pH conductivity meter. The pH conductivity meter was standardized and then inserted into the water sample. The pH was recorded accordingly (APHA, 2012). The materials used for this study include: Sterile Sampling Bottles, Gloves, Disinfectants (e.g. Ethanol, 95% Alcohol), Test tubes and racks, Beakers, Conical flasks, Measuring cylinder, Inoculating loop, Sterile needles and Syringes, Sterile swap sticks, McCartney bottles, Hand gloves, Nonabsorbent cotton wool, Aluminum foil, Hydrogen peroxide, Immersion oil, Paper tapes, Sterile water, Petri dishes, Microscopic slides, Gram staining reagents (Crystal violet, Iodine, 70% Alcohol, Safranin), Spatula, Sterile distilled water, Nutrient agar, Mueller-Hinton's agar, Face mask, Lab coat. The equipment used for this study include: Autoclave, Incubator, Hot air oven, Hot plate, Microscope, Weighing Balance, Bunsen burner, Refrigerator, Centrifuge, Mercury -in-glass Thermometer, pH meter.

The equipment used for this study were sterilized by appropriate technique. All glass wares such as Petri dishes, conical flasks, test tubes, beakers, McCartney bottles, etc., were thoroughly washed and sterilized in the hot air oven at 170°C for about 2 hours. The inoculation loop was sterilized by flaming in the Bunsen burner until it turns red hot. Similarly, working surface was sterilized by the application of disinfectant solution (95% ethanol) (Olajide and Anthony, 2013).

The Nutrient agar and Mueller-Hinton's agar were prepared according to manufacturer's instructions. For proper dissolution and homogenization, the media were shaken vigorously and melted using a stirrer before sterilizing in an Autoclave at 121°C for 15 minutes (Olajide and Anthony, 2013). Media were aseptically dispensed into sterile Petri dishes and allowed to solidify. Workbenches were also cleaned using ethanol to ensure adequate sterility.

Nine milliliters of sterile water was transferred into 5 sterile test tubes labeled 10^1 to 10^5 for each water sample. One milliliter of the sample was aseptically transferred into the first test tube (10^1) with sterile syringe and mixed. From the first test tube, one milliliter was equally transferred to the test tube labeled 10^2 and mixed using great sterile syringe. This was repeated until the test tube labeled 10^5. This method was equally requested for the other samples (Cullen *et al.*, 2016).

Total bacteria population in the fish pond water sample was assessed for Total Bacteria Count (TBC). Dilution was selected so that total number of colonies on a plate was between 30 and 300 for TBC. This was obtained and isolated by adopting the standard plate counts technique using pour plate method. This was done by carrying ut serial dilution of the water sample using sterile distilled water. Aliquots of 0.1 ml from 10^3 and 10^5 dilutions of each sample were aseptically transferred into properly labeled sterile Petri dishes. Twenty milliliters of the culture medium (Nutrient agar) was poured into the Petri dishes and swirled to allow homogenization. This was done in duplicates. A control was equally prepared, but without adding the sample. The plates were labeled, allowed to solidify, inverted and finally incubated at 37°C for 24 hours, after which, the plates were observed for development of bacterial colonies. The colonies on the plates were enumerated and sub-culturing of bacterial isolates was done to obtain pure culture. Bacterial colonies were picked with sterile inoculating loop and were streaked on freshly prepared nutrient agar plates. The plates were incubated at 37°C for 24 hours. Pure cultures of microorganisms were also inoculated in slants using McCartney bottles for storage (Ugochukwu *et al.*, 2020).

Calculation of colony forming unit (CFU) per milliliter for the bacteria was based on the formula:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume plated}}$$

A loopful of sterile distilled water was dropped on a clean grease-free slide by using a sterile inoculating loop after which an inoculum from the culture was mixed with the water on the slide. The smear was allowed to air dry and then heat fixed gently by passing it quickly over bursen flame. The smear was flooded with crystal violet solution for 60 seconds (one minute) and rinsed with water. The smear was again flooded with Lugol's iodine for 30 seconds and rinsed with water, 70% alcohol was poured on the slides for 15 seconds until the crystal violet had been completely washed off. It was then

counterstained with Safranin for 60 seconds and allowed to dry. The slides were then observed under oil immersion objective. Gram positive cells remained purple while Gram negative cells appeared red or pink (Fawole & Osu, 2007).

The following biochemical tests were carried out for the identification of the bacterial isolates: catalase test, oxidase test, motility test, indole test, voges-proskauer test, methyl red test, urease test, starch hydrolysis, citrate utilization test and sugar fermentation test, following standard procedures with reference to Bergey's Manual of Systematic Bacteriology (Sneath, 1986).

A drop of hydrogen peroxide solution was placed on a clean grease free slide. A flamed inoculating loop was used to place a loopful of an inoculum on the slide and gently mixed after which it was observed for bubbles or effervescence which is an indication of catalase positive organism (Fawole and Oso, 2007).

The Oxidase test is used to identify bacteria that produce Cytochrome C oxidase, an enzyme of the bacterial electron transport chain. A commercially prepared strip (filter paper packed with the substrate tetramethyl-p-phenylenediamine dihydrochloride) was used. The colony tested was picked with a inoculating loop and smeared on the strip after which the inoculated area were observed for a colour change to deep blue or purple within 10-30 seconds. A positive oxidase test gives a dark purple colouration (indophenols) within 10 seconds while a negative test results gives no colouration. While performing oxidase test, Nickel-base alloy wires containing chromium and iron (nichrome) should not be used to pick the colony and make smear as this may give false positive results (Fawole and Oso, 2007).

A sterile needle was used to pick a well-isolated colony and stabbed the medium to within 1 cm of the bottom of the tube. Made sure to keep the needle in the same line it entered as it was removed from the medium. It was then incubate at 35°C for 24 hours until growth is evident. A positive motility test was indicated by a red turbid area extending away from the line of inoculation. A negative test was indicated by red growth along the inoculation line but no further (Patricia and Laura, 2011).

Three millimeters of 1% Tryptone broth was placed into different tubes after which a loopful of the bacterial isolates were inoculated into different test tubes leaving one of the tubes uninoculated to serve as the control. The test tubes were then incubated at 37°C for 48 hours after incubation, 0.5 mi of Kovac's reagent was added and shaken gently after which it was allowed to stand for 20 minutes te permit the reagent to rise to the top. A red colour at the surface of the tubes indicated a positive result while a yellow colouration of the surface layer indicated a negative result (Fawole and Oso, 2007).

Using a Pasteur's pipette, 10 drops of Methyl red pH indicator was added to each tube, and tube was swirled gently to mix the drops into the broth. Each tube was examined for colour change. Bacteria that produce many acids from the breakdown of Dextrose (Glucose) in the MR-VP medium cause the pH to drop to 4.2. At this pH, methyl red becomes red. A red colour represents a positive test. Bacteria that produce fewer acids from the breakdown of glucose drop the pH to 6.0. At this pH methyl red was yellow and this represented a negative test (Olutiola *et al*., 2000).

This test is best carried out by inoculating MR VP medium and incubating at 30°C for 5 days or 17°C for 2 days. Test with methyl red and then add 0.6ml of alpha napthol solution (about 15 drops) and 2ml of 40% KOH (about 10 drops). Shake and examine for the red colour of a positive reaction after 15 minutes and 1 hour. A positive result is the development of a red colour after 15-60 minutes. Under alkaline conditions and in the presence of oxygen, acetyl-methyl-carbinol was oxidized to diacetyl which reacts with creatine to give a red colour, creatine is present in poptone (Olutiola *et al.,* 2000).

Some organism produce urease, an enzyme which break down urea to release ammonia, sufficient ammonia may be produced to give an alkaline reaction indicated by a red colour when the organisms are grown on a medium containing urea and phenol red indicator. It is necessary to set up a colour for the basal medium containing no added medium but inoculated with the same organisms. This is to check that ammonia is not produced from the peptone (which is a component of the medium) but

from the urea. However, the presence of glucose in the medium counteracts the slight alkaline reactions which may result from poptone break down. The medium was dispensed into test tubes and sterilized in an Autoclave at 121°C for 15 minutes and then cooled to 45-50°C (Sagar, 2022).

Starch agar media was prepared and sterilized using autoclave as 121°C for 15minutes. The media was poured into Petri dishes and allowed to solidify, after which, the test organisms were inoculated on to the plate with a sterile inoculating loop. The plates were incubated at 35°C for 48hrs. After incubation, the plates were flooded with Gram's iodine and they were observed for clear zone around the test organism (Fawole and Oso, 2007).

Media used in Citrate Utilization Test is the Simmon's Citrate Agar. The salts were dissolved in deionized water and the pH was adjusted to 6.9. The agar and Bromothymol blue were added. The mixture was heated gently, with mixing; to boiling until agar is dissolved 5.0 ml was then dispensed into tubes and put in an Autoclave at 121°C under 15 pai pressures for 15 minutes. It was allowed to cool in slanted position (long slant, shallow butt). The uninoculated medium was a deep forest green due to the pH of the sample and the bromothymol blue. The slant was streaked with a light inoculum picked from the center of a well-isolated colony and incubated aerobically at 35 to 37°C for up to 4-7 days. A color change from green to blue along the slant was observed. Growth with color change from green to intense blue along the slant indicated a positive reaction while no growth and no color change indicates a negative reaction (Fawole and Oso, 2007).

There are several substances that can give certain organisms to yield H_2S (gas) amongst the product of microbial degradation. In this test, peptone water was prepared and introduced into each test tube and sterilized. After cooling, inoculums were introduced into each test tubes and incubated for 24 hours. The following day, triple sugar iron was prepared and introduced into another test tube and sterilized. It was allowed to cool in a slanting position. A sterile inoculating needle was used to dipped in to broth containing the inoculum aseptically and was used to inoculate the surface of solidified slanted TSI in test tube and incubated. The following day the production of H_2S was determined by dark colour changes in the medium produced by the organism (Hajna, 1980).

Two separate drops of saline were dropped on a slide. Using a sterile inoculating loop, one colony of organism was emulsified in one drop to make thick suspension of bacteria. A loopful of plasma was added to both the suspension and saline drop and mix gently. Coarse clumping of the mixture within 10-15 seconds was observed. The formation of clumps within 10-15 seconds was positive test result. Saline and plasma mixture should showed no clumping (Tankeshwar, 2023).

The fermentation of sugar by the test organism was demonstrated by acid and gas production, phenol red (0.01 g), sodium chloride (1.0 g) and fermentable sugar (1.10 g) were weighed into a Conical flask containing 100ml. of peptone water. The mixture was swirled so that all components in it could dissolve. A 9 mL of the preparation was dispensed in test tubes containing inverted Durham tubes. The tubes were covered with cotton wool and aluminum foil which were sterilized in the autoclave at 121°C for 15minute. Each sugar such as, glucose, mannitol, sucrose, lactose, arabinose, inositol, xylose, maltose or fructose was used. All test tubes were inoculated with respective test organisms aseptically and were incubated at 37°C for 3-5days depending on how fast the organism could make use of the sugar. Change in colour of indicator (phenol red) from red to yellow indicates the use of sugar present (Patil, *et al*., 2014).

The antibiotic sensitivity of isolates was determined by the disc agar diffusion method. This test was performed to determine the phenotypic resistance trait of the bacteria isolates to the commonly used antibiotics. This test was carried out following the Kirby-Bauer disc diffusion method (2005). Inoculums from culture bacteria isolates on the nutrient agar slant were introduced into test tubes containing 9 ml of sterile nutrient broth and it was incubated at 37°C for 18 hours, after incubation, 1ml of 18 hours old culture were transferred into a freshly prepared sterile nutrient broth and Incubated at 37°C for 5 hours to achieve the 0.5 McFarland standard. Mueller Hinton Agar was prepared and poured in a Petri dish

and allow to solidify. A sterile swap stick was dipped into 5 hours' culture broth and it's used to streak the entire surface of the dried surface of agar plates. The plates were left to dry for about 15 minutes to avoid any excess surface moisture before applying the impregnated antibiotic discs. The antibiotics discs were placed and pressed firmly on the agar plate with sterile forceps to ensure complete contact with the agar. The plates were inverted and incubated for 18 hours at 37°C for 15 minutes.

The susceptibility patterns of each isolates to antibiotics were indicated by the clear zone of inhibition around the disc, this was examined after 18 hours of incubation. The diameter of zone of inhibition were measured and recorded from other side of plate by using a transparent calibrated ruler (Nawaz *et al.,* 2009; Mailafia, *et al.,* 2017). The isolates were said to be resistant or sensitive depending on the diameter of zone of the inhibition, results were interpreted according to the CLSI standard method of interpretation (CLSI, 2023).

4 DATA ANALYSIS AND DISCUSSION OF FINDINGS

4.1 Analysis of Data

Table 4.1: Fish Pond Sample Locations, and their Sample Identification Codes

Sample Locations	Sample Identification Code
Adekunle Ajasin University Akungba-Akoko, School Farm Fish Pond 1	SF 1
Adekunle Ajasin University Akungba-Akoko, School Farm Fish Pond 2	SF 2
Dr Ajayi's fish pond (1) along Foursquare Gospel Church street, Permanent-site Akungba-Akoko.	AHP 1
Dr Ajayi's fish pond (2) along Foursquare Gospel Church street, Permanent-site Akungba-Akoko.	AHP 2
Dr Ajayi's fish pond (3) along Foursquare Gospel Church street, Permanent-site Akungba-Akoko	AHP 3
Fish Pond at Department of Mass Communication's Building, Adekunle Ajasin University, Akungba-Akoko	MCP

Source: Author's Analysis, 2024

Table 4.1 shows the location of the fish ponds where the samples were collected in Akungba-Akoko and their identification codes. The first and second samples were collected from two different fish ponds in Adekunle Ajasin University Akungba-Akoko with SF 1 and SF 2 as the identification codes respectively. The third, fourth and fifth samples were collected from three different fish ponds at Dr. Ajayi's compound, along Foursquare Gospel Church street, Permanent-site, Akungba-Akoko, having their identification codes as AHP 1, AHP 2 and AHP 3, respectively.

Table 4.2: Sample Identification Codes and Ph and Temperature Values

Sample Identification Codes	pH value	Temperature Values (°C)
SF 1	8.2	31
SF 2	8.3	31
AHP 1	8.1	29

AHP 2	8.6	30
AHP 3	8.4	30
MCP	8.5	32

Source: Author's Analysis, 2024

Table 4.2 shows the pH of the samples ranged from 8.1 to 8.6. The water sample from AHP2 had the highest pH value of 8.6 while AHP1 had the lowest pH value of 8.1. And the temperature of the samples ranged from 29°C to 32°C. The water sample from MCP had the highest value of 32°C while AHP1 had the lowest value of 29°C.

Table 4.3: The Total Bacteria Count Water Sample Cultured on Nutrient Agar.

Sample Codes	Bacterial count (cfu/ml)
SF 1	1.7×10^3 3.5×10^4
SF 2	0.8×10^3 2.5×10^4
AHP 1	0.9×10^3 6.5×10^4
AHP 2	1.7×10^{-3} 1.3×10^5
AHP 3	3.7×10^3 8.5×10^4
MCP	3.2×10^3 2.4×10^5

Source: Author's Analysis, 2024

Table 4.3 shows the total bacteria count and colony forming unit per millimeter of the water samples from six different major fish ponds in Akungba-Akoko, Ondo State. The colony count ranged from 3.7×10^3 to 2.5×10^4 cfu/ml. Table 4 shows the morphological and biochemical characteristics of bacterial isolates. Out of the 16 bacteria obtained, 7 were Gram positive while 9 were Gram negative. The morphological or cultural characteristics recorded include colour (white, pale white of creamy), surface (dry, wet, smooth, rough or shiny), elevation (flat, raised or convex), shape (circular or moderate), edge (entire or web-like) and mode of spread (swarm or moderate). Table 5 shows the biochemical characteristics of the sixteen bacterial isolates, various tests were carried out so as to determine the positive and negative results.

Table 4.4: Cultural and Morphological Characteristics of Bacterial Isolates

Isolates	Cultural Characteristics	Gram Staining	Morphology
AHP 1A	cream, irregular, entire, raised	-	long rod
AHP 1B	cream, circular, entire, raised	+	Rod
AHP 1C	cream, Rhizoid, entire, flat	+	long rod
AHP 1D	cream, filamentous, entire, flat	-	Cocci
AHP 2A	cream, irregular, entire, raised	+	Rod
AHP 2B	cream, filamentous, entire, flat	-	Rod
MCP 1	cream, irregular, undulate, flat	-	Rod
MCP 2	cream, irregular, undulate, flat	-	Rod
AHP 3A	cream, circular, entire, raised	+	Cocci
AHP 3B	cream, irregular, entire, flat	-	long rod
AHP 3C	yellow, circular, entire, flat	-	long rod

AHP 3D	cream, circular, entire, raised	-	Rod
AHP 3E	cream, circular, entire, raised	+	Cocci
SF 1C	cream, circular, entire, flat	+	Cocci
SF 2B	cream, circular, entire, flat	+	Cocci
SF 2A	yellow, circular, entire, flat	-	Cocci

Source: Author's Analysis, 2024

4.2 Discussion of Findings

Fish has become increasingly important source of protein and other elements necessary for the nourishing of the body, and fish aquaculture is a practice in Akungba-Akoko, which serves as a means of ensuring all year supply of fishes. Temperature and pH value of water contribute to quality of water, and fish pond water is known to affect the activities and well-being of the fishes (Makori *et al*., 2017). Table 2 shows the results for the temperature and pH analysis of the different major pond water samples gotten from Akungba-Akoko.

The temperature (29-32°C) and pH (8.1-8.6) recorded in all the ponds were within the optimum range necessary for African fish aquaculture (Britz and Hecht, 1987). pH water quality parameter is vital in fish aquaculture as it affects the toxicity of other compounds to fish (Alam *et al*., 2006). Temperature is often referred to as the principal factor among environmental factors affecting aquatic life (Kitty, 1987), as it affects biochemical processes with direct consequences, including, food requirements and conversion efficiency, and fish growth. Optimum temperature and pH directly stabilize the physicochemical parameters of pond water, enhancing fish health and productivity, and maintaining a proper balance of microbial ecology in pond water (Makori *et al*., 2017).

In this study, seven (7) out of the sixteen (16) isolated bacteria from various fish ponds water were Gram-positive bacteria while the remaining nine (9) were Gram-negative. However, the total bacteria count could be an underestimation due to viable but non-culturable (VBNC) state of some microbial population. The bacterial isolated were *Proteus mirabilis, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella enterica, and Staphylococcus epidermidis.* The bacterial load observed in water could be a result of the effect of anthropogenic activities in the location where the ponds are situated. Moreover, the use of chicken manure as feed by some fish farmers could serve as easy biodegradable organic matter which may increase the total bacterial count (Elsaidy, *et al*., 2015). The coliforms isolated were an indication of the contamination of the pond water with faecal materials. Some reports have indicated high incidence of antibiotic resistant bacteria in integrated fish farming; a practice that combines aquaculture with other livestock farming operations and use of their manure for fish feeding (Watts *et al*., 2017). Faecal contamination of pond water may result in the introduction of pathogenic organisms into the pond m. Such pathogens have been shown to lead to fish diseases or food borne disease (Li *et al*., 2019). The presence of pathogenic microorganisms, especially *E. coli, Salmonella,* and *Staphylococcus* spp. can lead to the transmission of water borne diseases such as Typhoid fever (Elbashir *et al*., 2018).

The AST of this study shows that 57% of the Gram positive isolates were 100% resistant to erythromycin, amoxicillin, cefixime, and 71.4% resistant to ceftazidime. Also, 85.7% of the Gram-positive isolates were susceptible to levofloxacin, gentamycin and azithromycin. Up to 100% of the Gram-positive isolates were susceptible to rifampicin. Also, 57.1% of the Gram-positive isolates were susceptible to streptomycin and ciprofloxacin. Similarly, Gram-negative isolates, up to 56% of the Gram negative isolates were resistant to ceftazidime and ceftriaxone. And about 77.7% of the Gram negative isolates were susceptible to ciprofloxacin, ofloxacin, peflacine, gentamycin, and cyprofloxacin.

Antimicrobial resistance is a global public health problem sustained by multifactorial antibiotic usage. The spread of antibiotics resistance in the marine environment is poorly understood. Less attention has been given to the aquatic ecosystem in the transmission of AMR bacteria to humans. This has created

inadequate information on the antimicrobial drug susceptibility of the aquatic environment, which is vital in the epidemiology of AMR (Wamala *et al*., 2018).

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The result showed that there was no fish pond water sample that was free from bacteria, an indication that the entire fish pond water samples were contaminated by microorganisms. The contamination could have arisen from different sources which include air, source of water and fish feeds could have been responsible for the introduction of these organisms into the fish pond.

5.2. Recommendations

It is hereby recommended that:

i. Microbiological analysis of wastewater from fish ponds be regularly conducted to check for signs of possible infections.

ii. The ministry of agriculture should ensure that the fish farmers are supplied with healthy fry for their stock.

iii. Good quality water such as borehole should be used in the fish pond rather than water from questionable sources such as river stream, and surface-runoff.

iv. The fish feeds should be sourced from reputable manufacturers.

v. Water in the fish pond should be changed completely at regular intervals.

vi. Proper construction of fish pond should be ensured and the environment where the fish ponds are located should be protected from pollutants and weeds which can harbour microorganisms that find their way into fish pond by themselves or by passive process through wind, and rainfall, thereby affecting the fishes negatively.

vii. Also water supply to the fish pond should be clean and free of contamination. Sample of the fish pond should be taken and examined in the laboratory for its microbiological quality before stocking. This will also give an insight to the possible presence of certain types of microorganisms.

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