ISOLATION, CHARACTERIZATION OF ANTIBIOTIC RESISTANCE BACTERIA IN SELECTED FISH POND ECOSYSTEMS IN OSUN STATE

Abstract

The increasing prevalence of antibiotic-resistant bacteria in aquatic ecosystems poses a significant threat to public health and environmental sustainability. This study aimed to isolate and characterize antibiotic-resistant bacteria from selected fish pond ecosystems in Osun State, Nigeria. Sixty (60) fish water samples and Sixty (60) fish sediment samples were collected from selected fish ponds located in different regions of the state using purposive sampling technique. The samples were processed for the isolation of bacterial strains using standard microbiological techniques. Isolates were identified through morphological, convectional biochemical method using Bergey's manual of determinative bacteriology while their antibiotic resistance profiles were determined using the disc diffusion method against a panel of twelve (12) commonly used antibiotics. The results revealed the presence of a diverse range of bacterial species, including *Escherichia coli, Klebsiella spp., Salmonella spp., Pseudomonas aeruginosa,* and *Staphylococcus aureus*, with a high prevalence of antibiotic-resistant strains. Resistance was most commonly observed against ceftazidime, cefotaxine, vancomycin, cefuroxime and gentamicin and ciprofloxacin. The finding revealed that there was a growing issue of antibiotic-resistant bacteria in aquatic ecosystems showed a complex interplay between antibiotic use, environmental factors, and the emergence of resistance which was as a result direct consequence of anthropogenic activities such as the misuse of antibiotics in aquaculture from agricultural practices and improper disposal of antibiotics. This study highlights the urgent need for the implementation of effective monitoring and management strategies to mitigate the spread of antibiotic-resistant bacteria in aquatic ecosystems. Furthermore, it underscores the importance of promoting sustainable and responsible use of antibiotics in aquaculture to safeguard both human health and the integrity of aquatic environments.

Keywords: Antibiotic resistance, bacteria, fish ponds, Osun State, isolation, characterization, aquaculture.

Introduction

The development of antibiotics represents a milestone in modern medicine, credited with saving countless lives since their introduction in the 1940s. The discovery of the first antibiotics, streptomycin and penicillin, transformed the way bacterial illnesses were treated and made medical procedures that were previously too dangerous or impractical possible. Antibiotics are now essential in numerous medical fields, such as organ transplantation, intensive care, chemotherapy, and premature newborn care. Effective antibiotics have a critical role in preventing and treating infections that could otherwise be fatal in these procedures (Ventola, 2018; Cox & Wright, 2019). But as more pathogenic organisms develop resistance to widely used antibiotic treatments, antibiotic resistance has become a major global public health concern. According to Holmes *et al.* (2018), this resistance increases the chance of fatality and causes longer illnesses.

Antibiotic resistance has emerged as one of the greatest threats to global public health, exacerbated by its proliferation across diverse environments, including aquaculture ecosystems. Fish farming, a rapidly expanding sector worldwide, has contributed significantly to food security and the economy. However, the sector's reliance on antibiotics to combat bacterial infections poses considerable risks due to antibiotic-resistant bacteria (ARB) development in aquatic environments (Cabello *et al.,* 2018). In particular, fish pond ecosystems, with their closed or semi-closed environments and frequent antibiotic exposure, provide ideal conditions for bacterial adaptation and resistance. The presence of ARB in aquaculture settings not only threatens fish health but also has significant public health implications due to the potential for resistance gene transfer to human pathogens (Schar *et al*., 2020).

Aquaculture's contribution to antibiotic resistance is especially concerning in developing regions, where monitoring and regulation of antibiotic use in fish ponds remain limited (Kalyoncu et al., 2020). This situation is evident in countries such as Nigeria, where fish farming has grown rapidly as a response to protein demand and economic opportunity. However, the widespread and often indiscriminate use of antibiotics in these settings is leading to increased resistance profiles among aquatic bacteria, raising concerns about environmental contamination and potential human exposure (Narayanasamy *et al*., 2019). Antibiotics are commonly used in fish farming to prevent and control infections caused by bacterial pathogens, which can devastate fish populations and compromise productivity. In many fish farms, antibiotics are administered through feed or water, often in quantities that exceed what is therapeutically necessary (Sharma *et al*., 2019). The persistence of ARB in fish ponds, alongside other anthropogenic pollutants, underscores the urgent need for comprehensive studies to isolate, characterize, and understand the antibiotic resistance mechanisms present in these ecosystems.

In Nigeria, fish farming contributes significantly to local food production, but studies indicate that ARB prevalence in aquaculture is increasing, largely due to insufficient regulation of antibiotic use (Ajibola *et al.,* 2020). The issue is further compounded by contamination from other anthropogenic sources, including agricultural runoff, wastewater discharge, and improper waste disposal, all of which contribute to the concentration of antibiotics in aquatic environments. The urgent need to mitigate the spread of ARB in these settings has spurred research to isolate, characterize, and understand resistance patterns among bacteria in fish ponds, thereby providing insight into effective management strategies.

The development of antibiotic resistance in bacteria within fish pond ecosystems occurs primarily through genetic mutations or the acquisition of resistance genes via horizontal gene transfer (HGT). HGT, facilitated by mobile genetic elements such as plasmids, transposons, and integrons, allows bacteria to rapidly acquire and disseminate resistance traits across species (Lachmayr *et al.,* 2019). This genetic exchange is particularly concerning in aquaculture environments where bacterial populations are under constant selective pressure due to the presence of antibiotics. For instance, studies have documented the presence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and carbapenem-resistant *Klebsiella pneumoniae* in aquaculture settings, highlighting the diversity and complexity of resistance mechanisms at play (Zhang *et al.,* 2019).

The potential for ARB to persist and accumulate in fish pond sediment further complicates the situation. Sediments act as a repository for both antibiotic residues and ARB, creating conditions for sustained resistance gene exchange among microbial communities. Sediments in fish ponds have been shown to harbor high levels of ARGs, which can persist even in the absence of antibiotic exposure, indicating the resilience and adaptability of ARB in these environments (Wang *et al.,* 2019). This persistence is particularly problematic in semi-closed pond systems, where water exchange is limited, and bacteria are more likely to encounter and acquire ARGs over time.

The presence of ARB in aquaculture settings has direct and indirect implications for public health. The consumption of fish contaminated with ARB poses a direct risk, as these bacteria may be transferred to human microbiota, increasing the likelihood of antibiotic-resistant infections. Additionally, aquaculture environments facilitate the spread of ARGs to human-associated bacteria via waterborne transmission or through the handling and processing of fish (Almeida *et al.,* 2021). Studies have demonstrated that ARB such as *Salmonella*, *Aeromonas*, and *Pseudomonas* isolated from fish ponds exhibit resistance to multiple antibiotics, including those commonly used in human medicine, thus complicating treatment options and increasing the risk of morbidity and mortality (Zhou *et al.,* 2021).

Moreover, the discharge of water from fish ponds into natural water bodies can disseminate ARB and ARGs beyond aquaculture facilities, impacting broader aquatic ecosystems and public water supplies (Narayanasamy *et al.,* 2019). The environmental transmission of ARGs underscores the interconnectedness of human, animal, and environmental health, as addressed under the One Health framework, which seeks to address antibiotic resistance from a holistic perspective (FAO, 2019).

The primary objective of this study is to isolate and characterize antibiotic-resistant bacteria from fish pond ecosystems, focusing on both water and sediment samples.

Materials and Methods

The Study Location

The study was conducted in Osun State, in the South western geopolitical zone of Nigeria. It covers a total land area of about 14,875 km^2. It is bounded in the East by Ondo and Ekiti States, in the West by Oyo State while Kwara and Ogun States are respectively its boundaries in the North and the South (Osun State Government diary, 2005). Federal Office of Statistics (FOS) (2007) reported that Osun State has a population of 3,423,535 people as at 2006 National Census. Osun State has thirty Local Government Areas (LGAs) with several towns, villages and settlements. Osun State housed the Yoruba ethnic group with some dwellers from other parts of the country and even outside the country. The Southern part of Osun State is a rain forest zone with a mean annual rainfall of 1420 mm; the North is a derived Savanna with a mean annual rainfall of 1133 mm. The primary occupation of the people in Osun State is farming as obviously seen in its numerous villages. The climatic condition of Osun State favours the growth of varieties of plants including arable and permanent crops such as locust bean, maize, cassava, groundnut, sweet potatoes, palm trees, cocoa trees etc. Also, it accommodates rearing of livestock and fishery products. (Figure 1).

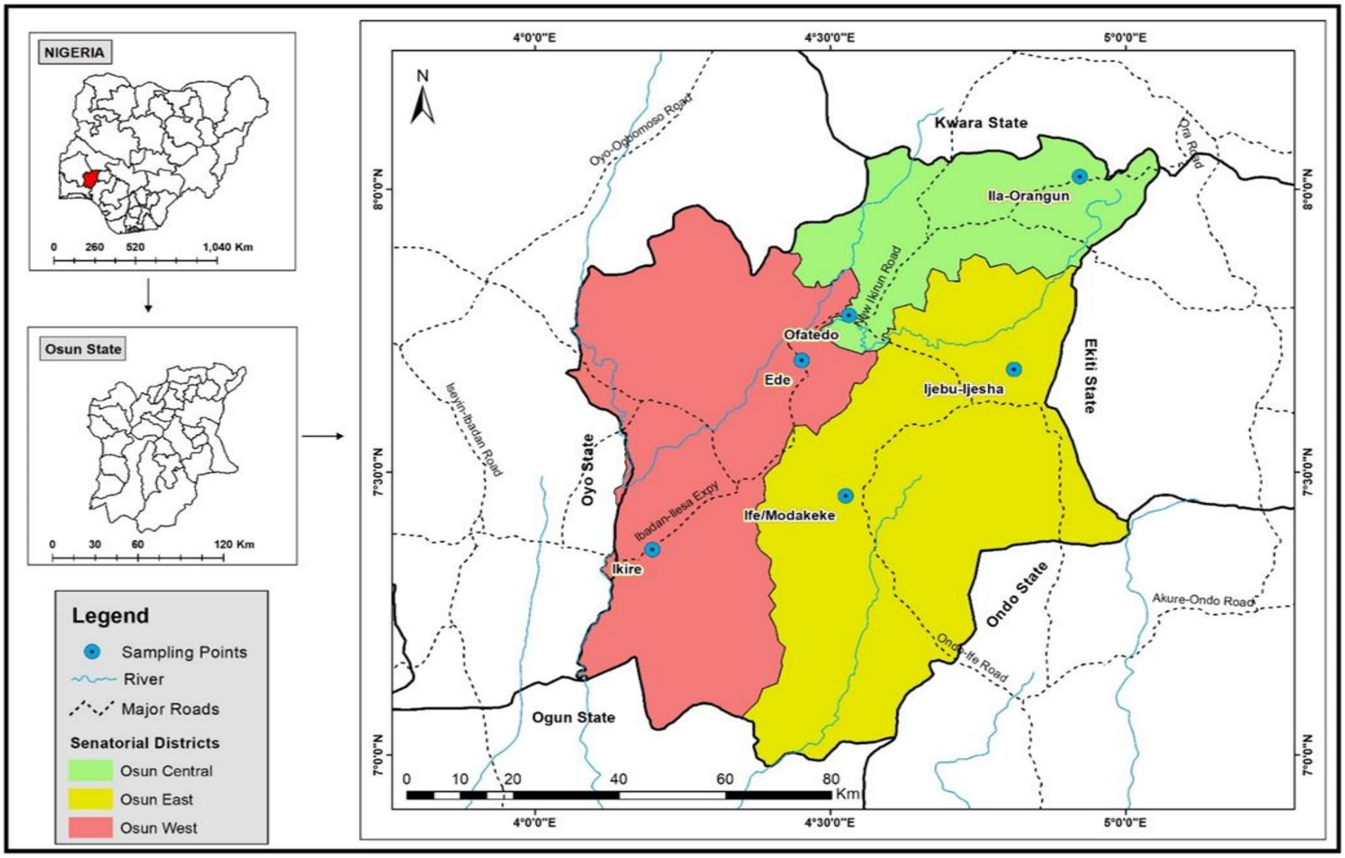


Figure 1: Map of Osun State Showing Sampled Locations

Study Design and Sample Collection

Sample Collection for Laboratory Analysis

The study focused on small-to-medium sized fish farms that were actively producing fish, purposively selected and situated in Osun State's three (3) main senatorial districts (Osun Central, Osun East, and Osun West). Two Local Government Areas were carefully selected from each of the senatorial districts designated as A to F. (With an average stocking density of 5–10 fingerlings/, Cat fish and Tilapia/ m^2, the surface areas of the ponds were 6–20 m^2 and 2.16 m^2, respectively, for concrete and plastic tanks and 5 00m^2 for the earthen pond.). A total of Sixty (60) water samples and Sixty (60) sediment samples were aseptically collected from the ponds using labelled-sterile screw-sample bottles, which includes ten (10) sampling areas within each of the six (6) selected Local Government Areas in Osun State. Composites samples were obtained by collecting at depths of 30 cm below the water surface for water samples and 60 cm below for sediment from concrete ponds, earthen ponds, and plastic ponds, respectively. The sampling was conducted between 9:00 am and 12:00 pm on each day of sampling. Sampling activities spanning between months of December, 2022 to March, 2023 respectively. The water and sediment samples were transported in a box containing ice packs to the laboratory for microbiological and biophysical analysis.

Laboratory Analysis

Serial Dilution

One millilitre (ml) of the water sample was dispensed with a sterile pipette and transferred aseptically into 9 ml of sterile distilled water. For each water sample, four test tubes containing 9 ml of sterile distilled water arranged in test tube racks was used. The test tubes were labelled 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. From the test tube 10^{-}

^{1}, 1 ml was aseptically transferred into the test tube labelled 10^{-2} using another sterile pipette. The tube was agitated for even distribution and 1 ml was also transferred aseptically from tube 10^{-2} into tube 10^{-3}. This was repeated for test tube labelled 10^{-4}. From each water sample, 1 ml of 10^{-2} – 10^{-4} dilutions were poured into sterile plate (Pour Plate Method).

Media

The following were the main media used for investigation: Salmonella/Shigella agar (SSA) and MacConkey Agar, Eosin Methylene Blue (EMB), Nutrient Agar (NA). They were used for Enumeration of the microbial load of the fish pond water and sediment samples for colony count by serial dilution and spread plate technique. Also, Mueller Hinton Agar (MHA) for antibiotic susceptibility. After preparation, the plates of Agar inoculated with samples were incubated at 37ºC for 24h. The isolates were then sub-cultured on a prepared sterile nutrient agar slant and stored in a refrigerator.

Isolation on Selective Media

The selected colonies were sub cultured on fresh nutrient agar plates by streaking carefully on the medium using a sterile inoculating loop. For each set of streaks made, the inoculating loop was flamed so that distinct colonies of the organisms were obtained. The plates were inverted and incubated at 37 ºC for 24 h, after which the plates were checked for growth. Uniformity in growth as well as appearance of distinct colonies on the plates indicated pure isolates. Once pure isolates were obtained, fresh nutrient agar slants are prepared in McCartney bottles. After the sterilization, the bottles were arranged such that they lay on their sides so that slants are formed, a colony of each isolate was aseptically transferred unto the slants by streaking it on the slant bottles and then placed in an incubator for 24 h which they were kept in the fridge at 4 ºC.

Identification of the Isolates

The bacterial isolates were characterized using colonial, morphological and biochemical tests. The isolated colonies were further identified using Bergey's manual of determinative bacteriology (2012). (Whitman *et al.,* 2012). The *in vitro* antibiotic susceptibility test was carried out using the disc diffusion assay by Bauer *et al.* (1966), and the zones of inhibition were measured, compared and interpreted using manual of Clinical Laboratory Standard Institute, (CLSI, 2023).

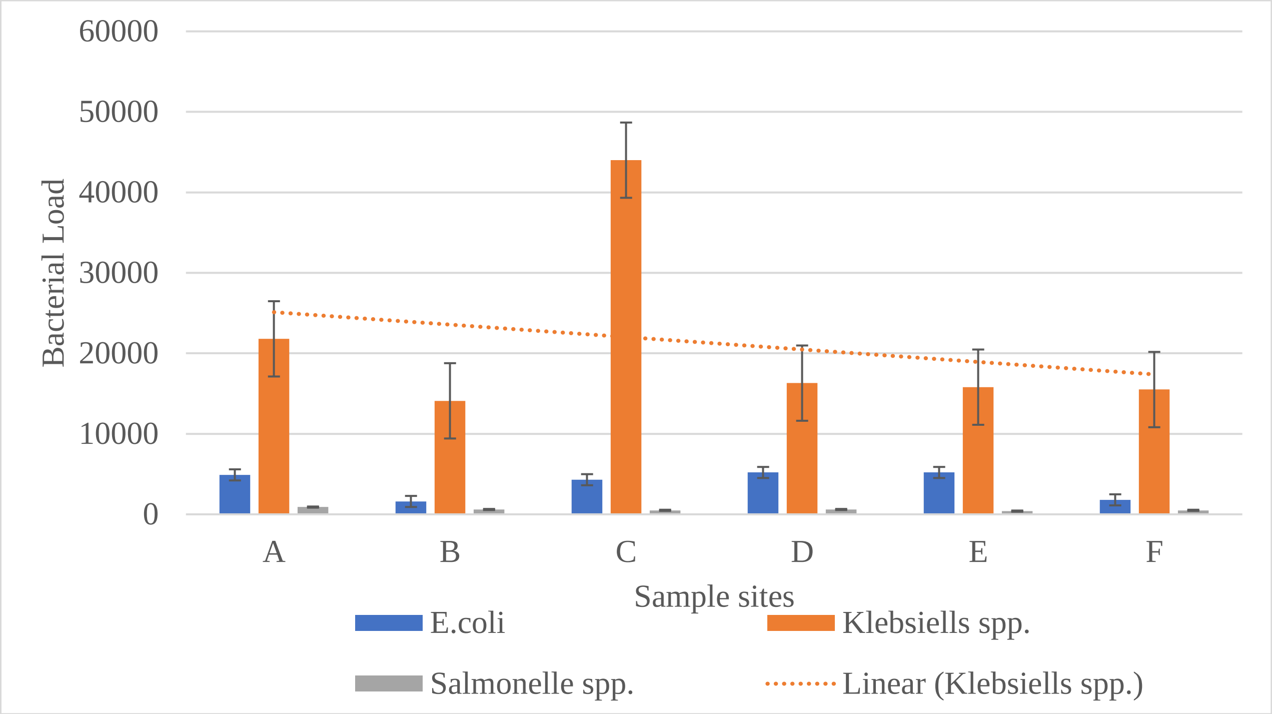
Statistical Analysis of Data

The data obtained were analyzed using appropriate statistical methods including descriptive statistics, inferential statistics and cross tabulation using SPSS (version 17). Chi-square test was used to determine association between categorical variables at a significant level of $p < 0.05$.

Results

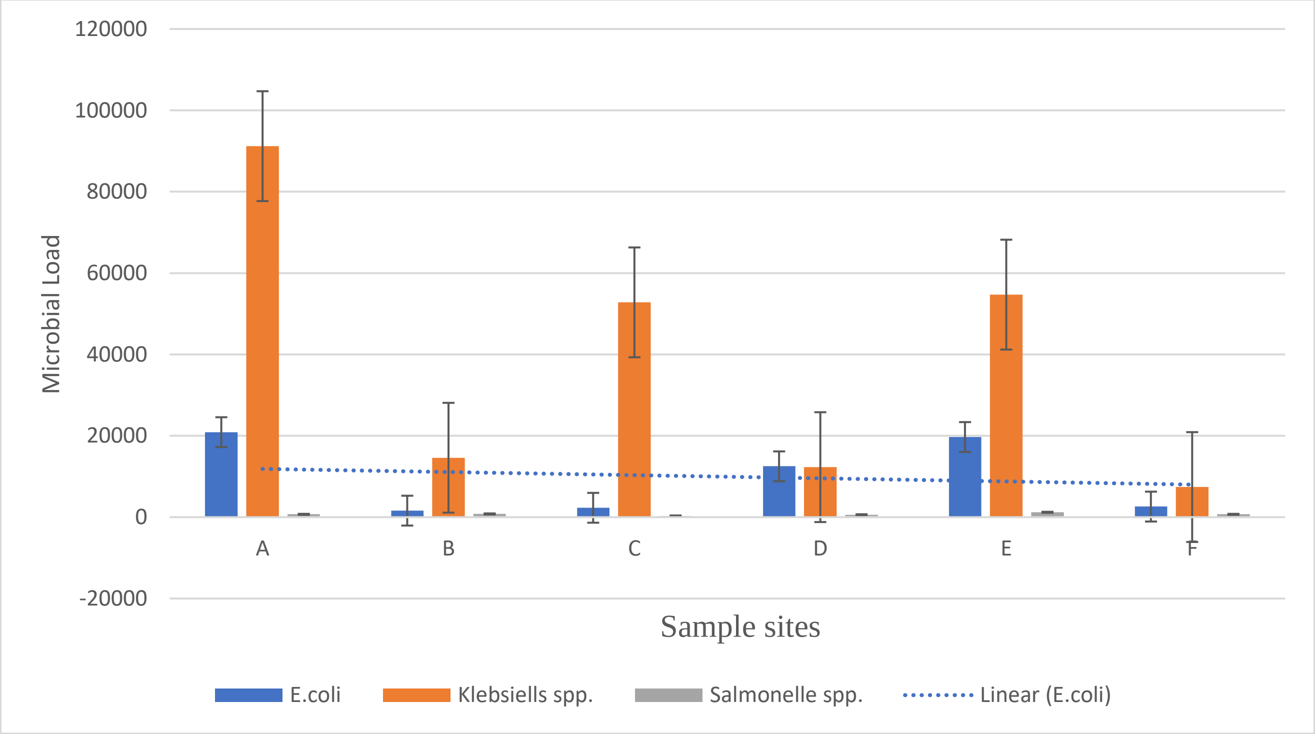
The microbiological analysis of water samples collected from various fish ponds indicated a high microbial load that varied across different locations. The study also detected the presence of coliform and fecal coliform bacteria in the fish pond water samples. As shown in Figure 2 the distribution of bacterial loads demonstrated significant differences among the bacterial species across the six sampling sites, with a statistical significance indicated by ANOVA ($p = 6.66954E-06$).

At each sampling site, the quantity of *E. coli* varied, with counts ranging from 1,600 CFU/mL to 5,200 CFU/mL. Sample site D had the highest load of *E. coli* at 5,200 CFU/mL, while sample site F had the lowest count at 1,800 CFU/mL. The levels of *Klebsiella spp*. ranged from 14,100 CFU/mL to 44,000 CFU/mL across the different sites. Sample site C recorded the highest load of *Klebsiella spp*. at 44,000 CFU/mL, while sample site B had the lowest at 14,100 CFU/mL. *Salmonella spp.* showed relatively stable levels across the sampling sites, with counts ranging from 400 CFU/mL to 900 CFU/mL. Sample site A had the highest count of *Salmonella spp.* at 900 CFU/mL, whereas sample site E recorded the lowest at 400 CFU/mL



Key: A (Ife East LGA), B (Oriade LGA), C (Ede South LGA), D (Irewole LGA), E (Olorunda LGA), F (Ila LGA)

Figure 2: Bacteria Load Distribution in Fish Pond Water Sample in Selected Local Government Areas in Osun State



Key: A (Ife East LGA), B (Oriade LGA), C (Ede South LGA), D (Irewole LGA), E (Olorunda LGA), F (Ila LGA)

Figure 3: Bacteria Load Distribution in Fish Pond Sediment Samples in Selected Local Government Areas in Osun State

Significant differences in the microbial load in the fish pond sediment across the bacterial isolates at the sample sites were found in this study (Figure 3). The six sample locations' average bacterial counts (CFU/mL) for *Salmonella spp., E. coli, and Klebsiella spp*. Sample sites B and A had mean counts of 1.60E+03 CFU/mL and 2.09E+04 CFU/mL, respectively, for *E. coli.* Sample site B had the lowest mean *E. coli* count while sample site A had the highest mean count. Sample sites F and A had mean counts of 7.40E+03 CFU/mL and 9.12E+04 CFU/mL, respectively, for *Klebsiella spp.* Sample site F displayed the lowest mean count, whereas sample site A had the highest. Regarding *Salmonella spp*., the mean counts varied from 3.00E+02 CFU/mL (sample site C) to 1.20E+03 CFU/mL (sample site E). Sample site E exhibited the highest mean count, while sample site C had the lowest count. An analysis of variance (ANOVA) was performed. The ANOVA results indicated a statistically significant difference in the microbial load among the three bacteria (*E. coli, Klebsiella spp*., and *Salmonella spp*.) with a p-value of 0.002464. It is important to note that the presence of these bacteria in the pond sediment ecosystem raises concerns for human and environmental health.

The macroscopic examination for physical, morphology and Biochemical characterization of isolates from both fish pond water and sediment were performed based on the size, colour, texture, pigmentation, odour and consistency while microscopic examination was carried out through Gram staining and biochemical tests were also carried out according to Cheesbrough (2006) and Forbes *et al.* (2002) of the bacterial isolates from the samples are presented. The bacterial species identified include species of *E. coli* (*Escherichia coli*) *Klebsiella spp*., *Salmonella*, *Proteus*, *Pseudomonas spp., Enterobacter spp*., *Citrobacter, Serratia, Shigella*, *Vibrio* and finally *Providencia spp.*

The frequency analysis of bacterial isolates as observed in Table 1 revealed variations in their presence across the six sample sites. A total of eleven (11) genera of Gram-negative bacteria were isolated from the ponds and were identified using the using Bergey's manual of determinative bacteriology (2012 & Whitman *et al.*, 2012) with the following percentage occurrence. *E. coli* exhibited the highest frequency, being present in all six sample sites, with a frequency of 22 isolates, accounting for 22.92 % of the total bacterial isolates. *Klebsiella spp*. also showed a high frequency, being present in all sample sites, with a frequency of 18 isolates, accounting for 18.75 % of the total bacterial isolates. *Salmonella spp*. was found in five out of the six sample sites, with a frequency of 14 isolates, representing 14.58 % of the total isolates. Other bacterial species showed varying frequencies. *Proteus spp*. and *Pseudomonas spp*. were found in multiple sample sites, with frequencies of 13 isolates (13.54 %) and 7 isolates (7.29 %), respectively. *Enterobacter spp*., *Vibrio spp*., *Citrobacter spp*., *Serratia spp*., and *Shigella spp*. were less prevalent, with frequencies ranging from 2 to 7 isolates (2.08 % to 7.29 %)

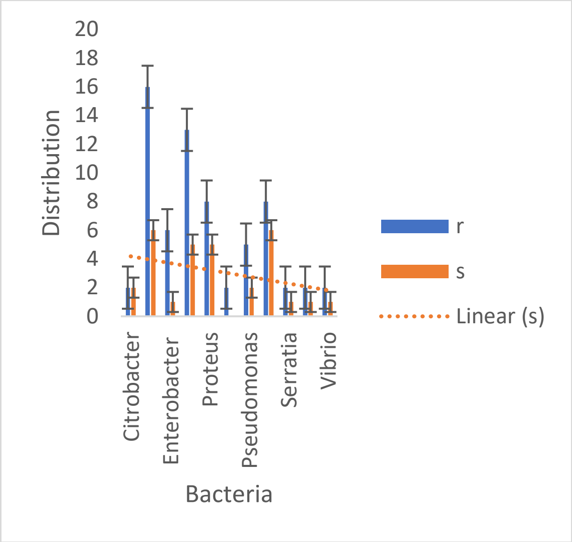
Table 1: Frequency of Bacteria Isolated from the Fish Pond Water and Fish Pond Sediment Samples

Sampled site Bacteria	1	2	3	4	5	6	Frequency	% Frequency
Vibrio spp	+	-	-	+	-	-	3	2.88
Citrobacter spp	-	-	-	+	+	-	4	3.84
E. coli	+	+	+	+	+	+	22	22.92
Enterobacter spp	+	-	+	-	-	-	7	7.29
Klebsiella spp	+	+	+	+	+	+	18	18.75
Proteus spp	+	+	-	-	+	-	13	13.54
Providencia spp	-	-	-	+	-	+	2	2.08
Pseudomonas spp	+	+	-	-	-	-	7	7.29
Salmonella spp	+	+	-	+	-	+	14	14.58

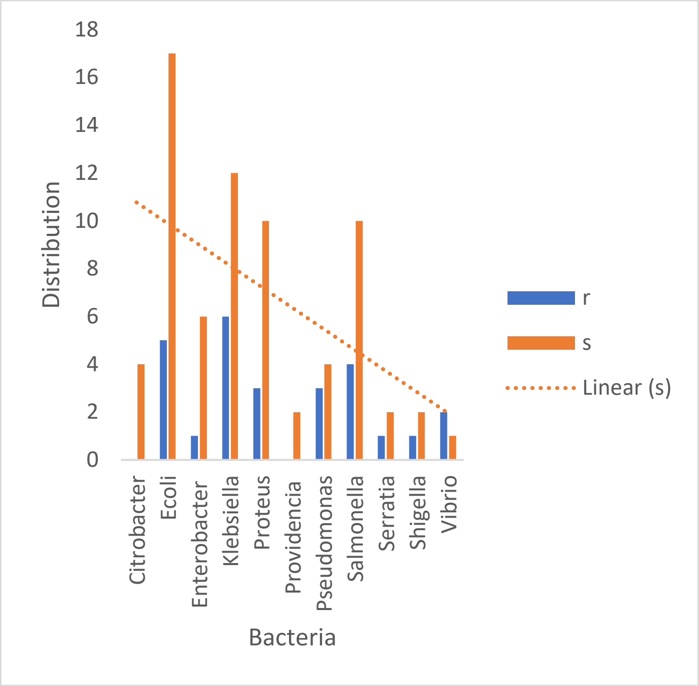
Serratia spp	-	+	+	-	-	-	3	3.13
Shigella spp	-	-	+	+	-	+	3	3.13

According to Figure 4, the bacteria with the lowest rates of resistance to the antibiotic Cefotaxime were *Shigella spp., Vibrio spp., Citrobacter spp., Providencia spp.* and *Serratia spp.,* but *Escherichia coli* followed by *Klebsiella spp., Proteus spp., and Salmonella spp* exhibited higher resistance to Cefotaxime. The 90 % bacteria isolated showed high susceptibility to Tetracycline as shown the least susceptible organisms were *Vibro spp.,* but *Klebsiella spp*. demonstrated the highest resistance to the drug, followed by *E. coli* and *Enterobacter Shigela spp.* and *Seratia spp. Proteus spp., Salmonella spp., Klebsiella spp*., and *E. coli* had the greatest resistance to Ceftazidime, whereas *Providencia spp*. and *Seratia spp.* had the lowest resistance. However, the organisms' susceptibility to the antibiotics was reduced. *E. coli* was more susceptible to Cotrimoxazole than *Klebsiella, Samolnella,* and *Proteus species,* with the least amount of susceptibility to *Vibrio species*. However, only certain strains of *Klebsiella, Enterobacter*, and *Providencia* did not exhibit resistance. Furthermore, *E. coli* has the highest gentamicin resistance, followed by *Klebsiella and Salmonella* species, however *Klebsiella* species also demonstrated the highest gentamicin susceptibility. The *E. coli* showed highest susceptible to Cefuroxime followed by *Salmonella spp* and *Klebsilla spp***.** with least in *Seratia spp***.** But *Klebsilla spp*. has highest resistance to Cefuroxime followed by *E. coli* and lowest showed in *Vibrio spp.* The most vulnerable to chloramphenicol is *Escherichia. coli,* which is followed by *Salmonella* and *Klebsiella* species, and the least susceptible are *Prividencia* and *Seratia* species. However, the bacteria with the greatest resistance to chloramphenicol were *Klebsiella spp., E. coli,* and *Vibrio spp*. The *E. coli* showed highest susceptibility pattern against Ceftriaxone followed by *Klebsiella spp and Proteus spp*. with the least in *Vibrio spp*. But *Salmonella spp* showed highest resistance against Ceftriaxone followed by *Klebsiella spp*. and least were in both

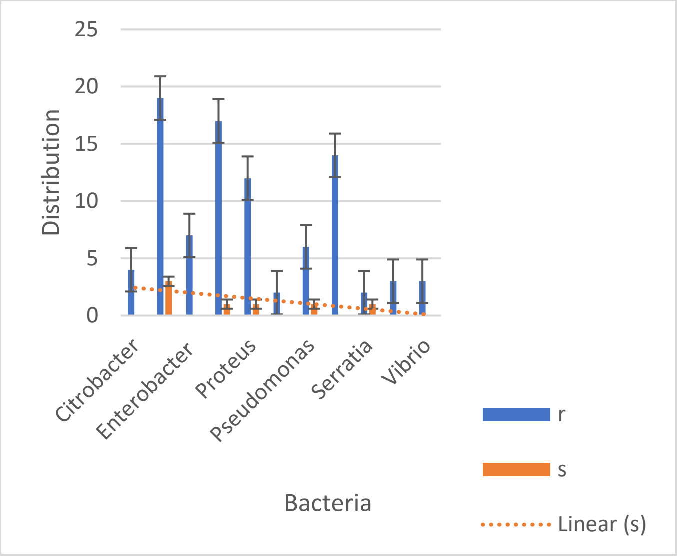
Fig 4 : *Citrobacter spp* a nd *Providencia spp*.



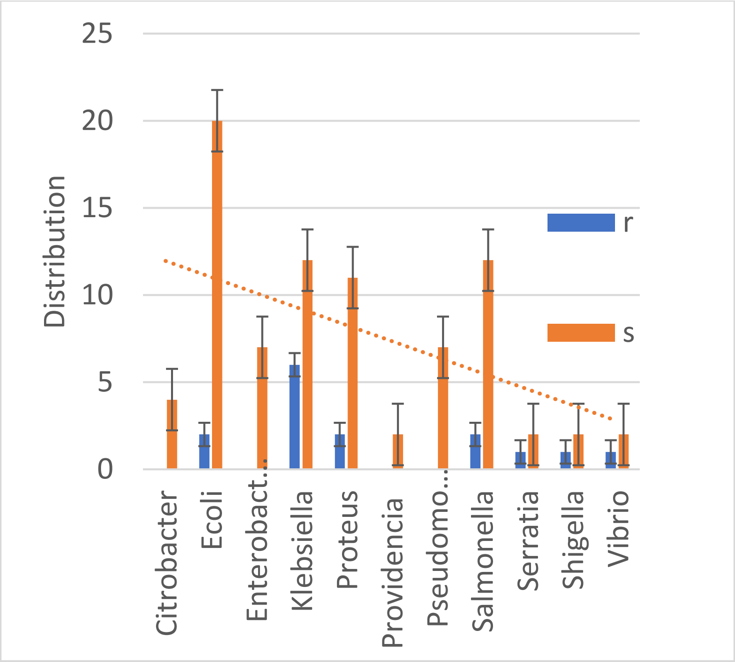
Cefotaxime

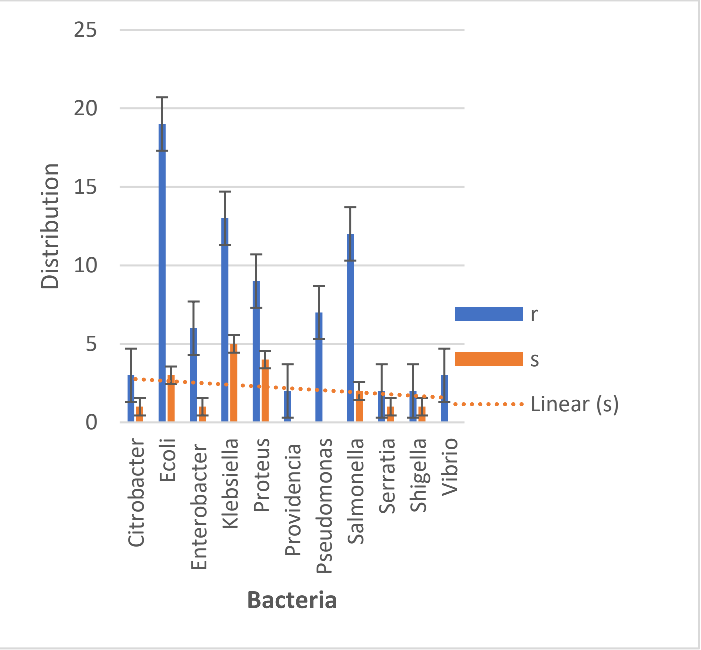


Tetracycline



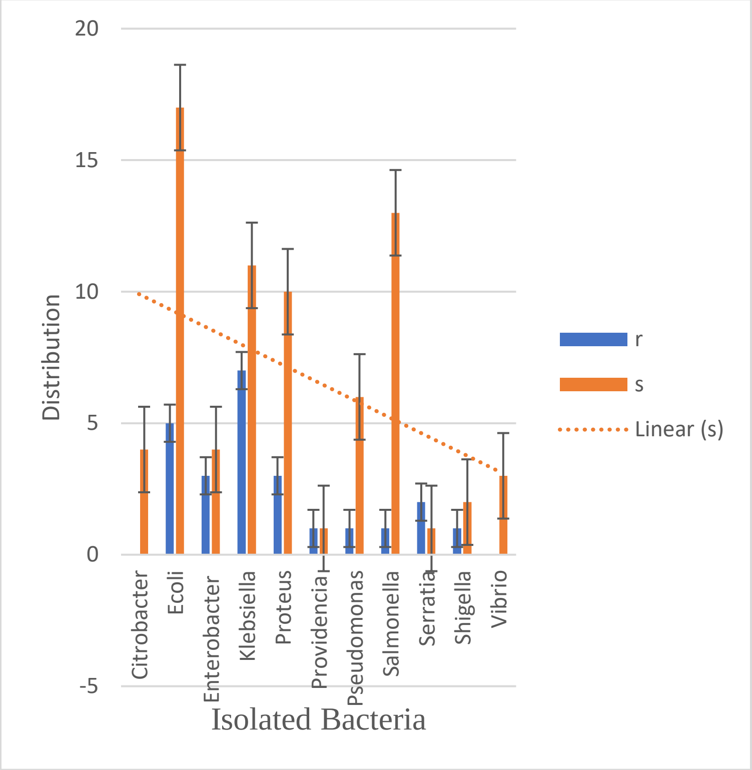
Ceftazidime

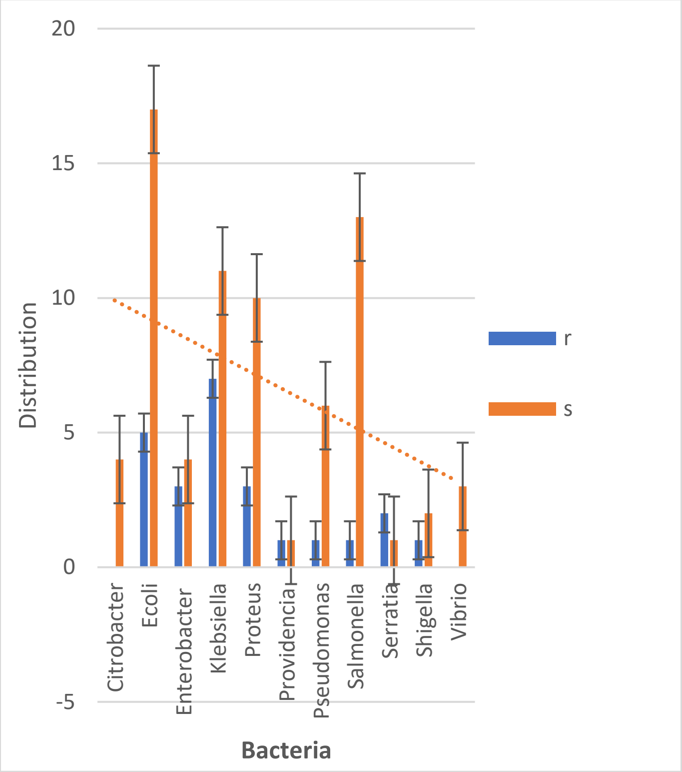




Cotrimoxazole

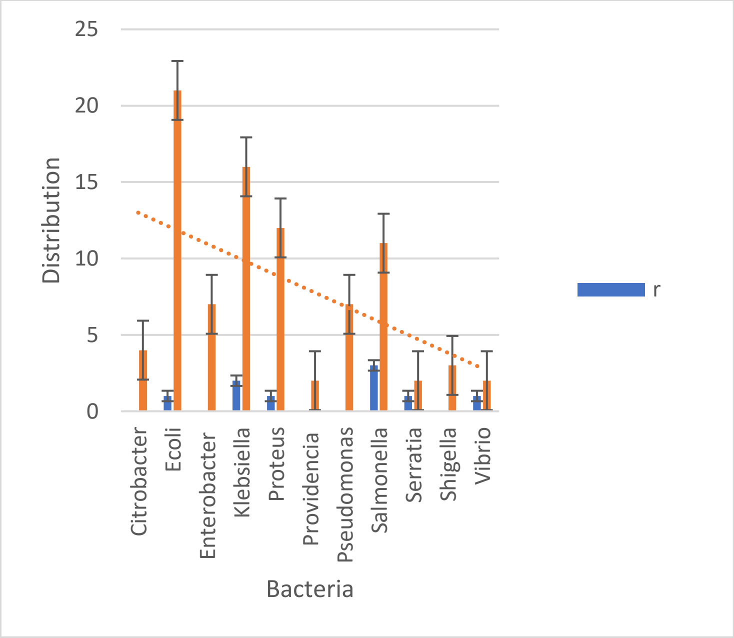
Gentamicin

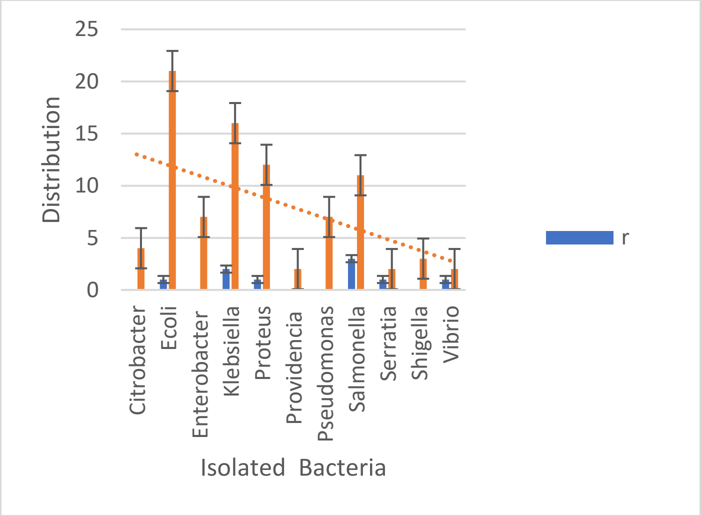




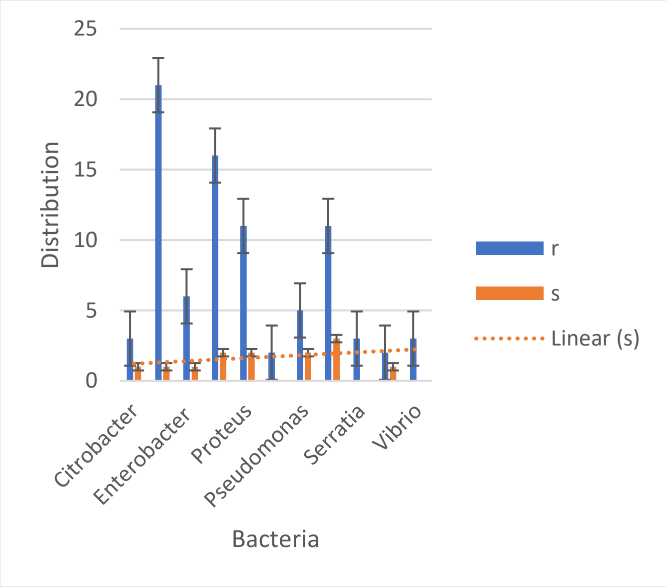
Cefuroxime

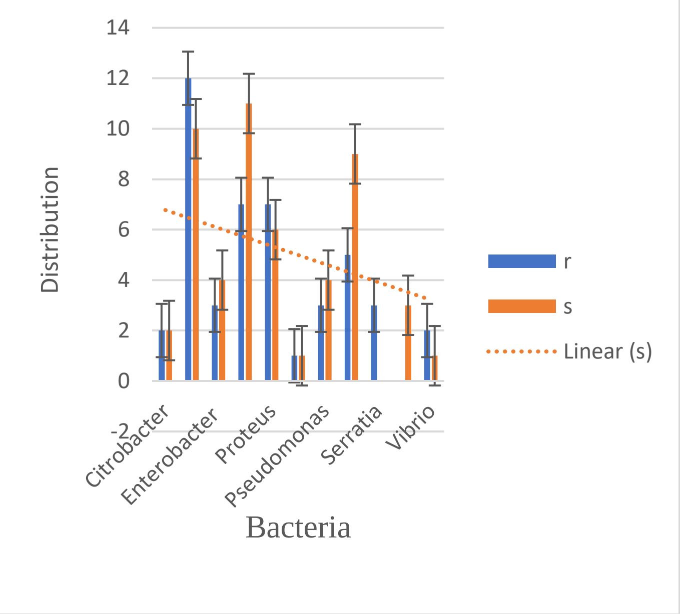
Chloramphenicol





Ceftriaxone **Ciprofloxacin**





Vancomycin Amikacin

The *E. coli* has highest Susceptible pattern against Ciprofloxacin followed by *Klebsiella spp, Proteus spp and Salmonella spp.* and least was in *Vibrio spp*. Although, *Salmonella spp* still showed highest resistance against Ciprofloxacin but least resistance in *Citrobacter spp.*The resistance of all the isolated bacteria to vancomycin was evident, where *E. coli* had the strongest pattern of resistance, followed by *Klebsiella spp.* and *Shigella spp.* with the lowest. However, *Salmonella* species showed the highest susceptibility to vancomycin, whereas *Vibrio* species showed the lowest. *Escherichia coli* has highest resistance pattern against Amikacin followed by *Klebsiella spp* and *Proteus spp*. and least resistance was recorded in *Providencia spp* while *Klebsiella spp* showed highest susceptibility and least was in *Seratia spp*.

DISCUSSION

This high bacterial load of fish pond water indicated that a huge number of bacteria were present in the water and deteriorated the water quality to a great extent. The total coliform count observed throughout the study period was always much higher than the standard values (WHO 2018). The results of water samples from different fish ponds within the three senatorial districts indicate high enteric bacterial counts which reflects those coliform counts per unit sample sources showed high levels of contamination. According to Verbyla *et al.* (2018) prevalence of enteric bacteria, including coliforms, in water from aquaculture systems, highlights the high contamination levels observed. Besides, the presence of waterborne pathogens, including enteric bacteria and coliforms, in aquaculture environments globally, emphasizes the high levels of contamination (Xu, 2021). The factors are likely to be fish feed on some microorganisms, high level of contamination with the presence of these indicator organisms could be alarming and could be linked to poor fish pond practices management. Based on the anthropogenic activities observed in the study area the increased microbial infiltration is possibly due to faecal contamination either of animal or human origin. According to Khan and Ghazali (2021), good water quality for fish pond management practices is vital to get improved quality fish yields, decreased mortality and lessen possible disease outbreak. Kasing Apun *et al.* (2010) reported that higher counts could be attributed to the possibility of higher input of domestic and human sewage into the rivers that supplied water to these ponds. From this study, it was noted that buildings were situated upstream of most of the rivers, and there is no doubt that there might be a certain amount of contamination such as domestic and human sewage, especially from the traditional toilets and also seepage from septic tanks into the rivers that flowed into the some of the earthen ponds. Sun *et al.,* 2021 revealed that upstream land use, including the presence of buildings and inadequate waste management practices, can lead to the contamination of rivers and impact downstream water bodies, such as ponds.

The microorganisms isolated from the ponds were *E. coli, Salmonella typhi, Staphylococcus aureus, and Klebsiella pneumoniae* which may be pathogenic to various aquatic life and may pose a threat to the health of the fishes, aquatic systems, and to the consumers. Recent research highlights the long-term persistence of bacterial pathogens in fish pond water and sediments, such as *Vibrio species, Salmonella species,* and *Aeromonas* species. Through direct fish ingestion or indirect water source contamination, these diseases can spread throughout the food chain (Carvalho *et al.,* 2018; Santos *et al.,* 2019; Vasconcelos and Aguiar, 2020).

It is important to note that the presence of these bacteria in the pond sediment ecosystem raises concerns for human and environmental health. The high counts of *E. coli* and *Klebsiella spp*., indicate a potential health hazard, as these bacteria were known pathogens. *Salmonella spp*., although present at lower counts, should not be overlooked due to its pathogenic nature. These findings underscore the heterogeneity of microbial load across the sample sites within the fish pond sediment ecosystem i.e. the possibility of bacterial contamination in aquatic environments is indicated by higher microbial counts for *Salmonella spp., E. coli, and Klebsiella spp.* This result aligns with recent research findings that suggest the rise in bacterial loads in pond sediments is frequently ascribed to the quantity of organic debris, which functions as a source of nutrients for a variety of bacteria. For example, Nguyen *et al.,* (2019) study discovered that nutrient-rich sediments in aquaculture ponds greatly enhance the growth of pathogenic bacteria, raising the possibility of bacterial contamination. Furthermore, Das *et al.* (2020) highlighted how organic matter in pond sediments fosters the growth of bacteria, including gastrointestinal diseases like *Salmonella spp.* and *E. coli* that are dangerous to human health as well as aquatic life. According to recent research, introducing organic enrichment into fish ponds can alter the benthic environment significantly and boost the number of bacteria living there. This is consistent with research done by Kumar *et al.* (2018) showed that adding more organic matter to aquaculture ponds drastically changed the properties of the sediment, which in turn encouraged the growth of bacteria, especially harmful species. Chen *et al.* (2019) found a direct correlation between the amounts of organic carbon and the multiplication of microorganisms in sediments. This phenomenon can result in elevated bacterial counts and pose a threat to the safety of food and seafood.

Escherichia coli was found to be the most common bacterium in fish pond water and sediment at all six sampling locations in the study area, with a total frequency of 22.92%, according to the analysis. Significant questions concerning water quality and possible pollution in aquaculture areas are brought up by this common occurrence. The use of contaminated water sources for fish farming, fecal contamination, and inadequate hygiene procedures are frequently linked to the occurrence of *E. coli* in fish ponds. Akinbowale *et al*. (2018) have reported similar results in previous research, where *E. coli* has been regularly isolated from aquaculture habitats as a result of anthropogenic activities such as inappropriate waste disposal and runoff from nearby agricultural lands. Notably, several studies have reported the occurrence of *E. coli* in aquaculture environments. For example, a study in China demonstrated that *E. coli* was present in fish ponds due to the extensive use of organic fertilizers and poor wastewater management, leading to the contamination of water bodies (Zhou *et al.,* 2020).

The presence of *Klebsiella* species in aquaculture environments has been linked to fecal contamination and poor hygiene in pond management. For example, a study by Igbinosa and Okoh (2019) in Nigeria found high levels of *Klebsiella* in fish ponds due to the inflow of untreated wastewater and runoff from nearby livestock farms, further contaminating water sources. This aligns with other international research, such as Singh *et al*. (2020) in

India, who reported frequent isolation of *Klebsiella* in aquaculture systems due to poor sanitation and water recycling practices.

Salmonella species are also commonly associated with contamination in aquaculture environments. These bacteria are pathogenic to both fish and humans, raising serious public health concerns. A study in Bangladesh by Rahman *et al.* (2019) confirmed the presence of *Salmonella* in fish ponds, primarily due to contamination from surrounding agricultural lands and improper handling of fish feed. Besides, Onyango *et al*. (2021) in Kenya reported similar findings, where fish ponds were contaminated by runoff from nearby farms using untreated manure as fertilizer, a common practice in many rural areas.

Another pathogenic bacterium that shows high frequency in both fish pond water and sediment was *Pseudomonas* species, known for their environmental adaptability and resistance to antibiotics, which were also frequently detected. Studies show that *Pseudomonas* can thrive in polluted waters, with a high organic load contributing to their prevalence. Nwosu *et al.* (2020) reported that a significant presence of *Pseudomonas* species in fish ponds is attributed to inadequate water filtration systems and the accumulation of organic matter. Similarly, Wang *et al*. (2020) in China reported that the frequent isolation of *Pseudomonas* in aquaculture environments is linked to nutrient-rich wastewater discharge from nearby industries.

Proteus and *Citrobacter* species are opportunistic pathogens commonly associated with environmental contamination. In Nigeria, a study by Adebayo and Ojo (2018) revealed high levels of *Proteus* and *Citrobacter* in fish ponds due to inadequate sanitation and the use of untreated water from surrounding communities. Chang *et al.* (2019) reported a similar in Taiwan linking the contamination to agricultural runoff and poor waste disposal practices in nearby aquaculture facilities.

The work of Mukherjee *et al.* (2019) revealed that the widespread use of third-generation cephalosporins like Cefotaxime in both human and veterinary medicine, most especially in aquaculture, has contributed to the development of resistant bacterial strains. This is consistent with the high resistance of *Escherichia coli* to Cefotaxime, which is followed by *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. because antibiotics are frequently used in fish ponds to treat and prevent infections, which puts resistant bacteria like *Escherichia coli* and *Klebsiella spp.* under selective pressure to survive. As per the findings of Xiong *et al.* (2018), the emergence of resistant bacterial strains in fish ponds has been further facilitated by the absence of stringent limits on antibiotic usage in aquaculture. Uncontrolled use of antibiotics, such as cefotaxime, in fish farming has been associated with the emergence of multidrug-resistant bacteria, such as *Escherichia* coli and *Klebsiella* spp., that are resistant to third-generation cephalosporins.

According to a study done in Southwest Nigeria, *Salmonella spp., Klebsiella spp.,* and *Escherichia coli* bacterial isolates from fish pond habitats showed a high susceptibility to Tetracycline. Tetracycline has been the go-to medication for fish illnesses among fish farmers as a result (Ogunbanwo *et al.,* 2020). Tetracycline in fish pond water and sediment environments, leading to its frequent use by fish farmers to treat infections may be as a result of tetracycline is known for its broad-spectrum activity against various Gram-negative and Gram-positive bacteria, making it highly effective against common fish pathogens such as *Shigella* spp., *Vibrio* spp., and *Citrobacter* spp. and others. The continued susceptibility of these bacteria to tetracycline in fish pond environments has reinforced its use as a first-line treatment for bacterial infections by fish farmers (Li *et al.,* 2020). Tetracycline is frequently used because fish farmers frequently see a quick response to its therapy in managing bacterial infections, according to Kumar *et al.* (2019).

The frequent use of ceftazidime in aquaculture may cause resistant bacterial strains to emerge. Because they can acquire resistance genes through mechanisms like horizontal gene transfer (HGT) and selective pressure from continuous antibiotic exposure*, Proteus spp., Salmonella spp., Klebsiella spp.,* and *E. coli* are among the most frequently identified resistant pathogens (Li *et al.* , 2020; Adebayo *et al.* , 2019). Furthermore, biofilm formation in fish ponds is another important factor that increases bacterial resistance to antibiotics like ceftazidime, according to Zhang *et al.* (2020). Because of its potency against a wide range of bacterial pathogens, including *Escherichia coli, Klebsiella spp., Proteus spp., Salmonella spp., Vibrio spp.,* and others, ciprofloxacin, a fluoroquinolone, has been used extensively in aquaculture. Environmental elements including pH, temperature, and the amount of organic matter in pond water and sediment can affect how effective ciprofloxacin is, according to Chukwu *et al. 's* (2019) research. The high resistance of common enteric bacteria such as *Escherichia coli*, *Salmonella spp.*, *Vibrio spp.*, and *Citrobacter spp.* against antibiotics like gentamicin and vancomycin in fish pond water and sediment is an area of significant concern in aquaculture. According to the study of Adeyanju *et al.* (2020) who stated that the abuse and misuse of antibiotics such as vancomycin and gentamicin in fish farming is one of the main causes of high resistance patterns. These antibiotics are frequently used without sufficient dosage restriction or veterinary oversight in an indiscriminate manner to treat illnesses, promote growth, or avoid disease.

CONCLUSION

The fact that these bacterial species are present in all sites highlights the necessity of better aquaculture management practices to avoid contamination. The results show that several issues, such as fecal contamination, runoff from agricultural lands, inadequate waste management, and antibiotic usage, are compromising the quality of the water in fish ponds. This emphasizes how crucial it is to monitor and regulate to protect aquatic ecosystems and public health.

The finding revealed that there was a growing issue of antibiotic-resistant bacteria in aquatic ecosystems showed a complex interplay between antibiotic use, environmental factors, and the emergence of resistance which was as a result direct consequence of anthropogenic activities such as the misuse of antibiotics in aquaculture from agricultural practices and improper disposal of antibiotics.

RECOMMENDATION

Based on these observations, therefore, the safety of the public in this regard through the following recommendations:

- There should be proper construction of fish pond. The environment where the fish ponds are located should be protected from pollutants and weeds which can harbour microorganisms that find ways into fish pond by themselves or by passive process through wind, rainfall, etc. thereby affecting the fishes negatively.
- There is increased need for the regulatory agencies such as National Agency for food and Drug Administration and Control (NAFDAC) to set standard guidelines for the fish industry in Nigeria. The enforcement of such guidelines will also assist the farmers to meet international set standards which can enhance their capabilities to export fish stocks to other countries.
- The sanitary conditions under which fishes are reared or cultured in ponds should be improved by following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things.
- Sanitation within the fish farm should be improved through the provision of wastewater management facilitates for collection and disposal of waste water.

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