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Research Article

Bacteriological Quality of Pond Water Used in Aquaculture in Enugu State, Nigeria

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Abstract: The bacteriological quality of fish pond water is an essential consideration in fish cultivation. Poor quality water can lead to outbreaks of bacterial infections in fish. Fish pond water can be contaminated due to improper location, inadequate sewage treatment, effluents, and agrochemicals from nearby industries and farms, as well as exposure to poor sanitation systems from various households leaching into water sources used in fish farms. Water from a total of ten fish pond was evaluated for the presence of bacterial pathogens. Microbial isolation, identification, and antibiogram screening were performed using standard methods. The result indicates that microbial bacteria were found in all fish ponds water that was assessed. Fecal coliform counts (cfu/ml) show that pond water was contaminated with pathogens due to pollution from fecal sources. The bacterial isolates were identified and the frequency was recorded as *Vibrio* spp. (26.2%), *Klebsiella* spp. (21.4%), *Salmonella* spp. (9.5%); *Staphylococcus* spp. (16.7%); *Escherichia coli* (9.5%) and *Shigella* spp. (16.7%). The isolates were examined for susceptibility against twelve antibiotics namely; Ciproflox, Norfloxacin, Gentamycin, Amoxil, Streptomycin, Rifampicin, Erythromycin, Chloramphenicol, Amicloxz, Levofloxacin, Augumentin and Cotrimoxazole. The result showed the bacterial isolates had varying levels of susceptibility, and were resistant to the antimicrobials. Therefore, it is imperative to assess pond water used in aquaculture systems periodically to ensure the production of quality and safe fish for human consumption.

Keywords: African catfish, fish farming, food safety, microbial contamination, water quality

1. Introduction

Fish farming is a form of aquaculture whereby fish are grown in a controlled environment for commercial and dietary purposes. Fish production by aquaculture is growing, because of the increasing demand for rich sources of protein and the decline of undomesticated catch. There are nutritional benefits from the consumption of fish such as protein, vitamins, and omega-3 fatty acids. Ademola et al. [1] noted that the rising rate of investment in fish production exceeds 8% annually. This production model when sustained would increase food productivity, thereby contributing to ensuring food security.

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The inflow of environmental contaminants into the aquatic system is a frequent occurrence. The pollutants could arise from human activities such as the application of pesticides on crops and the introduction of feeds in the ponds. The increasing introduction of nutrients from farmlands, drains, or inadequate sewage treatment and surface water run-off into water bodies could lead to eutrophication and pollution of such water bodies [2, 3].

Ponds are crucial in the cultivation of fish. The rate of fish production mainly relies on the quality of water used in fish cultivation [4]. Bacteria pathogens are implicated as the most common agents causing diseases in fish which brings immense loss in fish farming [5]. Arifin et al. [6] stated that bacterial pathogens are the main cause of fish infections and mortalities. Bacterial diseases usually come with huge economic losses due to high morbidities and mortalities [6, 7]. Bacterial diseases in fish do not largely occur as primary pathogens in fish but rather as opportunistic pathogens in immunosuppressed hosts [8]. Zaher et al. [8] noted that a significant number of fish diseases are caused by stress factors due to unfavorable environments such as poor water quality. Manifestations of bacterial infections in fish may include deep wounds on the skin penetrating the muscles, head disintegrating, wounds in the stomach, and internal bleeding [9]. The water used in the cultivation of fish usually harbors numerous microorganisms that can endanger the fish, plankton, and humans upon consumption [10]. Pond water is known to contain different strains of bacterial pathogens which can cause diseases [11]. Dietze et al. [12] noted that the administration of antibiotics through feed could lead to incomplete metabolism of fish feed which may leach into the environment and accumulate, resulting in resistance. There are severe public health and economic implications due to the development and spread of resistance to antibiotics by pond bacteria. The infections caused by resistant bacteria could escalate the incidence of disease transmission and death arising due to their adjustment towards different Aqua media. The resistant pathogens may spread to persons and domesticated livestock causing illnesses that cannot be managed by the usual antibiotics. Abuse in the administration of antibiotics and the presence of drug-resistant plasmids is a leading cause of bacteria resistance to antibiotics [13].

Due to the land topography of Enugu state in Eastern Nigeria, there is a scarcity of water supply for both domestic and industrial use. The 9th-mile area is the main source within the Enugu metropolis a reliable source of water for domestic, industrial, and agro-business purposes. This factor has made the 9th-mile area an agricultural and industrial hub within the state of Enugu.

Clarias gariepinus (African giant catfish) is the popular species cultivated in this environment. The successful rearing of *Clarias gariepinus* in Nigeria has been attributed to its ability to survive difficult environmental conditions, and its omnivorous nature [14]. However, numerous fish farms that are sited within the metropolis suffer from poor water quality because water in aquaculture here is contaminated due to animal dung from livestock farming, inadequate sewage treatments, effluents, and agrochemicals, in addition to exposure to poor sanitation systems from various households leaching into water sources used in fish farms thereby leading to the outbreak of bacterial infections among fish. There is a need for regular monitoring of pond water for bacteriological contamination. Additionally, regular examination of pond waters for factors such as heavy metals, nitrate, nitrite, ammonia, pH, watercolor, carbon dioxide concentrations, and plankton population is essential to ensure the quality of water used in fish cultivation. This practice would ensure increased fish production and provision of safe fish which is devoid of various forms of contamination and toxins which could cause harm to the human system upon consumption [15].

Therefore, the importance of pond construction in fish farming cannot be overemphasized. There are six types of ponds constructed for fish farming worldwide which include earthen ponds, concrete ponds, tar paulin ponds, plastic or rubber ponds, fibreglass tanks, and cage or pen ponds. The main sources of water for earthen ponds are frequently contaminated and are commonly surface water runoffs from streams, rivers, and lakes [16]. Meanwhile, underground water is mostly used for fish farming in most concrete ponds. Therefore, the evaluation of the bacteriological quality of water is imperative in fish cultivation.

2. Materials and methods

2.1 *List of materials*

An ice pack, universal bottles, Petri plate, autoclave, inoculating loop, forceps, Bunsen burner, conical flask, electronic weighing balance, plastic pipette, volumetric cylinders, microscope, incubator, beakers, glass slide, meter rule, sterile cotton wool, test tubes, and racks.

2.2 *List of reagents*

Nutrient Agar, MacConkey Agar, *Salmonella*, *shigella* Agar (SSA), Mueller-Hinton agar, Kovac's reagent, Crystal violet stain, Acetone, Safranin, Hydrogen peroxide, Peptone water, distilled water, and normal saline.

2.3 *Study area*

The study area was the 9th-mile Ngwo town of Enugu state. Ten fish ponds from different communities in this zone were investigated. The samples were collected into sterile bottles and labeled and transported to the laboratory immediately for analysis.

2.4 *Collection of water samples*

Three samples of water were harvested from the center point of each of the ten fish ponds that were under investigation. Samples of water were harvested by aseptic procedures at the center points of the pond with the aid of liter sterile screw-capped bottles. The bottles were quickly transferred into an ice pack. The samples were taken to the Microbiology laboratory at Coal City University Enugu and analyzed within 6 hours of collection. The samples were labeled accordingly with the names of the communities (sites) where they were collected.

2.5 *Isolation of bacteria*

The isolation of bacterial pathogens from the water samples was done by spread plate technique using nutrient agar and other selective media such as Eosin methylene blue (EMB) agar, Selenite F broth, Thiosulphate citrate bile sucrose (TCBS) agar, *Salmonella-shigella* (S-S) agar. One milliliter of each water sample was aseptically withdrawn with a sterile pipette and serially diluted in physiological saline to the fourth dilution using a ten-fold serial dilution. A 0.1 ml aliquot of each dilution was inoculated into a duplicate set of nutrient agar and Eosin methylene blue (EMB) agar, to determine the total aerobic heterotrophic culturable bacterial (THCB) population and fecal coliforms respectively. The water samples were also introduced on alkaline peptone water (pH 8.3) and in Selenite F broth and then spread plated on thiosulphate citrate bile sucrose (TCBS) agar and *Salmonella-shigella* (S-S) agar respectively for isolation of *Vibrio* species, *Salmonella* and *Shigella* species. Aliquots from diluted water samples were also plated on Mannitol salt agar (MSA) for the isolation of *Staphylococcus* species. Culture plates were incubated at 35 ℃ for 24 hours. Meanwhile, the EMB plates used for the isolation of fecal coliforms were incubated at 45 ℃ for 24 hours.

2.6 *Purification of isolates*

Distinct colonies were picked from culture plates. Sub-culturing of selected colonies was on nutrient agar plates and subsequently stored on nutrient agar slants in the refrigerator at 4 ℃ for further use.

2.7 *Identification and characterization of bacterial isolates*

The identification of isolates was determined according to standard methods using Bergey's manual of determinative bacteriology [17].

2.8 *Antimicrobial screening tests*

The isolates were examined for their sensitivity to some selected antibiotics on Mueller-Hinton agar using the Kirby-Bauer disc diffusion method. A wide range of antibiotics equivalent to drugs most frequently used in the management of human and animal diseases caused by Gram-negative and Gram-positive bacteria were employed in this study. The concentrations of the different antibiotics were recorded as follows, Ciproflox 10 µg, Norfloxacin 10 µg; Gentamycin 10 µg; Amoxil 20 µg; Streptomycin 30 µg; Rifampicin 20 µg; Erythromycin 30 µg; Chloramphenicol 30 µg; Amicloxz 20 µg; Levofloxacin 20 µg; Augumentin 30 µg and Cotrimoxazole 30 µg. Cultures of the bacterial isolates

were allowed to stand overnight inoculated into peptone water and incubated at 37 ℃ for 3-4 hours. The density of the bacterial culture required for the assay was adjusted to 0.5 McFarland standards. The Mueller-Hinton agar plates were uniformly inoculated by spotting 0.1 ml of the broth culture of each isolate and streaking over the entire plate, in at least three planes, using a swab stick. The plates were allowed to dry for 10 min; with sterile forceps, antibiotic-embedded paper discs were aseptically placed on the surface of the Mueller-Hinton agar medium at equidistance to each other and plates were incubated at 37 ℃ for 24 hours. A clear zone of inhibited growth around each antibiotic-impregnated disc was measured using a meter rule. The extent of sensitivity of the test organism to each antibiotic was determined and inferred as either sensitive (S), intermediate susceptible (I), or Defiant (D) in line with the clinical laboratory standard institute (CLSI).

2.9 *Statistical analysis*

The graph showing the percentage frequency of bacteria isolated from the different ponds was prepared using GraphPad Prism version 8.

2.10 *Results*

Isolation, Characterization, and Identification of Bacteria from Fish Pond.

The nature of bacterial growth and total bacterial counts and fecal coliform counts of the water samples was within the range of 0.40×10^6-1.97×10^6 cfu/ml and 1.15×10^4-5.90×10^4 cfu/ml respectively (Table 1). Water samples from the ten different ponds investigated were plated on both nutrient agar and eosin methylene blue agar.

A Total of six bacteria were isolated and identified from the ten fish ponds including: *Vibrio* spp.; *Klebsiella* spp.; *Salmonella* spp.; *Staphylococcus* spp.; *Escherichia coli*; and *Shigella* spp. Table 2 shows the total bacterial counts and fecal coliform counts of water samples from fish ponds investigated within 9th Mile Enugu.

Table 1. Nature of bacterial growth in media (incubation with nutrient agar)

Pond water sample	Fish density (g/mL)	Nature of bacterial growth
Sample 1 + NA (E-A)	500-700	Moderate growth
Sample 2 + NA (E-B)	600-700	Moderate
Sample 3 + NA (E-C)	≥ 1,000	Heavy
Sample 4 + NA (E-D)	≤ 500	Scanty
Sample 5 + NA (C-A)	600-700	Moderate
Sample 6 + NA (C-B)	500-700	Moderate
Sample 7 + NA (C-C)	≤ 500	Scanty
Sample 8 + NA (P-A)	500-700	Moderate
Sample 9 + NA (P-B)	≤ 500	Scanty
Sample 10 + NA (P-C)	600-700	Moderate

Key: NA = Nutrient agar. Sample 1-10, denotes water samples from various ponds as recorded in Table 3. E = Earthen pond A, B, C and D. C = Concrete pond A, B and C. P = Plastic pond A, B and C

Table 2. Nature of bacterial growth in media (Incubation with EMB Agar)

Pond water sample	Fish density (g/mL)	Nature of bacterial growth
Sample 1 + EMB (E-A)	500-700	Heavy growth
Sample 2 + EMB (E-B)	600-700	Moderate
Sample 3 + EMB (E-C)	≥ 1,000	Moderate
Sample 4 + EMB (E-D)	≤ 500	Moderate
Sample 5 + EMB (C-A)	600-700	Scanty
Sample 6 + EMB (C- B)	500-700	Moderate
Sample 7 + EMB (C-C)	≤ 500	Scanty
Sample 8 + EMB (P-A)	500-700	Moderate
Sample 9 + EMB (P-B)	≤ 500	Scanty
Sample 10 + EMB (P-C)	600-700	Moderate

Key: EMB = Eosin methylene blue agar. Sample 1-10, denotes water samples from various ponds as recorded in Table 3. E = Earthen pond A, B, C and D. C = Concrete pond A, B and C. P = Plastic pond A, B and C

Table 3. Total bacterial count and faecal coliform bacterial (FCB) counts

Locations (community)	Total bacterial count (cfu/ml)	Faecal coliform counts (cfu/ml)	Type of bacteria
Earthen pond A (Amaebo)	0.60×10^6	5.50×10^4	*Klebsiella* sp, *Vibrio* sp, *S. aureus*
Earthen pond B (Ukaka)	0.50×10^6	5.13×10^4	*S. aureus*, *E. coli*, *Klebsiella*, *Salmonella*, *Vibrio* spp
Earthen pond C (Okwojo)	0.70×10^6	5.10×10^4	*Vibrio* sp, *Shigella* sp, *Klebsiella* sp, *Salmonella* sp, *Vibrio* sp
Earthen pond D (Umuase)	0.90×10^6	5.90×10^4	*Vibrio* sp, *Shigella* sp, *Klebsiella* sp, *Vibrio* sp
Concrete pond A (Amadiukwu)	1.59×10^6	3.50×10^4	*Salmonella* sp, *S. aureus*, *Klebsiella* sp, *Vibrio* sp
Concrete pond B (Obinagu Okwe)	1.35×10^6	3.37×10^4	*E. coli*, *Salmonella* sp, *Shigella* sp
Concrete pond C (Amuba)	1.90×10^6	3.50×10^4	*Vibrio* sp, *Shigella* sp, *Klebsiella* sp,
Concrete pond D (Uwani Ngwo)	1.97×10^6	3.00×10^4	*Klebsiella* sp, *Shigella* sp, *S. aureus*
Plastic pond A (Akama)	0.40×10^6	1.15×10^4	*S. aureus*, *Shigella* sp, *E.oli*, *Salmonella* sp, *S. aureus*
Plastic pond B (Amaeke)	0.7×10^6	2.00×10^4	*E. coli*, *Klebsiella* sp, *Shigell*a sp, *Vibrio* sp

3. Antibiotic susceptibility

The result of the antibiotic susceptibility pattern of the seven bacterial genera isolated from pond water within 9th mile Enugu was presented in Table 4. The isolates were examined for susceptibility against twelve antibiotics namely; Ciproflox; Norfloxacin; Gentamycin; Amoxil; Streptomycin; Rifampicin; Erythromycin; Chloramphenicol; Amicloxz; Levofloxacin; Augmentin; Cotrimoxazole.

Table 4. Morphological and biochemical uniqueness of isolates

Limitations	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Colony characters	Milkish irregular shape with flat elevation	Pinkish, circular with flat elevation	Yellowish circular with flat elevation	Whitish irregular shape with flat elevation	Yellowish irregular shape with flat elevation	Whitish irregular shape with flat elevation
Cell characters	Cocci in clusters	Long rods in singles	Short rods in singles	Rods in clusters	Cocci in clusters	Rods in clusters
Gram's test	+	_	_	_	+	_
Motility test	_	_	_	+	+	+
Catalase	+	+	+	+	+	+
Coagulase	_	_	_	_	+	_
Citrate	_	_	_	_	+	_
Indole	_	+	+	_	+	_
Oxidase	_	_	_	+	_	+
Urease	+	+	+	_	+	_
Feasible organism	*Klebsiella* spp	*Salmonella* spp	*Shigella* spp	*E. coli*	*S. aureus*	*Vibrio* sp.

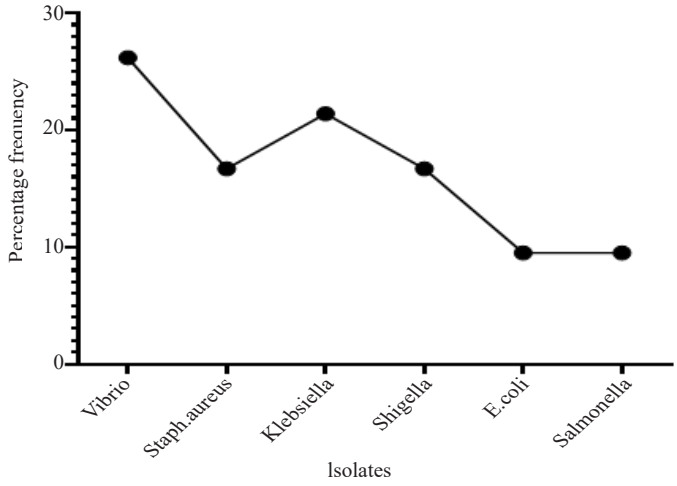

Figure 1. Graph showing the percentage frequency of bacterial isolated from the fish ponds

4. Discussions

The observed nature of the growth of isolates from the pond water sample (Tables 1 and 2) is evidence that the pond water ideally supports the growth of microorganisms. The observed growth of coliforms in the fish pond water (Table 3) shows the extent of bacterial contamination of the waters used in fish cultivation. This could be attributed to the presence of macrobiotic matter in the pond water. The isolation of bacteria from the water pond is consistent with the findings of [18] who noted that bacteria can be introduced into the water ponds through the feeds supplied in the ponds. The isolation of coliforms from the pond waters indicates fecal contamination of the pond waters. The fecal contamination of ponds could arise due to runoff from fecal sources, introduction of animal manure in fish ponds, and excretes by the fish into its ponds [16]. This implies that the fish pond is an essential culture medium for the growth and propagation of pathogens implicated in bacterial infections among fish in the ponds. Fish ingest pathogenic bacteria

from water, sediment, and food sources through their gut and gills [19]. Pathogens and poor water quality have been noted as major causes of fish mortalities [20].

This study found six genera of microorganisms in the fish pond water. The microorganisms found include; *Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *E. coli*, *Staphylococcus* spp, and *Vibrio* spp (Table 4). The isolates were identified using Bergey's manual of determinative bacteriology [17]. Figure 1 shows percentage frequency of bacterial isolated from the fish ponds. The occurrence of pathogenic bacteria such as *Klebsiella* sp and *E. coli* implied fecal contamination of the pond water from animal dung [21]. Coliforms especially *Escherichia coli* are used as indicators of water quality [21]. The total bacteria count (cfu/ml) indicates that there were enough bacteria to contaminate the pond water. Similarly, the presence of fecal coliform counts (cfu/ml) as seen in Table 3 shows that pond water was contaminated with pathogens due to pollution from fecal sources.

It is ideal to perform a bacteriological analysis of pond water to identify the presence of microorganisms that can cause illness and mortality of fish in the ponds. The result of these findings showed that *Vibrio* spp (26.2%) was the most frequently isolated microorganism from the fish ponds. The presence of microorganisms especially *E. coli*, *Salmonella*, *Shigella*, and *Vibrio* as identified in the water pond (Table 5) can bring about disease outbreaks and transmission of waterborne illnesses such as food poisoning and gastroenteritis upon ingestion of insufficiently cooked fish harvested from the ponds. Other bacteria are naturally occurring in aquaculture environments and are not harmful but can easily infect fish in stressed conditions. Studies have shown that the presence of *Pseudomonas* in fish ponds can hinder the growth and survival of fish in the fish ponds [22]. Seversal factors that may predispose the fish pond water to microbial contamination include the location of ponds near sewage and, the introduction of contaminated food or material into the pond water [23].

Table 5. General characters of the observed ponds

Pond id	Pond liner	Community	Fish density (g/mL)	Total bacterial count (cfu/ml)	Faecal coliform counts (cfu/ml)
E-1	Earthen	Amaebo	500-700	0.60×10^6	5.50×10^4
E-2	Earthen	Ukaka	600-700	0.50×10^6	5.13×10^4
E-3	Earthen	Okwojo	≥ 1,000	0.70×10^6	5.10×10^4
E-4	Earthen	Umuase	≤ 500	0.90×10^6	5.90×10^4
C-1	Concrete	Amadiukwu	600-700	1.59×10^6	3.50×10^4
C-2	Concrete	Obinagu Okwe	500-700	1.35×10^6	3.37×10^4
C-3	Concrete	Amuba	≤ 500	1.90×10^6	3.50×10^4
C-4	Concrete	Uwani Ngwo	500-700	1.97×10^6	3.00×10^4
P-1	Plastic	Akama	≤ 500	0.40×10^6	1.15×10^4
P-2	Plastic	Amaeke	600-700	0.7×10^6	2.00×10^4

Meanwhile, the use of antibiotics is not recommended in aquaculture cultivation but could assist in preventing mortalities of diseased fish. Miranda et al., [24] reported that antibiotics are very essential in the treatment of bacterial diseases in human medicine and also useful for the treatment of bacterial infections in livestock. The antibiotic susceptibility patterns of the isolated microorganisms are shown in Table 6. The outcome showed varying results such as resistance, susceptibility, and intermediate susceptibility of the isolates to the antibiotics. The observed resistance to some antibiotics as shown in Table 6 could be as a result of excessive use of antibiotics. This observation is in line with World Health Organization (WHO) reports of [13] which noted the overuse of antibiotics in aquaculture as a principal basis of resistance by bacteria. The observed resistance of some pathogens to bacteria is in line with the reports of some researchers [22, 24]. The dataset obtained in Table 6 was tested for correlation using the Pearson coefficient of correlation for antimicrobial drugs against bacteria. The letters were rated: D = 0, I = 1, and S = 2. We observed a strong

positive correlation between the effects of all drugs tested against all bacteria found in both earthen and concrete ponds except for Norfloxacin and Gentamycin. Across the three ponds studied, *Klebsiella* and *Salmonella* species showed a higher average correlation resistance value at 0.74 when compared to other bacteria. *Staphylococcus* and *Shigella* species further showed a positive correlation in their resistant to the drugs of interest. However, the result is more significant for the earthen pond than for concrete and plastic ponds.

Table 6. Antibiotic sensitivity to the bacterial isolates

Pond id	Organisma	Drug susceptibility profile											
		CPX	NB	CN	AML	S	RD	E	CH	APX	LEV	AU	SXT
E-1	Earthen pond A												
	Klebsiella sp.	S	S	D	S	D	I	D	D	D	D	D	I
	Vibrio sp.	S	S	I	S	I	D	S	S	S	D	D	D
	Staphylococcus sp.	D	D	D	D	D	D	D	D	S	D	D	D
E-2	Earthen pond B												
	Staphylococcus sp.	D	D	S	I	D	D	D	D	I	S	S	D
	Escherichia coli	S	D	S	S	D	D	S	I	S	S	D	S
	Klebsiella sp.	S	D	D	D	D	D	D	D	S	S	D	D
	Salmonella sp.	D	S	D	S	D	D	I	D	S	S	S	S
	Vibrio sp.	I	D	D	S	D	D	S	D	S	I	S	D
E-3	Earthen pond C												
	Vibrio sp.	S	S	I	S	D	S	I	I	S	S	S	D
	Shigella sp.	D	S	D	D	D	D	D	D	S	D	D	D
	Klebsiella sp.	S	S	I	S	D	S	S	S	S	S	S	S
E-4	Earthen pond D												
	Vibrio sp.	S	S	I	S	D	S	I	I	S	S	I	D
	Shigella sp.	D	S	D	D	D	D	D	D	S	D	D	D
	Klebsiella sp.	S	S	I	S	D	S	S	S	S	S	S	S
C-1	Concrete pond A												
	Salmonella sp.	D	S	I	S	D	D	S	D	I	S	I	S
	Staphylococcus sp.	D	D	D	S	D	D	D	D	S	S	D	D
	Klebsiella sp.	I	S	I	D	S	S	S	S	S	S	S	S
	Vibrio sp.	S	S	I	S	D	S	I	I	I	S	D	D
C-2	Concrete pond B												
	Escherichia coli	S	D	D	I	D	S	S	I	S	S	S	S
	Salmonella sp.	D	S	D	S	D	D	D	D	I	D	S	D
	Shigella sp.	D	S	D	D	D	D	D	D	S	D	D	D

Table 6. (cont.)

Pond id	Organisma	Drug susceptibility profile											
		CPX	NB	CN	AML	S	RD	E	CH	APX	LEV	AU	SXT
C-3	Concrete pond C												
	Vibrio sp.	S	D	D	I	D	D	S	D	S	S	D	D
	Shigella sp.	D	S	D	D	D	D	D	D	S	D	D	D
	Klebsiella sp.	S	S	I	S	D	S	S	S	S	S	S	S
	Vibrio sp.	S	S	I	S	D	S	I	I	I	D	D	D
C-4	Concrete pond D												
	Klebsiella sp.	I	I	S	S	D	S	S	S	S	S	D	S
	Shigella sp.	I	D	D	S	D	S	I	D	S	S	S	D
	Staphylococcus sp.	D	D	I	S	I	S	D	D	S	S	D	D
P-1	Plastic pond A												
	Staphylococcus sp.	D	D	S	S	1	S	D	D	S	S	S	D
	Shigella sp.	S	D	D	I	D	D	D	D	S	S	S	S
	Escherichia coli	D	S	D	S	D	S	S	I	S	S	D	S
	Salmonella sp.	S	S	I	S	D	I	D	D	D	I	D	D
	Staphylococcus sp.	D	D	S	S	I	S	D	D	S	S	S	D
P-2	Plastic pond B												
	Escherichia coli	S	D	D	I	D	S	S	S	S	I	I	S
	Klebsiella sp.	S	S	S	I	D	I	S	S	S	S	S	S
	Shigella sp	I	D	S	D	S	I	D	D	S	D	D	S
	Staphylococcus sp.	D	D	S	S	D	S	D	D	S	S	D	S
	Vibrio sp.	S	S	I	S	D	S	I	I	I	D	D	D

Key: D = Defiant, I = Intermediate, S = Susceptible; CPX-Ciproflox; NB-Norfloxacin; CN-Gentamycin; AML-Amoxil; S-Streptomycin; RD-Rifampicin; E-Erythromycin; CH-Chloramphenicol; APX-Ampicloxz; LEV-Levofloxacin; AU-Augumentin; SXT-Cotrimoxazole

Figure 2 shows the determination of antimicrobial resistance rate. Microbial reactions to the antimicrobial drugs were tested for their resistance (defiant) and susceptibility, the reactions which were rated D = 0, I = 1, and S = 2 were analyzed. The analyses indicate that Amoxil (AML) and Augmentin (AU) had the highest activities against most bacteria that were investigated. Amoxil showed a mean susceptibility of 1.46, while Augmentin followed closely with a mean susceptibility of 1.42. Ampiclox had a mean value of 1.38. Generally, bacteria had the greatest resistance to Streptomycin with the lowest mean value at 0.25. This was followed by Chrolamphenicol and Gentamycin with similar mean values at 0.83. Vertical bars represent the standard errors for the means of each drug. Meanwhile, some authors [24, 25] noted that antimicrobials are mostly prescribed in fish through medicated feed. There are reports of resistance bacteria such as *Vibrio* spp and *Salmonella* spp, which usually occur as part of natural flora in pond water but can be transmitted and cause infections in humans [26]. The information about the use and resistance of bacteria to antimicrobial agents does not undermine the value of aquaculture in the food supply system but rather seeks better ways

of identifying and resolving some challenges facing the system.

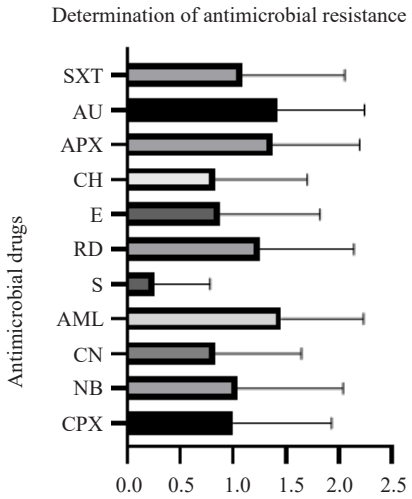

Figure 2. Effect of antimicrobial drugs on bacteria ratings, D = 0, I = 1, S = 2

5. Conclusions

Fish ponds are medium that could support the growth of microorganisms when fish are cultivated in an unhygienic environment. It is ideal to perform bacteriological analysis of fish pond water to identify the presence of microorganisms that have the potential to cause illness and mortality of fish in the ponds. Antimicrobial studies could help in choosing the most potential drugs that would assist in eliminating microorganisms causing illness in pond water. Periodic removal and replacement of water used in fish cultivation would reduce contamination of pond water from diverse sources including contaminants from uneaten feed. The maintenance of good water hygiene in fish ponds could reduce illness among fish and prevent the tendency to introduce antibiotics in feed used for the cultivation of fish. This research showed that Amoxil and Augmentin could be more effective in managing bacterial infections in pond water.

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Conflict of interest

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