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Bacteriological Examination of Fish Pond Effluents in Enugu State Metropolis

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Abstract **Original Research Article**

Due to diseases associated with fish pond effluents, the bacteriological examination of bacteria present in fishpond effluents in Enugu state was studied using standard methods. The total bacterial counts were 2.4 to 3.8x10^2 cfu/ml, coliform counts ranged from 2.1 to 3.0x10^2, and faecal coliform counts ranged from 1.5 to 2.7x10^2. The bacterial counts were identified as gram negative and gram positive bacteria. Escherichia coli and Staphylococcus sp had the highest frequency (17.24%), while Micrococcus had the least (3.44%). The species of Escherichia and Staphylococcus were isolated from all samples. This showed that the pond effluents do not meet standard. Fishes are unsafe for consumption and effluents require treatment before release.

Keywords: Bacteriological, Examination, Fish, Pond, Effluents, Metropolis.

Introduction

Aquatic food products are globally significant, serving not only as a nutrient source but also as a major international trade item, generating substantial revenue for many countries (Danba et al., 2014).

The occurrence of infectious diseases in fish depends on how pathogens interact with their aquatic environment (Noga, 2000).

Microorganisms play a crucial role in water ecosystems, impacting fish health in aquaculture settings, often leading to infectious disease outbreaks affecting entire farms (Ikpi, 2011).

In sub-Saharan Africa, Nigeria leads in aquaculture fish production, contributing over half of the region's farmed fish output (WorldFish, 2020).

The presence of microorganisms in fish and fish ponds poses significant risks to human health (Li, 2019).

Some microorganisms have resistance factors that increase their ability to cause infections in consumers. For example, Escherichia coli can thrive in water environments and are proficient at transferring genes, including those that resist antibiotics, to other bacteria (Fakorede, 2019).

Poor water quality, overcrowding, and the use of contaminated feed and animal waste have been linked to the contamination of these fish farming systems (Mukwabi 2019).

Fish can get infected with bacteria and heavy metals through their gills, skin, digestive tract, or even from contaminated eggs. Infected fish may show signs like loss of appetite, damaged fins, pale gills, and swollen bellies. They might also swim weirdly, float on their backs, or crowd near water inlets. Other symptoms include cloudy eyes, open sores, and scale loss. Escherichia coli is a common bacterium that can contaminate food and water, posing a significant health risk (Dutta, 2010). This study aimed to identify bacteria in pond effluents.

Materials and Methods

Study area

Enugu Metropolitan, Enugu state. The five fishpond effluents collected were in this area. Four of the fishponds were constructed with concrete, while one was an earthen pond. The fishpond effluents were collected from five different areas which are Uwani, Achara Layout, Maryland, New Haven and Coal camp.

Sample Collection

Effluents from five fishponds in Enugu State, Nigeria, were collected in sterile containers and sent to the Microbiology lab at Enugu State University of Science and Technology for testing.

Total bacterial count

Serial dilutions were carried out.

For total bacterial count, samples were serially diluted (10-fold), and 1ml of dilution 10^2 was inoculated onto nutrient agar in duplicates using pour plate method. Plates were incubated at 37°C for 24 hours, and visible colonies were counted as colony-forming units per milliliter (CFU/ml).

Total coliforms

1ml of a 10-fold serial dilution (10^x) of the samples was inoculated onto MacConkey agar in duplicates using pour plate method. Plates were incubated at 37°C for 24 hours, and visible colonies were counted as CFU/ml.

Faecal coliforms

1ml of a 10^4 dilution of the samples was inoculated onto Eosin Methylene Blue (EMB) agar in duplicates using pour plate method. Plates were incubated at 37°C for 24 hours, and visible colonies were counted as CFU/ml.

Characterization and Identification of the isolates

Isolates were identified based on cultural, morphological, and biochemical characteristics, compared to Bergey's Manual of Determinative Bacteriology. Identification involved subculturing on Nutrient agar, followed by tests like gram staining, motility, indole, methyl red, citrate utilization, oxidase, catalase, coagulase, and sugar fermentation (modified Cheesbrough, 2010).

Gram staining

The process involves making a smear on a slide, heat-fixing it, and then staining with crystal violet (1 min), Lugol's iodine (1 min), decolorizing with acetone (5 sec), and washing.

Oxidase Test

This involves mixing the organism with oxidase reagent on filter paper, with a purple color change indicating a positive result, while the motility test involves inoculating semi-solid agar, incubating for 24 hours, and observing for migration away from the inoculation line to indicate motility.

The motility test

This involves inoculating a semi-solid nutrient agar with a straight wire loop, incubating for 24 hours, and observing for migration away from the line of inoculation to indicate motility.

The coagulase test

Making a smear of the organism on a slide, adding human serum, and rocking for 10 seconds; clumping indicates a positive result.

The catalase test

Smear the organism on a slide, adding 3% hydrogen peroxide, and observing for bubbles (positive result).

The indole test

This procedure involves growing a bacterial culture in peptone broth (24 hours, 37°C), adding Kovacs reagent, and observing for a red colour (positive result).

The citrate utilization

This procedure involves inoculating Simmon citrate agar, incubating (24 hours, 37°C), and observing for a color change from green to deep blue (positive result).

The methyl red test

This procedure involves inoculating glucose phosphate peptone water, incubating (48 hours, 37°C), adding methyl red reagent, and observing for a red color (positive result) or yellow colour (negative result).

The sugar fermentation test

This involves preparing peptone water broth with sugar (glucose, fructose, lactose) and bromothymol blue, inoculating with the test organism, and incubating (24 hours); acid production is indicated by a color change, and gas production is indicated by a bubble in the Durham tube.

The spore test procedure

This involves heat fixing a bacterial film on a slide, staining with malachite green over boiling water (1 minute), washing, counterstaining with safranin (30 seconds), and viewing under a microscope; spores appear green, non-spore forming bacteria appear red.

Results

Table 1: Bacterial Counts of the Fish Pond Effluents

Sample	Total bacterial count (cfu/ml)	Total coliform count (cfu/ml)	Faecal coliform count (cfu/ml)
1	3.4×10^2	2.7×10^2	1.5×10^2
2	2.4×10^2	2.1×10^2	1.6×10^2
3	3.8×10^2	2.2×10^2	1.5×10^2
4	3.7×10^2	3.0×10^2	1.8×10^2
5	3.6×10^2	3.0×10^2	2.7×10^2

Table 2: Morphological and biochemical characteristics of the bacterial isolates from the fish pond effluents

Isolates	Gram staining	Oxidase Test	Congulase Test	Catalase Test	Citrate Test	Motility Test	Methyl Red Test	Indole Test	Sugar Fermentation Test			Spore Test	*Probable Identity*
1	-ve	-	-	+	-	+	+	+	A/G	A/G	-	-	*Escherichia sp*
2	-ve	-	+	+	+	-	+	-	A/G	A/G	A/G	-	*Staphlococcus sp*
3	-ve	-	-	+	-	+	+	-	A/G	-	A/G	-	*Salmonella sp*

4	-ve	-	+	+	-	-	+	+	A/G	-	A/G	-	Shigella sp
5	-ve	+	+	+	-	-	-	-	-	-	-	-	*Micrococcus sp*
6	-ve	-	-	+	+	+	-	-	A	-	A	-	*Enterobacter sp*
7	-ve	+	-	+	+	+	-	+	A/G	A/G	A/G	-	*Bacillus sp*
8	-ve	-	+	-	-	+	-	-	A	A	A	-	*Streptococcus sp*
9	-ve	+	-	+	+	+	-	+	A/G	A/G	A/G	-	*Aeromonas sp*
10	-ve	+	-	+	+	-	+	+	A/G	A/G	A/G	-	*Flavobacterium sp*

Key: **+ve = Positive reaction**
-ve = Negative reaction
+ = Positive result
- = Negative result
A = Acid production
G = Gas production

Table 3: Distribution of the bacterial isolates in the fish pond effluents

Isolates	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Bacillus sp	+	-	+	+	-
Staphylococcus sp	+	+	+	+	+
Streptococcus sp	-	-	-	+	+
Escherichia sp	+	+	+	+	+
Enterobacter sp	+	+	+	-	-
Flavobacterium sp	+	-	+	-	+
Klebsiella sp	-	+	+	+	-
Salmonella sp	+	+	-	-	-
Aeromonas sp	-	+	+	-	-
Micrococcus sp	+	-	-	-	-

+ = Detected
- = Undetected

Table 4: Frequency of occurrence of the bacterial isolates in the fish pond effluents

Bacterial isolate	Number of colonies isolated	Frequency of occurrence (%)
Bacillus sp	3	10.35
Staphylococcus sp	5	17.24
Streptococcus sp	2	6.89

Escherichia sp	5	17.24
Enterobacter sp	3	10.35
Flavobacterium sp	3	10.35
Klebsiella sp	3	10.35
Salmonella sp	2	6.87
Aeromonas sp	2	6.89
Micrococcus sp	1	3.44

Discussion

Table 1 shows sample 3 had the highest microbial load, while sample 4 had the lowest. Microbes in fishponds can be a food source for fish and help with nutrient cycling, matching findings by Amuneke et al. (2020) on bacteria counts in similar ponds.

Table 2 shows diverse bacteria genera like Escherichia, Staphylococcus, and Salmonella, matching Dabor's findings (2008) in similar fish ponds. Pathogens like Salmonella Typhi, Shigella flexneri, E. coli, and Vibrio cholerae pose risks of waterborne illnesses. Salmonella Typhi was prevalent, likely due to contamination from feeds, personnel, or environment. Coliforms suggest fecal contamination, possibly from water source or poor sanitation. Findings suggest bacteria likely originated from feed added to the ponds, aligning with Okpokwasilili et al. (1999).

Tables 3 and 4 show Escherichia spp was present in all samples, indicating fecal contamination of the fishponds. Staphylococcus sp was also highly frequent (17.24), while Micrococcus sp had the lowest occurrence (3.44). The presence of E. coli suggests possible contamination with gastrointestinal disease-causing agents (Ampoo and Clerk, 2010). The isolated coliforms indicate fecal contamination, likely from fertilization with animal manure, fish excretion, or poor sanitation practices (Kay et al., 2008). These findings are consistent with Raji and Ibrahim (2011), who reported pathogenic bacteria in water and fish samples, posing health risks to consumers. The presence of these pathogens highlights the need for improved sanitation and hygiene practices in fish farming.

Conclusion

The evaluation of Enugu State fish ponds indicates severe contamination, rendering the water unfit for producing fish for human consumption. Effluents also pose significant health risks to humans, requiring avoidance. Urgent action is crucial to ensure safe fish production, protect public health through regular checks, sanitation upgrades.

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