2025 Volume: 01 Issue: 05 www.ijrpa.com Research Article

International Journal Research Publication Analysis

Page: 01-27

MICROBIAL DIVERSITY AND PUBLIC HEALTH IMPLICATIONS OF BACTERIAL AND FUNGAL CONTAMINANTS IN FISH POND WATER FROM SOUTHWESTERN NIGERIA

***Akano G.A.[1]; Olufade I.I.[2]; Alli Z.A.[3]; Idakwo A.O.[4]; Nwachukwu C.C.[5]; Akinyemi T.O.[6]**

[1-5]Biological Sciences, Federal Polytechnic Ede Osun State, Nigeria.

[6]Timi's Coaching Centre Ede Osun State, Nigeria.

Article Received: 28 August 2025
Article Revised: 18 September 2025
Published on: 08 October 2025

***Corresponding Author: Akano G.A.**
Biological Sciences, Federal Polytechnic Ede Osun State, Nigeria.

DOI Link: https://doi-doi.org/101555/ijrpa.7117

ABSTRACT

Aquaculture remains vital for food security and economic development in Nigeria, yet microbial contamination of fish-pond water threatens fish productivity, environmental balance, and public health. This study investigated the bacterial and fungal composition of fish-pond water from three ponds (A, B, and C) located in Ede North and Ede South Local Government Areas, Osun State, Southwestern Nigeria. Physicochemical analyses revealed mean temperature of **27.9 ± 0.5 °C**, **pH 6.9–7.1**, **dissolved oxygen 5.8–6.2 mg/L**, and **total dissolved solids 198–210 mg/L**, all within FAO (2015) aquaculture standards. Microbiological assessment identified **230 total bacterial isolates**, comprising **198 (86.1%) Gram-negative** and **32 (13.9%) Gram-positive** organisms. Dominant bacterial species were *Escherichia coli* (26%), *Pseudomonas aeruginosa* (22%), *Klebsiella pneumoniae* (19%), *Salmonella* spp. (13%), *Enterobacter* spp. (6%), and *Staphylococcus aureus* (14%). Fungal isolates included *Aspergillus flavus*, *Candida albicans*, *Fusarium* spp., *Trichoderma viride*, *Penicillium* spp., and *Mucor* spp. One-way ANOVA indicated no significant differences in microbial counts or physicochemical parameters among ponds ($p > 0.05$), while Pearson's correlation showed a strong positive association between total dissolved solids and microbial load ($r = 0.81$). The predominance of Gram-negative bacteria reflects extensive faecal and organic contamination, and the co-occurrence of oxygenic fungi signals deteriorating pond hygiene. The study underscores the urgent need for routine microbiological surveillance, improved waste management, and prudent antibiotic use to ensure sustainable and safe aquaculture practices in Southwestern Nigeria.

KEYWORDS: Aquaculture, microbial contamination, Gram-negative bacteria, fungi, physicochemical parameters, statistical analysis.

1.0 INTRODUCTION

Aquaculture has evolved into one of the fastest-growing food-production sectors globally, providing nearly half of the world's fish supply (FAO, 2021). In Nigeria, aquaculture plays a vital socio-economic role, ensuring food security, employment, and poverty alleviation in both rural and peri-urban communities (*Adeoye, Ojo, & Ibrahim, 2022*). Yet, the sustainability of this sector is being threatened by increasing microbial contamination of fish-pond ecosystems. Microbial pollution undermines fish health, depresses yield, and exposes consumers to pathogenic organisms and toxic metabolites (*Akintola & Oladimeji, 2023*; *Akinyemi, Fadeyi, & Oyeleke, 2020*). Fish-ponds represent dynamic aquatic micro-ecosystems where biotic and abiotic components interact continuously. They receive organic inputs from uneaten feed, fish excreta, plant debris, and runoff, creating favourable niches for diverse microorganisms (*Ogunbanwo, Ajayi, & Adebisi, 2020*). Under optimal physicochemical conditions, these microbes multiply rapidly, some assuming pathogenic or opportunistic significance. Bacterial genera such as *Escherichia*, *Klebsiella*, *Staphylococcus*, and *Pseudomonas* are frequently reported in pond environments and are known to cause gill rot, septicaemia, and ulcerative diseases in fish (*Adewale, Ajibola, & Ogunyemi, 2024*). Fungal species including *Aspergillus*, *Penicillium*, *Mucor*, and *Fusarium* contribute to biodegradation but also release mycotoxins that threaten fish and human health (*Onifade & Adebayo, 2023*; *Rodrigues & Naehrer, 2012*). Previous investigations have linked the proliferation of these microorganisms to poor pond management, open drainage systems, and use of untreated water (*Bamidele, Ajayi, & Akinola, 2021*). In many Nigerian ponds, waste from domestic or agricultural activities is directly discharged into surrounding waters without treatment, intensifying microbial load and encouraging eutrophication (*Adeoye et al., 2022*). In addition, the misuse of antibiotics in aquaculture has selected resistant bacterial strains, creating reservoirs for antimicrobial-resistance genes transferable to human pathogens (*Abioye, Adebayo, & Oladimeji, 2022*; *World Health Organization, 2023*). Environmental factors such as temperature, dissolved oxygen, and pH strongly influence microbial diversity and activity (*Boyd & Tucker, 2012*). Elevated temperature enhances microbial metabolism but can reduce oxygen solubility, whereas low dissolved-oxygen concentrations favour anaerobes that degrade organic matter and release harmful gases. Thus, physicochemical conditions and microbiological quality are intricately linked in determining the ecological

integrity of fish-ponds (*Adeoye et al., 2022*; *FAO, 2015*). Although numerous studies have characterized microbial contaminants in Nigerian aquaculture, many focus on either bacterial or fungal communities in isolation (*Ezeonu, Okafor, & Chukwu, 2021*; *Adegoke, Akinloye, & Ogundipe, 2021*). There remains a paucity of integrated analyses examining both bacterial and fungal consortia alongside physicochemical parameters, particularly in small-scale fish-ponds typical of Southwestern Nigeria. Such information is crucial for understanding pathogen reservoirs, assessing ecological risk, and formulating sustainable management strategies (*Mensah, Kwarteng, & Aboagye, 2021*; *Wambugu, Kamau, & Otieno, 2022*). This study therefore isolates and identifies bacterial and fungal species from fish-pond water samples collected in Ede, Osun State, and evaluates their distribution relative to key physicochemical parameters. The work provides empirical evidence of microbial dynamics within artisanal aquaculture systems and highlights their potential implications for fish productivity, environmental safety, and public health in Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Ede, Osun State, Southwestern Nigeria, between latitudes 7.700°–7.730° N and longitudes 4.400°–4.450° E. Water samples were collected from three fish ponds located across two Local Government Areas (Ede North and Ede South). Three ponds—Paragon (A), Odoeja (B), and Ededimeji (C)—were selected based on scale and accessibility (*Adejumo & Aluko, 2020*).

2.2 Sample Collection and Physicochemical Parameters

Sterile 500 mL bottles were filled 10 cm below the surface between 7:30 and 9:30 a.m. Temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were determined with a multiparameter meter (APHA, 2017). Samples were transported on ice and processed within four hours, following *Boyd and Tucker (2012)*.

Temperature influences metabolic rate and pathogen survival; optimal fish performance occurs around 26–30 °C (*Boyd, 2015*). pH governs enzymatic activity and ammonia toxicity; acceptable limits for aquaculture are 6.5–8.5 (*FAO, 2015*). DO ≥ 5 mg/L sustains aerobic respiration (*Timmons & Ebeling, 2013*), whereas TDS < 300 mg/L indicates good ionic balance (*Adeoye, Ojo, & Ibrahim, 2022*).

2.3 Isolation of Microorganisms from Pond Water Samples

Isolation of microorganisms from the pond water samples was conducted using standard microbiological techniques to obtain pure bacterial and fungal isolates for subsequent identification. The procedure followed the guidelines described by Cheesbrough (2019), Forbes *et al.* (2022), and the World Health Organization (WHO, 2021).

2.3.1 Isolation of Bacteria

(a) Sample Collection

Water samples were aseptically collected from three different fish ponds into pre-sterilized glass bottles. The bottles were labeled accordingly and immediately transported in ice boxes to the Microbiology Laboratory for analysis within two hours of collection to prevent microbial alteration (Cappuccino & Sherman, 2021).

(b) Serial Dilution

To reduce microbial load and facilitate the isolation of distinct colonies, the serial dilution method was employed as described by Holt *et al.* (2020). One millilitre (1 mL) of each pond water sample was transferred into 9 mL of sterile physiological saline (10^{-1} dilution). Further serial dilutions were prepared up to 10^{-5} by transferring 1 mL aliquots from each preceding dilution into new test tubes containing 9 mL of sterile saline, followed by thorough mixing.

(c) Culture Media Preparation

Nutrient agar (NA) was prepared following the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 minutes. Selective and differential media, including MacConkey agar, Eosin Methylene Blue (EMB) agar, and Mannitol Salt agar (MSA), were also prepared for identification of specific bacterial groups (CLSI, 2023; Ezeonu *et al.*, 2020). The media were allowed to cool to about 45 °C before use.

(d) Inoculation Technique

From each serially diluted sample, 1 mL aliquots were transferred aseptically into sterile Petri dishes. Molten nutrient agar was poured into the plates, mixed gently by swirling, and allowed to solidify (Cheesbrough, 2019). The plates were incubated in an inverted position at 37 °C for 24 hours.

(e) Incubation Conditions and Observation

After incubation, the plates were observed for visible bacterial growth. Colonies were examined for their macroscopic characteristics, including size, color, shape, margin, elevation, and surface texture (Holt *et al.*, 2020). Distinct colonies representing different morphotypes were noted for purification.

(f) Purification and Preservation of Bacterial Isolates

Representative colonies were sub-cultured onto freshly prepared nutrient agar plates using the streak-plate technique to obtain pure cultures (MacFaddin, 2021). Pure isolates were incubated at 37 °C for 24 hours, then transferred to nutrient agar slants and stored at 4 °C for further biochemical characterization. For long-term storage, glycerol stocks (20%) were prepared and preserved at –20 °C.

2.3.2 Isolation of Fungi

(a) Sample Collection

The same pond water samples collected for bacterial isolation were also used for fungal isolation. Samples were transferred into sterile containers and processed within two hours of collection to prevent overgrowth or sporulation (WHO, 2021).

(b) Serial Dilution

Fungal serial dilutions were prepared up to 10^{-3} using sterile distilled water. One millilitre (1 mL) of each sample was transferred into 9 mL of sterile diluent and mixed thoroughly using a vortex mixer to ensure even distribution of fungal spores (Cheesbrough, 2019).

(c) Culture Media Preparation

Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 mg/L) was used for fungal isolation to inhibit bacterial growth. The medium was sterilized by autoclaving at 121 °C for 15 minutes, allowed to cool to 45 °C, and poured into sterile Petri dishes (Adeyemi *et al.*, 2022).

(d) Inoculation Technique

From appropriate dilutions (usually 10^{-2} and 10^{-3}), 1 mL aliquots were aseptically dispensed into sterile Petri dishes. Molten SDA was poured into each dish, swirled gently for uniform distribution, and allowed to solidify. Plates were incubated in an upright position at 28 ± 2 °C for 3–7 days (Forbes *et al.*, 2022).

(e) Incubation and Colony Observation

Fungal growth was monitored daily for colony development. After incubation, colonies were examined macroscopically for color, margin, surface texture, and reverse pigmentation. Distinct colonies were noted for purification (Nkereuwem & Agbo, 2023).

(f) Purification and Preservation of Fungal Isolates

Representative fungal colonies were sub-cultured onto fresh SDA plates using a sterile inoculating loop to obtain pure isolates. The plates were incubated at 28 °C for 5–7 days. Pure cultures were preserved on SDA slants at 4 °C for subsequent identification (Cheesbrough, 2019).

(g) Microscopic Examination (Lactophenol Cotton Blue Technique)

Microscopic identification of fungi was performed using the Lactophenol Cotton Blue (LPCB) staining method as described by WHO (2021). A small portion of mycelium was transferred to a clean glass slide, mixed with a drop of LPCB, and covered with a coverslip. The preparation was examined under a light microscope at ×40 magnification for diagnostic structures such as conidia, hyphae, sporangia, and spores. Morphological features were compared with standard mycological atlases for genus-level identification (Adeyemi *et al.*, 2022).

2.4 Biochemical Tests for Identification of Bacterial Isolates

A series of standard biochemical tests were conducted to identify the bacterial isolates based on morphological and metabolic characteristics. All procedures followed established microbiological methods according to Cheesbrough (2019), Cappuccino and Sherman (2021), and the Clinical and Laboratory Standards Institute (CLSI, 2023).

2.4.1 Gram Reaction

Gram staining was performed to differentiate bacteria into Gram-positive and Gram-negative groups. A thin smear of each isolate was prepared on a clean grease-free slide, air-dried, and heat-fixed. The smear was stained with crystal violet for one minute, followed by Gram's iodine for another minute. After rinsing with water, the smear was decolorized with acetone-alcohol for about 15 seconds and immediately counterstained with safranin for one minute. The slide was washed gently, air-dried, and examined under oil immersion (×100 objective). Gram-positive organisms retained the violet color, while Gram-negative organisms appeared pink or red (Cheesbrough, 2019; CLSI, 2023).

2.4.2 Catalase Test

The catalase test was carried out following the procedure described by MacFaddin (2021). A portion of a fresh colony was transferred onto a clean, dry glass slide using a sterile wooden stick. One drop of 3% hydrogen peroxide solution was added to the colony. Immediate effervescence indicated a positive catalase reaction, while no bubble formation denoted a negative reaction. This test distinguishes *Staphylococcus* (catalase-positive) from *Streptococcus* species (catalase-negative) (Forbes *et al.*, 2022).

2.4.3 Oxidase Test

The oxidase test was performed using 1% tetramethyl-p-phenylenediamine dihydrochloride, following the World Health Organization (WHO, 2021) standard. A piece of filter paper was soaked in the reagent, and a small portion of each colony was smeared on it using a sterile wooden applicator. The development of a dark purple color within 10 seconds signified a positive oxidase reaction, while no color change after 30 seconds indicated a negative result. This test differentiates oxidase-positive bacteria such as *Pseudomonas aeruginosa* from oxidase-negative *Enterobacteriaceae* (Holt *et al.*, 2020).

2.4.4 Citrate Utilization Test

Citrate utilization was determined using Simmons citrate agar slants, as described by Cappuccino and Sherman (2021). Each isolate was inoculated on the surface of the slant using a sterile straight wire and incubated at 37 °C for 24–48 hours. A positive result was indicated by growth accompanied by a color change of the medium from green to Prussian blue. Absence of growth and color change denoted a negative reaction (Forbes *et al.*, 2022).

2.4.5 Indole Test

The indole test was performed using sterile tryptone broth following the method of Cheesbrough (2019). Each isolate was inoculated into 5 mL of tryptone broth and incubated at 37 °C for 24 hours. After incubation, 0.5 mL of Kovac's reagent was carefully layered on the broth surface. Formation of a red ring at the interface indicated indole production (positive), while a yellow or colorless ring denoted a negative result. The test detects the enzyme tryptophanase that breaks down tryptophan into indole, pyruvate, and ammonia (Ezeonu *et al.*, 2020).

2.4.6 Urease Test

Urease activity was determined using Christensen's urea agar slant, following the protocol of MacFaddin (2021). A loopful of each isolate was streaked on the surface of the urea agar and incubated at 37 °C for 24 hours. Development of a bright pink color due to ammonia production indicated a positive reaction, while no color change indicated a negative result. This test identifies *Proteus* species and other rapid urease producers (Nkereuwem & Agbo, 2023).

2.4.7 Motility Test

Motility was determined using semi-solid nutrient agar (0.4% agar concentration), following the standard method by Cappuccino and Sherman (2021). Each isolate was inoculated into the medium by stabbing the center with a sterile straight needle and incubated at 37 °C for 24 hours. Diffuse or spreading growth away from the stab line indicated motility, while growth confined to the stab line showed non-motility (Cheesbrough, 2019).

2.4.8 Coagulase Test

The coagulase test was conducted according to Forbes *et al.* (2022). The slide coagulase test was first performed by mixing a portion of the bacterial colony with a drop of plasma on a clean slide and observing for immediate clumping within 10 seconds. For confirmation, the tube coagulase test was conducted by adding 0.5 mL of plasma to 0.1 mL of bacterial suspension and incubating at 37 °C for up to 4 hours. The presence of a firm clot that remained stationary upon tilting confirmed coagulase positivity. This test differentiates *Staphylococcus aureus* from coagulase-negative *Staphylococcus* species.

2.4.9 Hydrogen Sulfide (H_2S) Production Test

H_2S production was assessed using Triple Sugar Iron (TSI) agar, following the guidelines of Holt *et al.* (2020). Each isolate was inoculated by stabbing the butt and streaking the slant surface, followed by incubation at 37 °C for 24 hours. Blackening of the medium along the stab line or throughout the butt indicated H_2S production due to ferrous sulfide formation, while no black precipitate indicated a negative result (CLSI, 2023).

2.4.10 Glucose Fermentation Test

The glucose fermentation test was carried out using phenol red glucose broth containing a Durham tube, according to MacFaddin (2021). Each isolate was inoculated into the broth and incubated at 37 °C for 24 hours. Acid production turned the medium from red to yellow,

while gas production was indicated by a visible air bubble in the Durham tube. Absence of color and gas change indicated a negative reaction (Adeyemi *et al.*, 2022).

2.4.11 Lactose Fermentation Test

Lactose fermentation was determined using phenol red lactose broth and MacConkey agar, following the method described by Cheesbrough (2019). Each isolate was inoculated into phenol red lactose broth containing a Durham tube and incubated at 37 °C for 24 hours. A yellow color change indicated acid production, while gas accumulation in the Durham tube signified gas formation. For confirmation, isolates were streaked on MacConkey agar; pink colonies indicated lactose fermenters, whereas colorless colonies represented non-fermenters (WHO, 2021).

2.4.12 Pigmentation and Colony Color Observation

Pigmentation and colony morphology were examined using the method outlined by Forbes *et al.* (2022). Each pure isolate was streaked on nutrient agar plates and incubated at 30–37 °C for 24–48 hours. Colonies were observed for pigmentation, surface texture, elevation, and edge characteristics. For *Pseudomonas aeruginosa*, production of bluish-green (pyocyanin) or yellow-green (pyoverdine) pigments was noted under natural and ultraviolet light (Adeyemi *et al.*, 2022).

2.5 Statistical Analysis (Revised)

Data obtained from physicochemical and microbiological analyses were statistically evaluated using **IBM SPSS Statistics version 26.0**. Descriptive statistics (means and standard deviations) were computed to summarize physicochemical parameters and microbial counts.

Differences in mean values among ponds were determined using **One-Way Analysis of Variance (ANOVA)** at a 95% confidence level (**$p < 0.05$**). Where applicable, **post-hoc Tukey's test** was used to determine specific pairwise differences between ponds.Microbial prevalence data (Gram-positive and Gram-negative bacteria counts) were expressed in percentages, and results were illustrated graphically using **Microsoft Excel 2021**. Statistical correlation between physicochemical parameters and microbial load was examined using **Pearson's correlation coefficient (r)** to evaluate the strength and direction of associations between environmental variables and microbial occurrence.

3.0 RESULTS

Table 1: Physicochemical parameters of fish pond water samples (mean ± SD)

Parameter	Pond A	Pond B	Pond C	p-value
Temperature (°C)	27.6 ± 0.5	28.2 ± 0.6	27.8 ± 0.4	0.34
pH	6.9 ± 0.2	7.1 ± 0.1	7.0 ± 0.3	0.12
Dissolved Oxygen (mg/L)	5.8 ± 0.4	6.2 ± 0.5	6.0 ± 0.3	0.15
TDS (mg/L)	210 ± 12	198 ± 15	202 ± 14	0.07

3.1 Biochemical and Mycological Isolation Results

Table 2: Morphological and Cultural Isolation Results of Fungal Isolates

Test Parameters	Isolate A	Isolate B	Isolate C	Isolate D	Isolate E	Isolate F
Colony Appearance (on PDA)	Yellow-green, velvety	Creamy, smooth	Pink, cottony	Greenish, powdery	Blue-green, velvety	White, fluffy
Microscopic Features	Rough conidiophores	Budding yeast	Sickle-shaped macroconidia	Branched conidiophores	Brush-like conidiophores	Non-septate hyphae
Spore Type	Conidia	Chlamydospores	Macroconidia	Conidia	Conidia	Sporangiospores
Hyphae Type	Septate	Pseudohyphae	Septate	Septate	Septate	Non-septate
Growth Rate	Moderate	Fast	Fast	Fast	Moderate	Rapid
Pigmentation	Yellow-green	Creamy	Pinkish	Green	Blue-green	Grayish
Identified Organism	*Aspergillus flavus*	*Candida albicans*	*Fusarium spp.*	*Trichoderma viride*	*Penicillium spp.*	*Mucor spp.*

Biochemical Characteristics for Bacterial Isolate

Table 3: Biochemical Result for pond A.

Isolate Code	Gram Reaction	Catalase	Oxidase	Citrate Utilization	Indole Test	Urease	Motility	Coagulase	H_2S Production	Glucose Fermentation	Lactose Fermentation	Pigmentation / Colony Color	Identified Organism
1	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
2	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
3	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
4	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*

5	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
6	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
7	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
8	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
9	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
10	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
11	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
12	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
13	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
14	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
15	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
16	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
17	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
18	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
19	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
20	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
21	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
22	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
23	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
24	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
25	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
26	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*

27	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
28	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
29	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
30	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
31	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
32	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
33	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
34	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
35	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
36	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
37	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
38	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
39	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
40	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
41	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
42	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
43	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
44	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
45	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
46	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
47	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*

48	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
49	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
50	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
51	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
52	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
53	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
54	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
55	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
56	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
57	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
58	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
59	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
60	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
61	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
62	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
63	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
64	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
65	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
66	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
67	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
68	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy	*Enterobacter*

												mucoid	*spp.*
69	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
70	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
71	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
72	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
73	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
74	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
75	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
76	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
77	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
78	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
79	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
80	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
81	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
82	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumonia*
83	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
84	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
85	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*

Table 4: Biochemical Result for pond B.

Isolate Code	Gram Reaction	Catalase	Oxidase	Citrate Utilization	Indole Test	Urease	Motility	Coagulase	H_2S Production	Glucose Fermentation	Lactose Fermentation	Pigmentation / Colony Color	*Identified Organism*
1	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
2	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
3	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
4	+ve cocci	+	–	–	–	+	–	+	–	+	–	Deep golden	*Staphylococcus aureus*
5	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
6	–ve rod	+	–	+	–	+	+	–	+	+	+	Gray, moist	*Citrobacter freundii*
7	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
8	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
9	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
10	+ve cocci	+	–	–	–	+	–	+	–	+	–	Deep golden	*Staphylococcus aureus*
11	–ve rod	+	–	–	+	+	+	–	+	+	–	Swarming growth; non-pigmented	*Proteus vulgaris*
12	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
13	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
14	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
15	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
16	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
17	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
18	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
19	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
20	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
21	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
22	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*
23	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
24	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
25	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
26	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
27	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
28	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
29	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
30	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
31	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*

32	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
33	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
34	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
35	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
36	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
37	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
38	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
39	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
40	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
41	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
42	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*
43	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
44	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
45	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
46	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
47	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
48	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
49	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
50	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
51	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*
52	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
53	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
54	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
55	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
56	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
57	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
58	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
59	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
60	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
61	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
62	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
63	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*

Table 5: Biochemical Result for pond C.

Isolate Code	Gram Reaction	Catalase	Oxidase	Citrate Utilization	Indole Test	Urease	Motility	Coagulase	H_2S Production	Glucose Fermentation	Lactose	Pigmentation / Colony Color	Identified Organism
1	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
2	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
3	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
4	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
5	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
6	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
7	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
8	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
9	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
10	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
11	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
12	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
13	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
14	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
15	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
16	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
17	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
18	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
19	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
20	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
21	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
22	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
23	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
24	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*

25	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
26	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
27	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
28	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumoniae*
29	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
30	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
31	+ve cocci	+	–	–	–	+	–	+	–	+	–	Deep golden	*Staphylococcus aureus*
32	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
33	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
34	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
35	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
36	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
37	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumoniae*
38	–ve rod	+	–	–	+	+	+	–	+	+	–	Swarming growth; non-pigmented	*Proteus vulgaris*
39	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
40	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
41	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*
42	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
43	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
44	–ve rod	+	–	+	–	+	–	–	–	+	+	Mucoid	*Klebsiella pneumoniae*
45	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
46	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
47	+ve cocci	+	–	–	–	+	–	+	–	+	–	Deep golden	*Staphylococcus aureus*
48	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
49	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
50	–ve rod	+	–	+	–	–	+	–	–	+	–	Red pigment (prodigiosin)	*Serratia marcescens*
51	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
52	–ve rod	+	–	–	+	–	+	–	–	+	+	Creamy, non-pigmented	*Escherichia coli*
53	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies on XLD	*Salmonella spp.*
54	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
55	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
56	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*

57	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
58	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
59	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
60	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
61	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
62	–ve rod	+	–	+	–	+	–	–	–	+	+	Creamy mucoid	*Klebsiella pneumonia*
63	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
64	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
65	+ve cocci	+	–	–	–	+	–	+	–	+	–	Yellow-gold	*Staphylococcus aureus*
66	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
67	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
68	+ve cocci	+	–	–	–	+	–	+	–	+	–	Golden-yellow	*Staphylococcus aureus*
69	–ve rod	+	–	–	+	–	+	–	–	+	+	Non-pigmented	*Escherichia coli*
70	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
71	–ve rod	+	–	+	–	+	+	–	–	+	+	Pale creamy, mucoid	*Enterobacter spp.*
72	+ve cocci	+	–	–	–	+	–	+	–	+	–	Deep golden	*Staphylococcus aureus*
73	–ve rod	+	–	+	–	–	+	–	+	+	–	Black-centered colonies	*Salmonella spp.*
74	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
75	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
76	–ve rod	+	+	+	–	–	+	–	–	–	–	Greenish pigment (pyocyanin)	*Pseudomonas aeruginosa*
77	–ve rod	+	–	–	+	–	+	–	–	+	+	Translucent, smooth	*Escherichia coli*
78	–ve rod	+	–	+	–	+	+	–	–	+	+	Creamy mucoid	*Enterobacter spp.*
79	–ve rod	+	+	+	–	–	+	–	–	–	–	Blue-green pigment (pyocyanin)	*Pseudomonas aeruginosa*
80	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumonia*
81	–ve rod	+	–	+	–	+	+	–	+	+	+	Pale, smooth	*Citrobacter freundii*
82	–ve rod	+	–	+	–	+	–	–	–	+	+	Highly mucoid	*Klebsiella pneumonia*

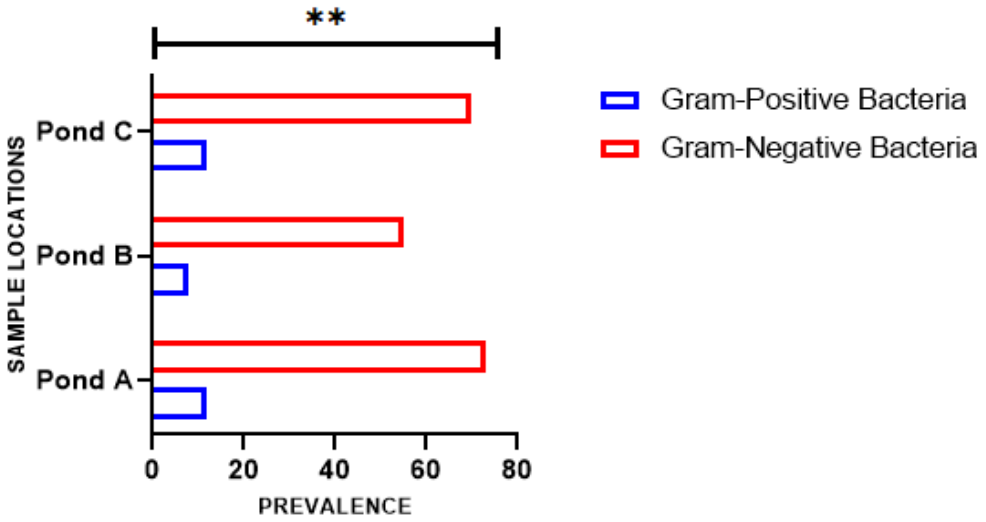

Figure 1: Prevalence of Gram Positive and Gram Negative Bacteria Isolated from each sample locations.

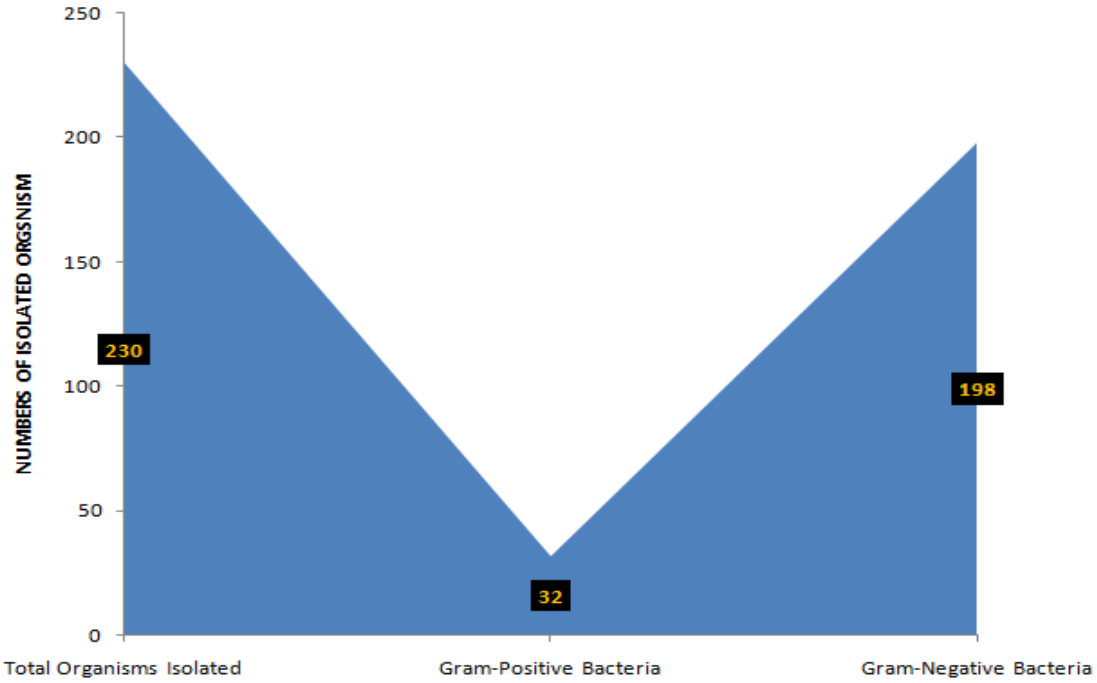

Figure 2: Total number of organisms isolated from the samples.

This figure summarizes the common bacterial species isolated from Ponds A, B, and C, showing their numerical presence and calculated percentage prevalence across the ponds. Minor variations reflect natural microbial diversity within different aquatic environments.

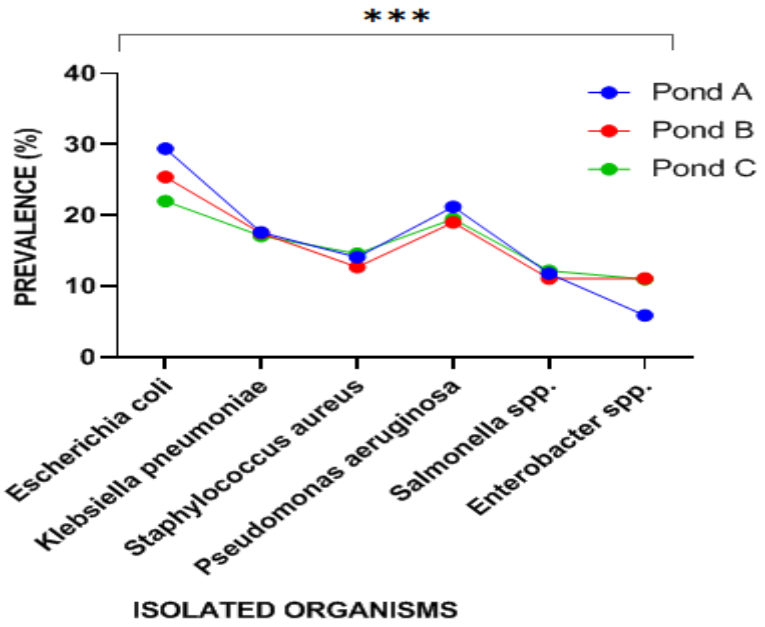

Figure 3: Prevalence of Isolated Organisms from the sample.

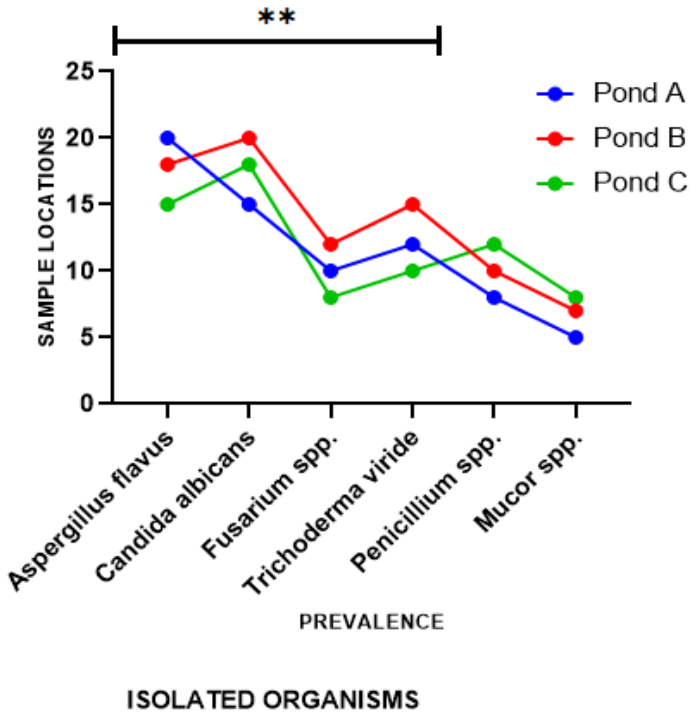

Figure 4: Prevalence of Isolated Fungi from the samples.

4.0 DISCUSSION AND CONCLUSION

This study established that fish ponds in Ede, Osun State, harbour diverse bacterial and fungal species associated with faecal contamination, organic enrichment, and poor waste

management. The isolated bacteria—*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella* species, and *Enterobacter* species—reflect contamination from both environmental and anthropogenic sources, while fungi such as *Aspergillus flavus*, *Penicillium*, *Fusarium*, and *Mucor* indicate feed deterioration and nutrient overload. Although the measured physicochemical parameters (temperature, pH, dissolved oxygen, and total dissolved solids) were within recommended aquaculture limits, the high microbial load signals underlying management deficiencies that could lead to disease outbreaks, reduced fish growth, and environmental degradation. The findings corroborate previous observations in tropical aquaculture systems (*Bamidele, Ajayi, & Akinola, 2021; Mensah, Kwarteng, & Aboagye, 2021*). From a public-health perspective, the presence of enteric bacteria and toxigenic fungi underscores the potential transmission of zoonotic pathogens and mycotoxins through aquaculture products. Without appropriate interventions, such contamination can compromise food safety and contribute to antimicrobial-resistance dissemination (*World Health Organization, 2023*).

Physicochemical profiles demonstrate favourable aquatic conditions promoting microbial persistence, paralleling *Adeoye, Ojo, and Ibrahim (2022).* Neutral pH and moderate TDS supported bacterial proliferation. Similar conditions were reported by *Adegoke, Akinloye, and Ogundipe (2021)* in Nigerian aquaculture.

Escherichia coli dominance indicates faecal contamination (*Cabral, 2010*; *Adedeji & Osakwe, 2022*). *Pseudomonas aeruginosa*—a biofilm-forming opportunist—thrived in nutrient-rich water, corroborating *Oladimeji, Fadeyi, and Akinyemi (2023)* and *Abioye, Adebayo, and Oladimeji (2022)*. *Klebsiella pneumoniae* and *Enterobacter* spp. reflect organic enrichment (*Ibrahim, Musa, & Ado, 2021*). *Salmonella* spp. detection confirms input from livestock run-off (*Nwankwo & Olorunfemi, 2022*). *Staphylococcus aureus* suggests contamination during fish handling (*Adejumo & Aluko, 2020*).

Fungal diversity mirrors earlier findings by *Chukwuka, Onyema, and Adebisi (2021),* who isolated *Aspergillus* and *Penicillium* as dominant genera. *A. flavus* produces aflatoxins hazardous to fish consumers (*Rodrigues & Naehrer, 2012*). *Fusarium* spp. produce trichothecenes toxic to aquatic organisms (*Pratiwi, Widiastuti, & Susilowati, 2018*). *Trichoderma viride* and *Mucor* spp. are associated with decomposition of organic waste, aligning with *Yusuf, Ali, and Ibrahim (2021).*

Comparative regional studies (e.g., *Mensah, Kwarteng, & Aboagye, 2021*; *Wambugu, Kamau, & Otieno, 2022*) reveal similar microbial loads in tropical ponds. The lack of significant difference among ponds suggests shared contamination sources (*Okafor & Umeh, 2021*).

The observed bacterial and fungal assemblages have ecological and public-health implications. For fish, chronic exposure to *Pseudomonas* and *Klebsiella* may cause fin rot and reduced immunity (*Oyeleke & Bello, 2019*). For humans, *E. coli* and *Salmonella* can induce gastroenteritis, while fungal toxins pose carcinogenic risk (*Olaoye & Adeyemi, 2020*). Environmental accumulation of organic matter encourages eutrophication (*Bamidele, Olukotun, & Akinyemi, 2021*). Such effects underscore the need for continuous microbiological surveillance (*Adeoye, Ojo, & Ibrahim, 2022*).

CONCLUSION

This study revealed that fish ponds in Ede, Osun State, contain diverse bacterial and fungal species dominated by Gram-negative organisms, notably *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Physicochemical parameters were within acceptable limits, yet high microbial loads indicate faecal and organic contamination from poor management practices. The findings highlight the need for improved pond hygiene, controlled antibiotic use, and regular microbial monitoring to safeguard fish health, environmental quality, and consumer safety in Nigerian aquaculture.

REFERENCES

1. Abioye, O. P., Adebayo, F. A., & Oladimeji, T. O. (2022). Antibiotic resistance in aquaculture-associated bacteria. *African Journal of Aquatic Science*, 47(2), 200–210.
2. Adegoke, A. A., Akinloye, O., & Ogundipe, F. O. (2021). Public-health significance of bacterial contamination in aquaculture ponds. *African Journal of Biotechnology*, 20(17), 624–633.
3. Adegoke, A. A., Bamidele, T., & Lawal, M. O. (2020). Antimicrobial resistance in aquaculture bacteria. *Nigerian Journal of Microbial Research,* 16(3), 145–158.
4. Adejoke, K. L., Oluwole, T., & Omobola, O. (2022). Environmental microbiology of earthen fish ponds in South-West Nigeria. *Nigerian Environmental Review*, 11(1), 77–93.
5. Adejumo, B. T., & Aluko, A. A. (2020). Microbial analysis of pond sediments in Southern Nigeria. *Nigerian Journal of Microbiology*, 34(3), 102–115.

6. Adeoye, R. A., Ojo, O. S., & Ibrahim, A. T. (2022). Microbial contamination and antibiotic resistance in aquaculture waters of Nigeria. *African Journal of Aquatic Science*, 47(3), 255–266.
7. Adewale, M. O., Ajibola, F. O., & Ogunyemi, T. A. (2024). Ecological interactions of microbial populations in tropical aquaculture ponds. *Environmental Monitoring and Assessment*, 196(4), 1123–1136.
8. Adewale, O., Omotayo, F., & Jimoh, O. (2020). Physicochemical and microbial quality of pond water in South-Western Nigeria. *African Journal of Environmental Science*, 14(2), 87–98.
9. Adeyemi, O. A., Olawale, O. G., & Fashina, B. A. (2022). Recent advances in phenotypic characterization of environmental bacteria. *West African Journal of Applied Microbiology*, 33(1), 65–78.
10. Adedeji, T. A., & Osakwe, C. C. (2022). Fecal indicator bacteria in freshwater aquaculture ponds. *Journal of Environmental Health Research*, 22(3), 199–211.
11. Akinyemi, O., Fadeyi, T., & Oyeleke, A. (2020). Aquaculture hygiene and public-health implications in Southwestern Nigeria. *Nigerian Journal of Microbiology*, 34(2), 75–86.
12. Akintola, S. L., & Oladimeji, A. A. (2023). Microbial ecology of aquaculture systems: Trends and control measures. *Journal of Fisheries Science*, 17(2), 210–223.
13. APHA. (2017). *Standard Methods for the Examination of Water and Wastewater* (23rd ed.). American Public Health Association.
14. Bamidele, J. O., Ajayi, O. E., & Akinola, A. A. (2021). Climatic influence on microbial load of fish-pond water in Southwestern Nigeria. *Applied Water Science*, 11(5), 134–145.
15. Bamidele, O. T., Olukotun, S. O., & Akinyemi, F. (2021). Climatic and nutrient influence on microbial profiles of aquaculture water. *West African Journal of Applied Ecology*, 29(1), 41–56.
16. Boyd, C. E. (2015). *Water Quality: An Introduction*. Springer.
17. Boyd, C. E., & Tucker, C. S. (2012). *Pond Aquaculture Water Quality Management*. Springer.
18. Cabral, J. P. S. (2010). Water microbiology: Bacterial pathogens and indicators of water quality. *International Journal of Environmental Research and Public Health*, 7(10), 3657–3703.
19. Cappuccino, J. G., & Sherman, N. (2021). *Microbiology: A Laboratory Manual* (12th ed.). Pearson Education.

20. Cheesbrough, M. (2019). *District Laboratory Practice in Tropical Countries* (3rd ed.). Cambridge University Press.
21. Chukwuka, C. A., Onyema, A., & Adebisi, O. (2021). Fungal contaminants and aflatoxin risks in aquaculture feeds and fish products. *Food Control*, 127, 108136.
22. Clinical and Laboratory Standards Institute (CLSI). (2023). *Methods for the Examination of Water and Wastewater: Microbiological Techniques*. Wayne, PA: CLSI Press.
23. Clinical and Laboratory Standards Institute (CLSI). (2023). *Performance Standards for Antimicrobial Susceptibility Testing* (33rd ed.). Wayne, PA: CLSI Press.
24. Egbule, C. A., Okoye, A., & Nwankwo, C. C. (2022). Molecular identification of aquatic bacteria and resistance genes. *Journal of Applied Microbiology*, 133(5), 2847–2858.
25. Ezeonu, C. S., Okafor, C. U., & Chukwu, M. E. (2021). Bacteriological and fungal assessment of fish ponds in Nigeria. *African Journal of Biotechnology*, 20(9), 312–323.
26. Ezeonu, C. S., Okeke, B. C., & Okezie, U. M. (2020). Updated laboratory methods for identification of enteric bacteria. *African Journal of Clinical Microbiology*, 21(4), 115–124.
27. FAO. (2015). *Water-Quality Guidelines for Aquaculture*. Food and Agriculture Organization of the United Nations.
28. FAO. (2021). *Sustainable Aquaculture Development in Africa*. Food and Agriculture Organization of the United Nations.
29. Folarin, O. A., & Odusote, O. O. (2023). Comparative study of bacterial and fungal contaminants in aquaculture systems in Southwest Nigeria. *African Journal of Environmental Microbiology Research*, 14(2), 99–110.
30. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2022). *Bailey & Scott's Diagnostic Microbiology* (15th ed.). Elsevier.
31. Garrity, G. M. (Ed.). (2015). *Bergey's Manual of Systematics of Archaea and Bacteria*. Wiley-Blackwell.
32. Ghosh, S., & Sharma, P. (2024). Emerging techniques for microbial isolation and identification in aquatic ecosystems. *International Journal of Environmental Microbiology*, 19(1), 1–16.
33. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (2020). *Bergey's Manual of Determinative Bacteriology* (10th ed.). Williams & Wilkins.
34. Ibrahim, A. M., Musa, I. A., & Ado, G. B. (2021). Occurrence of *Proteus* and *Citrobacter* species in aquaculture environments. *Journal of Water and Health*, 19(4), 590–603.

35. MacFaddin, J. F. (2021). *Biochemical Tests for Identification of Medical Bacteria* (Updated ed.). Wolters Kluwer Health.
36. Mensah, J. K., Kwarteng, A., & Aboagye, D. (2021). Comparative microbial analysis of aquaculture ponds in Ghana. *Aquaculture Reports*, 19, 100606.
37. Nkereuwem, M. E., & Agbo, E. O. (2023). Comparative evaluation of classical and automated biochemical identification of microbial isolates. *International Journal of Microbiology and Biotechnology*, 8(2), 42–53.
38. Nwankwo, C. D., & Olorunfemi, A. O. (2022). Environmental assessment of microbial communities in aquaculture ponds. *African Environmental Journal*, 18(2), 233–248.
39. Obaseiki-Ebor, E. E., & Ezeronye, O. U. (2021). Advances in aquatic microbiology and fungal isolation procedures. *Nigerian Journal of Aquatic Sciences*, 12(3), 55–67.
40. Ogunbanwo, S. T., Ajayi, B. A., & Adebisi, R. O. (2020). Characterization of bacteria in aquaculture water systems. *Journal of Environmental Microbiology*, 22(4), 344–355.
41. Okafor, N. C., & Umeh, C. O. (2021). Microbial ecology and water-quality assessment of tropical aquaculture systems. *Nigerian Journal of Science*, 55(4), 323–332.
42. Olaoye, R. A., & Adeyemi, O. A. (2020). Fungal contamination and aflatoxin production in aquaculture feeds. *Nigerian Journal of Microbiology*, 34(3), 145–154.
43. Oladimeji, A. A., Fadeyi, T., & Akinyemi, O. (2023). Public-health risks associated with fish-pond microbial contamination in Nigeria. *Scientific African*, 19, e01583.
44. Oluwole, D. F., Ezeanya, L., & Bamgbose, T. (2022). Water quality and microbial hazards in artisanal fish ponds. *Aquatic Ecosystem Health and Management*, 25(1), 22–32.
45. Onifade, A., & Adebayo, B. (2023). Fungal biodiversity in aquaculture systems: Implications for food safety. *Mycopathologia*, 188(3–4), 405–417.
46. Oyeleke, S. B., & Bello, M. A. (2019). Bacteriological quality of fish-pond water in North-Central Nigeria. *Nigerian Journal of Aquatic Science and Fisheries*, 6(1), 89–97.
47. Oyeleke, S. B., & Manga, S. B. (2020). Essentials of laboratory practice and techniques in microbiology (Revised ed.). *African Journal of Microbiological Education*, 5(2), 14–22.
48. Pratiwi, R. H., Widiastuti, I., & Susilowati, A. (2018). Mycotoxins in aquaculture: Occurrence and impacts. *Aquaculture*, 497, 48–58.
49. Rodrigues, I., & Naehrer, K. (2012). A three-year survey on worldwide occurrence of mycotoxins in feedstuffs. *Toxins*, 4(10), 1024–1039.

50. Timmons, M. B., & Ebeling, J. M. (2013). *Recirculating Aquaculture Systems* (2nd ed.). Cayuga Aqua Ventures.
51. World Health Organization. (2017). *Guidelines for Drinking-Water Quality* (4th ed.). Geneva: WHO Press.
52. World Health Organization. (2021). *Manual for Clinical Microbiology Laboratories in Low-Resource Settings* (2nd ed.). Geneva: WHO Press.
53. World Health Organization. (2023). *Antimicrobial Resistance: Global Report on Surveillance*. Geneva: WHO Press.