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ASSESSMENT OF GRAM-NEGATIVE BACTERIAL PROFILE ASSOCIATED WITH SOME SELECTED FISH POND WASTEWATER IN BALI METROPOLIS, TARABA STATE

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ABSTRACT

This study focused on the assessment of the profile of Gram-negative bacteria associated with some selected fish pond wastewater in Bali metropolis, Taraba state. Water samples were collected from three fish ponds namely Sky fish pond, Natural fish pond and Eastern fish pond and analyzed using standard microbiological techniques. Isolation of bacteria was carried out using Nutrient agar, MacConkey agar, and Blood agar, while identification was based on cultural characteristics, Gram staining, and biochemical tests including catalase, coagulase, and oxidase reactions. The isolates were presumptively identified as Pseudomonas sp., Staphylococcus sp., and Klebsiella sp., based on their cultural, morphological, and biochemical characteristics. Pseudomonas sp., was isolated from Sky fish pond and Natural fish pond, while Klebsiella sp., was isolated from Eastern fish pond. The presence of these pathogenic Gram-negative bacteriain fish pond wastewater highlights the possible risks to fish health and public health, especially where wastewater is discharged without proper treatment. The study concludes that fish pond wastewater may serve as an important reservoir for microorganisms of public health importance. Regular microbiological monitoring, improved wastewater management, and strict hygienic aquaculture practices are recommended.

KEYWORDS: Fish pond wastewater, Bacteria, Gram-negative, Pseudomonas sp., Klebsiella sp.

INTRODUCTION

Bali metropolis is a significant hub for aquaculture activities, including fish pond farming. The growth of the aquaculture industry in Bali has led to an increase in fish pond wastewater generation. Contamination of these culture systems has been attributed to poor water quality, high stocking density, and the use of animal manure and contaminated feed, posing significant environmental and health risks to the local community (Mukwabi*et al.,* 2019). Water is an essential natural resource that plays a vital role in aquaculture production and ecosystem sustainability. The most significant resource for aquaculture is water. Water has been known to be one of the pathogen-transmitting components. Water of good quality is essential for the viability of aquatic life such as fish (Onajobi*et al*., 2015). The aim of this study was to investigate the profile of Gram-negative bacteria isolated from fish pond wastewater in Bali metropolis.

Fish pond wastewater can contaminate surface and groundwater resources, harming aquatic ecosystems and human health. The wastewater can contain high levels of nutrients, such as nitrogen and phosphorus, which can lead to eutrophication (Zhang *et al*., 2018; Purnomo*et al*., 2022). The contamination of fish pond water has been attributed to feed (animal manure) which contains organic matter and introduces a wide range of microorganisms into the pond (Okpokwasili&Ogbulie, 1993; Njoku*et al*., 2015). Fish pond wastewater can also harbor pathogenic microorganisms, including bacteria, viruses, and parasites, which can cause waterborne diseases in humans. Effective fish pond wastewater management is crucial to mitigate the environmental, health, and economic risks associated with aquaculture activities in Bali metropolis. Proper management practices, including wastewater treatment and disposal, can help to reduce the environmental impacts of fish pond wastewater, protect human health by minimizing the risk of waterborne diseases, support sustainable aquaculture practices, and maintain economic viability.

Pathogenic organisms are capable of diminishing or reducing fish production (Buján*et al*., 2018). The presence of these pathogens in wastewater poses a significant risk to the health and wellbeing of local communities, particularly those involved in aquaculture activities, and increases the mortality of the cultured species (Buján*et al*., 2018). Microorganisms found in fish ponds can be bacteria, fungi, archaea, or protists. Some of these bacteria found in fish ponds are well known for causing mycobacteriosis, which can be transmitted to humans when infected fish are consumed. Most of these pathogens involved are resistant to antibiotics (Håstein*et al.,* 2006). As they are consumed, these resistant pathogens are transmitted among humans (Olayemi*et al*., 1991). Poor fish pond wastewater management can also have economic implications. The contamination of water sources can lead to a decrease in fish production, reduced tourism revenue, and increased costs for wastewater treatment and environmental remediation (Dauda*et al*., 2019).

Gram-negative bacteria are common inhabitants of aquatic environments, including fish pond wastewater. These bacteriacan pose significant health risks to humans and animals exposed to contaminated water (Fapohunda*et al*., 1994). Several species of Gram-negative bacteria have been isolated from such systems (Maduwuba, 2024), including*Aeromonashydrophila*, whichcan cause gastroenteritis, wound infections, and septicemia in humans.

Pseudomonas aeruginosa: can cause respiratory infections, wound infections, and septicemia in humans.*Escherichia coli*: which causes urinary tract infections, gastroenteritis, and septicemia in humans.*Salmonella* spp.: can cause gastroenteritis, typhoid fever, and paratyphoid fever in humans.*Vibrio* spp.: Can cause gastroenteritis, wound infections, and septicemia in humans.

Exposure to Gram-negative bacteria in fish pond wastewater can lead to various health risks (Fapohunda*et al*., 1994), including:Waterborne diseases: Consumption of contaminated water can lead to diseases such as gastroenteritis, cholera, and typhoid fever.

Skin infections: Contact with contaminated water can lead to skin infections such as wound infections and dermatitis.

METHODOLOGY

Description of the Study Area

Bali is one of the largest Local Government Areas in Taraba State, located in the North-Eastern region of Nigeria. It lies within the tropical savannah zone, characterized by a distinct wet and dry season, with annual rainfall ranging between 1000–1500 mm and temperatures often exceeding 30°C during the hot season. The Local Government Area headquarters is geographically positioned at latitude 7°51′N and longitude 10°58′E, with the entire LGA covering an expansive land area of approximately 9,146 square kilometers, making it one of the largest administrative landmasses within Taraba State (Adebayo &Tukur, 2020). The climatic conditions favor agricultural activities such as crop cultivation and livestock rearing, which constitute the major occupations of the inhabitants (Kefas*et al*., 2025). The study area is characterized by a culturally diverse population including ethnic groups such as the Tiv, Fulani, Jukun, and Hausa, which collectively shape its social structure and cultural dynamics (Garba&Dankano, 2017). Bali also hosts various streams, wells, and boreholes, which serve as major sources of water supply for both domestic and agricultural purposes.

Sample Collection

The water samples were collected from three different fish ponds in Bali metropolis into sterile bottles at a depth of 10–15 cm from the surface of the water. The samples were labeled accordingly as Sky Pond, Natural Pond, and Eastern Pond. The samples were transported to the Microbiology Laboratory of the Federal Polytechnic Bali and were analyzed within one hour of collection.

Isolation of the Bacteria

The workbench was disinfected using standard aseptic techniques. Nutrient agar, MacConkey agar, and blood agar media were prepared in strict accordance with the manufacturer's instructions to isolate and differentiate the target pathogens. Isolation of the bacterial pathogens from the collected fish pond wastewater was performed using standard serial ten-fold dilutions in sterile normal saline, followed by inoculation onto the agar plates using the pour plate technique (Maduwuba, 2024).

The inoculated nutrient agar plates were allowed to solidify and incubated at an optimal temperature of 35°C–37°C for 24–48 hours. Similarly, MacConkey agar plates were prepared, inoculated with the diluted water samples, and incubated at 35°C–37°C for 24–48 hours to selectively isolate and differentiate Gram-negative coliforms based on standard diagnostic frameworks (Maduwuba, 2024; Wamala*et al*., 2018).

Identification of the Isolates

Gram Staining Procedure

Colonies of presumptive *Pseudomonas*sp., *Staphylococcus*sp., and*Klebsiella*sp.,were picked subsequently and smears were made on clean, grease-free glass slides, heat-fixed by passing them over a flame 3 times, and allowed to air dry. The standard Gram staining method described by Beveridge (2001) was employed. Crystal violet was applied to the slide for 30 seconds, and the slide was rinsed gently with tap water. Lugol's iodine was then applied to the slide and allowed to act for 30 seconds before being rinsed with water. A decolourizer (95% ethanol) was applied briefly and washed off immediately. Safranin counterstain was applied to the slide for 30 seconds, rinsed, and the slide was allowed to air dry before microscopic examination (Beveridge, 2001).

Catalase Testing

Colonies of presumptive *Pseudomonas* sp.,*Staphylococcus* sp., and *Klebsiella*sp., were picked subsequently using a sterile wire loop and mixed with drops of 3% hydrogen peroxide inside test tubes, and the reaction (effervescence) was observed (Cheesbrough, 2006).

Coagulase Testing

Samples of the bacterial cultures were emulsified in a small amount of sterile saline in a test tube. A few drops of rabbit plasma (with anticoagulant) were added to the bacterial suspension, mixed gently, and observed for clumping within 4–24 hours of incubation at 37°C, following standard biochemical screening methodologies (Cheesbrough, 2006).

Oxidase Testing

A few drops of freshly prepared oxidase reagent (tetramethyl-p-phenylenediaminedihydrochloride) was placed on a filter paper. Pure colonies of the bacterial isolates were rubbed onto the reagent using a sterile glass rod, and a distinct color change to dark purple within 10–30 seconds was verified (Cheesbrough, 2006).

RESULTS

The tables below show the results of the preliminary identification of bacterial isolates (A, B, C) from the three fish ponds (Sky Pond, Natural Pond, and Eastern Pond) based on morphological characteristics and biochemical tests. Isolate A was found to be positive in Sky Pond and Natural Pond but negative in Eastern Pond. Isolate B and C showed negative growth in Sky Pond and Natural Pond, appearing exclusively as positive cultures in Eastern Pond (Table 1).

Table 1Cultural characteristics of bacterial isolates from fish pond waste water.

Isolates	Cultural characteristics	Sky Fish Pond	Natural Fish Pond	Eastern Fish Pond
Isolate A	Large, creamy colony	Positive	Positive	Negative
Isolate B	Creamy, smooth colony	Negative	Negative	Positive
Isolate C	Large, dome-shaped, mucoid colony	Negative	Negative	Positive

Table 2 shows the results of the identification of the isolates using Gram staining and differential biochemical testing, which include catalase, coagulase, and oxidase tests, alongside presumptive identifications.

Isolate A was found to be a Gram-negative rod that tested positive for catalase and oxidase, but negative for coagulase, confirming its presumptive identification as*Pseudomonas* sp. Isolate B was found to be a Gram-positive coccus, which tested positive for both catalase and coagulase but negative for oxidase, confirming its presumptive identification as *Staphylococcus* sp.

Isolate C was identified as a Gram-negative rod that tested positive for catalase, but negative for both coagulase and oxidase, confirming its presumptive identification as*Klebsiella*sp.

Table 2Biochemical identification of the isolates found in the fish ponds.

Isolates	Gram stain	Catalase test	Coagulase test	Oxidase test	Presumptive Organisms
A	Negative	Positive	Negative	Positive	*Pseudomonas* sp
B	Positive	Positive	Positive	Negative	*Staphylococcus* sp
C	Negative	Positive	Negative	Negative	*Klebsiella*sp

DISCUSSION

Isolate A, identified as *Pseudomonas* sp., exhibited Gram-negative reactions, appearing as pink, slender rods under the microscope after Gram staining. This characteristic staining result is due to their thin peptidoglycan layer and the presence of an outer membrane, which retains the counterstain (safranin) rather than the crystal violet-iodine complex (Cheesbrough, 2006). These findings align with previous reports that describe *Pseudomonas* species as aerobic, non-fermenting Gram-negative bacilli widely distributed in nature and known for their role in opportunistic infections, particularly in immunocompromised individuals (Holt, 2000).

Isolate C appeared as Gram-negative, short plump rods, with mucoid colonies suggestive of *Klebsiella* sp. The mucoid nature of the colonies is consistent with the presence of extracellular polysaccharide material commonly associated with *Klebsiella* spp. Such characteristics have been linked to increased environmental persistence and pathogenic potential (Koneman*et al.*, 2017). The presence of these encapsulated, mucoid *Klebsiella* sp. strains across the ponds, highlights the elevated public health risks associated with discharging untreated pond water into surrounding domestic water sources, as these features help them survive in the environment and easily cause infections in humans.

CONCLUSION

The studied fish ponds harbored two Gram-negative bacteria (*Pseudomonas* sp. *and Klebsiella*sp.). The presence of these potentially pathogenic bacteria in the sampled fish ponds suggests that fish pond wastewater may serve as a reservoir of microorganisms of public health significance.

RECOMMENDATIONS

1. Access to fish ponds should be strictly restricted to authorized personnel to minimize external contamination by microbial pathogens.
2. Fish pond wastewater should be regularly screened for pathogenic Gram-negative bacteria and properly treated before disposal to avoid potential zoonotic disease transmission.
3. Further studies on specific virulence factors and antibiotic resistance profiles of *Pseudomonas*sp.and *Klebsiella*sp. isolated from fish pond wastewater are highly encouraged.

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