Isolation and Identification of Bacteria and Fungi from Selected Rivers and Lake

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ABSTRACT

The aquatic environment especially the rivers and lake within the State of Anambra, Nigeria have been such that serves as avenue for different activities ranging from domestic to religious, agricultural, flood, etc. Thus, its ability to encourage the proliferation of microorganisms. This study was aimed at isolating and identifying bacteria and fungi from rivers and lake located at Anambra Central Senatorial District, Anambra State. Forty-two water samples were collected in triplicates from seven (7) water bodies randomly selected from each town representing the seven (7) Local Government Area in Anambra Central Senatorial District. Bacteria and fungi were isolated from these samples using standard isolation techniques. The isolates were identified phenotypically through morphological, microscopic and biochemical characteristics.A total of two hundred and sixty-six (266) isolates were recovered from them. Bacteria isolates represented 241(90.60%) of the total number of microbial isolates compared to the fungi isolates 25(9.40%). The bacteria isolated were Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae and Salmonella Typhiwhile the fungi isolates wereAspergillus niger, Aspergillus flavus, Candida albicans etc.Bacillus subtilis was observed to be predominant among the bacteria isolates while Candida albicans was predominant among the fungi isolates. In this study, the results established the presence of both bacteria and fungi in varying degrees. The frequency of occurrence of bacteria isolates was significantly higher than the fungi isolate in all the sample locations. This shows that bacteria greatly exceed fungi numerically in all habitat including aquatic habitat as equally reported by most researchers.

KEY WORDS: *Aquatic environment, Bacteria, Fungi*.

INTRODUCTION

Aquatic ecosystems, encompassing rivers and lakes, serve as critical habitats supporting diverse microbial communities crucial for ecosystem functioning and human well-being. Much of the earth's surface is occupied by water, and in environmental health monitoring, rivers and lakes are susceptible to pollution from various anthropogenic sources, leading to alterations in microbial community composition and water quality (Agu*et al.*, 2014; Savio *et al*., 2015; Agu *et al*., 2017;Agu and Odibo, 2021). The presence of pathogenic bacteria and fungi in these water bodies poses risks to ecosystem health and human populations through waterborne diseases.Water covers approximately 71% of the Earth's surface, with continents and islands accounting for the remaining 29% (Victor-Adulojo*et al*., 2023; Agu *et al*., 2023).Aquatic environment host a wealth of bacterial and fungal species, adapted to diverse ecological niches and environmental conditions. Recent studies have highlighted the high microbial diversity in rivers and lakes, with unique taxa thriving in different habitats within these ecosystems (Wang *et al*., 2020). Understanding microbial biodiversity provides insights into ecosystem dynamics, resilience, and responses to environmental changes.

Bacteria and fungi play vital roles in aquatic ecosystems, driving essential ecological processes such as nutrient cycling, organic matter decomposition, and pollutant degradation. For instance, bacteria belonging to the genus *Pseudomonas* have been identified as key players in the degradation of hydrocarbons in polluted aquatic

environment (Yousaf *et al*., 2010). Fungi also contribute significantly to organic matter breakdown and nutrient recycling, influencing ecosystem productivity and stability.The isolation and identification of bacteria and fungi from these environment are pivotal in understanding their ecological roles, biodiversity, and potential applications. This research is of paramount importance due to its implications for environmental management, public health, and biotechnological advancements.In light of these factors, the isolation and identification of bacteria and fungi from selected rivers and lakes represent a critical research area with far-reaching implications for environmental science, public health, and biotechnology. By advancing our understanding of microbial communities in aquatic ecosystems, this research contributes to sustainable management practices and the development of innovative solutions to environmental challenges. This research was aimed at isolation and identification of bacteria and fungi from selected rivers and lake within Anambra Central Senatorial District.

RESEARCH METHODOLOGY

Study Area

This study was carried out in Awka, the capital of Anambra State. Awka is located within the Coordinates 6°12′25″N7°04′04″E. The area covers 522 km^2 with an estimated population of 399,300. The city is located about 400 miles east of Lagos in the center of the densely populated Igbo heartland, South Eastern Nigeria(Ezenwaji*et al.*, 2014). According to the National Population Commission (2010), the State had a population of 2,796,475 in 1991, but rose to 4,182,032 in 2006 and 4,461,942 in 2011.

Study Design

Seven (7) Different water bodies were selected randomly from 7 Local Government Areas in Anambra Central Senatorial District. The names of the water bodies and the Local Government Area selected are: Obizi river (Awka South), Ezu river (Awka North), Agulu Lake (Anaocha), Ndibe (Dunukofia), OnuNgene River (Njikoka), Ukwuakpu River (Idemili North) and MmiliMgbo (Idemili South).

Sample collection

1.5 liters of water was collected from each sample point from about 30cm from the river surface from three sample points of each river sampled in each town representing the Local Government Area. The same sample collection pattern was done at both climate seasons (Rainy and Dry), giving a total of 42 samples collected. These samples were collected and transported to the Laboratory immediately for microbial examination.

Microbial Isolation

Nutrient agar (NA) andPotatoe Dextrose agar (PDA) used were prepared according to the manufacturer's instruction and sterilized in an autoclave at 121ºC at 15Psi pressure for 15 minutes. Then, allowed to cool at warm touch before they are dispensed into petri dishes. PotatoeDextrose Agar (PDA) supplemented with*Chloramphenicol*were used for the isolation of fungi while Nutrient agar (NA) was used to isolate bacteria.

The 50ml of each water samples collected was inoculated into 50ml of sterilized peptone water and incubated for 24hours at 37ºC to maintain the viability of the microbes in the sample. After which the water samples were serially diluted with sterile water to a 10- and 100-fold dilutions. Aliquots (0.1ml) from each water sample was spread onto respective agar plates for isolation of bacteria and fungi.

The bacteria and fungi culture plates were incubated in an inverted position at 37^0C and 28 ± 2^0C respectively, till visual growth of culture is observed. All isolates were maintained on NA and PDA agar slants at 4 °C until use (Kumar *et al.*, 2012; Thiyagarajan *et al.*, 2014).

Cultural Characterization: Culture plates of pure colonies of the isolates were examined and characterized. The distinct colonies were Gram stained using the standard Gram staining procedures as described in Cheesbrough, 2002. The staining was followed by biochemical tests and culturing in selective and differential medium for suspected organisms.

Identification of Isolate

Identification of the bacteria isolate was done using ABIS (Advanced Bacteria identification software) online software (https://www.tgw1916.net/bacteria_logare_desktop.html).

Pure cultures of common fungi were tentatively identified by comparing the characteristic features of fungi described in Atlas of Fungi accessed online (https://universe84a.com/atlas-of-fungi/). The isolated fungal strains were identified at genus level on the basis of macroscopic characteristics like color, colony, morphology, shape, texture, diameter, and appearance of colony and by microscopic characteristics like mycelium, presence of specific reproductive structures, structure and shape of conidia and presence of mycelium. The microscopic morphology was accessed by the improved slide culture technique of Agu and Chidozie (2021). A sterile glass slide was placed on the bottom of a sterile petri dish. With the aid of a sterile 2 ml syringe, 0.5 ml of the molten Sabouraud Dextrose Agar (SDA) maintained at 45 ^{O}C in a water bath was dispensed on the sterile glass slide. The cover of the petri dish was replaced and the molten agar allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus at the center of the bump. Thereafter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The plates were incubated at room temperature for 3 to 5 days depending on the growth rate of the fungus. When desired growth was observed, few drops of Lactophenol cotton blue stain was dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases.

RESULTS

Forty – two (42) water samples randomly collected from different water bodies (rivers and lake) in Anambra Central Senatorial District revealed the presence of bacteria and fungi colonies. The cultural, microscopic and biochemical characteristics of both the bacteria and fungi isolates are presented in Tables 1 and 2 respectively. The characteristics suggests the identities of the bacteria isolates to be *E. coli*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Shigella* spp., *Serratia* spp., *Proteus* spp. and *Salmonella Typhi.* The characteristics of the fungi colonies represent that of *Aspergillus niger*, *Aspergillus flavus*, *Geotrichum* spp., *Candida albicans*, *Rhizopus* spp. and *Penicillium* spp.

A total of two hundred and sixty-six isolates were recovered from the samples. The distribution of these isolates with respect to culture type and sample locations are shown in Figures 1 and 2. Bacteria isolates represent 90.60% of the total number of microbial isolates compared to the fungi isolates (9.40%). *Bacillus subtilis* and *Proteus* spp. are shown to be the predominantly occurring bacteria across the sample locations, with Nibo and Enugwu-ukwu recording the highest prevalence of this species. *Pseudomonas aeruginosa*and *Shigella* spp. are the least occurring bacteria with low prevalence (below 10%) across all sample locations except in Nnobi where *Pseudomonas aeruginosa*recorded a prevalence above 10%. The most occurring fungi in this study is *Rhizopus* spp, followed by *Aspergillus flavus* and *Aspergillusniger*. The result showed the absence of fungal growths in samples from Nibo and Eziowelle. Also, no fungi isolate except for *Rhizopus* was reported for samples from Nnobi.

Table1: Morphological and Cultural Characteristics of Bacteria Isolates from Water Bodies

Isolates	Grams Reaction/shape	Cell arrangement	Appearances	Colony shape/ margin	Texture/ consistency	Pigmentation/odor	Opacity	catalase	Citrate	oxidase	Indole	glucose	Lactose	maltose	sucrose	Suspected Organism
DS1	- rod	singly	Milky on NA. Red on MCA	Round/ entire	Smooth and Shiny	None/odor present	Translucent	+	-	-	+	+G	+	-	+	***E. coli***
DS2	- rod	singly	Milky on NA Pinkish on MCA	Irregular/ lobate	Smooth and shiny	None/pungent smell	Opaque	-	+	-	-	+	+	+	+	***Enterobacter* spp**
DS3	- rod	Single short rods	White on NA Colorless on MCA	Irregular/ lobate	Smooth/ mucoid	green/pungent smell	Translucent	+	+	+	-	-	-	-	-	***Pseudomonas aeruginosa***
DS4	+ rod	singly	White on NA	Irregular/ undulate	Smooth/ Viscid	odorless	Opaque	+	+	-	-	+	-	-	-	***Bacillus subtilis***
DS5	+ cocci	Clustered	Milky on NA Yellow on MSA	circular/ entire	Smooth/ Viscid	odorless	Opaque	+	-	-	-	+	+	+	+	***Staphylococcus aureus***
DS7	- rod	singly	White on NA. Pinkish on MCA	Round/ entire	smooth/ mucoid	None/pungent smell	Opaque	-	+	-	-	+	+	+	+	***Klebsiella pneumonia***
DS8	- rod	pairs	Colorless on MCA	Round/ entire	Mucoid	None/odor present	Transparent	+	-	-	-	+	-	-	-	***Shigella* spp**
DS9	- rods	Singly	Purple on NA	Round/ entire	Smooth	None	Opaque	+	-	-	-	+	-	-	-	***Serratia* spp.**
DS11	- rods	Short singly rods	Colorless and swarming on NA	Irregular	Smooth	None/ odor present	translucent	+	+	-	-	+G	-	+	+	***Proteus* spp**
DS12	+ rods	Rods in chains	Milky on NA	Round/ lobate	Rough	none	Opaque	+	+	-	-	+	-	-	-	***Bacillus subtilis***
DS16	- rod	Short single rods	Colorless	irregular	Smooth/ mucoid	none	Transparent	+	-	-	-	+G	-	-	-	***Salmonella typhi***
RS3	+ rod	singly	White on NA	Irregular/ undulate	Smooth/ Viscid	odorless	Opaque	+	+	-	-	+	-	-	-	***Bacillus subtilis***
RS5	+ rod	singly	Grayish white on NA	Small circular/ entire	Rough	None/fruity smell	Opaque	+	+	+	-	+	+	+	-	***Bacillus subtilis***
RS23	+ rod	singly	Milky on NA	Large circular/ entire	Rough	None	Opaque	-	-	+	-	+	+	+	-	***Bacillus subtilis***

Table 2: Cultural and Microscopic characteristics of fungi isolates from different water bodies

ISOLATE NO	COLOUR (R/F)	TEXTURE	Microscopy	Suspected Organism
1	Black / white	Mycelia (filamentous)	Conidial fungi, Septate, biseriate	*Aspergillus Niger*
2	Yellow to green/ White	Smooth, raised	Septate, biseriate with rough conidiophore	*Aspergillus flavus*
3	Black / white	Filamentous rough edge	Septate mycelium	*Geotrichum*spp
4	Yellow	Mucoid smooth	Blastoconidia singly/clusters, pseudohyphae	*Candida*spp
5	White/Milkfish yellow	Filamentous smooth	Non-septate sporangiophore	*Rhizopus*spp
6	Green / white	Filamentous	Conidiophore like brush, septate	*Penicillium*spp
7	Milky	Smooth	Blastoconidia singly/clusters, pseudohyphae	*Candida*spp

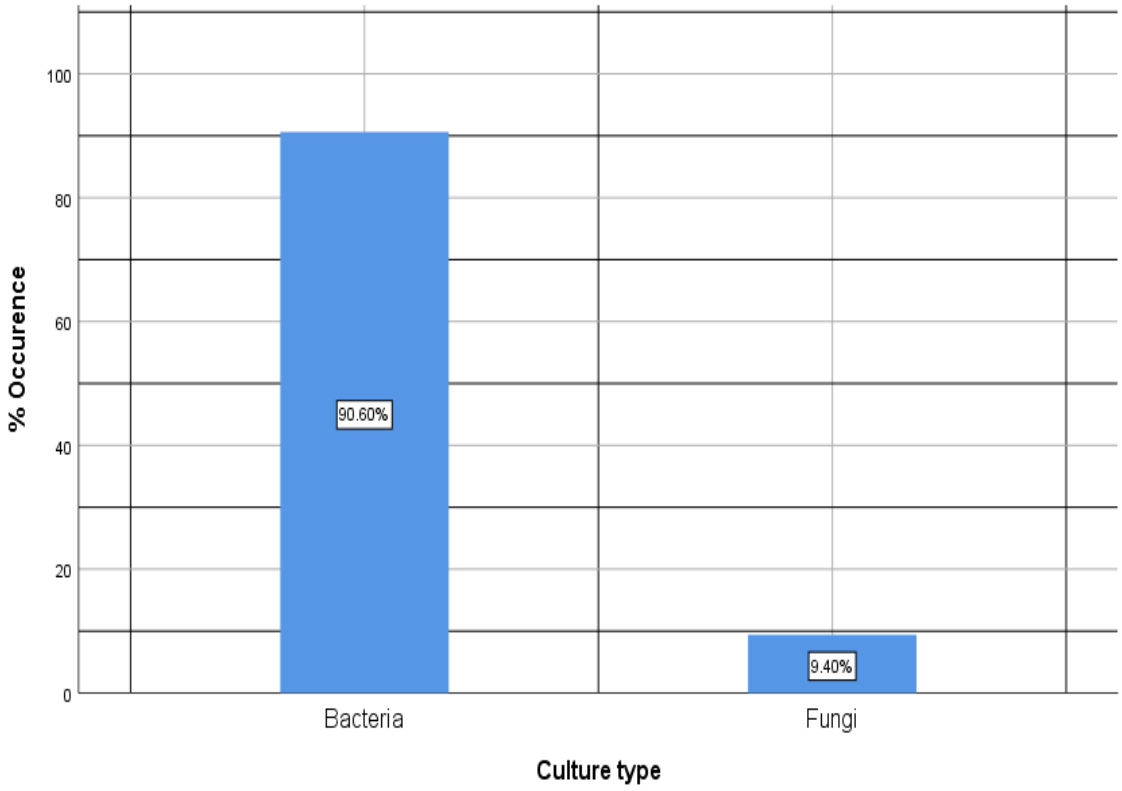

Figure 1: Microbial type occurrence

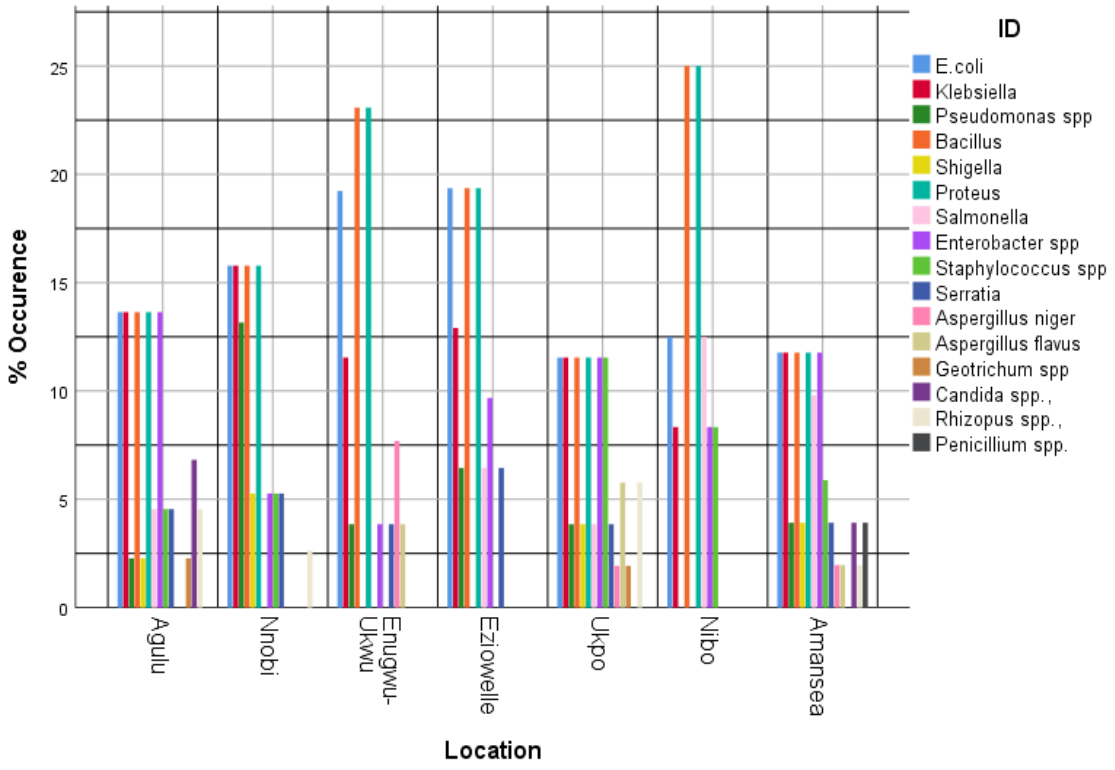

Fig. 2: Distribution of isolates across study sample sites

DISCUSSION

The aquatic environment especially the rivers and lake within the State of Anambra, Nigeria have been such that serves as avenue for different activities ranging from domestic to religious, agricultural, flood, e.t.c. Thus, its ability to encourage the proliferation of microorganisms. In this study, the results established the presence of both bacteria and fungi in varying degrees. The frequency of occurrence of bacteria isolates was significantly higher than the fungi isolate in all the sample locations. This is consistent with the recent report suggesting that bacteria greatly exceeded fungi numerically in all habitat including aquatic habitat (Bahram *et al.*, 2021). Also, Dai and his colleagues reported that bacteria were significantly more abundant than fungi in both sediment and water, in all their sampling areas (Dai *et al.*, 2021). Bacteria, are some of the smallest and oldest organisms on the planet, are prevalent in all water systems and practically every environment, present in the millions per milliliter (mL), and in the hundreds of millions per milliliter in waterways resulting to contamination and threat to great public health (Wang *et al.*, 2019). As part of the biofilm, bacteria find their way to surface water samples, through decaying matter (such as dead wood or leaves), or covering on surface of rocks, stones, and sand grains (the slippery coating on hard surfaces in rivers). The presence of fungi in aquatic environment play a crucial role in the decomposition of plant matter in aquatic systems, as they are among the only creatures capable of decomposing plant structural components such as cellulose and lignin (Osono*et al.*, 2021).

Our investigation revealed the presence of *E. coli*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus.*, *Klebsiella pneumonia*, *Shigella* spp., *Serratia* spp., *Proteus* spp. and *Salmonella Typhi*, across the water samples from study sites. These organisms have been implicated in samples of aquatic source in Southern Nigeria. Ogbonna and Inana reported the presence of *Escherichia coli*, *Pseudomonas putida*, *Salmonella Typhi*, *Shigella* spp, *Staphylococcus aureus*, *Enterobacter* sppand *Enterococcus faecalis* from fishes a potential source of contamination in water samples from Port Harcourt Rivers State Nigeria (Ogbonna and Inana 2018). Similarly, Oku and Amakoromo, 2013, reported the presence of these organisms from samples emanating from fresh water. In Anambra State, similar bacteria species have been reported to be present in water samples in Otuocha river

(Amunekeet *al.*, 2020).*Bacillus subtilis* and *Proteus* spp. among others bacteria isolated in this study are shown to be the predominantly occurring bacteria across the sample locations.

The findings of this research showed a low frequency of fungi compared to reports of other researchers suggesting a higher frequency of fungi in surface water samples (Al-gabret *al.*, 2014). Al-gabr and colleagues reported the presence of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp., *Mucor* sp., and the most dominant being *Rhizopus* spp. In agreement to the findings of Al-gabr and colleagues, our finding suggested *Rhizopus* spp. as the most dominant fungi specie, while on the contrary, we did not detect the presence of *Fusarium* spp., *Trichoderma* spp., *Mucor* spp. The presence of fungi in rivers has been associated with increased organic matter concentration (Pietryczuket *al.*, 2018). We detected the presence of bacteria and species of fungi that are well-known for causing infectious diseases and the production of mycotoxins and therefore may directly cause human health problems.

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