SCREENING AND ISOLATION OF POTENTIAL ANTIBIOTIC-PRODUCING BACTERIA FROM WATER BODY SAMPLES IN KWARA STATE POLYTECHNIC, ILORIN

BY

OLADOKUN MARIAM CYNTHIA

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CERTIFICATION

This is to certify that this project research is the original work carried out and reported by **ND/23/SLT/PT/0825** to the Department Of Science Laboratory Technology (SLT), I nstitute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin and it has been Approv ed in Partial fulfillment of the Requirement for the Award of National Diploma (ND) in Sc ience Laboratory Technology (SLT).

_____ _____

MISS. AHMED K. H. **DATE**

(PROJECT SUPERVISOR)

_____ _____

MR. LUKMAN I.A. **DATE**

(SLT PART-TIME COORDINATOR)

_____ _____

DR. ABDULKAREEM USMAN

(HEAD OF DEPARTMENT) **DATE**

DEDICATION

This project report is dedicated to Almighty God who gave me the privilege to start and complete the research work. May He forever be praised.

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Immeasurable thanks are due to Almighty God, who has counted me worthy of th ose that will undergo the program and for seeing me through successfully.

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My special appreciation goes to my incomparable, loving, caring and understandi ng parents for their moral, financial, social and spiritual support, I will always be grateful to you. May Almighty God bless you in all your endeavors. And may you live long to reap the fruits of your labour. (Amen)

My appreciation also goes to my friends and relatives, God bless you all.

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ABSTRACT

The escalating threat of multidrug-resistant bacteria demands exploration of new antimicrobial sources. This study screened and isolated antibiotic-producing bacteria from water samples collected at a dam within Kwara State Polytechnic, Ilorin. Total viable bacterial counts ranged from 4.1×10^5 to 19.9×10^5 cfu/g, with Sample C exhibiting the highest microbial load. Bacterial isolates were identified through Gram staining and biochemical tests, and their antimicrobial activities were evaluated against Escherichia coli and Staphylococcus aureus. Among four isolates tested, Bacillus spp. demonstrated the strongest inhibition against E. coli (17 mm zone of inhibition) and moderate activity against S. aureus (6 mm). Proteus spp. and Micrococcus spp. showed smaller zones of inhibition, while Pseudomonas spp. exhibited selective activity against S. aureus (7 mm). These results confirm that freshwater environments harbor diverse antibiotic-producing bacteria with promising bioactivity against clinically relevant pathogens. This study underscores the potential of local aquatic microbial communities as reservoirs for novel antimicrobial agents to address antimicrobial resistance.

CHAPTER ONE

INTRODUCTION

1.1 Introduction and Literature Review

The relentless rise of antibiotic resistance represents a critical global health crisis, undermining our ability to treat common infections and threatening to reverse dec ades of medical progress (World Health Organization, 2020). The widespread and often indiscriminate use of antibiotics in human and animal health has driven the selection and proliferation of bacteria that have developed sophisticated mechani sms to evade the effects of these life-saving drugs (Alekshun & Levy, 2020). This surge in multidrug-resistant organisms (MDROs) is associated with increased mor bidity, mortality, prolonged hospital stays, and escalating healthcare costs, posing a significant burden on healthcare systems worldwide (O'Neill, 2016).

In the face of this escalating crisis, the discovery and development of novel antim icrobial agents have become a paramount priority. However, the pace of new antib iotic discovery has significantly slowed in recent decades, creating a widening ga p between the emergence of resistance and the availability of effective treatments (Bush et al., 2020). This "antibiotic discovery void" necessitates the exploration of unconventional sources for novel antimicrobial compounds and alternative therap eutic strategies (Lewis, 2020).

Natural environments, particularly those teeming with microbial diversity, represen

t a rich reservoir of potentially novel bioactive compounds, including antibiotics (B altz, 2019). Bacteria, fungi, and other microorganisms have evolved intricate mec hanisms for survival and competition within their ecological niches, often involvin g the production of secondary metabolites with antimicrobial properties (Davies & Davies, 2010). Aquatic environments, with their diverse physicochemical conditio ns and microbial communities, are particularly promising sources for bioprospecti ng efforts (Goecke et al., 2020). The unique selective pressures within these envir onments may have driven the evolution of novel metabolic pathways and the pro duction of unique antimicrobial compounds.

Exploring indigenous microbial resources holds significant advantages, as locally adapted microorganisms may produce compounds effective against locally preva lent pathogens (Okeke et al., 2005).

1.2 Literature Review

The mechanisms of antibiotic resistance are diverse and include enzymatic inacti vation of the drug (e.g., beta-lactamases), modification of the target site (e.g., mut ations in ribosomal proteins), reduced permeability of the bacterial cell wall, and a ctive efflux of the antibiotic (Munita & Arias, 2016). The horizontal transfer of resi stance genes via plasmids, transposons, and bacteriophages further accelerates t he spread of resistance among bacterial populations (Carattoli, 2013). The emerg ence of MDROs, resistant to multiple classes of antibiotics, poses a significant thr eat to clinical practice, leaving few or no effective treatment options for serious in

fections (Tacconelli et al., 2018). Aquatic environments harbor a vast and largely untapped diversity of microbial life, encompassing a wide range of bacterial phyl a, including Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (Zhi et a l., 2020). These microbial communities engage in complex interactions, including competition for resources, which can drive the production of secondary metabolit es with antimicrobial activities as a form of chemical warfare (Cordova et al., 202 1). Historically, many clinically important antibiotics, such as penicillin and tetrac ycline, were originally discovered from environmental microorganisms, particularl y water-dwelling Actinomycetes (Watve et al., 2001). However, aquatic environme nts, with their unique ecological niches and selective pressures (e.g., salinity gradi ents, nutrient availability, pollution), offer the potential to discover novel microorg anisms producing structurally diverse and potentially more effective antimicrobial compounds (Fenical & Jensen, 2006; Bhatnagar & Kim, 2010). Recent bioprospect ing efforts have increasingly focused on underexplored aquatic habitats, includin g deep-sea sediments, hydrothermal vents, and even freshwater ecosystems, yield ing promising results in the search for novel bioactive molecules (Romanenko et a l., 2022; Zhang et al., 2023).

Bacterial antibiotics belong to diverse structural classes, including beta-lactams, aminoglycosides, tetracyclines, macrolides, and quinolones, each with a distinct mechanism of action targeting essential bacterial processes such as cell wall syn thesis, protein synthesis, DNA replication, and metabolic pathways (Silver, 2011).

The production of these secondary metabolites is often regulated by complex gen etic networks and is influenced by environmental factors such as nutrient availabi lity, pH, temperature, and the presence of signaling molecules (Davies, 2006). Anti biotic biosynthesis genes are often clustered together on bacterial chromosomes or plasmids, facilitating their coordinated expression and potential for horizontal gene transfer (Demain & Fang, 2000). Recent research continues to unravel the int ricate regulatory mechanisms governing antibiotic production in various bacterial species and explores strategies to enhance their yield through genetic engineering and optimization of culture conditions (van Wezel et al., 2020; Medema et al., 202 1).

Primary screening methods, such as the cross-streak assay and agar well diffusio n assay, are widely used for the rapid identification of microorganisms with antim icrobial activity. The cross-streak assay involves the direct co-culture of a test isol ate and an indicator strain on an agar plate, allowing for the visual detection of zo nes of inhibition. The agar well diffusion assay involves the application of cell-fre e supernatants of test isolates to wells in agar plates seeded with indicator strain s, and the subsequent measurement of the resulting zones of inhibition (Balouiri e t al., 2016). Secondary screening methods involve the further characterization of t he antimicrobial activity, including determining the spectrum of activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). A dvancements in high-throughput screening technologies and automation have ac

celerated the screening process, allowing for the evaluation of large microbial libr aries (Glukhov et al., 2020). Challenges in antibiotic screening include the redisco very of known compounds and the need for sensitive and robust assays that can detect novel mechanisms of action (Lewis, 2013).

The search for novel antimicrobial compounds has increasingly turned towards u nderexplored ecological niches, with aquatic environments emerging as particular ly promising frontiers. Water bodies, ranging from vast oceans and deep-sea trenc hes to freshwater rivers, lakes, ponds, and even human-impacted environments lik e wastewater treatment plants, harbor an extraordinary and largely unexplored mi crobial diversity (Dong et al., 2021). It is estimated that a significant proportion of microbial species in these environments remain uncultured and uncharacterized, r epresenting a vast untapped resource for novel bioactive molecules (Al-Dhabi et a l., 2021).

The unique physicochemical conditions and intense ecological pressures within a quatic ecosystems drive the evolution of diverse metabolic strategies among resi dent microorganisms. In these highly competitive environments, microbes frequen tly produce secondary metabolites, including antibiotics, to inhibit the growth of c ompetitors, defend against predators, or facilitate communication (Newman & Cra gg, 2020). These compounds often exhibit novel chemical structures and mechan isms of action, making them attractive candidates for new drug development.

Numerous recent studies have highlighted the potential of water bodies as source s of antibiotic-producing bacteria. Freshwater Environments such as rivers, lakes, and ponds are dynamic ecosystems influenced by terrestrial runoff and aquatic bi ota. Studies have successfully isolated antibiotic-producing bacteria from freshw ater sediments and water columns. For instance, Das et al. (2020) isolated variou s bacterial strains, including species of *Bacillus* and *Pseudomonas*, from freshwa ter sediments, demonstrating antimicrobial activity against both Gram-positive an d Gram-negative pathogens. Similarly, research on lake water samples has identifi ed *Actinobacteria* and other bacterial phyla producing compounds active against common clinical isolates (Wang et al., 2021). These findings suggest that freshw ater systems, often overlooked in favor of marine environments, hold significant p romise.

Marine ecosystems, covering over 70% of the Earth's surface, are renowned for th eir extreme conditions and unique biodiversity. Marine microorganisms, including bacteria, fungi, and actinomycetes, have evolved distinct metabolic pathways to a dapt to high salinity, pressure, and temperature variations, leading to the biosynth esis of structurally diverse and biologically active compounds (Al-Dhabi et al., 202 1). Recent discoveries from marine bacteria include novel polyketides, peptides, a nd alkaloids with potent antimicrobial, antiviral, and anticancer properties (Zhang et al., 2023). Deep-sea sediments, sponges, and corals are particularly rich source s of novel marine microbial strains that produce a wide array of secondary metab

olites (Li et al., 2022).

While often associated with pollution, wastewater treatment plants (WWTPs) and their associated sediments represent unique microbial ecosystems under constant anthropogenic pressure, including exposure to various chemicals and low concentrations of antibiotics. This selective pressure can drive the evolution of novel resistance mechanisms but also potentially stimulate the production of new antimicrobial compounds by competing microorganisms. Rahman et al. (2022) successfully isolated and characterized several antimicrobial-producing bacteria from WWTP sludge, demonstrating activity against common human pathogens. The microbial communities in these environments are highly dynamic and diverse, making them interesting candidates for bioprospecting.

1.3 Statement of the Problem

The increasing emergence of multidrug-resistant bacterial pathogens poses a serious threat to global public health. The overuse and misuse of existing antibiotics have led to diminished efficacy and the proliferation of resistant strains. Meanwhile, the discovery rate of new antibiotics has significantly declined in recent decades. Most current antibiotics are derived from water microorganisms, and their bioactive potential has been extensively exhausted. Therefore, there is a pressing need t

o explore untapped environments such as water bodies for novel antibiotic-produ cing bacteria. Unfortunately, these environments have not been adequately studie d in this regard, especially in local Nigerian contexts. The lack of local data on the antibiotic-producing capacity of aquatic bacteria represents a significant research gap in the fight against AMR.

1.3 Aims and Objectives

1.3.1 Aims

To screen and isolate potential antibiotic-producing bacteria from water body sa mples as a contribution toward combating antimicrobial resistance.

1.3.2 Objectives

1. To collect and analyze the physicochemical properties of water samples from selected water body location.

2. To isolate and culture bacteria from the water samples using standard microbi ological techniques.

3. To identify the isolated bacteria using Gram staining and biochemical charact erization.

4. To assess the antimicrobial activity of the isolated bacteria against clinically r elevant pathogens (*Escherichia coli* and *Staphylococcus aureus*).

5. To compare the findings with existing literature and evaluate the potential of t he isolates for further antibiotic development.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Water Sample Collection

Water samples were collected from the water dam within Kwara State Polytechnic, Ilorin by systematic random sampling using zig zag pattern. Approximately 50 ml of water were collected from three different sampling points into sterile bottles. The samples were properly labeled as (sample A,B and C) and immediately transported to the Microbiology laboratory, Kwara State Polytechnic, Ilorin for determination of physicochemical parameters and isolation of antibiotic producing bacteria respectively (Ismail et al., 2021).

2.2 Sources of Test Organisms

The method of Bala *et al.* (2012) was used for the collection of preserved culture of *E. coli* and *S. aureus* from the Microbiology laboratory of the Kwara State Polytechnic, Ilorin.

2.3 Isolation of Bacteria with Antibiotic Activity from Water Samples

Nutrient agar was prepared according to the manufacturer's specifications and the water samples were serially diluted upto six fold (i.e 10^{-6}). Three sets of petri dish for each sample were labeled as 10^{-4}, 10^{-5} and 10^{-6}. About 1ml of diluted samples from each test tube was dispensed aseptically into corresponding petri dish. 15 ml of molten nutrient agar was poured into the petri dishes, mixed gently and allo

wed to solidify. The plates were incubated at 37°C for 24 hours. After incubation, t he total viable bacterial counts of the water was conducted and recorded. All the p lates were incubated again at 37°C for 72 hours. The water bacteria that inhibited the growth of other bacterial colonies by producing inhibition zone were selected and subcultured on nutrient agar plate to obtain pure strain. The selected bacteria l isolates were inoculated by spotting on the lawn culture plates of the test bacteri a and incubated at 37°C for 24 hours, the one that produced inhibition zones arou nd test bacteria were stored as stock culture in nutrient agar slant at 4°C for furthe r usage.

2.4 Identification of the Bacterial Isolates with Antibiotic Activity

The method of Baltz (2006) was used for the identification of the bacterial isolate s. The identification was based on morphological description of colonies, Gram's reaction and biochemical tests and the results were compared with the standard d escription given in Bergey's manual of systematic bacteriology. The biochemical t ests performed were catalase, coagulase, citrate, sugar fermentation, methyl red, Voges-proskauer and motility test (Whitman et al., 2020).

2.4.1 Gram's Reaction

The Gram staining technique was employed to differentiate bacterial isolates into Gram-positive and Gram-negative groups, based on cell wall characteristics. A sm

ear of each isolate was prepared on a clean glass slide, heat-fixed, and sequentially stained with crystal violet, Gram's iodine, 95% ethanol (as a decolorizer), and safranin. The slides were then observed under a light microscope at 100× magnification using immersion oil. Gram-positive bacteria retained the crystal violet-iodine complex and appeared purple, while Gram-negative bacteria appeared pink due to safranin counterstaining (Alayande et al., 2021).

2.4.2 Catalase Test

Catalase activity was assessed by adding a few drops of 3% hydrogen peroxide to a fresh isolate colony on a clean glass slide. The immediate release of oxygen bubbles indicated a positive result, confirming the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen (Khan et al., 2020).

2.4.3 Coagulase Test

The coagulase test was performed to detect the production of coagulase enzyme. A drop of plasma was mixed with a bacterial suspension on a glass slide (slide test), and clot formation within 10–30 seconds indicated a positive result. For confirmation, a tube coagulase test was also performed by incubating the isolate suspension in plasma at 37°C for 4 hours and observing for clot formation (Uddin et al., 2022).

2.4.4 Citrate Utilization Test

Citrate utilization was tested using Simmons' citrate agar slants. The media were inoculated with isolate cultures and incubated at 37°C for 24–48 hours. A color c hange from green to blue indicated a positive result, demonstrating the ability of t he isolate to use citrate as a sole carbon source and produce alkaline by-products (Akinnibosun & Ogundahunsi, 2020).

2.4.5 Sugar Fermentation Test

Carbohydrate fermentation tests were carried out using phenol red broth containin g specific sugars (glucose). Durham tubes were included to detect gas productio n. The tubes were inoculated with the bacterial isolates and incubated at 37°C for 24–48 hours. A color change from red to yellow indicated acid production (positiv e fermentation), while gas bubbles in the Durham tube signified gas production (I gbinosa et al., 2020).

2.4.6 Methyl Red (MR) Test

The MR test was performed to assess the production of stable acidic end-product s from glucose fermentation. Isolate cultures were grown in MR-VP broth and incu bated at 37°C for 48 hours. After incubation, five drops of methyl red indicator wer e added. A red coloration indicated a positive result, signifying mixed acid ferment ation (Onuoha et al., 2021).

2.4.7 Voges-Proskauer (VP) Test

The VP test was carried out on the same MR-VP broth used above. After 48 hours of incubation, 1 mL of the culture was mixed with 0.6 mL of 5% α-naphthol and 0.2 mL of 40% potassium hydroxide. The mixture was shaken and allowed to stand for 15–30 minutes. A pink or red color indicated a positive result due to the presence of acetoin, a neutral fermentation end-product (Olajuyigbe & Falade, 2020).

2.4.8 Motility Test

Motility of the isolates was tested using semi-solid motility agar (0.4% agar). Tubes were stab-inoculated with isolate cultures and incubated at 37°C for 24–48 hours. Diffuse growth radiating from the stab line indicated a positive motility test, whereas growth confined to the stab line indicated non-motility (Eze et al., 2022).

CHAPTER THREE

RESULT

3.1 Total Bacterial Viable Counts

The total viable bacterial counts from the water samples are shown in Table 1. Sample C recorded the highest bacterial load at 19.9×10^5 cfu/g, followed by samples A and B, with counts of 4.2×10^5 and 4.1×10^5 cfu/g respectively. The significantly higher microbial load in sample C suggests that the water may be richer in nutrients or organic matter, providing a more favorable environment for bacterial growth.

3.2 Antimicrobial Activity of Bacterial Isolates

The antimicrobial activity of the bacterial isolates (B1–B4) against *Escherichia coli* and *Staphylococcus aureus* was evaluated using the agar well diffusion method, and the results are presented in Table 2. All isolates demonstrated varying degrees of inhibition against the test organisms. Isolate B1 exhibited the highest activity against *E. coli*, with a zone of inhibition measuring 17 mm, while the same isolate showed moderate activity (6 mm) against *S. aureus*. Isolate B3 showed no inhibition against *E. coli* but produced a 7 mm zone of inhibition against *S. aureus*, indicating possible species-specific activity. Overall, the data suggest that isolate B1 has the most potent broad-spectrum antimicrobial activity.

3.3 Morphological and Biochemical Characterization of Bacterial Isolates

The morphological, Gram staining, and biochemical test results for the bacterial is olates are summarized in Table 3. The isolates exhibited diverse colony morpholo gies and metabolic traits.

Table 1: Mean total bacterial viable counts of the bacterial isolates

Sample codes	Mean bacterial viable plate counts (cfu/g)
A	4.2×10^5
B	4.1×10^5
C	19.9×10^5