SCREENING AND ISOLATION OF POTENTIAL ANTIBIOTIC-PRODUCING BACTERIA FROM SOIL SAMPLES

BY

IBRAHIM ABDULSALAM AYINDE
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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/0156 to the Department Of Science Laboratory Technology (SLT), Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin and it has been Approved in Partial fulfillment of the Requirement for the Award of National Diploma (ND) in Science 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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ABSTRACT

The emergence and spread of antimicrobial resistance (AMR) among pathogenic microorganisms pose a significant threat to global public health. To address this challenge, the present study aimed to isolate and screen potential antibiotic-producing bacteria from soil samples collected from different environments. Three soil samples were collected and analyzed for physicochemical characteristics including color, texture, pH, moisture content, and porosity. The pH values ranged from 7.21 to 7.48, moisture content from 10.71% to 16.83%, and porosity from 10.64% to 15.47%. A total of four bacterial isolates were obtained, with sample C having the highest viable bacterial count (19.9 × 10⁵ cfu/g) compared to sample A (4.2 × 10⁵ cfu/g) and sample B (4.1 × 10⁵ cfu/g). The isolates were subjected to primary screening for antimicrobial activity against Escherichia coli *and* Staphylococcus aureus *using the agar well diffusion method. Among the isolates, B1 (Bacillus* spp.*) exhibited the highest zone of inhibition against* E. coli *(17 mm) and moderate activity against* S. aureus *(6 mm), indicating strong broad-spectrum antimicrobial potential. Biochemical characterization revealed diverse metabolic capabilities among the isolates, with B1(Bacillus* spp.*) showing positive results for catalase, coagulase, citrate, and methyl red tests. These findings suggest that soil is a rich reservoir of bioactive microbial agents, and isolate B1(Bacillus* spp.*), in particular, holds promise for the development of novel antibiotics to combat antimicrobial resistance.*

CHAPTER ONE

INTRODUCTION

1.1 Introduction And Literature Review

Antimicrobial resistance (AMR) stands as one of the most pressing global health challenges of the 21st century, threatening to reverse decades of progress in medicine and public health (WHO, 2020a). The ability of microorganisms, particularly bacteria, to resist the effects of antimicrobial drugs renders previously treatable infections difficult or impossible to cure, leading to prolonged illness, increased mortality rates, and significant economic burdens (CDC, 2023). This crisis is multifaceted, driven by the overuse and misuse of antibiotics in human medicine, agriculture, and animal husbandry, as well as inadequate sanitation, poor infection control practices, and a lack of new antibiotic development (O'Neill, 2016; Prestinaci et al., 2020).

The consequences of AMR are far-reaching. Common infections like pneumonia, tuberculosis, sepsis, and gonorrhea are becoming increasingly difficult to treat, leading to higher rates of treatment failure and the need for more expensive, toxic, and often less effective alternative therapies (Tacconelli et al., 2018; Laxminarayan et al., 2022). Surgical procedures, organ transplantation, and cancer chemotherapy, which rely heavily on effective antimicrobial prophylaxis, become riskier in the absence of reliable antibiotics (Ventola, 2015). The World Health Organization (WHO) has identified a list of priority pathogens, including

carbapenem-resistant *Acinetobacter baumannii,* extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, and methicillin-resistant *Staphylococcus aureus* (MRSA), for which new antibiotics are urgently needed (WHO, 2020b). The economic impact of AMR is equally staggering, with estimates suggesting billions of dollars in healthcare costs and lost productivity annually (OECD, 2018; Dadgostar, 2019). Without concerted global action, projections indicate that AMR could cause 10 million deaths per year by 2050, surpassing cancer as a leading cause of death (O'Neill, 2016). This grim outlook underscores the critical and urgent need for the discovery and development of novel antimicrobial compounds.

In the relentless pursuit of new antibiotics, environmental reservoirs, particularly soil, have emerged as paramount sources of novel antimicrobial agents. Soil is not merely an inert substrate; it is a dynamic, complex, and highly diverse microbial ecosystem, teeming with an astonishing array of bacteria, fungi, archaea, and viruses (Fierer, 2017; Trivedi et al., 2020). A single gram of soil can harbor billions of microbial cells belonging to thousands of different species, many of which remain uncultured and uncharacterized (Daniel, 2005; Hug et al., 2016). This immense biodiversity is a direct consequence of the intricate ecological interactions occurring within the soil matrix, where microorganisms compete for limited resources, engage in symbiotic relationships, and produce a vast repertoire of secondary metabolites to gain a competitive advantage (Berdy, 2020).

These secondary metabolites, which include antibiotics, antifungals, antivirals, and immunosuppressants, are not directly involved in the primary metabolic processes of growth and reproduction but play crucial roles in microbial communication, defense, and adaptation to environmental stresses (Davies & Davies, 2010; Genilloud, 2017). The constant evolutionary pressure within soil communities has driven the development of highly potent and structurally diverse bioactive compounds. This makes soil an unparalleled natural library for drug discovery, a fact historically validated by the origins of many clinically important antibiotics.

The golden era of antibiotic discovery, spanning from the 1940s to the 1960s, was largely fueled by the exploration of soil microorganisms. The seminal discovery of penicillin by Alexander Fleming in 1928 from the fungus *Penicillium notatum* laid the groundwork, but it was the systematic screening of soil microbes that truly revolutionized medicine (Aminov, 2010). Selman Waksman's pioneering work in the 1940s led to the isolation of streptomycin from *Streptomyces griseus*, a soil-dwelling bacterium, marking the beginning of the 'streptomycin era' and the recognition of actinobacteria as prolific producers of antimicrobial agents (Waksman, 1953). This discovery was particularly significant as streptomycin was the first effective treatment for tuberculosis.

Following streptomycin, numerous other life-saving antibiotics were isolated from soil bacteria, primarily from the genus *Streptomyces*, including chloramphenicol, tetracyclines, erythromycin, and neomycin (Berdy, 2005). Other

soil-dwelling genera, such as *Bacillus* (e.g., bacitracin, polymyxin) and certain fungi (e.g., cephalosporins from *Cephalosporium acremonium*), also contributed significantly to the antibiotic arsenal (Demain, 2009; Newman & Cragg, 2020). This historical success underscores the immense potential that still lies within the largely unexplored microbial diversity of soil. Despite the decline in new antibiotic approvals since the 1980s, driven by factors such as the ease of re-discovering known compounds and the increasing complexity of drug development, the fundamental principle of looking to nature, especially soil, for novel chemistry remains valid and urgent (Lewis, 2020).

To appreciate the significance of discovering new antibiotics, it is crucial to understand their modes of action and the mechanisms by which bacteria develop resistance. Antibiotics typically target essential bacterial processes, leading to cell death (bactericidal) or inhibition of growth (bacteriostatic) (Kohanski et al., 2010). Common targets include, cell wall synthesis, protein synthesis, nucleic acid synthesis, folic acid metabolism and cell membrane integrity.

Bacterial resistance to antibiotics arises through various mechanisms, often encoded on mobile genetic elements like plasmids, allowing for rapid dissemination among bacterial populations (Blair et al., 2020). The continuous evolution of the resistance mechanisms necessitates the discovery of antibiotics with novel targets or modes of action that can circumvent existing resistance strategies (Davies & Davies, 2010; Piddock, 2017).

While a vast array of microorganisms contribute to the soil's chemical diversity, certain bacterial genera are particularly renowned for their prolific production of antibiotics and other bioactive compounds. The genus *Streptomyces*, belonging to the phylum Actinobacteria, is arguably the most significant source of naturally derived antibiotics (Berdy, 2020; Varghese et al., 2021). These Gram-positive, filamentous bacteria are ubiquitous in soil and are characterized by their complex life cycle, involving the formation of mycelia and spores, and their distinctive earthy odor (Barka et al., 2016). *Streptomyces* species are responsible for producing over two-thirds of all known natural product antibiotics, including, aminoglycosides, macrolides, tetracyclines, glycopeptides, ansamycins and polyenes. The genetic capacity of *Streptomyces* to produce such a diverse array of secondary metabolites is attributed to their large genomes, which contain numerous gene clusters dedicated to the biosynthesis of these compounds (Challis & Hopwood, 2003; Liu et al., 2021). Research continues to explore novel *Streptomyces* species from underexplored environments and to activate cryptic biosynthetic gene clusters to discover new compounds (Rutledge & Challis, 2015; Wang et al., 2022).

Species within the genus *Bacillus* are Gram-positive, rod-shaped, spore-forming bacteria commonly found in soil and various other environments. While not as prolific as *Streptomyces* in terms of sheer number of discovered antibiotics, *Bacillus* species are significant producers of a variety of antimicrobial compounds, particularly peptides and lipopeptides (Shafi et al., 2021). Notable

examples include, bacitracin, polymyxins, iturins, fengycins, surfactins and bacteriocins .*Bacillus* species are also widely used in agriculture as biocontrol agents due to their ability to produce these antimicrobial compounds, which protect plants from pathogens (Shafi et al., 2021). Their ease of cultivation and genetic manipulability make them attractive candidates for industrial production of bioactive compounds.

Beyond *Streptomyces* and *Bacillus*, other soil-dwelling microorganisms contribute to the natural product landscape. Actinobacteria with genera like *Micromonospora*, *Nocardia*, and *Actinomadura* also produce clinically important antibiotics (e.g., gentamicin from *Micromonospora purpurea*, rifampicin from *Nocardia mediterranei*). Soil fungi, such as *Penicillium* (penicillins) and *Cephalosporium* (cephalosporins), have historically been crucial sources of antibiotics. While some species of *Pseudomonas* are pathogenic, others are known to produce antimicrobial compounds, including phenazines and pyrrolnitrin, which have antifungal and antibacterial properties (Gross & Loper, 2009).

Despite the historical success, antibiotic discovery has faced significant challenges in recent decades, leading to a period often referred to as the "discovery void" or "innovation gap" (Lewis, 2020). Extensive screening efforts often yield compounds that have already been identified, making it difficult to find truly novel chemical scaffolds. A vast majority of environmental microorganisms are "uncultivable" using standard laboratory techniques,

meaning their potential for producing novel compounds remains untapped (Ling et al., 2021). The journey from discovery to market for a new drug is long, expensive, and fraught with regulatory complexities, often deterring pharmaceutical companies from investing in antibiotic research and even newly discovered antibiotics face the inevitable challenge of resistance development, requiring a continuous pipeline of new drugs. To overcome these challenges, researchers are employing a combination of traditional and innovative strategies. Focusing on unique ecological niches (e.g., extreme environments, deep sea, symbiotic relationships) where microbial communities might have evolved novel chemical defenses (Zeng et al., 2020), directly extracting DNA from environmental samples and screening for biosynthetic gene clusters, bypassing the need for cultivation (Charlop-Powers et al., 2015; Rondon et al., 2000). This approach allows access to the genetic potential of uncultivable microbes. And Analyzing the genomes of known and newly isolated microorganisms to identify "cryptic" or silent biosynthetic gene clusters that might be activated under specific conditions (Medema & Fischbach, 2015; Weber et al., 2020). Also, growing different microbial species together to stimulate the production of secondary metabolites that might not be produced in monoculture (Bertrand et al., 2014), developing innovative methods to culture previously uncultivable microorganisms, such as the iChip, which allows for in situ cultivation in their natural environment (Ling et al., 2015) and Engineering microorganisms to produce specific compounds or designing novel antimicrobial peptides based on

known structures (Wang et al., 2020). Despite the advent of these advanced techniques, traditional culture-based screening remains a fundamental and cost-effective initial step for identifying culturable producers. It provides tangible isolates that can be further characterized and manipulated.

The escalating crisis of antimicrobial resistance necessitates a continuous and robust search for novel antimicrobial compounds. Soil, with its unparalleledO microbial diversity and historical significance as a source of antibiotics, remains a critical frontier in this endeavor. Many soil microorganisms are yet to be discovered, and their metabolic potential remains largely unexplored.

1.2 Statement of the Problem

The global crisis of antimicrobial resistance continues to grow, with alarming rates of resistance being reported in critical pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL) -producing *Enterobacteriaceae*, and carbapenem-resistant *Acinetobacter baumannii* (WHO, 2020b). Current antibiotic pipelines are insufficient to meet this challenge. While soil remains an abundant source of bioactive compounds, a significant portion of its microbial diversity remains untapped due to traditional culturing limitations.

Furthermore, most developing countries including Nigeria face additional challenges due to limited surveillance, poor antibiotic stewardship, and over-the-counter misuse. There is an urgent need to explore local soil microbial

communities for new antimicrobial agents to help replenish the global antibiotic pipeline and curb the escalating threat of AMR.

1.3 Aims and Objectives

1.3.1 Aims

To screen and isolate potential antibiotic-producing bacteria from soil samples as a contribution toward combating antimicrobial resistance.

1.3.2 Objectives

1. To collect and analyze the physicochemical properties of soil samples from selected locations.
2. To isolate and culture bacteria from the soil samples using standard microbiological techniques.
3. To identify the isolated bacteria using Gram staining and biochemical characterization.
4. To assess the antimicrobial activity of the isolated bacteria against clinically relevant pathogens (*Escherichia coli* and *Staphylococcus aureus*).
5. To compare the findings with existing literature and evaluate the potential of the isolates for further antibiotic development.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Soil Sample Collection

Soil samples were collected from diverse agricultural fields within Kwara State Polytechnic, Ilorin by systematic random sampling using zig zag pattern. Approximately 100 g of soil were collected from the top 5-10 cm depth from three different sampling points and placed into sterile polythene bag using sterile spatula. 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 Determination of Soil Physicochemical Parameters

The method of Donald *et al.* (2002) was used in determining the color, texture, pH, moisture content and porosity. The mean of the 3 samples collected at each point was then taken.

2.3 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.

2.4 Isolation of Bacteria with Antibiotic Activity from Soil Samples

Nutrient agar was prepared according to the manufacturer's specifications and the soil 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. 15ml of molten nutrient agar was poured into the petri dishes, mixed gently and allowed to solidify. The plates were incubated at 37°C for 24 hours. After incubation, the total viable bacterial counts of the soil was conducted and recorded. All the plates were incubated again at 37°C for 72 hours. The soil 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 bacterial isolates were inoculated by spotting on the lawn culture plates of the test bacteria and incubated at 37°C for 24 hours, the one that produced inhibition zones around test bacteria were stored as stock culture in nutrient agar slant at 4°C for further usage.

2.5 Identification of the Bacterial Isolates with Antibiotic Activity

The method of Baltz (2006) was used for the identification of the bacterial isolates. The identification was based on morphological description of colonies, Gram’s reaction and biochemical tests and the results were compared with the

standard description given in Bergey's manual of systematic bacteriology. The biochemical tests performed were catalase, coagulase, citrate, sugar fermentation, methyl red, Voges-proskauer and motility test (Whitman et al., 2020).

2.5.1 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 smear 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).

2.5.2 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).

2.5.3 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).

2.5.4 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 change from green to blue indicated a positive result, demonstrating the ability of the isolate to use citrate as a sole carbon source and produce alkaline by-products (Akinnibosun & Ogundahunsi, 2020).

2.5.5 Sugar Fermentation Test

Carbohydrate fermentation tests were carried out using phenol red broth containing specific sugars (glucose). Durham tubes were included to detect gas production. 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 (positive fermentation), while gas bubbles in the Durham tube signified gas production (Igbinosa et al., 2020).

2.5.6 Methyl Red (MR) Test

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

2.5.7 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).

2.5.8 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).

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CHAPTER THREE

RESULT

3.1 Physicochemical Parameters of Soil Samples

The physicochemical characteristics of the three soil samples (labeled A, B, and C) are summarized in Table 1. All soil samples exhibited a loamy texture, with colors ranging from dark brown to brown. The pH values were slightly alkaline, ranging from 7.21 in sample C to 7.48 in sample B. Moisture content (MC) varied among the samples, with the highest observed in sample B (16.83%) and the lowest in sample A (10.71%). Similarly, porosity values ranged from 10.64% in sample A to 15.47% in sample B. These variations in physicochemical properties could influence microbial diversity and activity in the respective soil environments.

3.2 Total Bacterial Viable Counts

The total viable bacterial counts from the soil samples are shown in Table 2. 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 soil may be richer in nutrients or organic matter, providing a more favorable environment for bacterial growth.

3.3 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 3. 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.

3.4 Morphological and Biochemical Characterization of Bacterial Isolates

The morphological, Gram staining, and biochemical test results for the bacterial isolates are summarized in Table 4. The isolates exhibited diverse colony morphologies and metabolic traits.

Table 1: Mean Physicochemical parameters of Soil Samples

SC	Colour	Texture	pH(µs/cm)	MC (%)	Porosity (%)
A	Dark brown	Loam	7.36	10.71	10.64
B	Brown	Loam	7.48	16.83	15.47
C	Dark brown	Loam	7.21	13.15	12.89

SC= Sample codes, MC= Moisture content.

Table 2: 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

Table 3: Antimicrobial activity of bacterial isolates against *E. coli* and *S. aureus*

S/N	Bacteria isolates	Zones of inhibition (mm)	
		E. coli	*S. aureus*
1	B1	17	6
2	B2	11	5
3	B3	0	7
4	B4	14	5

Table 4: The morphological and biochemical identification of the bacterial isolates

Bacteria isolates	Morphological Description of Colony	Gram's reaction	Biochemical tests							Inference
			Cat	Coa	Cit	Mer	Vop	Suf	Mot	
B1	Irregular, rough, large whitish colony	+ve rods	+	-	+	+	+	+	+	*Bacillus* spp.
B2	Circular, pinhead, convex yellowish colony	+ ve clusters	+	-	+	-	+	+	-	*Micrococcus* spp.
B3	Oval, mucoid, medium Greenish colony	–ve rods	+	-	+	-	-	+	+	*Pseudosomonas* spp.
B4	Small,round, convex yellowish brown colony	–ve rods	+	-	+	+	-	+	+	*Proteus* spp.

Cat= Catalase test, Coa= Coagulase test, Cit= Citrate test, Mer= Methyl red test, Vop= Voges-proskauer test, Suf= Sugar fermentation test, Mot= Motility test, – = Negetive result and + = Positive result.

CHAPTER FOUR

DISCUSSION, RECOMMENDATION AND CONCLUSION

4.1 DISCUSSION

The findings of this study provide insight into the microbial profile and antibacterial potential of bacteria isolated from different soil samples. The physicochemical characteristics of the soils, such as pH, texture, moisture content, and porosity, play a significant role in shaping microbial diversity and activity. In this study, all samples exhibited a loamy texture and slightly alkaline pH, ranging from 7.21 to 7.48. These conditions are favorable for the survival and metabolic activity of many soil bacteria, particularly those involved in organic matter decomposition and antibiotic production. Similar physicochemical conditions supporting microbial growth have been reported by Ateh et al. (2020), who found that loamy, near-neutral soils supported diverse bacterial communities in forest environments.

Moisture content and porosity varied among the samples, with Sample B showing the highest moisture content (16.83%). High moisture content often correlates with increased microbial activity due to improved substrate diffusion and microbial motility (Mohammed, 2021). Sample C recorded the highest bacterial load (19.9×10^5 cfu/g), suggesting that favorable environmental factors, such as balanced moisture and nutrient availability, may have contributed to enhanced microbial proliferation. This finding is consistent with reports by

Onyilokwu et al. (2021), who also observed that soil microbial counts are significantly influenced by the physical structure and water-holding capacity of the soil.

The antimicrobial screening of the bacterial isolates revealed variable zones of inhibition against *Escherichia coli* and *Staphylococcus aureus*, indicating differential antibacterial potentials. Notably, isolate B1(*Bacillus spp.*) exhibited the highest zone of inhibition (17 mm) against *E. coli*, signifying strong antibacterial activity. This aligns with the study by Omidoyin and Femi-Ola (2020), who isolated *Bacillus* spp. from soil samples that showed comparable inhibitory effects on a broad range of clinical pathogens. Isolate B3(*Pseudosomonas* spp.) showed no activity against *E. coli* but had a 7 mm inhibition zone against *S. aureus*, suggesting specificity in antimicrobial production, a pattern also reported by Umeokoli et al. (2022) in their study of *Pseudomonas* isolates from agricultural soils.

The morphological and biochemical identification of the isolates further revealed the presence of *Bacillus*, *Micrococcus*, *Pseudomonas*, and *Proteus* species. These genera are commonly associated with soil and are recognized for their diverse enzymatic and secondary metabolite profiles. The identification of *Bacillus* spp., a well-known producer of antimicrobial peptides such as bacitracin and subtilin, reinforces its significance in the development of alternative antibiotics (Ezebialu et al., 2020). Likewise, the isolation of *Pseudomonas* spp.,

known for producing pyocyanin and other bioactive metabolites, supports the biotechnological potential of these organisms, particularly in antimicrobial drug discovery (Ahmed et al., 2021).

The positive results obtained from catalase, citrate, and sugar fermentation tests, along with motility in most isolates, are indicative of robust metabolic capabilities. These traits not only aid in species identification but also suggest the potential functional roles of these bacteria in soil nutrient cycling and secondary metabolite synthesis. The biochemical traits observed in this study agree with the profiles reported in the studies by Obasi et al. (2020) and Nwankwo et al. (2023), where similar test results were used to successfully classify soil isolates with known antimicrobial properties.

The observed antimicrobial activity, particularly from *Bacillus* and *Pseudomonas* species, underscores the promising role of soil bacteria as sources of natural antibiotics. In the face of rising antimicrobial resistance, the exploration of indigenous soil microbiota offers a valuable avenue for discovering novel bioactive compounds. The present findings, therefore, contribute to the growing body of research advocating for soil-based bioprospecting for antimicrobial agents, as emphasized by studies such as those by Alhassan et al. (2020) and Adedayo et al. (2022).

4.2 RECOMMENDATION

Further Characterization of Active Compounds: Future research should focus on the extraction, purification, and structural characterization of the bioactive compounds produced by the identified bacterial isolates, particularly *Bacillus* spp. This will help determine their specific antimicrobial mechanisms and potential clinical applications.

Molecular Identification of Isolates: It is recommended to employ molecular techniques such as 16S rRNA gene sequencing for more accurate taxonomic identification and to better understand the genetic basis of antimicrobial production in these bacteria.

Expand Sample Locations: Sampling from a wider variety of environments, including extreme habitats and undisturbed soils, could uncover novel microorganisms with potent antimicrobial properties that have yet to be explored.

Antibiotic Resistance Profiling: The isolated bacteria should be subjected to antibiotic resistance screening to evaluate the potential for horizontal gene transfer and ensure safe use in pharmaceutical development.

Scale-Up Studies and Fermentation Optimization: Pilot-scale fermentation and optimization studies are essential to assess the feasibility of producing antimicrobial compounds at an industrial scale.

4.3 CONCLUSION

These findings emphasize the importance of soil as a valuable reservoir for antibiotic-producing bacteria. The results support ongoing efforts to explore natural microbial communities as sources of novel antimicrobial agents, especially in the wake of rising antimicrobial resistance. The study contributes to the growing body of research on microbial bioprospecting and lays the groundwork for further investigation into the purification and characterization of the active compounds produced by these isolates.

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