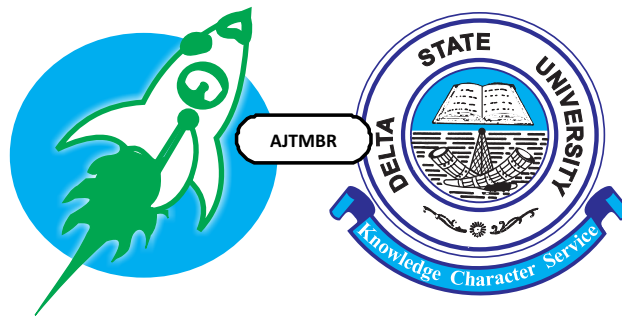


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Investigation of Antibacterial Potential of *Bacillus* and *Actinomycetes* Isolated from Soil against Drug-resistant Clinical Isolates

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ABSTRACT

Background: *Bacillus* and *Actinomycetes* are well known antibiotic-producing soil isolates. However, there is paucity of information against drug-resistant clinical isolates from Nigeria. This research was therefore designed to investigate the anti-bacterial potential of *Bacillus* and *Actinomycetes* isolated from soil against drug-resistant clinical isolates.

Methods: Soil samples were collected from the *rhizosphere* of Mango trees within Caleb University, Lagos, between January 2024 and March 2024. Targeted isolation of *Bacillus* and *Actinomycetes* from the soil samples was done. Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* were identified and subjected to antibiotic susceptibility testing. The crowded plate method was used to screen the antibiotic-producing soil isolates. Ultra-violet (UV) induced mutation was performed on the soil isolates to determine change in antibacterial activity at two different time exposures (2 hours and 4 hours).

Result: The distribution of the soil isolates showed *Bacillus* species (71.40 %) as the most prevalent isolates. Multi-drug resistance was observed among the clinical isolates. Prior to UV exposure, none of the *Bacillus* isolates exhibited antibiotic-producing activity against the tested clinical isolates unlike *Actinomycetes* with inhibitory activity against clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli*. However, after UV exposure at 2 hours and 4 hours, all soil isolates demonstrated inhibitory activity against at least one tested clinical isolate.

Conclusion: The inhibitory activity of antibiotic-producing soil bacteria against drug-resistant pathogenic clinical isolates can be enhanced using UV induction.

Keywords: *Actinomycetes*, antibacterial activity, clinical isolates, multidrug resistant, UV induction

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INTRODUCTION

Among the microorganisms habitats, soils represent a large reservoir and vital global health for the immense diversity of living organisms on earth^{1,2}, which serves as medium where plant and

microbial communities exchange substances^{3, 4}. One of the predominant organisms mostly found in the soil is the bacteria, which play vital roles in decomposing, processing, exchange of nutrients, and soil fertility regulation^{5,6,7}. Findings revealed

that about 30% of the available antibiotics are screened and cultured from the soil, and more than 80% antibiotics used clinically for the treatment of human are isolated from the soil⁸. Antimicrobial resistance (AMR) is one of the greatest world challenges, when antibiotics proven to be effective; has lost their potency due to microorganisms shift over a period⁹. The uncontrolled spread of AMR is a natural process which has evolved from abuse/overuse of antibiotics, and this has constituted to public health threat today¹⁰. Over time, AMR develops naturally as a result of genetic mutations that could pass from one generation to another. Bacteria develop different mechanisms of resistance which include: antigenic variation, target defense, and suppression of cellular antibiotics^{9, 11, 12}. Findings revealed that not all resistant bacteria are capable of producing diseases but achieve pathogenesis through dissemination of gene encoding AMR to new bacteria in a favourable environment¹². The common means of spreading AMR is the transfer of antibiotic resistant genes. Plasmids, transposons, or integrons and horizontal gene transfer are agents that enhance the transfer of resistant gene(s)¹³. Several natural sources have emerged since the pursuit of novel antibiotics¹⁴. Most clinically relevant secondary metabolites are best produced by *thermophilic* bacteria; are a biodiverse reservoirs of bioactive compounds. Antibiotics, heat-stable enzymes and cancer agents are valuable compounds of considerable global interest^{15, 16}. Thermostable enzymes such as *lipases*, *amylases*, *cellulases* are mostly produced in large amount by *thermophilic* bacteria with considerable industrial uses¹⁷.

The genus *Bacillus* are soil inhabitant bacteria, and producers of important antibiotics¹⁸. *Bacillus* species have a wide spectrum of antimicrobial substances such as *ribosomally* and non-*ribosomally* synthesized cyclic lipopeptides,

bacteriocins and other peptide based compounds^{19,20,21}. *Actinomycetes* are notable sources of bioactive compounds which are distributed naturally, and or man-made that play vital roles in organic degradation²².

Actinomycetes has long history in the production of secondary metabolites, and sources of promising new antibiotics^{23, 24, 25}. This research work focused on investigating antibacterial potential in *Bacillus* and *Actinomycetes* isolated from soil against drug-resistant clinical pathogens.

MATERIALS AND METHODS

Study Area

Caleb University main campus is located in Imota, Lagos, Nigeria. Imota's latitude and longitude coordinates are around 6.5833°N and 3.3500°E. The average annual rainfall in Imota is between 1,500 and 2,000 millimetres, with the rainy season typically lasting from March until October. The dry season last from November to February. Imota's average annual temperature ranges from 25°C to 30°C. Imota covers a portion of the Lagos metropolis, which comprises residential areas, commercial spaces, agricultural land, natural vegetation and educational institutions such as Caleb University.

Sample Collection

The soil samples were meticulously collected from *rhizosphere* of Mango trees within Caleb University, Lagos. Sampling was conducted at a depth of at least 10 cm using a calibrator and a sterile spatula. Each sample was carefully stored in a sterile transport box and promptly transferred to the laboratory at room temperature.

Isolation of soil Bacteria

The soil samples underwent air drying by exposing them to 50°C and 70°C for 1 hour in an

oven to target *Bacillus* and *Actinomycetes* respectively. Isolation of *Bacillus* species and *Actinomycetes* was carried out through a serial dilution technique, about 1 g of the soil samples were collected in a sterile container as described by Tabbene et al¹⁸ and Singh et al²³. A 5-folds serial dilution were performed, samples were diluted progressively: An average of 1 mL from the first tube was mixed with a total 9 mL of distilled water in the next tube, and this process continued until 5th tube. From 5th, an average of 0.1 mL was transferred onto a nutrient agar plate using the spread plate method. Streaking methods were employed for plating on nutrient broth and agar, followed by incubation at 37°C for 24 hours.

Collection of clinical isolates

Clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* were collected from urine, high vaginal swab, wound and stool samples respectively.

Identification of Isolates

Morphological and biochemical analyses was done to identify soil isolates (*Bacillus* and *Actinomycetes*) and the collected clinical isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*). Morphological analyses was done using Gram-staining and cultural methods. Using the cultural method, growth media: Eosin Methylene Blue Agar (EMB) was used for the isolation of *Escherichia coli* which was isolated from urine sample, and high vaginal swab. *Salmonella-Shigella* Agar (SSA) was used for the isolation of *Salmonella typhi* isolated from stool sample. Mannitol Salt Agar (MSA) was used for the growth of *Staphylococcus aureus* isolated from stool, and Nutrient Agar was used for the isolation of *Pseudomonas aeruginosa* obtained from wound sample. Biochemical tests, including the citrate utilization test, catalase test, coagulase

test, starch hydrolysis test, and urease test.

Antibiotic susceptibility testing of clinical isolates

Isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* were subjected to antibiotic susceptibility using fourteen (14) antibiotics. The antibiotics included: pefloxacin (10 µg), gentamicin (10 µg), amoxicillin (30 µg), cefuroxime (25 µg), ceftriaxone (25 µg), ciprofloxacin (20 µg), azithromycin (12 µg), levofloxacin (20 µg), erythromycin (10 µg), co-trimoxazole (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), amoxicillin/clavulanic acid (30 µg), and Ofloxacin (10 µg). The agar diffusion method as described by the Clinical Laboratory Standard Institute²⁶ was employed.

Primary Screening of Antibiotic-Producing Isolates using Crowded Plate Technique

The crowded plate method was used for the preliminary screening of antibiotic-producing isolates. A total of 0.1 suspension from dilutions 10⁻³ and 10⁻⁷ was aseptically spread on agar medium with a sterile swab stick. Each clinical test isolates were exposed on the same agar medium, which were then incubated at 37°C for 24 - 48 hours. Following incubation, a clear zone of inhibition found surrounding the test isolate and the soil isolate's colony responsible for the clear zone was then further isolated²⁷. Two incubation periods: 24 hours and 48 hours.

Mutation Induction on Antibiotic-Producing Isolates (API)

UV-induced mutation was employed on the API (*Bacillus* and *Actinomycetes*). The isolates were subjected to a UV radiation for 2 hours and 4 hours respectively. Antibiotic production activity was repeated to determine change in activity.

Statistical analysis

The IBM Statistical Package for Social Science (SPSS) version 20 was used to analyse data from the study. The result of antibiotic susceptibility was presented as mean $\pm$ standard deviation in bar chart. Results were also presented in percentage on a pie chart.

Ethical Considerations

Ethical approval was obtained from Caleb University Research Ethics Committee with approval number CUL 24/025 according to the declaration of Helsinki.

RESULTS

Isolation and Distribution of Bacterial soil Isolates

The distribution of the soil isolates as presented in Figure 1 showed *Bacillus* species (71.40 %) as the most prevalent isolates. Out of seven (7) bacteria isolates, five (5) of these isolates were identified as *Bacillus* species, and two (2) as *Actinomycetes* species as shown in Plate 1 and 2 respectively.

Antibiotic Susceptibility of Clinical Test Isolates

A panel of conventional antibiotics was used to test the antibiotic susceptibility of four clinical test isolates to determine multi-drug resistance, which included: *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Figure 2). The results showed that *E. coli* was susceptible to antibiotic; levofloxacin (LEV) with zone diameter of 14 mm. However, it exhibited resistance to antibiotics like ciprofloxacin (CPX), amoxicillin (AM), and amoxicillin/clavulanic acid (AU), with no observable zones of inhibition. *Pseudomonas aeruginosa* displayed resistance to all antibiotics such as ampicillin-clavulanic (AMP-CLAV) and ceftazidime (CEP). *Staphylococcus aureus* was

susceptible to antibiotics like azithromycin (AZ), levofloxacin (LEV), erythromycin (E), pefloxacin (PEF), gentamicin (CN), ceftriaxone (C), with zone diameters ranging from 6 mm to 20 mm, but exhibited resistance to antibiotics like cefuroxime (Z), vancomycin (VAN). *Salmonella typhi* demonstrated susceptibility to antibiotic levofloxacin (LEV), with zone diameter of 17 mm, but showed resistance to antibiotics like ciprofloxacin (CPX), amoxicillin (AM), and amoxicillin/clavulanic acid (AU).

Antibiotic-Producing soil isolates (API) using UV-induced mutation

The primary screening of antibiotic-producing isolates (API) was conducted before and after UV radiation exposure using the crowded plate method (Table 1). Prior to UV exposure, none of the *Bacillus* isolates exhibited antibacterial activity against the tested clinical isolates. Nevertheless, both of the *Actinomycetes* isolates were inhibitory to *Pseudomonas aeruginosa* and *Escherichia coli*. However, after UV exposure at different time intervals of 2 hours and 4 hours at two different incubation time of 24 hours and 48 hours; all soil isolates demonstrated inhibitory activity against at least one of the tested clinical isolates (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*). The results interpreted that UV radiation induced mutations in the isolates, leading to the production of antibiotics with inhibitory activity against the tested clinical isolates. The two (2) *Actinomycetes* isolates: Actino 1 and Actino 2 showed notable antibacterial activity against the tested clinical isolates before and after UV exposure, as shown in Table 1. Actino 1 showed inhibitory activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* after both 2 hours and 4 hours UV exposure with 24 hours and 48 hours incubation time (Plate 4.3). Actino 2 also showed inhibitory activity against *Pseudomonas aeruginosa*, *E. coli* and *Salmonella*

typhi after 2 hours UV exposure and only against *Staphylococcus aureus* at 4 hours UV exposure.

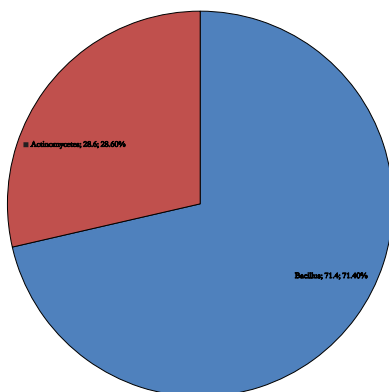


Figure 1: Percentage distribution of soil isolates among *Bacillus* and *Actinomycetes*



Plate 1: Colony morphology of *Actinomycetes*



Plate 2: Colony morphology of *Bacillus* Sp.

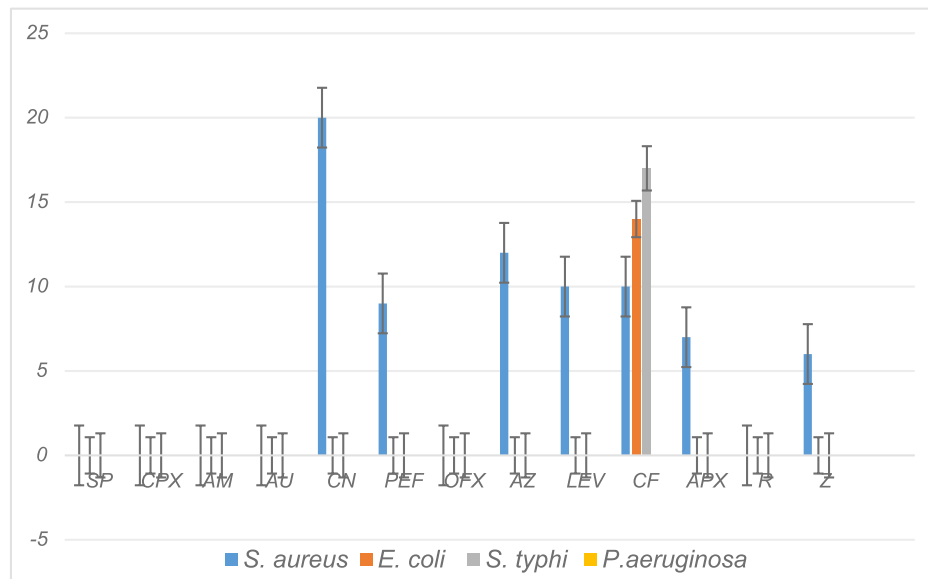


Figure 2: Antibiotic susceptibility of clinical test isolates

Table 1: Primary Screening of Antibiotic-producing Isolates (API) before and after UV exposure

Isolates	Before UV exposure	After 2 hours UV exposure		After 4 hours UV exposure		Interpretation
		24hrs	48hrs	24hrs	48hrs	
<i>Bacillus</i> 1	No activity	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Sensitive to UV
<i>Bacillus</i> 2	No activity	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i>	Inhibitory to <i>E. coli</i> , <i>Staph</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Sensitive to UV
<i>Bacillus</i> 3	No activity	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i>	Inhibitory to <i>Sal</i> , <i>Staph</i>	Inhibitory to <i>Sal</i> , <i>Staph</i>	Sensitive to UV
<i>Bacillus</i> 4	No activity	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>pseudo</i>	Inhibitory to <i>pseudo</i>	Sensitive to UV
<i>Bacillus</i> 5	No activity	Inhibitory to <i>pseudo</i> , <i>E. coli</i>	Inhibitory to <i>pseudo</i> , <i>E. coli</i>	Inhibitory to <i>pseudo</i> , <i>Sal</i>	Inhibitory to <i>Sal</i>	Sensitive to UV
Actino 1	Inhibitory to <i>Pseudo</i> , <i>E. coli</i>	Inhibitory to <i>Pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>Pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitor y to <i>Pseudo</i> , <i>E. coli</i> , <i>Staph</i> , <i>Sal</i>	Inhibitory to <i>Pseudo</i> , <i>scoli</i> , <i>Staph</i> , <i>Sal</i>	Sensitive to UV
Actino 2	Inhibitory to <i>Pseudo</i> , <i>E. coli</i>	Inhibitory to <i>Pseudo</i> , <i>E. coli</i> , <i>Sal</i>	No activity	Inhibitory to <i>Staph</i>	No activity	Sensitive to UV

Key: *Pseudo* – *Pseudomonas aeruginosa* *E. coli* – *Escherichia coli* *Sal* – *Salmonella typhi* *Staph* – *Staphylococcus aureus*

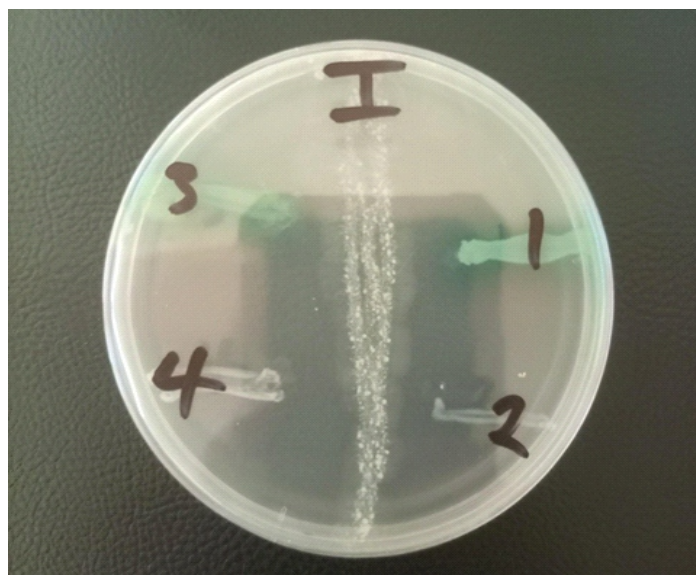


Plate 3: Actinomycetes Crowded plate method after 4 hours of UV exposure.

DISCUSSION

The results, from the study on the variety and distribution of bacteria in soil samples from Caleb University were consistent with Ismail and Ahmed²⁸. Higher occurrence of *Bacillus* species compared to *Actinomycetes* in similar environmental conditions²⁹. The prevalence of *Bacillus* species over *Actinomycetes* suggests the resilience and adaptability of *Bacillus* in environments possibly due to their ability to form spores. Comparing these findings with a study conducted, showed the similarities in colony characteristics between *Bacillus* and *Actinomycetes* isolates, such as creamy colonies and distinct enzyme profiles³⁰. The smooth white colonies of *Bacillus* spp. contrast with the filamentous appearance of *Actinomycetes* showing their growth patterns. Further biochemical tests revealed differences with *Actinomycetes* having Gram rods and distinct enzyme profiles compared to *Bacillus* isolates. The discovery of multidrug resistance among clinical isolates echoes trends seen in studies³¹. Clinically relevant bacteria like *Escherichia coli*,

Salmonella typhi, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed resistance to antibiotics used in this study. This evidence emphasizes the issue of antibiotic resistance and the critical need, for innovative treatment approaches^{32,33}.

UV-induced mutagenesis is a technique used to generate mutations in bacteria, potentially leading to enhanced antibiotic production. The discovery of UV triggered creation, in this research aligns, with the results discussed by Ibnouf³⁴ emphasizing how microbial communities adapt to challenges by altering their metabolic pathways and producing different bioactive compounds. A detailed comparison also sheds light on how UV radiation can boost the production of metabolites and but also highlights the potential of these metabolites to improve the combat capabilities of microorganisms against clinically relevant pathogens. In comparison to studies by Chaudhary et al³⁵, the *Actinomycetes* isolates exhibited comparable or superior antibacterial activity against clinically relevant pathogens both

before and after UV exposure. This reinforces the notion of Actinomycetes as prolific producers of bioactive compounds with therapeutic potential, warranting further exploration for future antimicrobials. The enhanced antibiotic activity observed after UV exposure suggests that these stress conditions may induce the production of novel or more potent antimicrobial compounds.

CONCLUSION

This study showed the efficacy of antibiotic-producing *Bacillus* and *Actinomycetes* against multi-drug resistance clinical isolates. By integrating these findings with existing literature, this study contributes to a comprehensive understanding of antibiotic-producing soil bacteria, antibiotic resistance dynamics, and the biotechnological implications of UV-induced antibiotic production. Future research endeavours may benefit from secondary metabolite production in natural environments such as soil.

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