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# PROBATION OF THE SOIL FROM CALEB UNIVERSITY, IMOTA LAGOS, NIGERIA AS A SOURCE OF ANTIBIOTIC-PRODUCING BACTERIA

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**ABSTRACT**

The importance of antibiotics in treating bacterial diseases cannot be overstated. However, the excessive use of antibiotics has led to the development of resistance in many bacteria, making the treatment of bacterial infections challenging. Therefore, it is essential to search for new strains of bacteria in different habitats that produce novel antibiotics. The aim of this study was to investigate the soil from Caleb University for antibiotic-producing bacteria. Soil samples were collected from seven locations within Caleb University, including Joshua Hall (J), Cafeteria (C), Dump site (DS), Lab garden (LAB G), Last Garden (LG), and School Inner Garden (SID). The samples were diluted, and standard procedures were followed for all experiments. The results of this study indicate that the isolated species did not show significant inhibitory activity against the test bacteria and were unable to produce antimicrobial compound. Therefore, it can be concluded that the species found in the soil samples from Caleb University are not effective in stopping bacterial growth.

**Keywords**: Antibiotics, Bacteria, Soil, Caleb University, Anti-microbial

## INTRODUCTION

Soil has been identified as a suitable habitat for a diverse range of microorganisms with high antibiotic-producing capabilities. The soil microbial population is influenced by various factors, making each soil a potential source of unique organisms (Anokhee *et al*., 2017) that could serve as promising candidates for developing antibiotics to combat antibiotic resistance (Makut and Owolewa, 2011).

Antibiotics are substances that can inhibit or eliminate bacterial growth (Pandey *et al*., 2018). The term "antibiotics" is derived from the word antibiosis, meaning "against life." They can be derived from microbes such as bacteria and fungi (Singh and Mishra, 2013). Antibiotics have been extensively used to treat bacterial infections like meningitis, tuberculosis, and pneumonia.

Unfortunately, the widespread misuse of antibiotics by users has led to a significant increase in cases of antibiotic resistance in recent years (Yuce, 2001). This alarming trend has prompted the scientific community to urgently address the need for developing more effective options to combat the growing threat posed by antibiotic resistance in the face of a rapidly expanding human population (Sandhya *et al*., 2015; Pandey *et al*., 2018). This study aims to address this critical need.

## MATERIALS AND METHODS

### Collection of soil samples

Dirt on the soil surface was removed using the method described by Anokhee *et al*. (2017) before randomly collecting soil samples from a depth of 1-5 cm below the soil surface (Sura *et al.*, 2017) with the assistance of a sterile spatula and a calibrated ruler. The samples were collected from seven locations within Caleb University, resulting in a total of seven samples for the study. The samples were placed in properly labeled sterile containers marked as Joshua Hall (J), Cafeteria (C), Dump site (DS), Lab garden (LAB G), Last Garden (LG), and School Inner Garden (SID).

### Sample and Media Preparations

The test tubes containing 9 mL of distilled water were sterilized at a temperature of 121ºC for 15 minutes (Rafeeq *et al*., 2018). To create the stock sample, 1 g of soil sample was added to 9 mL of distilled water, mixed thoroughly, and labeled as "J stock" (Rafeeq *et al*., 2018). Subsequently, 1 mL of the stock was transferred to the next tube labeled as $J10^{-1}$ to create the first dilution. This process was repeated for all test tubes to produce $J10^{-2}$. In a conical flask, 9g of starch casein agar was mixed with 500 mL of distilled water and thoroughly combined before sealing with cotton wool. The medium was then sterilized at 121ºC for 15 minutes.

### Bacterial Isolation

A sterile syringe was used to introduce exactly 0.5 mL of each of the dilutions $10^{-2}$ and $10^{-5}$ into separately labeled sterile plates. Subsequently, molten starch casein agar was poured into Petri dishes containing the test samples. The plates were then inverted and incubated at a temperature of 25°C for 7 days (Njenga *et al*., 2018).

### Sub-culturing

A sterile inoculating loop was used to collect each individual bacterial colony found on the starch casein agar for streaking on the surface of fresh sterile starch casein agar plates. The colonies were then incubated at 25°C for 7 days (Njenga *et al*., 2018). Subsequently, the pure colonies were preserved as stock cultures on agar slants at 4°C (Ismail and Ahmed, 2021).

**Gram Staining**

The method used by Rafeeq *et al*. (2018) was employed to perform Gram staining on the isolated and sub-cultured bacterial colonies. A smear was made on a clean glass slide, fixed with heat, and then air-dried. The smear was then immersed in crystal violet for 1 minute, followed by washing with distilled water. Iodine solution was applied to the smear surface for 30 seconds, washed with distilled water, rinsed with acetone, and then covered with the counterstain, safranine. The smear was allowed to dry for 60 seconds, washed, and dried. Once dried, the slide was viewed under a ×100 objective lens to observe the stained smear for the bacterial isolates.

**Biochemical Tests on Bacterial Isolates**

Biochemical tests (catalase, oxidase, urease, and citrate tests) were performed on the isolates following Bergey's Manual of Systematic Bacteriology.

**Screening for Antimicrobial Activity**

A mixture containing 5 mL of nutrient broth and isolated and sub-cultured bacterial colonies were incubated at 37°C for 24 hours. Next, 38 g of Mueller-Hinton agar was autoclaved in 1000 mL of distilled water at 121°C for 15 minutes and then allowed to cool before being dispensed into Petri dishes to solidify. The test organisms were inoculated in test tubes containing 2 mL of sterilized physiological saline. Using sterilized cotton swabs, a cell concentration equal to 0.5 McFarland standards of the test bacteria was inoculated on Muller-Hinton agar plates. Wells were created on the agar using a sterile cork borer. The control, a mixture of 0.1 mL of bacterial isolates and erythromycin (250 mg/mL), was introduced into the wells. The inhibition zones were observed and measured after the plates had been incubated at 37°C for 24 hours (Ismail and Ahmed, 2021).

**RESULTS AND DISCUSSION**

Table 1 shows the physical description of soil samples collected from Caleb University, including the texture and color of the soil samples, ranging from clay to loam soil and from light brown to dark brown respectively; and the mean viable bacterial plate count (CFU/g). Samples from Lab garden behind school building and school inner garden had colonies that were too numerous to count while sample from the lab garden had the lowest number of colonies.

Results from growth on plates show that bacteria are abundantly distributed in both clay and loam soils in Caleb University after appropriate incubation of the bacterial isolates on appropriate media, colonial morphology was observed Table 2. Gram reaction and biochemical characteristics of bacterial isolates are presented in Table 3. Suspected bacteria isolates include members of the actinomycetes group and *Bacillus* spp. Table 4 presents results for screening of bacterial isolates for antimicrobial activities against confirmed clinical isolates of *Staphylococcus aureus* and *Escherichia coli.* Results showed no antimicrobial activity by our isolates against *S. aureus* and *E. coli.*

**Table 1: Mean Viable Bacterial Count**

| S/N | Sample code | Colour | Texture | CFU/g |
|---|---|---|---|---|
| | J | Light brown | Clay soil | $1.5 \times 10^5$ |
| | CAF | Dark brown | Loam soil | $3.3 \times 10^5$ |
| | DS | Dark brown | Loam soil | $3.3 \times 10^5$ |
| | LAB G | Dark brown | Loam soil | $1.2 \times 10^5$ |
| | F | Light brown | Clay soil | $1.3 \times 10^5$ |
| | LG | Dark brown | Loam soil | TNTC |
| | SIG | Dark brown | Loam soil | TNTC |

Key: J=Joshua Hall, C=Cafeteria, DS=Dump site, LAB G = Lab garden, F=Field, LG= Garden behind school building and SIG = School Inner Garden. TNTC = too numerous to count

**Table 2: Colony Morphologies of Bacteria Isolated from Soil Samples**

| ISOLATE CODE | Colony Morphology on Starch Casein Agar | Differentiated Pigmentation on Agar | Aerial mycelium | Substrate mycelium | Elevation | Surface |
|---|---|---|---|---|---|---|
| DS102A | Bluish round dry colonies with entire margins with coloration on agar | +(brown) | Blue | Brown | Raised | Smooth |
| DS102D | Round, entire creamy dry colonies, with white powdery margin and characteristic coloration on agar | + (brown) | White | Brown | Raised | Smooth |
| SIG105A | Powdery white dry round colonies with entire margins | - | White | White | Flat | Smooth |
| CAF105A | Creamy round colonies with entire margins and characteristic coloration on agar plate | +(brown) | Cream | Brown | Raised | Smooth |
| LABG105D | Whitish-cream, colonies with entire margins | - | Cream | Cream | Flat | Smooth |
| SIG106A | Powdery grayish-white irregular-shaped colonies with creamy background and hollow centre | - | White | Cream | Flat | Smooth |
| J102F | Powdery white, round, filiform and conical appearance | - | White | White | Flat | Rough |

| | | | | | | |
|---|---|---|---|---|---|---|
| J102C | Small powdery white round colonies with entire margins | - | White | Cream | Flat | Rough |
| F102B | powdery white, round dry colonies | - | White | Cream | Upraised | Smooth |
| LG102C | Small round creamy, moist colonies with entire margins | - | Cream | Cream | Flat | Smooth |

**Table 3: Gram Reaction and Biochemical Characteristics of Isolates**

| Isolate Code | Gram Reaction | UT | CT | OT | CU | Suspected Organism |
|---|---|---|---|---|---|---|
| DS102A | Gram-positive filamentous Bacilli | + | - | - | + | Member of the Actinomycetes group |
| DS102D | Gram-positive thread-like filaments observed | + | - | - | - | Member of the Actinomycetes group |
| SIG105A | Gram-positive thread-like filaments observed | + | + | - | - | Member of the Actinomycetes group |
| CAF105A | Gram-positive branching filamentous Bacilli | + | - | + | - | Member of the Actinomycetes group |
| LABG105D | Gram-positive filamentous Bacilli | + | + | - | - | Member of the Actinomycetes group |
| SIG106A | Gram-positive thread-like branching filamentous Bacilli | + | - | - | - | Member of the Actinomycetes group |
| J102F | Gram-positive filamentous bacilli seen with beaded-like structure | + | + | - | - | Member of the Actinomycetes group |
| J102C | Gram-positive filamentous rods seen | + | - | + | + | Member of the Actinomycetes group |
| F102B | Gram-positive thread-like filamentous rods observed | + | + | - | + | Member of the Actinomycetes group |
| LG102C | Short gram-positive rods observed | + | + | - | + | *Bacillus* spp. |

**Key:** UT = Urease Test, CT = Catalase Test, OT = Oxidase Test, CU = Citrate Utilization Test

**Table 4: Screening of Isolates for antimicribial activities**

| ISOLATE CODE | E. coli | S. aureus |
|---|---|---|
| DS102A | R | R |
| DS102D | R | R |
| SIG105A | R | R |
| CAF105A | R | R |
| LABG105D | R | R |
| SIG106A | R | R |
| J102F | R | R |
| J102C | R | R |
| F02B | R | R |
| LG102C | R | R |

Key: R = resistant, S = sensitive

**Discussion**

The continuous effort targeted at ending the menace of antimicrobial resistance is justifiable by the fact that it has become a global threat that has significantly impacted the global economies, in addition to causing death, disabilities, prolonged illness, longer hospital stays, and financial burdens for those affected (Hernando-Amado *et al*., 2019). Soil has been identified as a haven for a plethora of microorganisms (Banerjee *et al*., 2023). Gram staining of the isolates revealed the presence of actinomycetes in the soil samples. This is consistent with the results of a study by Kaur and Teotia (2019), which also found actinomycetes in soil samples

collected from Benin, Edo State (South-South), Nigeria. The results also showed that the isolates did not inhibit the test organisms. This may be attributed to the fact that the isolates lack the genetic viability to produce antimicrobial metabolites. This is in line with the findings of Kaur and Teotia (2019), which showed that 10 out of 28 actinomycetes isolates exhibited varying degrees of antagonistic activities against 10 test organisms, with 80% of the isolates recovered from rhizospheric soils. The findings of this study are further supported by the fact that the soils were not rhizospheric, which are known to be good sources of antimicrobial substances (Dhawane and Zodpe, 2017). This is also supported by Abo-Shadi *et al*. (2010), who suggested that rhizospheric soils harbor diverse bioactive metabolites. It could also be due to the fact that the isolates had not reached the stationary phase of growth, where the bulk of secondary metabolites, including those with microbial inhibitory potentials, are produced. Therefore, it can be concluded from this study that the studied isolates cannot serve as a source of candidates for antibiotic production.

## CONCLUSION

Soil samples from various locations within Caleb University were screened for the presence of bacteria with antibiotic-producing potential. The study, which did not investigate the different microbial growth phases, could only determine that actinomycetes and *Bacillus spp*. which were the only organisms obtained from the soil samples, lacked the ability to produce metabolites with inhibitory activity against *S. aureus* and E. coli.

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