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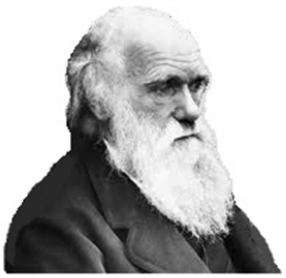

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# Screening of antibiotic-producing bacteria isolated from soil gotten within gaba, Bwari FCT, Abuja

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**ABSTRACT**

This investigation seeks to determine the antibiotic-producing potential of bacteria in inhibiting the growth of other organisms like *Staphylococcus aureus* and *Escherichia coli.* The bacterial isolates were identified using standard cultural, morphological, and biochemical characterization. These were further screened for their antibiotic-producing potential using the Mueller Hinton agar (MHA) media. The identified bacterial isolates had a mean viable count ranging between $1.38\times10^6$ / 1,380,000 cfu/ml and $1.52\times10^6$ / 1,520,000 cfu/ml. *Micrococcus roseus, Brevibacterium species, Bacillus subtilis, Bacillus anthracis*, and *Bacillus cereus* were the predominant bacterial isolates. Nonetheless, the antibiotic test revealed that *Bacillus subtilis* and *Bacillus anthracis* showed antimicrobial activity against *S. aureus*, whereas *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus subtilis* showed zones of inhibition against *E. coli.* This study shows that *Bacillus* species have the potential to produce antibiotics and can be used to control microbial growth in the future.

***Keywords*:** Antibiotics, Antimicrobials, Microorganisms, Bacteria

## 1. INTRODUCTION

Bacterial pathogens *(e.g. Mycobacterium tuberculosis, Staphylococcus aureus* etc.) have been reported to frequently acquire resistance to antibiotics, and exhibit multi-drug resistance. In recent years multi-drug resistance in bacteria has raised a serious concern among the scientific community.

This highlights a greater need to find more antibiotics as well as alternative antimicrobial substances that can be used for use in clinics, food preservation and dairy products (Gerits *et*

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*al*., 2016). The continuous quest for new antibiotics is due to a phenomenon known as antibiotic resistance. This is a situation whereby an antibiotic that once killed a harmful bacterium no longer works.

Thus, the microbe is said to have become resistant to the chemical. Antibiotic resistance against infectious diseases has increased in recent years (Llic *et al*., 2005).

This pathogenic resistance to antibiotics (as in the case of *Staphylococcus aureus*) has posed a dangerous threat to the treatment of human infections. Scientists and medical professionals are worried about this dangerous threat. This explains why multiple approaches have been taken by scientists, in recent times, to develop novel, potent, and less toxic antibiotics from natural sources against pathogenic bacteria (Adwan *et al*., 2008). Soil has been considered a natural source of obtaining bacteria with the ability to produce noble antibiotics. Fazly et al. (2005) reported that over 55% (representing a total of more than 5,000 compounds) of the antibiotics detected between 1945 and 1978 originated from a genus of a soil microorganism known as Streptomyces.

In 1944, a species of Streptomyces called *Streptomyces griseus* was discovered to produce streptomycin – an antibiotic which was very efficient in treating tuberculosis (Schallmey et al., 2004) The choice of soil microbes with antibiotic-producing potential stems from their ability to survive the highly competitive, harsh environment infested with other microorganisms by inhibiting the growth of the latter through the secretion of antibiotics (Hibbing, et. al., 2010).

Thus, in tandem with the ongoing search for new antibiotics produced by soil bacteria to ward off stubborn pathogens that can make an infection untreatable by scientists, the present investigation becomes very important (Aghamirian et al., 2009).

This investigation aims to identify novel antibiotic-producing microbial strains with unprecedented (never seen or done before) importance and to check the ability of the antibiotic-producing microorganisms to inhibit the growth of other organisms like *Staphylococcus aureus* and *Escherichia coli.*

## 2. MATERIALS AND METHODS

### 2. 1. Equipment

Sterile spatula, sterile plastic bags, test tubes, beaker, autoclave, petri plates, incubator, sterile loop, glass slides, cover slips, electric microscope, laminar air flow, sterilised cotton buds, centrifuge machine, sterile borer, distilled water, normal saline, and colony counter.

### 2. 2. Media and Reagents

Nutrient agar, nutrient broth, Mueller Hinton agar, Simon citrate agar, urea agar, 30% hydrogen peroxide, 1% paradimethyl amino cinnamaldehyde, 1% tetramethyl-para-phenylenediamine, Crystal violet, Gram's iodine, alcohol, safranine, and malachite green. All media were prepared according to the manufacturer's instructions.

### 2. 3. Sample Collection and Preparation

Approximately 1 kg of soil samples was collected from the micro and macro environment (including waste-polluted soil, normal street soil, and agricultural soil) within Bwari Area Council, Abuja. The soil samples were collected from a depth of 6 inches at the study site using

a sterile spatula. The samples were then conveyed to the laboratory within 24 hours in sterile plastic bags for analysis. To commence the analyses, a ten-fold serial dilution in a lamina airflow was conducted. 0.1 ml of each soil sample from selected dilutions (usually 1:100 and 1:1000) was plated out using the pour plate techniques on labelled nutrient agar plates. The inoculated Petri dishes were incubated for 24 h at 37 °C to obtain the isolated colonies.

### 2. 4. Identification and Characterisation

Following the 24 h incubation at 37 °C, a heterotrophic plate count was conducted and the number of colonies formed was calculated and expressed as Colony Forming Unit per millilitre (CFU/ml). Discrete bacterial colonies were sub-cultured on fresh nutrient agar plates and incubated for 24 h at 37 °C. Meanwhile, test organisms *i.e.*, *Escherichia coli* and *Staphylococcus aureus* obtained from the school's laboratory were tested for antimicrobial activity of antibiotic-producing bacteria isolates using the agar well diffusion method.

### 2. 5. Screening for Antimicrobial Activity

Swab cultures of *Escherichia coli* and *Staphylococcus aureus* were made on Petri dishes containing Mueller Hinton agar (MHA) media. Wells were made on MHA plates using a sterile borer. To these wells, supernatants of isolated and sub-cultured bacterial colonies centrifuged at 6000 rpm for 10 min were poured into these wells and incubated for 48 h. The observation was made for zones of inhibition after incubation.

### 2. 6. Staining and Biochemical Characterisation

Gram staining and spore staining of the isolated and sub-cultured bacterial colonies were done and the identification of bacterial isolates biochemical tests were performed as described by Bergey's manual *i.e.*, IMViC tests (citrate utilization test, catalase test), oxidase, urease, nitrate reduction and blood hemolysis.

## 3. RESULTS

The results of the analysis of 1 kg of soil samples collected from the micro and macro environment (including waste-polluted soil, normal street soil, and agricultural soil) within Bwari Area Council, Abuja are presented as follows:

**Table 1** depicts the number of discrete colonies formed from a plated 0.1 ml of a $10^4$-dilution factor of the respective soil samples after sub-culturing.

**Table 2** shows the morphological, cellular, and biochemical characteristics of the five culture strains of bacteria isolated from the various soil samples. The results of the biochemical tests for the isolates were checked using Bergey's manual of systematic bacteriology. The five isolated culture strains included *Micrococcus roseus, Brevibacterium* sp., *Bacillus subtilis, Bacillus anthracis,* and *Bacillus cereus.*

**Table 3** and **Fig. 1** illustrate the antibiotic production activities of the selected culture strains of bacteria using the agar well diffusion method. The zones of inhibition were observed against the test bacteria – *Staphylococcus aureus* and *Escherichia coli*.

**Table 1.** Determination of the Colony Forming Unit

| Samples | Number of Colonies | Diluted Factor | Amount Inoculated (ml) | Cfu/ml |
|---|---|---|---|---|
| Plate 1 | 138 | $10^4$ | 1.0 | $1.38\times10^6$ / 1,380,000 cfu/ml |
| Plate 2 | 152 | $10^4$ | 1.0 | $1.52\times10^6$ / 1,520,000 cfu/ml |
| Plate 3 | 146 | $10^4$ | 1.0 | $1.46\times10^6$ / 1,460,000 cfu/ml |
| Plate 4 | 147 | $10^4$ | 1.0 | $1.47\times10^6$ / 1,470,000 cfu/ml |
| Plate 5 | 141 | $10^4$ | 1.0 | $1.41\times10^6$ / 1,410,000 cfu/ml |

**Table 2.** Identification of Bacterial Isolates Collected from Various Soils

| **Colonial Morphology** | | | | | **Cellular Characterization** | | | | **Biochemical Characterization** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates codes** | **Whole colony** | **Surface colony** | **Edge** | **Pigment** | **Elevation** | **Gram stain** | **Spore stain** | **Motility** | **Citrate utilization** | **Catalase** | **Oxidase** | **Urease** | **Nitrate reduction** | **Hemolysis** | **Isolated organism** |
| N1B | Circular | Smooth | Entire | Pink | Slightly Convex | + | - | - | - | + | + | - | + | - | ***Micrococcus roseus*** |
| N2A | Irregular | Smooth | Entire | Yellow | Convex | + | - | - | + | + | - | + | - | + | ***Brevibacterium specie*** |
| N2B | Irregular | Dry | Undulate | Whitish – cream | Rounded elevation | + | + | + | + | + | - | - | + | + | ***Bacillus subtilis*** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N3A | Irregular | Granular | Entire | Cream | Raised | + | + | – | + | + | - | - | + | - | ***Bacillus anthracis*** |
| N3B | Irregular | Dry | Undulate | Cream | Flat | + | + | + | + | + | + | - | + | + | ***Bacillus cereus*** |

**Table 3.** Inhibition Zone Shown by Different Bacterial Isolates Against Test Organisms

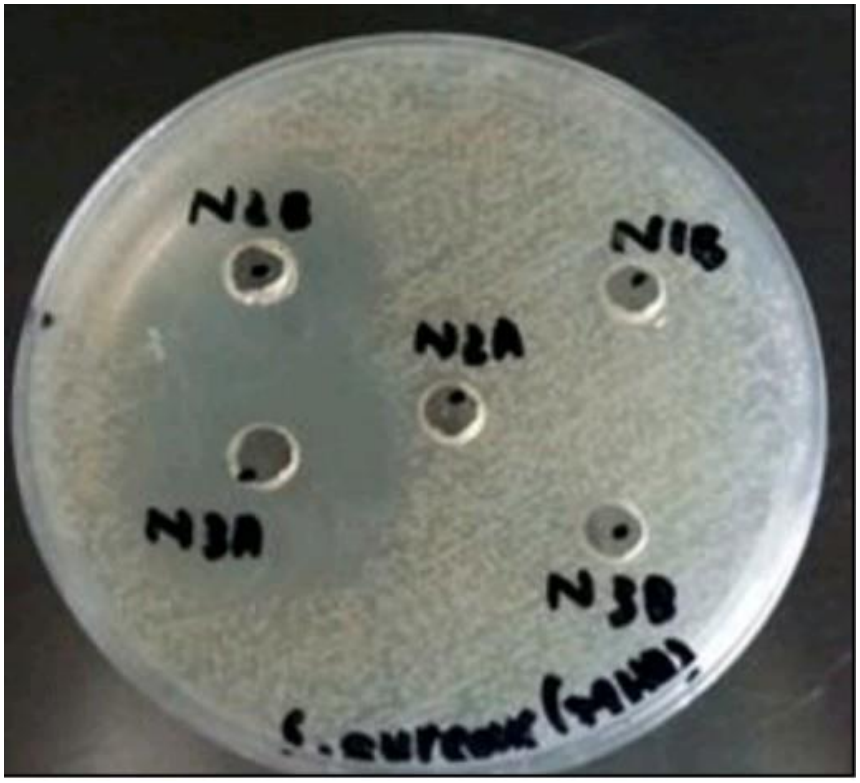
| ISOLATE CODES | TEST ORGANISMS | |
|---|---|---|
| | *S. aureus* | *E. coli* |
| N1B | - | - |
| N2A | - | - |
| N2B | + | + |
| N3A | + | + |
| N3B | - | + |

NOTE: + means active against the target bacteria, - means no activity

## 4. DISCUSSION

In searching for new antibiotics, screening of microorganisms through relatively rapid and simple methods has been done for antibiotic production. Antibiotic production is a main feature of several kinds of soil microorganisms i.e., bacteria and fungi and may thereof represent a survival mechanism. Temperature variations may also affect the synthesis of antibiotic production. The bacteria isolated from soil show antibiotic activity under normal growth conditions and were found to inhibit some gram-positive as well as some gram-negative organisms. The isolates of bacteria were not organic acid producers but they were antibiotic producers (Chandrashekhara, 2010). In the present study, bacteria were isolated from soil samples (waste-polluted soil, normal street soil and agricultural land soil). The selected culture strains of bacteria were then identified by techniques, like gram staining, spore staining and biochemical characterization tests and bacterial isolates were subjected to test microorganisms (*Staphylococcus aureus* and *Escherichia coli*) to check their ability to produce antibiotics using agar well diffusion method. The results indicate that *Bacillus species* (with the highest number of isolates) produces a clear zone of inhibition against test microorganisms.


**(A)**




**(B)**

**Fig. 1(A,B).** Clear Zones of Bacterial Isolates

*Bacillus species* were dominant in showing antibiotic activity against *S. aureus* and *E. coli*. This finding corroborates that of Ahmed *et al*. (2013) who screened soil microorganisms for antibiotic production and revealed that only *Bacillus* species exhibited antibacterial activity of all bacteria isolated. Interestingly, an average of about 145 cells per gram of Rhizobacteria were found in the soil. This is similar to the findings of Amin *et al*. (2015) who isolated similar species of Rhizobacteria from soil samples they worked on.

The antimicrobial activity from a sediment habitat and resistance to antimicrobials of bacteria can easily explain the persistence and selection of such strains in this particular ecology. On agar media, cultural characteristics displayed by bacteria were used to identify bacteria because of their specific and different growth patterns (Sousa *et al*., 2013). It has been reported that *Bacillus species* and other spore-forming bacteria carry genes for the production of antibiotics and the breakdown of diverse carbon sources. It has also been reported that Bacitracin produced by *Bacillus species* inhibits both *E. coli* and *S. aureus*. Hassan *et al*. (2014) identified fourteen isolates of antibiotic-producing *Bacillus species* from soil. For the synthesis of secondary metabolites *Bacillus species* are well known with remarkable diversity both in its function and structure.

There is an argument with Aslim *et al*. (2002) who documented that strains of *Bacillus* had greater effects on gram-positive bacteria as compared to gram-negative bacteria. *B. subtilis* has the potential to produce antibiotics and has been recognized for the past 50 years. *B. subtilis* is an endospore forming *Rhizobacterium*. Sonenshein *et al*. (2002) collected several wild types of *B. subtilis*, which have the potential to produce more than two dozen antibiotics. *B. subtilis* C126 strain from sugar cane fermentation has the potential to produce a polypeptide antibiotic, Bacitracin.

Production of Bacitracin by *B. subtilis* is pH dependent which gave maximum production at pH of 7.8 - 8. Strains of *B. cereus* from a soil sample can produce Bacteriocin and be active against most gram-positive but not against gram-negative bacteria. M15 strain of *B cereus* possesses an inhibitory effect against both gram-positive and gram-negative bacteria. *Bacilli* are predominant soil bacteria widely used in industrial applications, particularly antibiotics production having medical, agricultural, and veterinary importance (Akbar *et al*., 2016). *Bacillus species* are preferred hosts for the production of many improved and new products used in genomic and proteomics (Schallmey *et al*., 2004). To enhance the yield of Bacitracin it is possible to clone and amplify the gene coding for some key enzymes in the biosynthetic pathways of Bacitracin (Rafiq, et al., 2018).

## 5. CONCLUSION

The present study was an attempt to identify and characterize versatile strains of bacteria and to check their ability for antibiotic production. Several different bacterial isolates were found producing clear zones of inhibition against test organisms i.e. *Staphylococcus aureus* and *Escherichia coli*. The study revealed that bacterial species have the potential for antibiotic production. The potential antibiotic-producer bacterial species identified include *Bacillus* strains i. e. *Bacillus subtilis, Bacillus anthacis* and *Bacillus cerus.* This study may contribute to providing information on the antibiotic-producing microorganisms in soil. Nonetheless, Further characterization, purification, and structural elucidation are recommended to know the novelty, quality and commercial value of these antibiotics.

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