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Antibiotic-producing bacteria isolated from some natural habitats in the Federal Capital Territory (FCT), Nigeria

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High global prevalence of multidrug-resistant bacteria due to antibiotics misuse has prompted the need for novel antibacterial compounds to replace the failing antibiotics. This study investigated some natural habitats in Abuja, Nigeria, for antibiotics-producing bacteria. Thirty-six soil samples from termite mounds, river banks and rhizospheres of *Anacardium occidentale* L. (cashew tree), *Gmelina arborea* Roxb. ex Sm. (beechwood), *Ageratum conyzoides* L. (goat weed) including *Cymbopogon citratus* (DC) Stapf. (lemon grass) were cultured on nutrient media. Twelve potential antibiotic-producing isolates were identified by crowded plates method and characterized using Bergey's manual. The antimicrobial activities of the filtrates from the isolates against some pathogenic strains namely *Streptococcus pneumonia, Salmonella typhi, Escherichia coli* (ATCC 25922), *Proteus mirabilis, Staphylococcus aureus* (ATCC 25923), *Candida albicans, Bacillus subtilis* and *Pseudomonas aeruginosa* (ATCC 27853) were determined by agar-well diffusion method. Only 5 eventually inhibited at least 1 test microorganism; 4 showed activities against both Gram-positive and Gram-negative bacteria (broad spectrum) and 2 among the 4 also inhibited *Candida albicans*, while the remaining 1 inhibited only 1 Gram-positive bacterium (narrow spectrum). The 5 potent antibiotics-producers were *Bacillus* spp. In conclusion, some natural habitats in the FCT are important sources of antibiotic-producing bacteria. Their antimicrobial lead compounds could be extracted and developed locally for pharmaceutical applications.

Key words: Antibiotics-producing bacteria, *Bacillus* species, natural habitats.

INTRODUCTION

The discovery of antibiotics in the 20th century was a global success resulting in the improvement of treatment outcomes from infectious diseases thereby saving millions of lives (Shatzkes et al., 2017). However, this

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success is being threatened by antimicrobial resistant microorganisms as antibiotics are showing weaknesses to previously susceptible bacterial infections (Ventola, 2015).

Antibiotic resistant crises dated back to the introduction of antibiotics in the clinics and have continued with every new antibiotic developed for medical purposes (Rossolini, 2015). Every new class of antibiotics introduced also results in the evolution of resistant bacteria pathogens due to overuse, inappropriate prescription, extensive agricultural use and horizontal gene transfer which provide an evolutionary drive for bacteria to develop resistance (Nadeem et al., 2020).

The emergence of new infectious diseases including multidrug resistant pathogens is on the rise worldwide (Sautter and Halstead, 2018). The global morbidity and mortality cases as a result of the rise in antibiotic resistant bacteria are increasing as the effective-treatment options decline (Rather et al., 2017). Antibiotic-resistant bacteria infections account for about 33,000 annual human death globally (Cassini et al., 2019), and estimated to rise to about 10 million by 2050 without check of which Africa will record 40% of cases (O'Neill, 2017). Developed countries like the United States records thousands of antibiotic-resistant microbial infections cases of which about 23,000 die because of limited treatment options (Rather et al., 2017). The World Health Organization (WHO) published the first global surveillance report on antibiotic resistance (ABR) in 2014 attributing multi-drug resistant (MDR) bacteria to be responsible for 45% of deaths in both Africa and South-East Asian (WHO, 2014).

Antimicrobial resistance constitutes a significant threat resulting in prolonged treatment, expensive therapy, morbidity, mortality, and economic loss to both the patient and nation (Ahmad and Khan, 2019). The clinical isolates like Methicillin Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, enterococci especially vancomycin-resistant enterococci (VRE), and members of Family Enterobacteriaceae, like *Proteus sp.*, *E. coli*, and *Klebsiella pneumoniae* have all displayed rapid antibiotic resistance and spread in the hospital domain (Basak et al., 2016).

Soil is the reservoir for novel antibiotic-producing microbes (Rafiq et al., 2018) and majority of the antibiotics in clinical use were discovered by screening for antimicrobial activities of microorganisms isolated from the soil (Rolain et al., 2016). The success recorded by soil microbes is attributable to their ability to produce antibiotics in the natural ecosystem as a mechanism of survival and dominance over other competing bacteria (Dwivedi and Sisodia, 2019).

The emergence of new diseases and reemergence of multidrug-resistant including pan drug-resistant pathogens that neutralizes the effectiveness of existing antibiotics has prompted the need for novel antibiotics (Rolain et al., 2016; Sautter and Halstead, 2018). Some new drugs are

currently undergoing development; however, they fall below increasing medical needs. Furthermore, most of these drugs are derivatives of established classes of antimicrobials currently in use and therefore prone to existing bacterial resistance mechanisms (Chopra, 2013).

Consequently, the present study investigated biotechnologically important strains of antibiotic compounds-producing bacteria from soil samples in the Federal Capital Territory (FCT).

MATERIALS AND METHODS

Study area

The study area was in the FCT (Figure 1) which lies between 8° 15'N to 9° 12'N latitude and 6° 27'E to 7° 23'E longitude and located in the central region of Nigeria. The FCT has six area councils and occupies a landmass of approximately 713 km^2 (Okiemute et al., 2018). It is situated within the savannah region with moderate climatic conditions.

Study design and sample collection

Randomized experimental design was adopted for the collection of soil samples in the FCT. Six (6) soil replicates were collected from some natural habitat for analyses. Soil samples were collected from selected natural habitats in each of the 6 area councils in the FCT, North Central Nigeria. Between November 2 and December 14, 2019 a total of thirty-six (36) samples from six (6) soil replicates were collected from each selected natural habitat at the six (6) Local government Area Councils by randomized sampling. The six Area Councils include Abaji, Kuje, Abuja Municipal, Gwagwalada, Kwali and Bwari respectively. The samples were aseptically collected from 5 to 15 cm soil depth into labelled zip-lock bags using sterile garden trowel and transported in cold-chain (4 to 8°C) to the laboratory for analyses. One (1) natural habitat was mapped to each area council for the collection (Table 1). The habitats include termite mounds, rhizosphere of *Anacardium occidentale* L. (family: Anacardiaceae; popular name: cashew tree), rhizosphere of *Gmelina arborea* Roxb. ex Sm. (family: Lamiaceae; popular name: beechwood), rhizosphere of *Ageratum conyzoides* L. (family: Asteraceae; popular name: goat weed), rhizosphere of *Cymbopogon citratus* (DC) Stapf. (family: Poaceae; popular name: lemon grass) and river bank.

The plant species (of which the rhizospheres were examined) were also collected, identified and deposited at the Herbarium & Ethnobotany Unit of National Institute for Pharmaceutical Research and Development, Abuja, Nigeria (NIPRD).

Screening for potential antibiotic-producing microorganisms

Potential antibiotic-producing bacteria were isolated following the crowded plate technique according to Bavishi et al. (2017) with slight modifications. For this purpose, 1.0 g of each soil sample was weighed into 9.0 ml of sterile normal saline (0.85% sodium chloride solution) to obtain 1:10 dilution. The tubes were vortexed for 5 min and sediment was allowed to settle before subjecting the supernatant to serial dilutions up to 1:10^5. By Spread plate technique, 0.1 ml of the inoculum from 1:10^2 dilutions and 1:10^3 dilutions were aseptically spread on nutrient agar (NA) plates (HiMedia, India). The inoculated agar plates had been seeded with
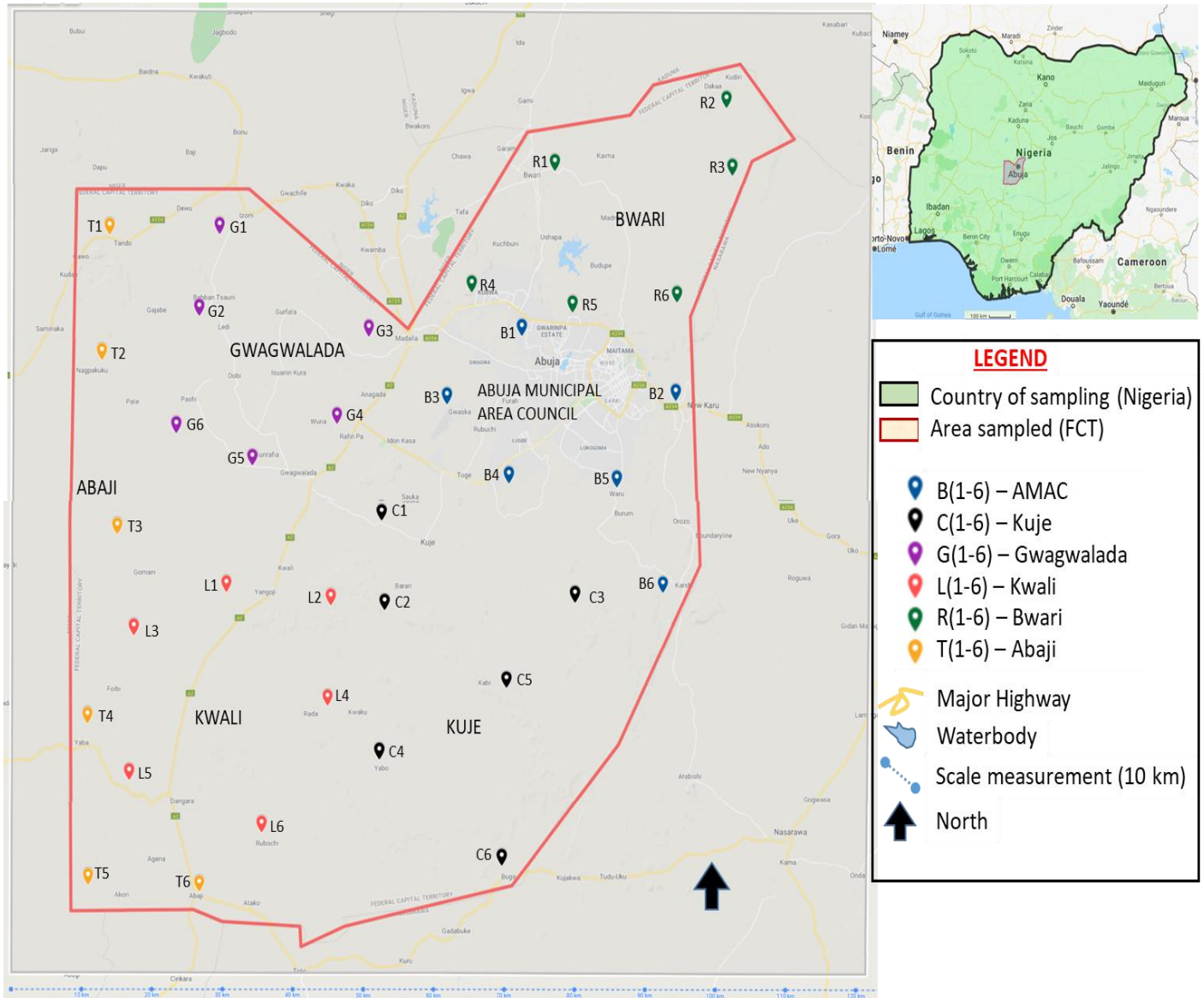
Figure 1. Map of FCT showing the study area.

nystatin (50 μg/ml) [Nanjing Sino Pharmaceutical] to inhibit fungal contaminant and were incubated at 37°C for 24 h in inverted positions. Culturing was performed in triplicate and colonies that displayed antagonism against other bacteria was identified by clear zones of inhibition around them were selected as potential antibiotic-producers. Selected colonies were sub-cultured on fresh NA plates and incubated at 37°C for 24 h to obtain pure cultures. The pure colonies were preserved on NA slants and stored in 4°C until further use.

Characterization of identified isolates

Potential antibiotic-producers were characterized morphologically and biochemically and confirmed according to the Bergey's manual of systematic bacteriology 3 (Vos et al., 2011). The morphological characteristics of isolates were determined by performing tests like Gram's staining, endospore staining, motility test, growth in 6.5% NaCl at 37°C, growth in anaerobic condition and growth at 55°C. Biochemical tests include catalase test, methyl red, Voges-Proskauer test (VP), Indole, citrate, oxidase, nitrate reduction, starch hydrolysis. Carbohydrate fermentation including glucose, lactose, sucrose, mannitol and sorbitol were performed.

Test for antibiotic-producing potential of isolated bacteria

The organisms used for susceptibility testing were obtained from the Department of Microbiology and Biotechnology, NIPRD. The organisms were some American Type Culture Collection (ATCC) strains and some clinical isolates namely Gram-positives like *Bacillus subtilis, Staphylococcus aureus* (ATCC 25923), and *Streptococcus pneumoniae*; Gram-negative bacteria including *Proteus mirabilis, Pseudomonas aeruginosa* (ATCC 27853),

Table 1. Habitats and coordinates of the study area within the FCT, Nigeria.

S/N	Habitat	Sample region	Code	1	2	3	4	5	6
1	Termite mounds	Abaji	T	N9°13'5.268" E6°49'55.488"	N9° 4' 37.668" E6° 49' 19.380"	N8° 53' 4.272" E6° 50' 32.316"	N8° 40' 11.136" E6° 48' 13.860"	N8° 29' 11.868" E6° 48' 17.352"	N8° 28' 42.636" E6° 56' 54.240"
2	Rhizosphere of *Anacardium occidentale* L. (cashew tree)	Kuje	C	N8° 53' 59.784" E7° 11' 0.744"	N8° 47' 53.556" E7° 11' 13.560"	N8° 48' 25.488" E7° 25' 57.432"	N8° 42' 36.756" E7° 20' 38.940"	N8° 37' 45.336" E7° 10' 47.640"	N8° 30' 31.248" E7° 20' 16.224"
3	Rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood)	AMAC	B	N9° 6' 14.832" E7° 21' 54.540"	N9° 1' 51.204" E7° 33' 51.372"	N9° 1' 39.144" E7° 16' 6.024"	N8° 56' 18.636" E7° 20' 54.564"	N8° 56' 3.192" E7° 29' 15.252"	N8° 49' 4.404" E7° 32' 46.644"
4	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	Gwagwalada	G	N8° 59' 42.072" E6° 55' 4.692"	N9° 7' 36.444" E6° 56' 52.296"	N9° 13' 8.256" E6° 58' 27.660"	N9° 6' 12.132" E7° 10' 1.416"	N9° 0' 17.676" E7° 7' 33.348"	N8° 57' 31.032" E7° 0' 59.364"
5	Rhizosphere *of Cymbopogon citratus* (DC) Stapf. (lemon grass)	Kwali	L	N8° 48' 13.140" E7° 7' 2.964"	N8° 49' 9.876" E6° 58' 59.340"	N8° 41' 22.704" E7° 6' 50.184"	N8° 46' 11.748" E6° 51' 49.464"	N8° 36' 20.052" E6° 51' 28.332"	N8° 32' 45.492" E7° 1' 43.104"
6	River bank	Bwari	R	N9° 21' 36.756" E7° 37' 47.064"	N9° 17' 5.100" E7° 38' 15.720"	N9° 17' 22.020" E7° 24' 29.268"	N9° 8' 30.228" E7° 33' 58.212"	N9° 9' 10.548" E7° 18' 2.160"	N9° 7' 51.132" E7° 25' 51.168"

Escherichia coli (ATCC 25922), *Salmonella typhi* and Yeast: *Candida albicans.* The identity of the supplied test microorganisms was further confirmed by morphological and biochemical tests.

Standardization of the test organisms

The microorganisms tested were standardized according to the Clinical and Laboratory Standards Institute (2016)**.** Gram positives and negatives tested bacteria from 24-hour culture plates were suspended in sterilized 2 ml of nutrient broth and incubated at 37°C for 2 h. The microbial suspension was adjusted to 0.5 McFarland turbidity standard equivalents to optical density of 0.08-0.13 for bacteria and 0.05 for yeast after measured at 600 nm in spectrophotometer (Jenway 6405 UV/VIS, UK). That corresponded to 1×10^8 colony-forming units per milliliter (CFU/ml) for the bacteria suspensions which was diluted 1:100 with nutrient broth to obtain 1×10^6 CFU/ml. The inoculum size for yeast (*C. albicans*) was 1×10^6 spore-forming unit/ml equivalents to 0.5 McFarland standards.

Susceptibility testing

Preliminary test for antibiotic-producing potential of the bacteria was carried out by agar-well diffusion technique as described by Rafiq et al. (2018) with slight modifications. For this purpose, pure culture from the potential antibiotic-producers were inoculated into sterile 10 ml Nutrient broth (NB) and kept in shaker incubator on 120 revolution/minute (rpm) at 37°C for 96 h. After incubation, the tubes were centrifuged at 6,000 rpm for 10 min to separate the partial cell-free supernatant from the cell pellet. The supernatant was finally filtered with 0.45 µm disposable membrane filter unit (Millipore brand, Massachusetts, USA) to obtain cell-free supernatant.

The standardized microbial suspension (10^6 CFU/ml) was swabbed with sterile cotton buds on Mueller-Hinton agar (MHA) **(**ThermoScientific, Massachusetts, USA)**.** Wells of 6mm diameter were aseptically bored on inoculated MHA plates, filled with 100 µl cell-free supernatants and allowed to diffuse at room temperature for 2 h. Sterile distilled water served as the negative control while standard antibiotics, 50 µg/ml of amoxicillin (GlaxoSmithKline) and antifungal, 50 µg/ml nystatin (Nanjing Sino Pharmaceutical) were the positive controls. The plates were incubated in the upright position at 37ºC for 24 h. The zones of inhibition (ZI) were observed after 24 h and the diameter of the zones were measured in millimeter (mm) using a Vernier caliper. The antibiotic-producing isolate was determined by the tested isolates inhibited after measuring the ZI displayed in the culture plates.

Molecular identification of the promising candidate isolates

The most potent antibiotic-producing isolates were further identified by molecular techniques as described by Pomastowski et al. (2019) with slight modifications. The five most active antibiotic producers (ASS02/01, AMP03/02, AMP03/05, AAH02 and AMR06/01) were cultured in tryptone-soy broth (TSB) (ThermoScientific, Massachusetts, USA) at 37ºC for 24 h. The genomic DNA was extracted using AxyPrep bacterial genomic DNA miniprep kit (Axygen Biosciences, California, USA) following the manufacturer's instructions and was checked for quality by using a NanoDrop device (ThermoScientific, Massachusetts, USA). The 16S rRNA gene fragment of the isolates were amplified by using the universal primers 27F(5′- AGAGTTTGATCCTGGCTCAG –3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) to yield the PCR products of approximately 1500 base pair. Polymerase Chain Reaction (PCR) was carried out in a GeneAmp 9700 PCR System thermal cycler (Applied Biosystem Inc., USA). The thermal cycling condition included a cycle of an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing of primers at 50°C for 30 s, extension at 72°C for 1.5 min and then a final extension at 72°C for 7 min. The amplified PCR products were checked on 1.5% agarose gel electrophoresis ran on
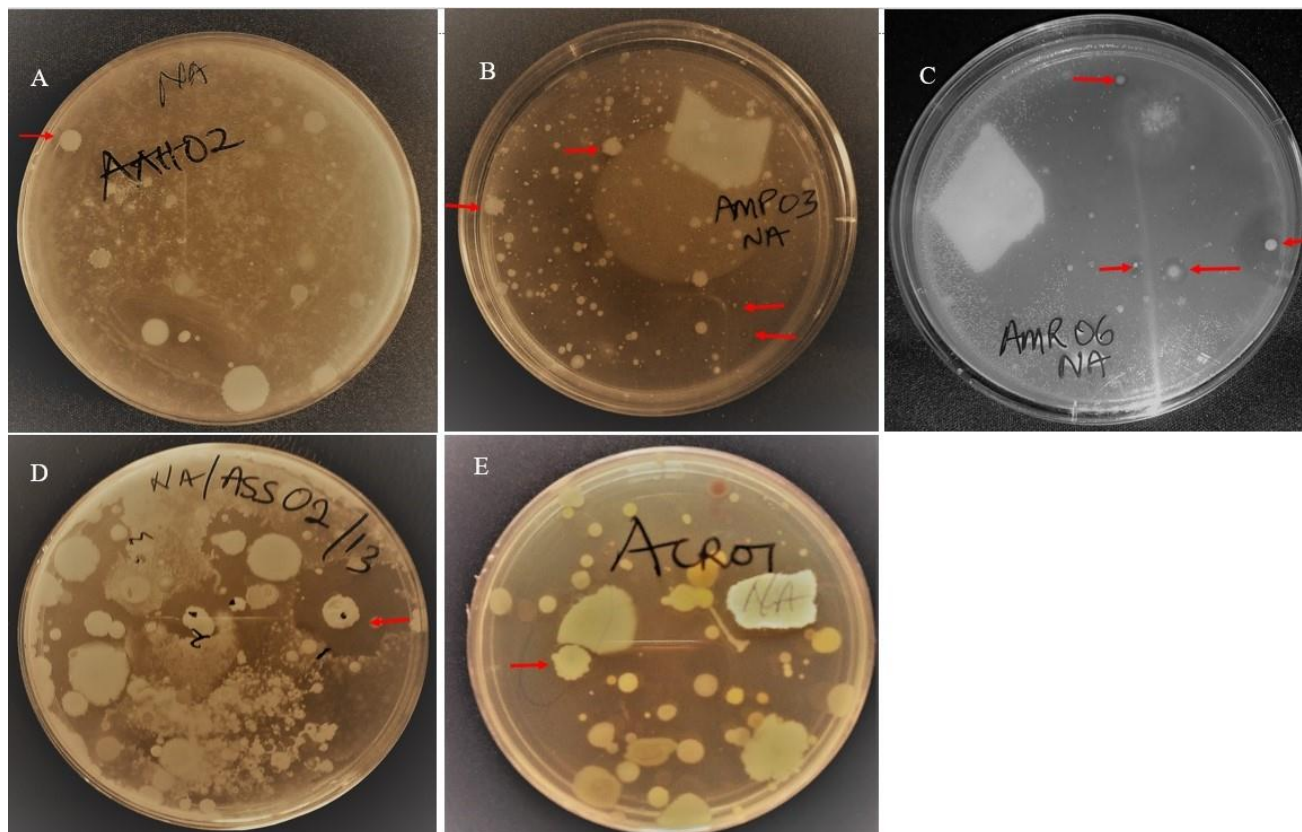
Figure 2. Inhibitory activity of the potential antibiotic-producing bacteria in Crowded plate for soil sample collected (A) from termite mound (B) from the rhizosphere of *Ageratum conyzoides* L.(goatweed) (C) from the rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood) (D) from the river bank (E) from the rhizosphere of *Anacardium occidentale* L. (cashew tree). Red arrows show the isolates with zones of inhibition.

a voltage of 120 V for 45 min. The DNA fragment was viewed under UV transilluminator and purified using QIAquick PCR purification kit (Qiagen, Germany) in preparation for sequencing. The sequencing was performed using ABI PRISM Big Dye Terminator cycle sequencer (Applied Biosystems, USA). The 16S rRNA gene sequences obtained were exported into the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information website (NCBI-http://www.ncbi.nlm.nih.gov) to identify matches with the annotated strains.

RESULTS

Isolation of antibiotic-producing microorganisms by crowded plate technique

Plates containing approximately 300 to 400 colonies with at least a colony showing halo around it were selected. A total of 12 bacteria colonies showing zones of inhibition following the crowded plate technique were recovered from soil samples from 5 of the 6 tested natural habitats (Figures 2A to E and Table 4). None of the isolates from samples from the rhizosphere *of Cymbopogon citratus* (DC) Stapf. (Lemon grass) showed zone of inhibition.

Termite mounds

Only 1 colony coded AAH02 displayed zone of inhibition of size 21 mm around it (Figure 2A).

Rhizosphere of Ageratum conyzoides L. (Goat weed)

Five colonies coded AMP03/01, AMP03/02, AMP03/03, AMP03/04 and AMP03/05 showed zones of inhibition of sizes 7, 7, 4, 3 and 8 mm respectively (Figure 2B).

Rhizosphere of Gmelina arborea Roxb. ex Sm. (Beechwood)

Three colonies coded AMR06/01, AMR06/02 and AMR 06/03 showed zones of inhibition of sizes 5, 16 and 6 mm around them respectively (Figure 2C).

River bank

Two colonies coded ASS02/01, ARS03/01 both showed zones of inhibition of sizes 22 and 16 mm respectively

(Figure 2D).

Rhizosphere of Anacardium occidentale L. (Cashew tree)

One colony coded ACR01 showed zone of inhibition of size 6 mm around it (Figure 2E).

Rhizosphere of Cymbopogon citratus (DC) Stapf. (Lemon grass)

None of the colonies displayed a zone of inhibition around it.

Characterization of potential antibiotic-producing isolates

The twelve potential antibiotic-producing bacteria isolated from diverse natural habitats were subjected to morphological and biochemical tests for identification as summarized in Table 2. The isolate coded AAH02 showed a whitish, rough, wrinkled and irregular form on a nutrient agar medium. The isolate was gram-positive rods with centrally located spores and showed positive reactions to motility, catalase, Voges-Proskauer (V-P), citrate, nitrate reduction, growth in 6.5% NaCl, growth in anaerobic condition, growth at 55°C, starch hydrolysis, carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). The isolate was negative to methyl-red test, indole and oxidase tests respectively. Considering the culture morphology and biochemical characteristics, bacteria isolate AAH02 was identified as *Bacillus licheniformis.*

Isolate coded ACR01 showed a yellow-greyish, umbonate, smooth, flat and irregular form on a nutrient agar medium. The isolate was gram-positive rods with sub-terminally located spores and showed positive reaction to motility, catalase, oxidase, nitrate reduction and growth at 55°C. However, the isolate displayed negative reaction to methyl red, V-P, indole, citrate, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). Based on the cultural characteristics and biochemical tests, the isolate was identified as *Bacillus brevis.*

Isolate coded AMR06/01 showed a greyish, wrinkled and irregular form on a nutrient agar medium. The isolate was gram-positive rods with centrally-located spores. It showed a positive reaction to motility, catalase, citrate, oxidase, nitrate reduction, growth in 6.5% NaCl at 37°C and starch hydrolysis; and weak positive reaction to carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). However, the isolate showed a negative reaction to methyl red, V-P, indole, growth in

anaerobic condition and growth at 55°C. Based on the cultural characteristics and biochemical tests, the isolate was identified as *Bacillus lentus.*

Isolate coded AMR06/02 was whitish, rough, wrinkle, and irregular form on a nutrient agar medium. The isolate was gram-positive rods with centrally-located spores. It showed a positive reaction to motility, catalase, V-P, citrate, nitrate reduction, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, growth at 55°C and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). The isolate showed a negative reaction to methyl red, indole and oxidase. Considering the cultural characteristics and biochemical tests, the isolate was identified as *Bacillus licheniformis.*

Isolate coded AMR06/03 showed a greyish, smooth, flat and circular form on a nutrient agar medium. The isolate was gram-positive rods with centrally-located spores. It showed a positive reaction to motility, catalase, V-P, citrate, nitrate reduction, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis, and some carbohydrate fermentation (glucose and sucrose). The isolate showed a negative reaction to methyl red, indole, oxidase, Growth at 55°C, and some carbohydrate fermentation (lactose, mannitol, and sorbitol). Given the cultural characteristics and biochemical tests results, the isolate was identified as *Bacillus cereus.*

Isolate coded AMP03/01 showed yellow-grey, umbonate, smooth, flat and irregular form on a nutrient agar medium. The isolate was gram-positive rods with sub-terminally located spores and showed positive reaction to motility, catalase, oxidase, nitrate reduction and growth at 55°C. However, the isolate displayed negative reaction to methyl red, V-P, indole, citrate, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). Based on the cultural characteristics and biochemical tests, AMP03/01 was identified as *B. brevis.*

Isolate coded AMP03/02 showed a greyish, smooth, flat and circular form on a nutrient agar medium. The isolate was gram-positive rods with centrally-located spores. It showed a positive reaction to motility, catalase, V-P, citrate, nitrate reduction, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis, and some carbohydrate fermentation (glucose and sucrose). The isolate showed a negative reaction to methyl red, indole, oxidase, Growth at 55°C, and some carbohydrate fermentation (lactose, mannitol, and sorbitol). Given the cultural characteristics and biochemical tests results, the isolate was identified as *B. cereus.*

Isolate coded AMP03/03 showed a yellowish, smooth, convex, and circular form on a nutrient agar medium. The isolate was gram-positive cocci with no spores. Isolate showed positive catalase reaction, V-P, citrate, oxidase and growth in 6.5% NaCl at 37°C. The isolate showed a

Table 2. Morphological and biochemical characterization of the potential antibiotic-producing isolates

Test	AAH02	ACR01	AMR06/01	AMR06/02	AMR06/03	AMP03/01	AMP03/2	AMP03/03	AMP03/04	AMP03/05	ASS02/01	ARS03/01
Colony	White, Rough, Wrinkle, and Irregular	Yellow-grey, Umbonate, smooth, flat and Irregular	Grey, wrinkle, Irregular	White, Rough, Wrinkle, and Irregular	Grey, Smooth, Flat and Circular	Yellow-grey, Umbonate, smooth, flat and Irregular	Grey, Smooth, Flat and Circular	Yellow, smooth, convex, and Circular	Yellow-grey, Umbonate, smooth, flat and Irregular	Yellow-grey, Umbonate, smooth, flat and Irregular	White, Rough, Flat, and Irregular	Grey, Smooth, Flat and Circular
Gram staining	+ rods	+ rods	+ rods	+ rods	+ rods	+ rods	+ rods	+ cocci	+ rods	+ rods	+ rods	+ rods
Spore staining	+; central	+; sub-terminal	+; central	+; central	+; central	+; sub-terminal	+; central	-	+; sub-terminal	+; sub-terminal	+; central	+; central
Motility	+	+	+	+	+	+	+	-	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	-	-	-	-	-	-	-	-	-	-	-	-
Voges-Proskaeur	+	-	-	+	+	-	+	+	-	-	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	+	+	-	+	+	-	-	+	+
Oxidase	-	+	+	-	-	+	-	+	+	+	-	-
Nitrate Reduction	+	+	+	+	+	+	+	-	+	+	+	+
Growth in 6.5% NaCl at 37°C	+	-	+	+	+	-	+	+	-	-	+	+
Growth in anaerobic condition	+	-	-	+	+	-	+	-	-	-	-	+
Growth at 55°C	+	+	-	+	-	+	-	-	+	+	+	-
Starch Hydrolysis	+	-	+	+	+	-	+	-	-	-	+	+
Glucose	+	-	±	+	+	-	+	-	-	-	+	+
Lactose	+	-	±	+	-	-	-	-	-	-	+	-
Sucrose	+	-	±	+	+	-	+	-	-	-	+	+
Mannitol	+	-	±	+	-	-	-	-	-	-	+	-
Sorbitol	+	-	±	+	-	-	-	-	-	-	+	-
CBI	*B. licheniformis*	*B. brevis*	*Bacillus lentus*	*Bacillus licheniformis*	*Bacillus cereus*	*Bacillus brevis*	*Bacillus cereus*	*Micrococcus luteus*	*Bacillus brevis*	*Bacillus brevis*	*Bacillus subtilis*	*Bacillus cereus*

+, Positive; -,negative; ±, weak positive; CBI, confirmed bacteria isolates.

negative reaction to motility, methyl red, indole, nitrate reduction, growth in anaerobic condition, growth at 55°C, starch hydrolysis, and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). Considering the cultural characteristics and biochemical tests results, the isolate was identified as *Micrococcus luteus.*

Isolate coded AMP03/04 showed yellow-grey, umbonate, smooth, flat and irregular form on a nutrient agar medium. The isolate was gram-positive rods with sub-terminally located spores and showed positive reaction to motility, catalase, oxidase, nitrate reduction and growth at 55°C.

However, the isolate displayed negative reaction to methyl red, V-P, indole, citrate, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). Given the cultural characteristics and biochemical tests, the isolate was identified as *B. brevis.*

Table 3. Summary of the susceptibility profile of potential antibiotic-producing bacteria against tested isolates.

Target organisms	Diameter of zones of Inhibition (mm) of the potential antibiotic- producing isolates											
	S01	S02	S03	S04	S05	S06	S09	S010	S011	S012	S013	S014
S. pneumoniae	0.0	0.0	0.0	0.0	10.0±0.4	0.0	0.0	0.0	11.4±0.4	0.0	0.0	0.0
S. typhi	0.0	9.4±0.4	0.0	0.0	0.0	11.1±0.4	0.0	0.0	10.2±0.2	0.0	0.0	0.0
E. coli (ATCC 25922)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.0±0.0	0.0	0.0	0.0
Proteus mirabilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. aureus (ATCC 25923)	0.0	10.3±0.3	0.0	0.0	9.1±0.4	0.0	0.0	0.0	10.3±0.4	0.0	0.0	0.0
C. albicans	0.0	0.0	0.0	0.0	0.0	9.3±0.4	0.0	0.0	9.3±0.3	0.0	0.0	0.0
B. subtilis	0.0	12.2±0.4	0.0	0.0	10.0±0.4	12.1±0.4	0.0	0.0	9.5±0.4	9.3±0.4	0.0	0.0
P. aeruginosa(ATCC 27853)	0.0	10.1±0.4	0.0	0.0	0.0	9.4±0.6	0.0	0.0	8.4±0.4	0.0	0.0	0.0

Mean ± Standard Deviation (SD); S01= filtrate (cell-free supernatants) from AMP03/01; S02 from AMP03/02; S03 from AMP03/03; S04 from AMP03/04; S05 from AMP03/05; S06 from AAH02; S09 from ARS03/01; S10 from ACR01/01; S011 from ASS02/01; S012 from AMR06/01; S013 from AMR06/02; and S014 from AMR06/03.

Isolate coded AMP03/05 showed yellow-grey, umbonate, smooth, flat and irregular form on a nutrient agar medium. The isolate was gram-positive rods with sub-terminally located spores and showed positive reaction to motility, catalase, oxidase, nitrate reduction and growth at 55°C. However, the isolate displayed negative reaction to methyl red, V-P, indole, citrate, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). Based on the cultural characteristics and biochemical tests, the isolate was identified as *B. brevis.*

Isolate coded ASS02/01 showed a whitish, rough, flat, and circular form on a nutrient agar medium. The isolate was gram-positive rods with centrally located spores and showed positive reaction to motility, catalase, V-P, citrate, nitrate reduction, growth in 6.5% NaCl at 37°C, growth at 55°C, starch hydrolysis and carbohydrate fermentation (glucose, lactose, sucrose, mannitol, and sorbitol). It however displayed negative reaction to methyl-red, indole, oxidase, and growth

in anaerobic condition. Considering the cultural characteristics and biochemical tests, the isolate was identified as *Bacillus subtilis.*

Isolate coded ARS03/01 showed a greyish, smooth, flat and circular form on a nutrient agar medium. The isolate was gram-positive rods with centrally-located spores. It showed a positive reaction to motility, catalase, V-P, citrate, nitrate reduction, growth in 6.5% NaCl at 37°C, growth in anaerobic condition, starch hydrolysis, and some carbohydrate fermentation (glucose and sucrose). The isolate showed a negative reaction to methyl red, indole, oxidase, growth at 55°C, and some carbohydrate fermentation (lactose, mannitol, and sorbitol). Given the cultural characteristics and biochemical tests results, the isolate was identified as *B. cereus.*

Susceptibility test for antibiotic-producing potential of the isolated bacteria

The preliminary test for potential antibiotic-producing bacteria was carried out by Agar-well

diffusion technique (Table 3). Out of the 12 candidate bacteria isolate obtained from the crowded plates, 5 displayed inhibitory activities against at least 1 pathogenic microbe. Filtrates S01, S03, S04, S09, S10 S013, and S014 showed no activities against all the tested pathogens. Summary of the number of test pathogens inhibited by filtrates from the potential antibiotic-producing bacteria was also displayed in Table 4. Filtrate S02 showed activities of 12.2±0.4, 10.3±0.3, 10.1±0.4 and 9.4±0.4 mm against *B. subtilis, S. aureus, P. aeruginosa* and *S. typhi*, respectively; filtrate S05 showed activities of 10.0±0.4, 9.1±0.4 and 10.0±0.4 mm against *B. subtilis, S. aureus* and *S. pneumonia*, respectively; filtrate S06 showed activities of 12.1±0.4, 9.3±0.4, 9.4±0.6 and 11.1±0.4 mm against *B. subtilis, C. albicans*, *P. aeruginosa* and *S. typhi*, respectively. Filtrate S011 from ASS02/01 displayed the strongest activity as it inhibited seven of the eight (8) test pathogenic isolates as follows: 9.5±0.4, 9.3±0.3, 10.3±0.4, 11.4±0.4, 9.3±0.3, 8.4±0.4 and 10.2±0.2 mm activities against B. subtilis, *E. coli*, *S aureus, S. pneumoniae, C. albicans, P. aeruginosa*

Table 4. Summary of potential antibiotic-producing bacteria and the test isolates.

S/N	Specimen collection points	Habitats	Antagonistic isolate codes	Diameter of zone of Inhibition in crowded plates (mm)	Number of test microorganisms inhibited
1	Abaji	Termite mound	AAH02	21	4
2	Kuje	Rhizosphere of *Anacardium occidentale* L. (cashew tree)	ACR01	6	0
3	AMAC	Rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood)	AMR06/01	5	1
4	AMAC	Rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood)	AMR06/02	16	0
5	AMAC	Rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood)	AMR06/03	6	0
6	Gwagwalada	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	AMP03/01	7	0
7`	Gwagwalada	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	AMP03/02	7	4
8	Gwagwalada	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	AMP03/03	4	0
9	Gwagwalada	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	AMP03/04	3	0
10	Gwagwalada	Rhizosphere of *Ageratum conyzoides* L. (goat weed)	AMP03/05	8	3
11	Bwari	River bank	ASS02/01	22	7
12	Bwari	River bank	ARS03/01	16	0
13	Kwali	Rhizosphere *of Cymbopogon citratus* (DC) Stapf. (lemon grass)	No inhibitory bacteria	-	NA

AAH02: Cell-free supernatant (S) from *Bacillus licheniformis;* ACR01: S from *Bacillus brevis*; AMR06/01: S from *Bacillus lentus;* AMR06/02: S from *Bacillus licheniformis;* AMR06/03: S from *Bacillus cereus;* AMP03/01: S from *Bacillus brevis;* AMP03/02: S from *Bacillus cereus;* AMP03/03: S from *Micrococcus luteus;* AMP03/04: S from *Bacillus brevis;* AMP03/05: S from *Bacillus brevis;* ASS02/01: S from *Bacillus subtilis;* ARS03/01: S from *Bacillus cereus;* NA=Not applicable.

and *S. typhi* respectively. Filtrate S012 showed activities of 9.3±0.4 mm against only *B. subtilis*.

None of the filtrates from the potential antibiotic-producing strains displayed inhibitory activities against Proteus mirabilis.

Molecular identification of the promising candidate isolates

The PCR result was analyzed by using agarose gel electrophoresis as summarized in Figure 3. The observed bands validated the amplification of 16S rDNA fragments. The amplicons obtained from the genomic DNAs of the five candidate isolates were approximately 1500 base pair (bp). The homology search for the sequences in the BLAST revealed that isolates ASS02/01, AAH02,

AMP03/02, AMP03/05 and AMR06/01 had closest similarity to *B. subtilis* subsp. subtilis 168, *B. licheniformis* strain SCDB 34, *B. cereus* strain A1, *B. brevis* strain NCTC2611, and *B. lentus* strain NCTC4824 respectively. Table 5 illustrated the summary of the molecular result.

DISCUSSION

Isolation and screening for antibiotic-producing bacteria are vital to the global effort in curbing the challenges from antimicrobial-resistant pathogens. The conventional antimicrobials are showing weaknesses against the plethora of previously susceptible microbes including the emerging and re-emerging drug-resistant pathogens. To this end, screening natural habitats of soil origin for

lead compounds from candidate antimicrobial-producing microorganisms in order to replace the failing antimicrobials is crucial considering the successes previously recorded (Elkholy et al., 2019; Makut and Owolewa, 2011; Rajivgandhi et al., 2019). In this study, some natural habitats such as termite mounds, river bank and plant rhizospheres were investigated for potential antibiotic-producing bacteria in the FCT. Twelve potential antibiotics-producers were isolated and were subjected to morphological and biochemical tests for identification (Table 2).

The potential antibiotics producers were identified and confirmed according to the Bergey's manual (Vos et al., 2011). Isolate coded AAH02 isolated from termite mound sample was identified as *B. licheniformis*. Isolate ACR01 isolated from sample from the rhizosphere of *Anacardium*

occidentale L.(cashew tree) was identified as *B. brevis*. Isolate AMR06/01 isolated from the rhizosphere of *Gmelina arborea* Roxb. ex Sm. (beechwood) was identified as *B. lentus*. Isolate coded AMR06/02 isolated from the rhizosphere of *G. arborea* Roxb. ex Sm. (beechwood) was identified as *B. licheniformis*. Isolate coded AMR06/03 isolated from the rhizosphere of *G. arborea* Roxb. ex Sm. (beechwood) was identified as *B. cereus*. Isolate coded AMP03/01 isolated from the rhizosphere of *A. conyzoides* L. (goat weed) was identified as *B. brevis*.

Isolate coded AMP03/02 isolated from the rhizosphere of *A. conyzoides* L. (goat weed) was identified as *B. cereus*. Isolate coded AMP03/03 isolated from the rhizosphere of *A. conyzoides* L. (goat weed) was identified as *M. luteus*. Isolate coded AMP03/04 isolated from the rhizosphere of *A. conyzoides* L. (goat weed) was identified as *B. brevis*. Isolate coded AMP03/05 isolated from the rhizosphere of *A. conyzoides* L. (goat weed) was identified as *B. brevis*. Isolate coded ASS02/01 isolated from the river bank was identified as *B. subtilis*. Isolate coded ARS03/01 isolated from the river bank was identified as *B. cereus*.

Eleven among the twelve isolated potential antibiotic-producers were *Bacillus* species while one was *M. luteus*. This is similar to the study by Abdulkadir and Waliyu (2012) where the majority of antibiotic-producers screened from soil were *Bacillus* spp. (*B. luteus, B. alvei and B. pumillus*) including one *Micrococcus spp.* (*M. roseus*).

Of the twelve potential antibiotic-producing isolates, ten were rhizospheric bacteria, one from the termite mound and another 1 was from the river bank. Among these 12 isolates, only five inhibited the test microorganisms of which four showed activities against both Gram-positive and Gram-negative bacteria (broad spectrum of activities), while the remaining 1 inhibited only 1 Gram-positive bacterium (narrow spectrum of activities). Two among the 4 with broad spectrum of activities also showed activities against *Candida albicans* (yeast).

The 5 antibiotics-producing isolates belong to the genus *Bacillus*. *Bacillus* species are predominant in several habitats including soil and possess between 5 to 8% secondary metabolite-producing genes (Fira et al., 2018). The five organisms include: *Bacillus cereus* isolated from the rhizosphere of *Ageratum conyzoides* L. (goat weed). *Bacillus cereus* (AMP03/02 filtrate) showed inhibitory activities against *B. subtilis (*12.2±0.4 mm)*, S. aureus (*10.3±0.3 mm)*, P. aeruginosa* (10.1±0.4 mm) and *S. typhi* (9.4±0.4 mm) respectively. This is similar to the study by Yilmaz et al. (2006) where *Bacillus cereus* M15 strain isolated from soil showed inhibitory activities against both Gram-positive and Gram-negative pathogenic isolates.

Bacillus brevis was also isolated from the rhizosphere of *Ageratum conyzoides* L. (goat weed). *Bacillus brevis* (AMP03/05 filtrate) showed activities against *B. subtilis (10.0±0.4 mm), S. aureus (9.1±0.4 mm) and S.*

pneumoniae (10.0±0.4 mm) respectively. Ghai et al. (2007) isolated *B. brevis* (M116 and T122 strains) from soil and both isolates showed antibacterial activities against both gram-positive and gram-negative pathogenic bacteria.

Bacillus licheniformis isolated from termite mound. *B. licheniformis* (AAH02 filtrate) showed activities against *B. subtilis* (12.1±0.4 mm), *C. albicans* (9.3±0.4 mm), *P. aeruginosa* (9.4±0.6 mm) and *S. typhi* (11.1±0.4 mm) respectively. This is similar to the study by Al-Turk et al. (2020) where *B. licheniformis* isolated from the soil displayed inhibitory activities against some Gram-positive and Gram-negative bacteria.

Bacillus subtilis isolated from river bank and *B. lentus* from the rhizosphere of *G. arborea* Roxb. ex Sm. (Beechwood). *B. lentus* (AMR06/01 filtrate) inhibited only *B. subtilis (*9.3±0.4 mm) among the tested pathogens. A similar study by Abdulkadir and Waliyu (2012) also showed that *B. lentus* isolated from the soil displayed antibacterial activity against a gram-positive pathogenic strain.

Among the 5 antibiotic-producers, *Bacillus subtilis* (ASS02/01 filtrate) displayed the strongest inhibitory ability against the test pathogenic strains. It showed antimicrobial activities against seven (7) of the eight (8) test isolates namely *Streptococcus pneumonia* (11.4±0.4 mm), *Salmonella typhi* (10.2±0.2 mm), *Escherichia coli* (11.0±0.0 mm), *Staphylococcus aureus* (10.3±0.4 mm)*, Bacillus subtilis* (9.5±0.4 mm)*, Pseudomonas aeruginosa* (8.4±0.4 mm) and *Candida albicans* (9.3±0.3 mm)*.* This is contrary to the study by Kiesewalter et al. (2020) that reported that the environmental strain of *B. subtilis* P5_B1, a non-ribosomal peptides producer, showed a weak impact on the soil bacteria community. Non-ribosomal peptides antibiotics are among the secondary metabolites produced by *B. subtilis* that function by targeting bacteria protein synthesis, lysis of fungal membrane and enzyme inhibition (Kiesewalter et al., 2020; Wang et al., 2015).

The antimicrobial activities displayed by the Bacillus isolates is in agreement with the study by Fira et al. (2018) in which case multiple strains of *Bacillus* species exhibited strong antibacterial and antifungal activities *in vivo* and *in vitro*. *Bacillus cereus, Bacillus brevis, Bacillus licheniformis* and *Bacillus subtilis* all displayed broad-spectrum of activities while *Bacillus lentus* showed a narrow spectrum of activities.

The other seven (7) isolates that displayed no inhibitory activities (0.0 mm) against test organisms includes *B. brevis, M. luteus and B. brevis,* all of which were isolated from the rhizospheres of *A. conyzoides* L. (goat weed). Others include *B. cereus* isolated from the river bank*, B. brevis* isolated from the rhizosphere of *Anacardium occidentale* L. (cashew tree)*, B. licheniformis* isolated from the rhizosphere of *G. arborea* Roxb. ex Sm. (beechwood) and *B. cereus* also from the rhizosphere of *G. arborea* Roxb. ex Sm. (beechwood). Their inability to inhibit test pathogenic isolates may be due to the minimal

Table 5. Summary of molecular identification of the five antibiotic-producing isolates.

Sample ID	Source	Matches in the BLAST	Query covered	E-value	Percentage Identity	Accession number
ASS02/01	River bank	*Bacillus subtilis* subsp. *subtilis* 168	97	0.0	100	NC_000964.3
AAH02	Termite mound	*Bacillus licheniformis* strain SCDB 34	98	0.0	95.5	NZ_CP014793.1
AMP03/02	Rhizosphere[a]	*Bacillus cereus* strain A1	99	0.0	99.2	NZ_CP015727.1
AMP03/05	Rhizosphere[a]	*Bacillus brevis* strain NCTC2611	100	0.0	99.4	NZ_LR134338.1
AMR06/01	Rhizosphere[b]	*Bacillus lentus* strain NCTC4824	96	0.0	100	NZ_LS483476.1

[a]Of *Ageratum conyzoides* L. (goat weed); [b] Of *Gmelina arborea* Roxb. ex Sm. (beechwood)

competition from other microbes in their natural habitats, resulting in their indisposition to producing potent antimicrobial molecules.

P. mirabilis showed resistance to all the 12 potential antibiotic-producing isolates. This may be associated with the presence of transferrable resistant genes and mobile genetic elements common with multi-drug resistant *P. mirabilis* (Alabi et al., 2017).

Our study also demonstrates the uncertainty in the use of zones of inhibition obtained by the antibiotic-producing microorganisms in the crowded plates as predictor of the number of inhibitory test pathogenic strains (Table 4). For example, the filtrate from ASS02/01 which showed a zone of inhibition (ZI) 22 mm from crowded plate inhibited seven test microbes. However, AAH02 with ZI of 21 mm from the crowded plate inhibited only four (4) test isolates as similar to AMP03/02 with ZI 7 mm from the crowded plates. AMP03/05 with ZI 8 mm inhibited three (3) test isolates, AMR06/01 with ZI 5 mm inhibited only one (1) of the test microbes (Table 5). However, isolates ACR01, AMR06/02, AMR06/03, AMP03/01, AMP03/03, AMP03/04, ARS03/01 with ZI 6, 16, 6, 7, 4, 3 and 16 mm respectively in the crowded plates displayed no inhibition against all the test microbes. The inconsistencies could be as a result of the variable sensitivity levels displayed by the test pathogens, including the strengths of resistance already familiar with by the antibiotics-producing bacteria from other competing bacteria in their natural ecosystem.

Our findings suggest a noteworthy episode in the ecological niche. For example, the filtrate from *B. licheniformis* (AAH02) isolated from the termite mound showed broad spectrum of activities against some of the tested pathogenic strains. Termites are social insects that cohabitate in the termitarium. Among the mechanisms for survival adopted by termites is the release of antimicrobial compounds into the termitarium to inhibit pathogen microbes (Cole et al., 2021; He et al., 2018). Therefore, the persistent exposure of the isolate in this study to the antimicrobial metabolites in the termite mound may have primed the organism into antimicrobial-producing capabilities.

The antimicrobial activities displayed by *B. cereus* (AMP03/02), *B. brevis* (AMP03/05) and *B. lentus* (AMR06/01) isolated from the rhizospheres of some plants corroborate with some other ecological findings. Plants adopt several strategies to compete favorably within the resource-limited environ. Studies have shown that plants release bioactive compounds into the rhizospheres to modulate the soil environ in their favor, and also to attract rhizosphere microbial community required for their fitness in the ecosystem (Hu et al., 2018; Zhalnina et al., 2018). Some of the released metabolites attract antibiotic-producing microorganisms needed as weapons against existing plant pathogens (Babalola et al., 2021; Hu et al., 2018; Schulz-Bohm et al., 2018).

Conclusion

The outcome of this study indicates that some natural habitats in the FCT are important sources of antibiotic-producing bacteria. The 5 bacteria with filtrates showing inhibitory activities against tested pathogenic isolates, namely *B. cereus, B. brevis, B. licheniformis, B. subtilis* and *B. lentus* are potential candidates for antibiotic production. The strongest among the isolated antibiotic-producing isolates, *B. subtilis* displayed broad spectrum of antimicrobial activity haven inhibited 7 out of the 8 test isolates.

Recommendation

Further investigations like liquid chromatography mass spectrometry analysis, nuclear magnetic

resonance spectroscopy or fourier transform infrared spectroscopy (FTIR) will be needed to elucidate the potential antibiotic compounds in the candidate isolates. Development and purification
of the broad-spectrum antimicrobial active compounds from the antibiotic-producing isolates will assist in narrowing the gap of new antimicrobial agents. The discovery of indigenous candidate isolates with antimicrobial capabilities in the FCT has indicated that antimicrobial lead compounds can be sourced locally.

CONFLICT OF INTERESTS

The authors have no conflict of interests.

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