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Research Article

Characterization, Antibiotic Sensitivity Patterns, Plasmid Profile Analysis and Antagonistic Potentials of Microorganisms from Termitaria on Mango Trees in Ibule-Soro, Akure, Nigeria

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Abstract

Over the years it has been proven that a mutualistic relationship exists between termites and microorganisms inhabiting their gut. Microbiological analyses were carried out on termitarium, macerated dead termites and mango barks from farm settlements in Ibule – soro Akure, Ondo state, Nigeria. Bacteria isolates such as *Pseudomonas aeruginosa, Clostridium botulinum, Bacillus subtilis, Actinobacter* sp and *Microbacter* sp were identified while fungal isolates such as *Chrysosporium tropicum, Mortierella rolfi, Aspergillus niger, Fusarium oxysporum* and *Penicillium chrysogenum* were also identified from the analyzed samples. Isolates such as *Actinobacter* sp, *Aspergillus niger, Mortierella rofli* and *Penicillium chrysogenum* showed mild antagonism against selected clinical pathogens used; the selected pathogens include: *Staphylococcus aureus, Salmonella* spp and Streptococcus pyogenes respectively. The results obtained from antibiotic sensitivity tests showed that Microbacter sp and *Pseudomonas aeruginosa* were multiple antibiotic resistant isolates (MDRIs). However, molecular characterization of these multiple antibiotic resistant isolates was carried out via plasmid profiling to determine the size of the bacterial plasmids. These isolates were again subjected to antibiotics treatment after being cured of their plasmids to determine if their initial resistance to antibiotics is chromosomal or extra – chromosomal. The findings of this research revealed that termite nests serve as reservoirs for microbes which possess antagonistic properties and that the genetic basis for antibiotic resistance of screened multiple antibiotic resistant bacteria isolated from termite nests were plasmid based. It is therefore recommended that advanced culture independent approaches should be used to explore the microbial diversity of the termite nests in the near future for greater research focus.

Keywords: Termitarium; Antibiotic Resistance Patterns; Antagonistic Patterns; Plasmid Profile Analysis; Mango Tree

Introduction

Termites are social insects that live in nests (termitaria) of their own construction. Termite workers build and maintain nest to house their colonies. These are elaborate structures made using a combination of soil, mud, chewed wood and cellulose, saliva and feces [1]. Termites play integral roles in soil development being one of the primary soil producers in arid and semi-arid systems [1,2]. Termites are classified in the order Isoptera of pterygote insects [2,3].

Most of the approximately 2000 species are found in tropical and subtropical regions [4]. They reduce organic material to small pieces, thereby improving the nutrient contents of tropical soils, which are generally infertile due to nutrient poverty [5]. Bacteria and Fungi are found in the gut contents of termites but apparently do not contribute significantly to the digestion of cellulose that termites consume [6]. Fungi in the gut, therefore, may be transient and present only as a result of ingestion by the termites [4].

Mangoes are juicy stone fruit (drupe) from numerous species of tropical trees belonging to the flowering plants with genus *Mangifera*, cultivated mostly for their edible fruit (*Mangifera indica*) is distributed worldwide to become one of the most widely cultivated fruits in the tropics [7]. Other *Mangifera* species (e.g. horse mango, Mangifera foetida) are grown on a more localized basis [7].

Recent studies have shown that mutualism exist between the termites and microorganism inhabiting their gut [1]. These studies have laid a background basis for further research into the microbial biodiversity of termites as well as their nests (termitaria) with respect to whether the type of trees there are found on directly impact the nature, distribution and types of microorganisms that can be obtained from them [3,8]. Hence, this study aimed at investigating the identity of microorganisms associated with the termitarium environment on Mango trees in Ibule-Soro, Akure, Nigeria; the antagonistic features of the identified microorganisms against known selected pathogens, evaluation of the antibiotic resistance patterns of the bacterial isolates obtained and as well as investigating the genetic basis of antibiotic resistance observed in the bacterial isolates obtained.

Materials and Methods

Description study area

The study area Ibule-Soro, Akure is found in Ondo State, Nigeria with coordinates 7°13' N 7°14' N/ 5°8' E 5°10' E [9]. It is located at the extreme southern region of the Akure South Local Government Area of Ondo state, Ondo State capital with an estimated population of about 2,500 persons (inhabitants inclusive) [9].

Sampling points in the study area

The clusters of farm settlements with mango plantations were considered as case studies for comparative study at Ibule-Soro, Akure Ondo State between April to June 2017.

Sample collection

A total of 45 samples comprised of termitaria from Mango trees, Mango tree barks and as well dead termites were collected into sterile universal bottles using the guidelines described in Barreto., *et al* [10]. The samples were stored in ice packs before laboratory analyses were carried out and all the samples collected were analyzed in the laboratory within 6hr of sample collection [11].

Sample preparation, Standardization of Inoculum and Isolation of Microorganisms

The method described by Chouvenc., *et al.* [5] were adopted for sample preparation and Inoculum standardization in which sterile distilled water was used as diluents and a 1g of each sample stock was weighed into 10ml of sterile distilled water for a serial dilution procedure in sterile test tubes under aseptic conditions until four different dilutions were obtained for a pour plate culture technique Okhuma and Brune [12]. Thereafter, a 1 ml each of the dilution factors 3 and 4 were used for inoculating already prepared Nutrient Agar and Potato Dextrose Agar seeded with 250 mg Chloramphenicol (for total filamentous fungi counts). The Bacterial isolates were incubated at 37°C for 24hrs while Fungal isolates at 26 ± 2°C for 3 - 5 days [3]. Following incubation, the culture plates were observed for colony forming units of bacteria and spore forming mycelia units of fungi; thereafter, the fourth dilution factor was established as the standard for the isolation of the fungi due to easy numerical estimation of different colony and mycelia units [4].

Biochemical Characterization and Identification of Bacterial Isolates

The methods described by Rosengaus., *et al.* [1] and Mueller., *et al*. 2015 were adopted by subjecting the various obtained sub cultured distinct colonies to wide arrays of biochemical tests for characterization and identification. Gram staining technique, Catalase test, Motility test, Sugar fermentation (glucose, sucrose, lactose, mannitol and triple salt iron) tests, Methyl Red/Voges Proskauer test, Oxidase test, motility test, Spore staining and Catalase test were carried out on the distinct isolates obtained after sub culturing [6]. The distinct biochemically characterized colonies were then further sub cultured on freshly prepared MacConkey Agar, Yeast Extract Agar and Bile Esculin Agar respectively; incubated at 37°C for 24h [4]. Thereafter which the identity of the bacteria isolates was determined after their growth on these selective media.

Identification and Characterization of fungal isolates

The methods described by Fawole and Oso [11] were adopted for identification of the fungi isolates. The cultural characteristics (macro-morphology) and the microscopic morphological characteristics (micro-morphology) of the various distinct mycelia units obtained were compared with the available literature (Compendiums for Air, Soil, Food and Indoor fungi) [13]. The macro-morphological properties of the different mycelium clones were obtained by visual appearance of the mycelium units while micro-morphological properties of fungi mycelium clones were obtained via microscopic observations of stained mycelia with cotton blue in lacto phenol dye [13]. Photomicrographs of the different mycelium clones obtained were compared and juxtaposed for matching information contained in the available literature for air and soil fungi as described in [13].

Preservation of Isolates

The identified pure bacterial isolates of *Actinobacter* spp, *Bacillus subtilis, Pseudomonas aeruginosa, Clostridium botulinum* and *Microbacter* spp were preserved on Nutrient Agar Slants and stored at 4°C as described by Cheesebrough [14], while identified fungal isolates of *Aspergillus niger, Fusarium oxysporum, Chrysosporium tropicum, Mortierella rofli* and *Penicillum chrysogenum* were preserved on Potato Dextrose Agar Slants and stored at 4°C as described by Cheesebrough [14].

Antibiotic Sensitivity Test for Bacterial Isolates

The Kirby-Bauer test, also known as disc diffusion method was used to determine the effect of standard antibiotics on bacterial isolates on Mueller Hinton agar. The agar was seeded with 18 hold pure broth cultures of *Salmonella* isolates [15]. The discs were applied unto the seeded plates and incubated for 24h at 37°C [16]. The bacterial isolates were tested against a wide range of antibiotics namely; Ofloxacin (5µg), Amoxicillin (25 µg), Ciprofloxacin (10 µg), Tetracycline (30 µg), Pefloxacin (5 µg) [14]. Thereafter, a ruler was used to measure the diameter of the clear zones of inhibition noticed on the plates and this was noted as degree of antibiotic resistance as described. The isolates' zones of inhibition was classified into susceptible (17 mm and above), intermediate (13 mm - 17 mm), and resistant (0 - 12 mm) based on the specified standard of mean zone of inhibition for pathogenic gram positive and gram negative bacteria respectively [14].

Molecular Characterization of Multiple Antibiotic Resistant Bacteria

Plasmid profile analysis of the multiple antibiotic resistant bacteria isolates were carried out according to the modified alkaline lyses plasmid extraction protocols described in Chan., *et al*. [16] and Matsui., *et al* [17]. Thereafter, a 1% Agarose gel was prepared and loaded into electrophoresis chamber containing between 8 wells; this was buffered with 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA and then adjusted to pH 7.8 with acetic acid. The sample buffer contained 25% sucrose, 5 mM sodium acetate, 0.05% bromophenol blue and 0.1% SDS. Electrophoresis was allowed to proceed at room temperature until bands become visible at the positive end of the chamber. After electrophoresis, gels were stained with ethidium bromide (1 µl/ml) and viewed under UV transillumination. The molecular marker that was used was the bacteriophage *Hind III* digest and extrapolations were made from the Electrophorogram obtained. The multiple antibiotic resistant isolates were cured of their plasmid afterwards by exposing the overnight grown culture at 37°C and 10 mg/ml of ethidium bromide adopting the methods described in Birnboim and Dolly [18], as well as Brown [19].

Antibiotic Sensitivity Test after Plasmid Curing

The screened multiple antibiotic resistant bacteria isolates were subjected to antibiotic sensitivity test again after plasmid curing using broad spectrum antibiotics (CM128PR100) by adopting the methods described in Matsui., *et al* [17].

Antagonistic Assays

The fungal and bacterial isolates obtained from the samples collected were evaluated for possible antagonism against selected clinical pathogens obtained from the Department of Laboratory Services, Ondo State Specialist Hospital, Akure, Nigeria. The selected pathogens are standard clinical cultures of *Salmonella* sp, *Staphylococcus aureus* and *Streptococcus pyogenes* respectively; they were selected because they were of public health importance to pediatric health in Akure at the time of the research. The method described by Fokkema and Heuvel [20], was adopted for evaluation of antagonistic potentials of obtained fungal isolates against the selected clinical pathogens while the methods in Birnboim and Dolly [18]; Matsui., *et al.* [17] were adopted for evaluation of antagonistic potentials of bacterial isolates against these selected clinical pathogens; a standard broad spectrum antibiotic (Ofloxacin 50 µg) was used as control for the bacterial and fungal antagonistic assays respectively as described by Rosengaus., *et al* [1].

Data Analysis

Analyzed sample treatments were replicated thrice; data means obtained were subjected to a 2-way analysis of variance and treatment means were separated using Duncan's New Multiple Range test at P ≤ 0.05 level of significance [16].

Results

The characterization of the bacterial and fungal isolates obtained from the samples across the different farm settlements are represented in tables 1 and 2 respectively. A total of 22 bacterial isolates of *Microbacter* sp (4), *Pseudomonas aeruginosa* (5), *Bacillus subtilis* (4), *Clostridium botulinum* (6) and *Actinobacter* sp (3) were obtained from across the sampling points while a total of 14 fungal isolates of *Aspergillus niger* (4), *Fusarium oxysporum* (3), *Chrysosporium tropicum* (2), *Mortierella rofli* (3) and *Penicillium chrysogenum* (2) were also obtained from the isolates analyzed. The means of the zones of inhibition of the bacterial isolates subjected to antibiotic sensitivity tests which were subjected to statistical analysis using Duncan's New Multiple Range test at P ≤ 0.05 level of significance and as well as their antibiotic resistance patterns are represented in table 3. The resistance patterns were denoted by Comparison of analyzed data with accepted standards for Gram positive and Gram negative bacteria respectively as described in Cheesebrough [14].

I.	Gram Stain	Sugar Fermentation					O/C	M.t./ Sp. T.	MR/ VP	Growth on Media			N. I.
		Lac.	Glu.	Suc.	Mann.	TSI				NA	Mac. A	Y.E.A.	
M.S.	+ve (cluster cocci)	-ve	+ve	+ve	+ve	-ve	+ve /+ve	-ve /-ve	+ve /-ve	Cream/ raised	-ve	+ve (brown)	4
B.S	+ve (bacilli rods)	-ve	+ve	+ve	+ve	A/G	+ve /+ve	+ve /+ve	-ve /+ve	Cream/ raised	-ve	-ve	4
C.B.	+ve (bacilli rods)	+ve	+ve	+ve	-ve	A/G	+ve /+ve	+ve /+ve	+ve /+ve	Milky/ lobate	+ve (pale yellow)	-ve	6
P.A.	-ve (bacilli rods)	-ve	+ve	+ve	-ve	K/NF	+ve /+ve	-ve	-ve /-ve	Cream/ raised	+ve (pink)	-ve	5
A.S	+ve (cocco-bacilli)	-ve	+ve	+ve	-ve	-ve	+ve /+ve	-ve /-ve	-ve /+ve	Cream/ lobate	-ve	+ve (pale yellow)	3

Table 1: Identification of Bacterial Isolates from samples analyzed.

Keys: I: Isolates; M.S.: Microbacter sp. B.S.: Bacillus subtilis; C.B.: Clostridium botulinum; P.A.: Pseudomonas aeruginosa; A.S.: Actinobacter sp.; Lac.: Lactose; Glu.: Glucose; Suc.: Sucrose; Mann.: Mannitol; TSI: Triple Salt Iron; O/C: Oxidase/ Catalase test; M.t./Sp. T.: Motility test/ Spore test; MR/VP: Methyl Red/ Voges Proskauer; NA: Nutrient Agar; Mac. A.: MacConkey Agar; Y.E.A: Yeast Extract Agar; N.I.: Number of Isolates; -ve: Negative; +ve: Positive; A/G: Acid/ Gas; K/NF: Alkaline Slant/ No fermentation.

RIC	Cultural Characteristics	Morphological Characteristics	CFI
1	Fastidious white flattened mycelium which becomes brownish grey with age having tall and short projection centers	Sporangiophores branched in mixed sympodial and monopodial forms, appearance of non-septate hyphae with rough edged zygospores	*Chrysosporium tropicum*
2	Fastidious stained white mycelium with brownish black centers that spreads rapidly	Long thin walled hyaline conidiophores with globose radiate heads appear smooth with black bars; conidiophores are branched and lumped with cylindrical phalides	*Aspergillus niger*
3	Fastidious white fluffy mycelium with dark green velvety centers	A globose rough edged, non-septate conidia observed with monoserate radiate head; phalides appear short necked attached to pigmented conidiophores vesicles	*Penicilliium chrysogenum*
4	fastidious, powdery yellowish and creamy mycelium with loose furniculose tufts and large exudates droplets growing broadly on plates	Non-septate, thin hyphae containing spores with globose thick walled Asci loosely linked at the apex that appear flattened at the base.	*Mortierella rofli*
5	peachy velvety spreading aerial mycelium with brown edges that appear dark yellowish as culture ages	Long thin walled conidiophores with fusiform conidia that appears convex at the apex. Hyphae is hyaline with branched monophalides	*Fusarium oxysporum*

Table 2: Morphological and Cultural Characterization of fungi isolated from the samples.

Keys: RIC: Representative Isolate Clones from Different Sampling Points; CFI: Confirmed Fungal Isolate

A.t.	Zones of Inhibition (mm)						Resistance patterns					
	M.S.	P.A.	B.S.	C.B.	A.S.	C.t.	M.S.	P.A.	B.S.	C.B.	A.S.	C.t.
ERY	6.25 ± 1.22^{c}	5.50 ± 1.35^{a}	3.23 ± 0.46^{a}	4.34 ± 1.02^{a}	9.24 ± 1.28^{b}	00 ± 00^{a}	R	R	R	R	R	R
CPX	4.23 ± 0.56^{b}	7.23 ± 1.17^{b}	18.46 ± 1.22^{e}	19.22 ± 1.39^{d}	14.64 ± 1.09^{c}	00 ± 00^{a}	R	R	S	S	I	R
COT	3.33 ± 0.33^{b}	4.44 ± 1.22^{a}	5.08 ± 1.23^{a}	6.28 ± 1.69^{b}	7.98 ± 1.29^{b}	00 ± 00^{a}	R	R	R	R	R	R
AMX	00 ± 00^{a}	3.56 ± 0.87^{a}	6.26 ± 1.21^{b}	4.95 ± 1.26^{a}	8.67 ± 1.24^{b}	00 ± 00^{a}	R	R	R	R	R	R
OFL	00 ± 00^{a}	19.29 ± 1.22^{e}	18.46 ± 1.70^{e}	19.33 ± 0.68^{d}	20.59 ± 1.22^{e}	00 ± 00^{a}	R	S	S	S	S	R
STR	4.58 ± 1.24^{b}	6.13 ± 0.92^{b}	4.23 ± 0.85^{a}	6.56 ± 1.22^{b}	5.45 ± 1.22^{a}	00 ± 00^{a}	R	R	R	R	R	R
CHL	2.45 ± 0.33^{b}	15.78 ± 1.28^{e}	14.55 ± 1.45^{d}	12.89 ± 1.06^{c}	13.23 ± 1.71^{c}	00 ± 00^{a}	R	S	I	I	I	R
CEF	1.22 ± 0.14^{b}	7.28 ± 1.11^{c}	8.46 ± 1.42^{b}	4.24 ± 1.56^{a}	16.56 ± 1.21^{d}	00 ± 00^{a}	R	R	R	R	S	R
GEN	13.57 ± 1.18^{d}	12.59 ± 1.24^{d}	11.39 ± 1.29^{c}	14.26 ± 1.20^{c}	4.25 ± 1.23^{a}	00 ± 00^{a}	I	I	I	I	R	R
PEF	16.24 ± 1.20^{e}	18.50 ± 1.50^{e}	19.51 ± 1.95^{e}	20.43 ± 1.22^{d}	19.21 ± 1.21^{d}	00 ± 00^{a}	S	S	S	S	S	R
The isolates M.S. and P.A. are resistant to more than 6 antibiotics; hence MDRIs.												

Table 3: Zones of inhibition and Antibiotic resistance patterns of characterized bacterial isolates.

Keys: M.S: Microbacter sp; P.A: Pseudomonas aeruginosa; B.S.: Bacillus subtilis; C.A: Clostridium botulinum; A.S: Actinobacter sp C.t.: Control; A.t.: Antibiotics; COT: Cotrimoxazole; CPX: Ciprofloxacin; ERY: Erythromycin; AMX: Amoxycillin OFL: Ofloxacin; STR: Streptomycin; CHL: Chloramphenicol; CEF: Ceftriaxone; GEN: Gentamycin; PEF: Pefloxacin; R: Resistant (0 - 12 mm); I: Intermediate (13 mm - 17 mm); S: Susceptible (17 mm and above); MDRIs: Multiple Drug Resistant Isolates; values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

The already analyzed zones of inhibition and antagonistic patterns of the antagonistic assays of bacterial isolates against selected clinical pathogens of interest are represented in table 4 while analyzed zones of inhibition of and antagonistic patterns of obtained fungal isolates against selected clinical pathogens of interest are also represented in table 5. Antagonistic patterns in tables 4 and 5 were all denoted as either positive (+ve) at ≤ 16.00 mm and above, Intermediate (I) at ≤ 11.00 - 15.00 mm and negative (-ve) at ≤ 10.00 mm as described by Toledo*., et al.* [3] and Rosengaus*., et al* [1]. Conversely, the screened multiple antibiotic resistant bacteria isolates (MDRIs) which were resistant to more than 6 antibiotics were profiled for plasmid analysis are represented in figure 1; their analyzed zones of inhibition as well as resistance patterns after plasmid curing were also represented in table 6 below.

Bacteria Isolate	Observed antagonistic interaction (mm)			Deduced Antagonistic patterns		
	Salmonella sp	*Staph. aureus*	*Strep. Pyogenes*	*Salmonella* sp	*Staph. aureus*	*Strep. pyogenes*
M.S.	8.25 ± 1.22^{b}	9.89 ± 1.84^{b}	14.89 ± 1.22^{c}	-ve	-ve	I
P.A.	12.45 ± 1.58^{c}	11.66 ± 1.82^{c}	10.75 ± 1.22^{c}	I	I	I
B.S.	6.37 ± 1.82^{b}	5.58 ± 1.64^{b}	00 ± 00^{a}	-ve	-ve	-ve
C.B.	12.72 ± 1.59^{c}	00 ± 00^{a}	00 ± 00^{a}	I	-ve	-ve
A.S.	13.44 ± 2.53^{c}	13.44 ± 1.86^{c}	12.90 ± 1.10^{c}	I	I	I
C.At.	17.55 ± 1.46^{d}	17.78 ± 1.84^{d}	18.61 ± 1.19^{d}	+ve	+ve	+ve

Table 4: Antagonistic patterns of bacteria isolates against selected clinical pathogens.

Keys: M.S: Microbacter sp; P.A: Pseudomonas aeruginosa; B.A: Bacillus subtilis; C.A: Clostridium botulinum; A.S-Actinobacter sp; C.At.: Control Broad spectrum Antibiotic (Ofloxacin 50 µg); I: Intermediate (at ≤ 11.00 : 15.00 mm); -ve: Negative Antagonism or No Antagonistic Interaction (at ≤ 10.00 mm); +ve: Positive Antagonism (at ≤ 16.00 mm and above); values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

Fungal-Isolates	Observed antagonistic interaction (mm)			Deduced Antagonistic patterns		
	Salmonella sp	*Staph. aureus*	*Strep. Pyogenes*	*Salmonella* sp	*Staph. aureus*	*Strep. Pyogenes*
A.N.	16.81 ± 1.36^{c}	3.42 ± 1.84^{b}	17.89 ± 1.19^{c}	+ve	-ve	+ve
F.Oxy.	4.26 ± 1.58^{b}	00 ± 00^{a}	11.28 ± 1.56^{b}	-ve	-ve	I
C.Tr.	00 ± 00^{a}	4.58 ± 1.21^{b}	10.73 ± 1.25^{b}	-ve	-ve	-ve
M.R.	17.22 ± 1.38^{c}	6.84 ± 1.16^{b}	7.56 ± 1.28^{a}	+ve	-ve	-ve
P.C.	18.91 ± 1.33^{c}	8.44 ± 1.12^{b}	8.89 ± 1.87^{a}	+ve	-ve	-ve
C.At.	19.51 ± 1.69^{c}	17.74 ± 1.61^{c}	18.22 ± 1.74^{c}	+ve	+ve	+ve

Table 5: Antagonistic patterns of fungal isolates against selected clinical pathogens.

Keys: A.N.: Aspergillus niger; F.Oxy: Fusarium oxysporium; C.Tr.: Chrysosporium tropicum; M.R.: Mortierella rofli; P.C.: Penicillium chrysogenum; C.At.: Control Broad spectrum Antibiotic (Ofloxacin 50 µg); I: Intermediate (at ≤ 11.00 - 15.00 mm); -ve: Negative Antagonism or No Antagonistic Interaction (at ≤ 10.00 mm); +ve: Positive Antagonism (at ≤16.00mm and above); values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

B.S. At.	Zones of Inhibition (mm)			Resistance patterns		
	M.S.	P.A.	C.t.	M.S.	P.A.	C.t.
ERY	16.25 ± 1.52[b]	17.50 ± 1.35[b]	00 ± 00[a]	S	S	R
CXC	18.23 ± 0.56[b]	16.23 ± 1.10[b]	00 ± 00[a]	S	S	R
OFL	19.33 ± 2.33[b]	18.44 ± 1.21[b]	00 ± 00[a]	S	S	R
AUG	16.78 ± 1.24[b]	19.44 ± 1.87[b]	00 ± 00[a]	S	S	R
CAZ	20.55 ± 1.25[b]	19.89 ± 1.05[b]	00 ± 00[a]	S	S	R
CRX	19.64 ± 1.36[b]	18.32 ± 1.62[b]	00 ± 00[a]	S	S	R
GEN	17.45 ± 1.46[b]	18.78 ± 1.24[b]	00 ± 00[a]	S	S	R
CTR	17.62 ± 1.14[b]	17.28 ± 1.84[b]	00 ± 00[a]	S	S	R
The two MDRIs were susceptible to all the antibiotics used; hence initial antibiotic resistance was plasmid based						

Table 6: Zones of inhibition and Antibiotic resistance patterns of MDRIs after Plasmid curing.

Keys: M.S: Microbacter sp; P.A: Pseudomonas aeruginosa; C.t.: Control; B.S.At.: Broad Spectrum Antibiotics; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Augumentin; CAZ: Ceftrazidine; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; R: Resistant (0 - 12 mm); I: Intermediate (13 mm - 17 mm); S: susceptible (17 mm and above); MDRIs: Multiple Drug Resistant Isolates; values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

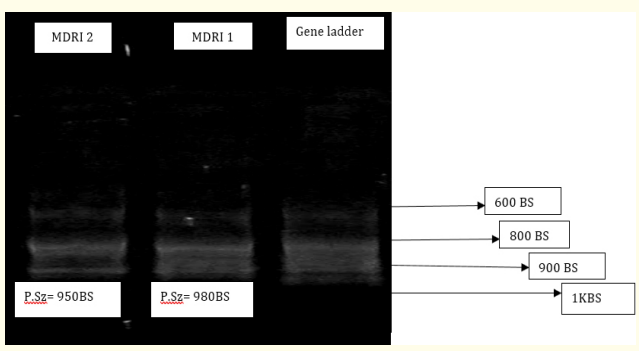

Figure 1: Electrophorogram of multiple drug resistant bacteria plasmids of screened isolates

Keys: MDRI 1: Microbacter sp; MDRI 2: Pseudomonas aeruginosa; B.S.: Base Pairs; K.B.S.: Kilo Base Pairs; P.Sz: Plasmid Size

Discussion

The arrays of isolated microorganisms gotten from the samples denoted the termitarium environment is a good microhabitat for microorganisms as also described in Bignell [21]. The isolated microorganisms; bacterial isolates which include *Pseudomonas aeruginosa, Bacillus subtilis, Actinobacter* sp, *Microbacter* sp, *Clostridium botulinum* have been implicated in recent investigations into the study of the termitarium micro flora notably in Fall., *et al.* [22] and Manjula., *et al* [15]. While Fungal isolates *Aspergillus niger, Fusarium oxysporum, Chrysosporium tropicum* and *Mortierella rofli* have also been implicated to have parasitic/pathogenic effects on Mango trees (*Mangifera indica*), humans and some insects alike while *Fusarium oxysporum* produce mycotoxins which have a negative economic effect on plants and subsequently animals and humans [22].

It has also been reported in studies that Termites harbor a consortium of aerobic, anaerobic and micro-aerophilic bacteria that are responsible for their large hemi-cellulose deposits and this is responsible for the different arrays of microorganisms that benefit from inhabiting their guts [21]. Ecological interactions between termite guts and organisms that inhabit them (bacteria and fungi) have been reported in both lower and higher termites according to Ohkuma and Brune [12]. Lo and Eggleton [23] reported that the fecal materials of these termites, consisting of digested plant materials is fashioned into small ventilated structures called the fungal combs which act as substrates for the growth of the symbiotic fungi.

In their findings, Rosengaus., *et al.* [1] reported that termites have accordingly developed several defensive strategies against pathogens and parasites and as such can control infections by pathogens or parasites by secreting antimicrobial substances in defensive glandular secretions, feces, and body exudates; This particularly explains why all the bacterial isolates obtained in this study have varying degrees of antimicrobial resistance as they might have been exposed to some of this antibiotic combinations and hence developed resistance to them. More so, the varying antibiotic sensitivity patterns of bacterial isolates to the antibiotics used could be due to the continuous overexposure of the termitarium to insecticide treatments from local farmers which may have predisposed these isolates to some active components contained in many of the antibiotics used; this was also described in the findings of Brune and Dietrich [8] and Toledo., *et al* [3].

The dual culture method which was adopted was used to determine which of the isolates obtained (bacteria and fungi) had antagonistic potentials of against other pathogenic microbes since Toledo., *et al.* [3] suggested that some microorganisms inhabiting the gut of many termites are capable of exhibiting mild antagonism against other pathogens and thereby keeping the termites resistant to many pathogens. The bacterial isolates obtained however did not show any significant antagonism at the specified level of significance, while fungal isolates showed mild antagonistic effects against selected clinical isolates of interest at the same level of significance, this result agrees slightly with the findings of Lavelle., *et al.* [24] and Toledo., *et al* [3].

Many research findings also reported that some microorganisms associated with termite tubes function as antagonists against various invasive bacterial, fungal and nematode pathogens of plants by secreting various kinds of antibiotics [25]. Therefore, the mild antagonism of fungal isolates to the selected pathogens in this study justifies the direct relationship between antibiotic producing fungi and protection the offer to the termites in a mutualistic relationship; agreeing with the findings in Rosengaus., *et al* [1].

Conclusion

The findings of this study have shown that the termitarium is a microbial habitat that is rich in many nutrients and enables optimum growth of many microbes. Many researchers have investigated the relationships of termites with their diverse eukaryotic intestinal and external symbiotic co-inhabitants. Nevertheless, various questions still awaits clarification as to how termites integrate their rich internal micro biota with their nests; this situation is doubtless due to the multitude of microorganisms present in termite nests. Hence, not only do a large number of organisms remain to be investigated, but divergent evolutionary pathways leading to different symbiotic systems and strategies must be scientifically investigated. This study revealed the mild antagonistic potentials of isolated microorganisms obtained from test samples against known selected pathogens and showed that the possession of resistant factor plasmids is responsible for the multiple antibiotic resistance patterns of isolated bacteria to antibiotics used.

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Conflict of Interest

There are no conflicts of interests.

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