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Evaluation Of Antibiotic Resistant Microorganisms From Selected Manured Farmlands In Parts Of Enugu State, Nigeria

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Abstract
Background: *A rising concern in recent years has been the entry of resistance genes into the food system through the amendment of soils with manure from animals treated with antibiotics. This study examined the prevalence of antibiotic-resistant microorganisms (ARM) from selected manured farmlands.*
Materials and Methods*: A total of 12 soil samples were collected randomly from farmlands (maize farm, yam farm, cassava farm and control soil) in 3 local governments (Enugu North, West and South) within Enugu State, Nigeria. Prior to collection of soil samples, the sterile soil probe method was employed by digging up 3-5cm of soil surface at different spots and the debris removed before soil collection. The soil samples were analyzed for their physical characteristics and cultured on nutrient and sabourand dextrose agar and characterized by standard microbiological methods. Antibacterial sensitivity test was done by disc diffusion method while Fungal sensitivity assay was done by agar well diffusion methods. Isolates were further characterized by polymerase chain reaction(PCR) and amplified fragments sequenced.*
Results: *From the study, total bacterial count were higher from maize farm at 4.5x10 while the control soil recorded the least mean value at $3.4x10^6$. The total fungi count was higher in the control soil with mean value ($2.9x10^6$) in the control farm while the least mean values were found in maize farm at $1.8x10^6$. Bacteria isolated from the farms were E. coli, Klebsiella sp, Pseudomonas aeruginosa and Staphylococcus aureus, Pantoea stewartii, Proteus mirabilis, Alcarigenes feacalis, Shigella sp, Salmonella sp. and Bacillus subtilis while fungi were Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus and Fusarium verticillioides. The bacterial and fungal isolates that were highest in occurence were Pantoea stewartia and Fusarium verticillioides at 8(67%) respectively. The isolates showed varied results in their susceptibility patterns but Klebsiella pneumoniae, Pantoea stewartii, Proteus mirabilis and Fusarium verticillioides were completely resistant to the test agents. PCR was used to detect the Klebsiella sp, Pseudomonas aeruginosa, Pantoea stewartii, and Proteus mirabilis specific 16SrRNA genes and Fusarium verticillioides specific ITS genes with ampilicon sizes of 1.5kpb and 650kpb respectively.*
Conclusion: Most bacterial isolates showed high resistance patterns to most of the commercially available antibiotics.
Keyword: *Soil, Resistant microorganisms, PCR, Antibiotics. Antifungal drugs*

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I. Introduction

One of the most important issues affecting human and animal health worldwide is antimicrobial resistance (AMR). Although the use of antibiotics and other antimicrobials is the primary cause of the rise in AMR, research reveals that the environment also plays a significant role in the spread of diseases (Collignon *et al.*, 2018; Graham *et al.*, 2018).

The dynamics of microbial communities in soil, which is a massive repository of microbial variety and a complex ecosystem, are still little understood. It is essential to comprehend these processes because soil management is essential to both agriculture and the overall health of ecosystems, but because soil is so complex, it also provides a good environment for the creation and development of microbes that are resistant to antibiotics. The high complexity of soil and the ongoing competition between microorganisms create conditions that promote the emergence and spread of antimicrobial resistance. A study by Julija *et al.* (2019) highlighted that soil management practices can inadvertently contribute to the proliferation of antimicrobial resistance by altering the

microbial community dynamics. This emphasizes the need for sustainable soil management practices that consider the potential consequences on microbial diversity and the spread of resistance.

A rising concern in recent years has been the frequency of antibiotic-resistant microorganisms (ARMs) in agricultural soils and their potential to spread to other environmental niches. Concerns have been expressed regarding these bacteria's potential to infect people and animals due to their capacity to survive and grow in the environment, especially in the absence of antibiotics. One concerning pathway for the entry of resistance genes into the food system is through the amendment of soils with manure from animals treated with antibiotics. These animals serve as reservoirs for antibiotic resistance genes, and when their manure is applied to soil, these genes can potentially be transferred to soil microorganisms and ultimately enter the food chain (Nikolina *et al*., 2014). The development of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in farmlands, can also be attributed to other anthropogenic activities in agriculture, such as irrigation with municipal wastewater and the use of bio solids in these farmlands. Like many anthropogenic activity, agricultural intensification has a negative influence on the environment. These effects include deforestation, land deterioration, and water and soil pollution. Through manure storage and the use of manure solids or wastewater as soil fertilizer, it has been demonstrated that the preventative and therapeutic use of antibiotics in animal farming contributes to an increase in AMR (Binh *et al.,* 2008; Negreanu *et al.,* 2012; Chantziaras *et al.,* 2014). AMR is a pre-clinical condition that occurs in nature (D'Costa *et al.,* 2011; Bhullar *et al.,* 2012). Although antibiotic resistance genes naturally exist in soils apart from human activity (Davies and Davies 2010), research indicates that their abundance in agricultural soils has been rising ever since antibiotics were introduced for growth promotion purposes in animal farming (Knapp *et al.,* 2010), making their way into agricultural fields via manure application. When compared to non-manured soils, the use of manure can introduce ARB and ARGs to soils, expanding antibiotic resistance reservoirs (Cytryn, 2013; Amarakoon *et al.,* 2016; McKinney *et al.,* 2018). Furthermore, numerous studies have demonstrated that soil pollution can directly impact human health. Soil contamination by heavy metals like arsenic, lead, and cadmium, as well as organic chemicals such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), poses risks to human well-being (Steffan *et al*., 2018). These pollutants can accumulate in the soil and subsequently enter the food chain, potentially causing adverse effects on human health. Importantly, soil pollution by heavy metals can trigger co-selection processes, whereby genes responsible for antibiotic resistance and decreased antibiotic susceptibility are co-regulated. This means that exposure to heavy metals in contaminated soil can enhance the survival and proliferation of bacteria carrying antibiotic resistance genes, leading to an increased risk of antibiotic resistance development (Steffan *et al*., 2018).

Justification

Antibiotic resistance is one of the most serious problems affecting global health. Although the increase in antibiotic resistance is mostly driven by antibiotic use. This study will help give an idea on how the natural environment especially the farmland plays a key role in the wider spread of resistance across microorganisms which poses a threat to human health.

Statement of Problem

The overuse of antibiotics in various human activities, including agriculture, has led to the accumulation of antibiotic residues in soil environments. This has created a selective pressure that promotes the emergence and proliferation of antibiotic-resistant microorganisms in the soil. The problem at hand is the increasing prevalence of antibiotic-resistant microorganisms in farmlands, which poses significant threats to human health, agricultural productivity, and environmental sustainability. The persistence of antibiotic residues in soil, coupled with the transfer of resistant genes among microorganisms, contributes to the spread of antibiotic resistance beyond clinical settings. If left unaddressed, this problem has the potential to undermine the effectiveness of antibiotics, limit agricultural productivity, and compromise the delicate balance of soil ecosystems. Therefore, it is crucial to investigate and understand the factors driving the development of antibiotic resistance in farmlands, as well as to explore effective strategies for mitigating its impact on human and environmental health.

II. Materials And Methods

Sample Collection

The sample collection was done in accordance with the work of Kieran *et al. (*2021). The soil samples were collected from different farmlands in Enugu North, West and South Local Government Area of Enugu State. Random collection of soil samples was carried out under sterile conditions to avoid contamination (using sterile soil probe). A total of 12 soil samples were collected within Enugu State at three (3) different local governments. From each local government, 4 samples were collected from 4 crops field (cassava farm, yam farm, maize farm and control soil). The soil surface was dug 3-5cm deep before collecting at different spots and the debris from the soil were removed before the soil sample were collected.

Determination of Physical Characteristics of Soil Samples

Moisture Content

Gravimetric method of soil moisture determination was used. A total of 30g of the soil samples were weighed and recorded. The samples were oven dried at 105^0C for 24h. Afterwards, the soil samples were weighed and recorded. The values obtained were used to calculate the moisture content of the soil sample according to the method of Reynolds, (2013).

Soil pH

The pH of the soil sample was determined using pH meter in accordance with method of Cheesbrough, (2012). A total of 20g of the soil sample was added into a 100ml beaker and mixed with 20ml of deionized (DI) water. The solution was mixed uniformly by stirring for 30 minutes using a stirrer. The mixture was allowed to stand for 1h and the pH meter was placed in soil samples for readings to be taken.

Sample Preparation

A total of 1g of each soil sample was suspended in 9ml of sterile normal saline in a test tube. The soil sample was serially diluted using ten-fold dilution method to decrease the microbial load of the sample. Ten-fold dilutions of the samples were made with sterile normal saline as diluent (Prescott *et al*., 2016).

Isolation of Organisms on Nutrient Agar

A total of 0.1ml of the different dilutions of the different soil samples were inoculated into the petri dishes, prepared nutrient agar media and were allowed to solidify. The plates were incubated at varied 37°C for bacteria and 28^0C for fungi for 24-72hrs. After incubation the representative colonies on the plates were counted and recorded (Prescott *et al*., 2016).

Determination of Colony Forming Unit

The method described by Prescott *et al*. (2016) for estimating bacterial counts was used to enumerate the total viable counts of the isolates. The number of colonies of the plates were multiplied by the reciprocal of the dilution factor and calculation was done for 0.1ml of the original sample and plating was done in duplicates for each dilution. An average count was taken to obtain the total counts.

Characterization and Identification of Bacteria Isolates

The isolates were characterized by Gram staining and biochemical test which include catalase, coagulase, citrate, oxidase, indole, urease, methyl red and sugar fermentation tests.

Identification of Fungal Isolates

Identification of fungal isolates was done according to the manuals of Oyeleke and Manga (2012). The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation. After isolation, fungal isolates were sub-cultured on the SDA slants. Later the isolates were primarily subjected to the Lacto phenol cotton blue staining, and then analyzed by Scanning through Electron Microscope under required magnifications to observe the morphology of mycelium and spore structures Tafinta *et al.,* (2013).

Molecular Analysis of the Isolates

Some bacterial and fungal isolates were further characterized by PCR.

DNA Extraction Using zr Fungal/Bacterial DNA Miniprep

A total of 2mls of bacterial cells broth was added to a ZR Bashing TM Lysis Tube. Then 750ul Lysis Solution was added to the tube. They were secured in a bead fitted with 2 ml tube holder assembly and processed at maximum speed for > 5mins. Thereafter, the ZR Bashing BeadTM Lysis tube was centrifuged at > 10,000 x g for 1 minute using a microcentirifuge. It was then transferred up to 400ul supernatant to a Zymo-Spin TM IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 x g for 1min. Thereafter, 1,200ul of Fungal/Bacterial DNA binding buffer was added to the filtrate in the collection tube. A total of 800ul of the mixture was transferred to a Zymo-Spin TM IIC Column in the collection tube and centrifuged at 10,000 x g for 1min. The flow through was discarded from the collection tube and repeated. A total of 200ul DNA pre-wash buffer was added to the Zymo-Spin TM IIC Column in new collection tube and centrifuged at 10,000 x g for 1min. Thereafter, a total of 500ul fungal/bacterial DNA Wash Buffer was added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 x g for 1min. The Zymo-SpinTM IIC Column was transferred to a clean 1.5ml microcentrifuge tube and 100ul DNA Elution Buffer was added directly to the column matrix and was centrifuged at 10,000 x g for 30 seconds to elute the DNA.

Electrophoresis for DNA and PCR

A total of 1g of agarose (for DNA), and 2g of agarose for PCR were weighed and the agarose powder mixed with 100 mL 1xTAE in a microwavable flask. It was microwaved for 3min until the agarose was completely dissolved. The agarose solution was allowed to cool down to 50 °C for 5mins. Then, 10µL EZ vision DNA stain was added. The agarose was poured into a gel tray with the well comb in place. Then, the newly poured gel was placed at 4°C for 20mins to solidify. Loading buffer was added to each of the DNA samples or PCR products and allowed to solidify. Then, the agarose gel was placed into the gel box (electrophoresis unit) and filled with 1xTAE (or TBE) until the gel was covered. The molecular weight ladder was loaded into the first lane of the gel and the samples were carefully loaded into the additional wells of the gel. The gel was run at 80-150 V for about 1h. The DNA fragments or PCR product were visualized under UV trans illuminator.

16SrRNA Gene Amplification of the Bacterial Isolates

The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (27F: AGAGTTTGATCMTGGCTCAG) and reverse (1525R: AAGGAGGTGWTCCARCCGCA) primer; 2µL of DNA template and then made up with 8.5µL nuclease free water. There was an initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec. Then, followed by annealing at 56˚C for 30secs and elongation at 72˚C for 45sec. This was followed by a final elongation step at 72˚C for 7mins and temperature was held at 10 ˚C.

Internal Transcribed Spacer (ITS) Gene Amplification of the Fungal Isolate

The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (ITS 1: TCC GTA GGT GAA CCT GCG G) and reverse primer (ITS4 TCCTCCGCTTATTGATATGS); 2µL of DNA template and then made up with 8.5µL Nuclease free water. There was an initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 50˚C for 30secs and elongation at 72˚C for 45sec. it was then followed by a final elongation step at 72˚C for 7 mins and temperature was held at 10 ˚C.

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X for all genetic analysis.

Antimicrobial Susceptibility Testing

The antibiotic sensitivity test was performed using the disc diffusion method (Cheesebrough, 2012) to determine the antibiotic resistance profile of the isolates. The isolates were tested against routinely used commercially available antibiotics.

Standardization of Inoculum

McFarland turbidity standard was prepared by dissolving 1ml of barium chloride ($BaCl_2$) into 9ml of sulphuric acid (H_2SO_4). Then, pure cultures of identified bacterial isolates from a 24hour plate culture were selected. Sterile wire loop was used to pick small colonies of each isolates and emulsified into test tubes containing 5ml of sterile saline, they were vortexed thoroughly. Adjustment was made with extra inoculums or diluents, until 0.5 McFarland turbidity standards were obtained (Meena *et al.*, 2015).

Antimicrobial Assay

A total of 0.1ml of the organisms already matched to McFarland turbidity standards were inoculated into Mueller Hinton agar plates. The inoculated plates were air dried at 37^{O}C to allow for any excess surface moisture to be absorbed before applying the antibiotic discs (NCCLS, 2004). After the inoculation, the antibiotic discs for bacteria were placed on the inoculated media with the different isolates respectively using sterile forceps. The disc was gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37^{O}C for 24 hours. Where there were zones of inhibition, they were measured using caliper to determine the level of sensitivity of the drugs. Results were recorded as sensitive, intermediate or resistant.

Preparation of Fungi for Antimicrobial Activities

Antifungal activity was determined by agar well diffusion method as described by Aleruchi *et al.,* (2019). The antifungals used were, posaconazole (100mg/ml), fluconazole (100mg/ml) and ketoconazole (100mg/ml). The tablets/capsules of original concentration of 200mg of each drug were weighed and crushed into powder. 100mg/ml of the drugs were obtained by dissolving with 2ml of solvent. The isolates were prepared by

transferring into test tubes containing Sabourand Dextrose agar broth. The suspension was vortexed and turbidity adjusted to yield 1 x 10^6– 5 x 10^6 cells/ml (0.5 McFarland standards).

Reconstitution of Drugs/Extract

After the concentration of the extract, a total of 1g of the extract was weighed and dissolved with 5ml of the dimethyl sulfate to obtain a stock extract of 200mg/ml in concentration. Then double fold serial dilution method was employed to create further dilutions of 100mg/ml, 50mg/ml and 25mg/ml.

Antifungal Assay of Fungal Isolates

Antifungal Assay was determined by the process of spread plate technique as described by National Committee for Clinical Laboratory Standards (NCCLS, 2000). A total of 0.1ml of the isolates was inoculated on the surface of a solidified Sabouroad Dextrose agar. A sterile 6mm diameter cork borer was used to create four uniform wells on the solidified Sabouroad Dextrose agar and incubated for 48h at room temperature (28^{0}C). The Zones of inhibition were measured in millimeters using caliper.

Statistical Analysis

Statistical Package for the Social Science (SPSS) was used for the data analysis. Analysis of variance (ANOVA) was used to compute and arrived at statistical decision.

III. Results

Moisture Content of the Soil Samples

From the study, the results showed that cassava farm recorded the highest mean value of moisture content (1.261 ±.465). While, the yam farm recorded the least mean value of moisture content (.529 ± .505). There was statistical difference in moisture content between the soil samples in the cassava farm and control farm at ($p<0.05$). (Table 1).

Table 1: Moisture Content of the Soil Samples Studied

Soil Samples	Weight Before	Weight After	Moisture content	P-value
Maize Farm	10.45±.509	9.66±.470	.791±.484	.0348
Yam Farm	10.11±.102	9.58±.419	.529±.505	.0159
Cassava Farm	10.42±.609	9.161±.144	1.261±.465	.7130
Control Soil	10.43±.244	9.21±.555	1.222±.792	.9360

pH of Soil Samples

From the study, the results indicate that maize farm recorded the highest mean value of pH (7.00 ±.205) while, the control farm recorded the least mean value of pH (6.881 ± .282). There is statistical difference at ($p<0.05$) in the pH values in the soil samples from various farmlands. (Table 2).

Table 2: pH of Soil Samples from Various Farmlands

Soil Samples	Mean±SD	P-value
Maize Farm	7.00±.205	.348
Yam Farm	6.86±.320	.159
Cassava Farm	6.88±.229	.713
Control Farm	6.81±.282	.936

Total Viable Bacterial and Fungal Counts from Different Farmlands.

From the study, the prevalence occurrence of bacterial count was higher than the fungal count in the farms with maize farm having the highest bacterial count (4.5x10^6). (Table 3).

Table 3: Total Viable Bacterial and Fungal Counts from Different Farmlands.

Samples	Mean Bacterial Count CFU/g	Mean Fungal Count CFU/g
Maize Farm	4.5x10^6	1.8x10^6
Yam Farm	3.4x10^6	2.6x10^6
Cassava Farm	3.8x10^6	2.3x10^6
Control Farm	3.2x10^6	2.9x10^6

Characteristics, Biochemical Test Results and Suspected Isolates from the Farmlands.

The isolates showed varied results in their biochemical analysis. *Bacillus subtilis* and *Staphylococcus aureus* were Gram +ve rod and Gram +ve cocci respectively. While *E. coli, Pseudomonas aeruginosa, Pantoea stewartii, Alcaligenes feacalis, Proteus mirabilis, Shigella sp., Salmonella* sp., *and Klebsiella pneumonia* were Gram -ve rods. (Table 4).

Table 4.: Characteristics, Biochemical Test Results and Suspected Isolates from the Farmlands.

S/N	Growth Appearance on Media	Gram reaction	Catest	Oxitest	Cittest	Coatest	Methyl red test	Indole test	Glu	Fru	Mal	Man	Lac	Suspected Organisms
1	Dark pink convex round colony on macconkey agar.	-v short rod in pairs	+v	-v	-v	-v	+v	+v	AG	AG	AG	AG	AG	*E. coli*
2	Round, flat, and creamy colonies on nutrient agar.	-v short rod in pairs	+v	+v	+v	-v	+v	+v	-v	A	A	A	-v	*Pseudomonas aeruginosa*
3	Yellow mucoid colonies on nutrient agar.	-v short rod in pairs	+v	-v	+v	-v	-v	-v	A	A	A	A	A	*Pantoea stewartii*
4	Very large whitish cottony colony on nutrient agar.	+v short rod	-v	-v	+v	-v	-v	-v	A	A	A	-v	-v	*Bacillus subtilis*
5	Large yellow colonies on mannitol salt agar.	+v cocci in clusters	+v	-v	+v	-v	+v	-v	A	A	A	A	A	*Staphylococcus aureus*
6	small, mucoid and whitish colony on nutrient agar.	-v short rod in pairs	+v	-v	+v	-v	+v	-v	A	A	A	-v	-v	*Alcaligenes feacalis*
7	Raise mucoid and whitish colony on nutrient agar.	-v short rod in pairs	+v	-v	+v	-v	+v	-v	A	A	A	-v	-v	*Proteus mirabilis*
8	Black convex round colonies on Salmonella-Shigella agar.	-ve short rod	+v	-v	+v	-v	+v	-v	AG	A	A	AG	-v	*Salmonella* sp.
9	Colorless convex round colonies on Salmonella-Shigella agar.	-v short rod	+v	-v	-v	-v	+v	+v	AG	A	A	AG	-v	*Shigella sp.*
10	Medium pinkish raised msucoid colonies on macconkey agar.	-v short rod in pairs	+v	-v	+v	-v	-v	-v	A	A	A	A	A	*Klebsiella pneumonia*

KEY: Cat=Catalasetest, Cit= Citratetest, Coa=Coagulasetest, Ind=Indoletest, Oxi=Oxidasetest, Gl=Glucose, F=D-Fructose, Ml=Maltose, Ma=Mannitol, La=Lactose, +=positive, **- =**negative, A=Acidic, AG=AcidicandGas, G=Gas, +ve=positive, —ve=negative

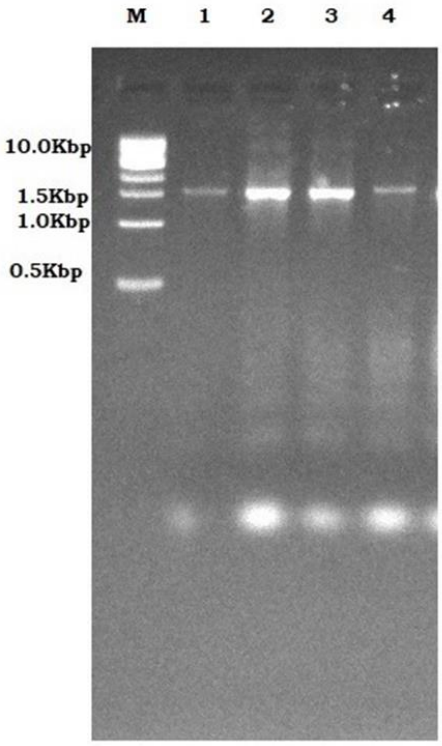

Fig. 1: Gel image showing positive amplification for the 16SrRNA Gene in *Pseudomonas aeruginosa* (lane 1); *Proteus mirabilis* (lane 2), *Pantoea sterwartii* (lane 3) and *Klebsiella pneumonia* (lane 4). Lane M shows 1Kpb DNA ladder

M Lane 1

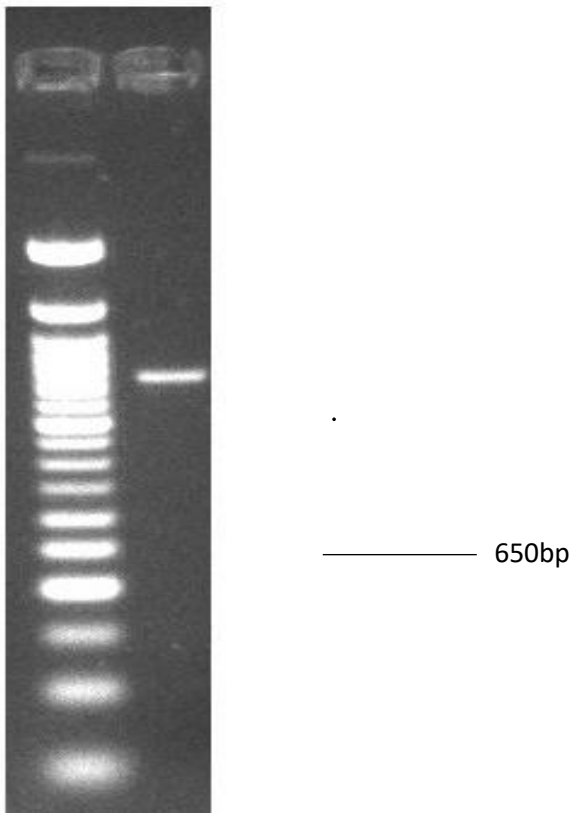

Fig. 2: Gel image showing positive amplification for the ITS Gene in *Fusarium vertiicillioides* (lane 1) Lane M shows 50Kpb DNA ladder

IV. Discussion

Soil is a complex ecosystem that harbors a diverse range of microorganisms. While soil management is crucial for agriculture, it also presents challenges related to antimicrobial resistance and soil pollution. In order to gain an insight into the levels of ARB in different land use soils in Enugu State, Nigeria, we investigated soils with distinct human activities, i.e. Farmlands from different crop farms (maize, yam, and cassava farms) and semi-natural soils which was referred to as 'control' in this study.

In the study, Cassava farm had the highest mean value of moisture content (1.261 ±.465), followed by Control soil (1.222±.792), Maize farm (.791±.484) and yam farm which had the least mean value of moisture content (.529 ± .505). (Table 1). These moisture content measurements provide insights into the water availability in the soil samples, which is crucial for understanding soil fertility, its suitability for various agricultural activities and the level of microorganisms that could thrive in that soil. This result is in agreement with the findings of Agata and Jadwiga (2016) on soil moisture as a factor affecting the microbiological and biochemical activity of soil. Their result demonstrated that the highest counts of microorganisms and the highest enzymatic activity occurred in soils with a moderate content of moisture.

In Table 2, The maize farm recorded the highest mean value of pH (7.00 ±.205), followed by Cassava Farm (6.88±.229), yam farm (6.86±.320) and the control soil recorded the least mean value of pH (6.881 ± .282). The pH levels of soil samples are important indicators of soil acidity or alkalinity, influencing nutrient availability and plant growth as well as the microbial profile of the soil.

In the study, the total bacterial count results indicate that maize farm had the highest mean value ($4.5x10^6$) and the control farm had the least mean value of ($3.4x10^6$). While the fungi count, result indicates that control soil had the highest mean value ($2.9x10^6$) and maize farm had the least mean value of ($1.8x10^6$)(Table 3).

In the study, bacteria isolated were *E. coli, Klebsiella sp, Pseudomonas aeruginosa* and *Staphylococcus aureus, Pantoea stewartii, Proteus mirabilis, Alcarigenes feacalis, Shigella sp, Salmonella sp. and Bacillus subtilis.* The occurrence of these microbes is an indication that manure which could constitute a huge number of enteric bacteria from livestock excretes were used for the agricultural produce. The presence of these aforementioned organisms cut across in all the farms in the study area. Fungi isolated from the farms were *Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus* and *Fusarium verticillioides.* This result is in correlation with the work of Chitra *et al.* (2014), who found *E.coli, Micrococcus sp, Escherichia sp* and *Staphylococcus sp* in soil used for cultivation. Several bacteria and fungi were also isolated from polyhouse soil

(Nakuleshwar *et al.,* 2013). In the study, *Pantoea stewartii* were highest in occurrence at 8(67%), while *Alcaligenes faecalis* had the lowest occurrence 3(25%). From fungi isolates *Fusarium verticillioides* occurred at 8(67%) while *Aspergillus terreus* were lowest in occurrence at 5(42%).

The PCR products of selected bacterial strains revealed predicted amplicon size of 1.5kpb. It was further used to detect the presence of 16SrRNA (*Klebsiella sp, Pseudomonas aeruginosa, Pantoea stewartii,* and *Proteus mirabilis*) while ITS genes (*Fusarium verticillioides*) showed ampilicon sizes of 650kpb.

In the study, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Pantoea stewartii* and *Proteus mirabilis* showed a high level of resistance to the various antibiotics, while others displayed varied susceptibility patterns. (Tables 8, 9,10,11 and 12). *Aspergillus fumigatus, Aspergillus niger*, *Aspergillus terreus* and *Fusarium verticillioides* showed low zones of inhibition to most of the test agents indicating high resistance to the test agents (Table 13). This shows high resistance to the test agents. Multiple research investigations have demonstrated that using animal dung in agricultural soil increases the amount of ARGs and hence ARB (Heuer *et al.,* 2011; Ruuskanen *et al.,* 2016; Sengeløv *et al.,* 2003; Udikovic-Kolic *et al.,* 2014), at least momentarily following their application (Marti *et al.,* 2014; Liu *et al.,* 2017). The buildup of ARB in manured agricultural soil is influenced by the input of ARB from animal guts, through horizontal gene transfer to soil bacteria, and the selective pressure of antibiotic residues and other chemical stressors present in the soil (Binh *et al.,* 2008; Udikovic-Kolic *et al.,* 2014; Marti *et al.,* 2013; Xie *et al.,* 2018). In a past study that analyzed ARB change over time in farmlands, it showed that more exposure to manure led to higher levels of ARB, but these levels decreased over time when the manure was removed (Udikovic-Kolica *et al*., 2014). The differences in the antibiotics could represent if the antibiotics were used in animal feed and the amount of antibiotics used. However, the areas from where the soils were sampled did not affect the resistance because the control areas had multidrug resistance without being exposed to antibiotics. This could also suggest how AMR genes are naturally present in bacteria (Shaikh *et al*., 2015). As shown in the study, ARB numbers throughout different sites vary, which adds inconsistency to their true prevalence. A review by Pepper *et al.,* (2018) that looked at the connection between soil ARBs and healthcare revealed a similar result.

This is an indication that in the midst of the challenge of ARBs that there could be alternative route to combating the presence and rise of ARBs in the environment. Hence more research should be conducted to produce and expand the industry of antibiotics production using other routes such as plants.

V. Conclusion

From the study, most of the microbial isolates were resistant to most of the test agents.

VI. Recommendation

Antibiotic resistance issues have been made even worse by the careless use of antibiotics in agricultural animals. Given this, taking actions to lessen the misuse of antibiotics in animal husbandry will significantly impact the level of antibiotic resistance. This is due to the interaction between the environment and animals, which in turn affects human health. Overall, the following recommendations are made since there is a widespread deficiency among farmers regarding awareness of antibiotic usage and storage, abuse, and resulting risks to the environment and public health.

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