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Evaluation of Antibiotic Resistant Bacteria from Organic Fertilized Farm Soils and Waterleaf (*Talinum triangulare*) in Aluu, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of antibiotics in agriculture is believed to contribute to the spread of antibiotic resistance, and consumption of vegetables represents a route of direct human exposure to resistant bacteria found in soil. This study was carried out to isolate and characterize antibiotic resistant bacteria from poultry dropping fertilized farm soils in Aluu community Rivers State. Thirty-six (36) soil and waterleaf (*Talinum triangulare*) samples were collected for a period of three months from the vegetable farms and subjected to standard microbiological procedures such as standard plate counts, identification, sensitivity testing using Kirby-Bauer disk diffusion method and molecular identification. The total heterotrophic bacterial (THB), *Staphylococcal*, and total coliform counts

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ranged from2.72±0.52x10^6 to 7.30±3.54x10^{6}Cfu/g; 0x10^{5}to 4.30±0.28x10^{5}Cfu/g; 0x10^5 to 21.40±2.26x10^{5}Cfu/g and 2.65±0.21x10^5 to 3.10±0.85x10^{5}Cfu/g for FarmsA, B and C respectively. There was a significant difference ($p<0.05$) in the total heterotrophic, coliform bacterial count but no significant difference in *Staphylococcal* and *Salmonella-Shigella* count between the different vegetable farms sampled in month one. THB, *Staphylococcal*, Faecal coliform (FC), coliform, *Salmonella-Shigella* counts ranged from3.80±1.83x10^6 to 4.69±1.69x10^{6}cfu/g; 1.08±0.04x10^5 to 1.40±0.11x10^{5}Cfu/g; 2.00±0.42x10^4 to 8.30±2.12x10^{4}Cfu/g; 3.95±0.21x10^5 to 5.95±0.21x10^{5}Cfu/g and 1.20±0.28x10^{4}to 2.40±0.85x10^{4}Cfu/g for FarmsA and B respectively. There was no significant difference ($p<0.05$) in the THB, *Salmonella-Shigella*, Total coliform (TC) counts, but there was a significant difference in the *Staphylococcal* and faecal coliform counts between the different vegetable farms sampled for month two. The bacterial isolates identified were as follows; *Escherichia coli*, *Enterobacter* spp, *Shigella* spp, *Salmonella* spp, *Pseudomonas* spp *Proteus* spp, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus licheniformis, Serratia marcesens* and *Serratia nematodiphila*. Forty-six (46) bacterial isolates were isolated from the soils and vegetables. *Escherichia coli* had the highest occurrence (75%) and *Bacillus* spp (9.09%) had the least occurrence. The susceptibility pattern of *Bacillus* spp, *Staphylococcus* spp, *Enterobacter* spp, *Salmonella* spp, *Escherichia* coli, *Shigella* spp, *Pseudomonas* sppand *Proteus* sppwere all susceptible to Ofloxacin, and gentamicin (100%) and resistant to Ceftazidime, Cefixime and Augmentin (100%). Hundred-percent (100%) of the bacterial isolates had multidrug resistance index greater than 0.2 and QnrA resistant gene were found in the resistant bacterial isolates. In conclusion, the use of antibiotic indiscriminately for agricultural purposes should be discouraged because of multiple antibiotic resistance.

Keywords: Soil microbiology; antibiotic resistant bacteria; talinum triangulare; salmonella-shigella.

1. INTRODUCTION

The antibiotic-resistant bacteria in soil is caused by the frequent use of antibiotics in animal production which encourages the development of resistant forms of bacteria. The most critical areas related to the growth and spreading of antibiotic resistant genes (ARGs) is livestock and poultry production. Manure is commonly used as a fertilizer due to its rich nutrient and organic matter contents. This analysis surveys the microorganisms and ARGs which may be found in animal manure and evaluates their outcome on human health through contact with soil and plant resistome [40-45].

"The spread of antibiotic-resistant bacteria (ARB) is a rising problem worldwide.It has been assessed that antimicrobial resistance (AMR) is accountable for 700,000 death/year worldwide. It has also been anticipated that by 2050, AMR will be accountable for more death than cancer" [1,46-52]. "Soil is a serious component of the environmental health systemand plays a vital role in human health and well-being, mainly because most food is derived from soil, that is,plants and signifies the major pathway for the transfer of essential nutrients, such as nitrogen, phosphorus and trace elements, to humans" [2,3]. "The role of soil from biological, chemical and physical perspectives consequently have direct effect on human health and well-being" [4,53-58]]. "For instance, human action can increase soil concentrations of pollutants to levels high enough to cause long-term health threats to humans consuming contaminated crop and animal products" [5].

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was carried out in three (3) different vegetable farms in Aluu Community viz; Farms A, B and C all in Ikwerre Local Government Area of Rivers State; where the soil samples were collected. The vegetable farms were selected due to the high level of poultry droppings there.

2.2 Sample Collection

Thirty-six (36) soil and waterleaf samples were collected for a period of three months from the vegetable farms in Aluu Community, Nigeria. The samples were labelled properly and transported aseptically to the Department of Microbiology Laboratory, Rivers State University, Port Harcourt for bacteriological analysis.

2.3 Sample Preparation

One gram (1g) each of soil and waterleaf were weighed aseptically and dispensed into test-tubes containing 9ml of diluent. "The test-tubes

were gently and repeatedly shaken as it is widely believed to facilitate the detachment of the adhered microbes from the soil and vegetables as much as possible into the solution" [6,59-63].

2.4 Bacteriological Analysis

2.4.1 Enumeration and isolation of bacteria

Tenfold serial dilution was carried out from dilution factor 10^{-1} to 10^{-6}. Aliquots (0.1 ml) of appropriate dilutions were spread plated in duplicates onto Nutrient, MacConkey, Mannitol salt, *Salmonella-Shigella*, and Eosin Methylene Blue (EMB) Agar plates. The plates were incubated at 37^{o}C for 24 hours and 44.5^{o}C for EMB plates (Faecal coliform counts). The colonies formed on the plates were counted and described morphologically. The colonies formed on Eosin Methylene Blue agar was used for the enumeration of the population of faecal coliformand MacConkey agar for other coliforms while*Salmonella-Shigella* agar for *Salmonella-Shigella* counts, and Mannitol salt agar for *Staphylococcal* count. Colonies formed on Nutrient Agar plates were used to estimate the total heterotrophic bacterial counts (THBC). Representative discrete colonies were sub-cultured onto freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hours to obtain pure cultures used for subsequent analysis.

2.5 Antibiogram

2.5.1 Agar disk diffusion method (kirby bauer disk diffusion)

"A sterile swab stick was dipped into the tube containing the bacterial suspension and its turbidity with equivalence of 0.5 McFarland Turbidity Standard and the swab was used to swab the surface of the petri dish evenly which contain already prepared Mueller Hinton agar in three dimensions and rotating the plates to about 60^{o} to ensure even distribution of the organism. The agar was allowed to dry for about 3-5minutes. With Sterile forceps, the impregnated antimicrobial discs were placed evenly on the surface of the inoculated plate and the disc was placed 15mm away from the edge of the plate. The head of the forcep was used to Press down each disc slightly to make contact with the agar. After applying the discs, the plates were incubated in an inverted position aerobically at 35^{o}C for 16-18h. After incubation, the test plates were examined to ensure growth or near confluence. The diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate and recorded for reference purpose" [7].

2.5.2 Determination of Multiple Antibiotic Resistance Index (MAR)

"Multiple antibiotic resistance is the resistance of bacterial isolate to three or more antibiotics" [8]. "Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where a stands for the number of antibiotics to which the test isolate depict resistance and b stands for the total number of antibiotics to which the test isolate has been evaluated for susceptibility" [9].

2.6 Molecular Identification

2.6.1 Extraction of DNA

The separation of DNA from proteins, membranes and other cellular materials contained in the cell is known as a phenomenon called DNA extraction (Kelly, 2013). Boiling method was used for the extraction process. A 24hours, old pure culture of the Bacterial isolates was put in Luria-Bertani (LB) Broth and incubated at 37^{o}C. Five millilitres (5ml) of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. This process was repeated 3 times. "The cells were re-suspended in 500ul of normal saline and heated at 95^{0}C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro centrifuge tube and stored at -20^{o}C for other downstream reactions" [10].

2.6.2 DNA quantification

The purity and concentration of the DNA can be determined by a process called DNA quantification. The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The Beer Lambert's principle which is used to evaluate the quality and quantity of the genomic DNA is used by the Nanodrop spectrophotometer. The Nanodrop spectro-photometer was connected to a computer with Nanodrop software installed. The software of the equipment was lunched by double clicking on the Nanodrop icon. The sample pedestals were properly cleaned. The equipment was initialized using 2µl of sterile distilled water and blanked using 2µl of Normal saline. About 2µl of the extracted DNA was loaded onto the lower pedestal to measure the concentration of the sample, and the upper pedestal was brought down to make contact with the DNA on the lower

pedestal. Then, DNA concentration was measured by clicking the "measure" button displayed on the computer screen [11].

2.6.3 16S rRNA amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: The X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV trans-illuminator for a 1500bp amplicons.

2.6.4 DNA sequencing

"Sequencing was carried out using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min" [12].

2.6.5 Phylogenetic analysis

"Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0" [13]. The bootstrap consensus tree inferred from 500 replicates [14] is taken to represent the evolutionary history of the taxa analysed. "The evolutionary distances were computed using the Jukes-Cantor method" [15].

2.6.6 Amplification of QnrAGene

"QnrA genes from the isolates were amplified using the QnrAF: 5′-GATCGTGAAAG CCAGAAAGG-3´ and QnrAR: 5′-CGATGCCTGGTAGTTGTCC-3´ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: The X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4uM and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 50ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue transilluminator" [10].

2.7 Data Analysis

Statistical analysis was carried out on the bacterial counts from the soil and vegetables obtained in the study. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for mean separation. This was done using a computer-based Programme-SPSS version 25 (Bewick et al., 2004).

3. RESULTS

3.1 Bacterial Population of Soil from the Vegetable Farms

Results of the bacterial population of the soil samples are presented in tables. The total heterotrophic bacterial counts of Farms A, B and C were as follows;7.30±3.54 $x10^6$, 2.92±0.95 $x10^6$ and 2.72±0.52 $x10^6$Cfu/g,respectively and control with 5.53±3.18$x10^6$Cfu/g. There was a significant difference (p≤0.05) in the total heterotrophic bacterial counts between the different vegetable farms sampled. The Total *Staphylococcal* counts of Farms A, B and C were as follows; 4.30±0.28 $x10^5$, 2.5±0.95 $x10^4$ and 0 $x10^5$Cfu/g respectively to control with 1.50±0.57 $x10^6$Cfu/g. The Total coliform counts of Farms A, B and C were as follows; 2.90±0.14 $x10^5$, 21.40±2.26 $x10^5$ and 0 $x10^5$Cfu/g compared to control with 8.40±0.85 $x10^5$Cfu/g. There was a significant difference (p≥0.05) in total coliform counts. Total *Salmonella-Shigella* counts of Farms A, B and C were as follows; 2.65±0.21$x10^5$, 2.80±0.85$x10^4$ and 3.10±0.85 $x10^4$Cfu/g compared to control with 2.20±0.57$x10^4$Cfu/g count all for month one (1). There was no significant difference (p≥0.05) in total *Staphylococcal* and*Salmonella-Shigella* counts, from the waterleaf farms.

The total heterotrophic bacterial counts for month two (2) of Farms A, B and C were as follows;4.69±1.69 $x10^6$, 8.80±1.83 $x10^6$ and 4.45±1.32 $x10^6$Cfu/g compared to control with 6.49±1.32$x10^6$Cfu/g. There was no significant difference (p≤0.05) in the total heterotrophic bacterial counts between the different vegetable farms sampled. The Total *Staphylococcal* counts of Farms A, B and C were as follows;1.40±0.11 $x10^5$, 1.08±0.04 $x10^5$ and 1.23±0.02 $x10^5$Cfu/g compared to control with 1.13±0.06$x10^5$Cfu/g. The faecal coliformcounts of Farms A, B and C were as follows;2.00±0.42 $x10^4$, 8.30±2.12 $x10^4$ and 4.90±0.71 $x10^5$Cfu/g compared to control with 12.25±0.64 $x10^4$Cfu/g. The Total coliform counts of Farms A, and C were as follows; 5.95±0.21$x10^5$, 4.00±0.57$x10^5$ and 3.95±0.21x 10^5Cfu/g compared to control 4.19±0.57 $x10^5$Cfu/g. Total *Salmonella-Shigella* count of Farm A, B and C is as follows;2.40±0.85 $x10^4$, 1.85±0.35 $x10^4$ and 1.20±0.28 $x10^4$Cfu/g compared to control with 10.65±0.83 $x10^4$Cfu/g count all for month two (2). There was a significant difference (p≥0.05) in total coliform*, Salmonella-Shigella*, *Staphylococcal* and faecal coliform counts from the vegetable farms.

The total heterotrophic bacterial counts for month three (3) of Farms A, B and C were as follows; 2.83±0.05$x10^6$, 2.71±0.21$x10^6$ and 1.81±0.67x 10^6Cfu/g compared to control 1.05±0.04 $x10^6$Cfu/g. There was a significant difference (p≤0.05) in the total heterotrophic bacterial count between the different vegetable farms sampled. The total *Staphylococcal* counts of Farm A, B and C is as follows; 42.00±8.08$x10^4$Cfu/g, 1.93±0.08$x10^4$Cfu/g and 1.50±0.28$x10^4$Cfu/g compared to control 7.15±0.21 $x10^4$Cfu/g. There was no significant difference (p≥0.05) in total *Staphylococcal* count from the vegetable farms. The total faecal coliformcounts of Farms A, B and C were as follows; 2.15±0.02$x10^4$, 1.72±0.21$x10^4$ and 9.15±0.21$x10^4$Cfu/g compared to control having 1.69±0.05 $x10^4$Cfu/g. The total coliform counts of Farms A, B and C were as follows; 2.58±0.35$x10^5$, 1.92±0.02$x10^5$ and 2.86±0.04$x10^5$Cfu/g compared to control 2.38±0.07 $x10^4$Cfu/g. Total *Salmonella-Shigella* counts of Farms A, B and C were as follows; 2.20±0.07$x10^4$, 1.25±0.05$x10^4$ and 5.70±0.71$x10^4$Cfu/g compared to control 1.94±0.01 $x10^4$Cfu/g. There was a significant difference (p≥0.05) in total coliform*, Salmonella-Shigella* and faecal coliform counts from the vegetable farms.

3.2 Prevalence of Bacterial Isolates from Soils in Aluu Community

A total of forty-six (46) bacterial isolates were isolated from the soils. For month one (1) in the soil samples; Proteus and *Salmonella*spp had the highest occurrence (66.67%) and *Bacillus*spp (9.09%) had the least occurrence. In month two (2) *Bacillus*spp (45.45%) had the highest percentage occurrence while *Staphylococcus aureus* had the least occurrence (22.22%). Formonth three (3) in the soil sample, *Proteus* and *Salmonella*spp had the highest occurrence (33.33%) while *Enterobacter*spp had the least prevalence (14.29%). In the vegetables, *Bacillus*spp occurred most in the vegetables from the different farms. Generally, in Farm A, *Escherichia coli* had the highest prevalence (75%) followed by *Enterobacter*spp (42.86%) in Farm B and *Salmonella*spp (66.67%) in Farm C.

Table 1. Bacterial populationinsoil from the farms (Month 1)

Farm	THB $x10^6$Cfu/g	TSC $x10^5$Cfu/g	TCC $x10^5$Cfu/g	TSS $x10^4$ Cfu/g
Control	5.53±3.18^b	1.50±0.57^a	8.40±0.85^b	2.20±0.57^a
A	7.30±3.54^c	4.30±0.28^a	2.90±0.14^a	2.65±0.21^a
B	2.92±0.95^a	0.25±0.05^a	21.40±2.26^c	2.80±0.85^a
C	2.72±0.52^a	0.00±0.00^a	0.00±0.00^a	3.10±0.85^a

*Keys: THB (Total Heterotrophic Bacteria count), TCC (Total Coliform Count), TSSC (Total Salmonella-Shigella counts), TSC (Total Staphylococcalcounts). *Means with same alphabet across the columns shows no significant difference (p≥0.05)*

Table 2. Bacterial populationinsoil from the farms (Month 2)

Farm	THB $x10^6$ Cfu/g	TCC $x10^5$ Cfu/g	TFC $x10^4$ Cfu/g	TSC $x10^5$ Cfu/g	TSS $x10^4$ Cfu/g
Control	6.47±0.11^a	4.19±0.57^a	12.25±0.64^c	1.13±0.06^a	10.65±0.78^b
A	4.69±1.69^a	5.95±0.21^b	2.00±0.42^a	1.40±0.11^b	2.40±0.85^a
B	3.80±1.83^a	4.00±0.57^a	8.30±2.12^b	1.08±0.04^a	1.85±0.35^a
C	4.45±1.32^a	3.95±0.21^a	4.90±0.71^a	1.23±0.02^{ab}	1.20±0.28^a

*Keys: THB (Total Heterotrophic Bacteria count), TCC (Total Coliform Count), TSSC (Total Salmonella-Shigella counts), TSC (Total Staphylococcalcounts), TFC (Total Faecal coliform count). *Means with same alphabet across the columns shows no significant difference (p≥0.05)*

Table 3. Bacterial population in the soil from the farms (Month 3)

Farms	THB x10^{6}Cfu/g	TCC x10^{5}Cfu/g	TFC x10^4 Cfu/g	TSC x10^{4}Cfu/g	TSS x10^{4}Cfu/g
Control	1.05±0.04^a	2.38±0.07^{bc}	1.69±0.05^a	7.15±0.21^a	1.94±0.01^{ab}
A	2.83±0.05^{bc}	2.58±0.35^{cd}	2.15±0.02^{cd}	42.00±8.08^a	2.20±0.07^{bc}
B	2.71±0.21^c	1.92±0.02^a	1.72±0.02^b	1.93±0.08^a	1.25±0.05^a
C	1.81±0.67^b	2.86±0.04^d	9.15±0.21^e	1.50±0.28^a	5.70±0.71^d

*Key: THB (Total Heterotrophic Bacteria count), TCC (Total Coliform Count), TSSC (Total Salmonella-Shigella counts), TSC (Total Staphylococcalcounts). *Means with same alphabet across the columns shows no significant difference (p≥0.05)*

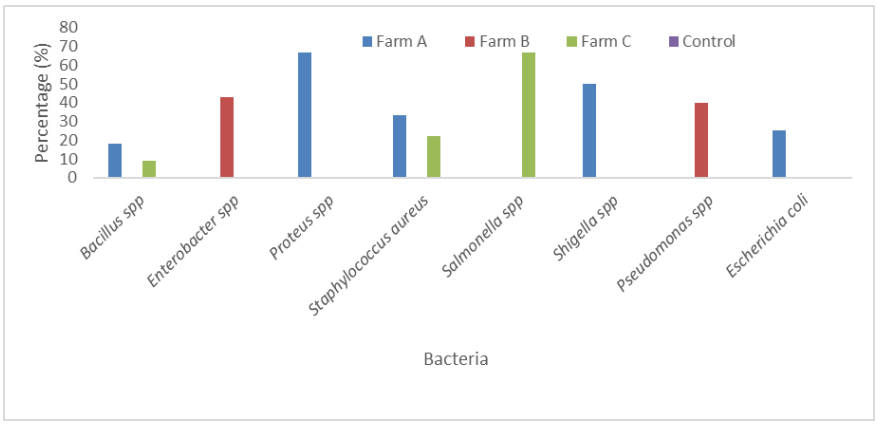

Fig. 1. Percentage Relative Abundance of Bacteria from soil from the Farms (Month 1)

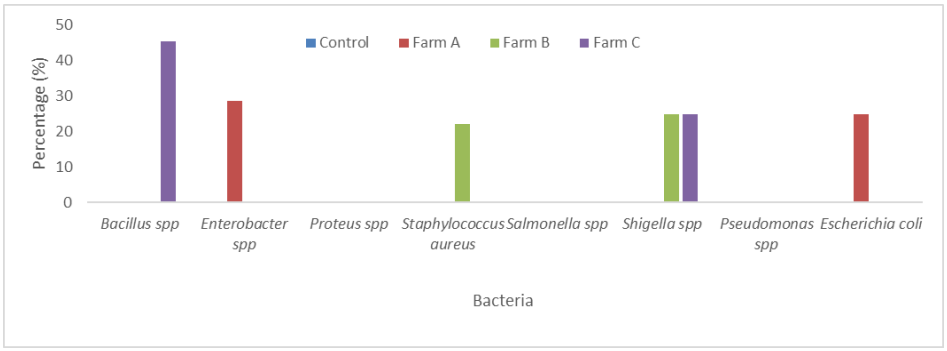

Fig. 2. Percentage Relative Abundance of bacteria from soil in the Farms (Month 2)

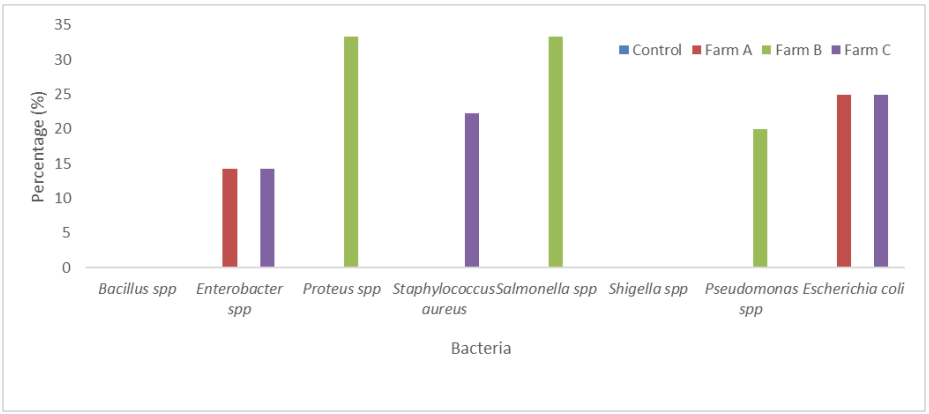

Fig. 3. Percentage Relative Abundance of Bacteria fromsoil from the Farms (Month 3)

Table 4. Summary of multiple antibiotic resistance index of bacteria isolate

Isolates	MAR Index					
	0.3	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
Bacillus spp (11)	10(90.91)	1(9.09)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Staphylococcus spp (9)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	9(100)
*Enterobacter*spp (7)	0(0.00)	0(0.00)	7(100)	0(0.00)	0(0.00)	0(0.00)
Salmonella spp(3)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	0(0.00)
Escherichia coli (4)	0(0.00)	0(0.00)	4(100)	0(0.00)	0(0.00)	0(0.00)
*Shigella*spp (4)	0(0.00)	0(0.00)	4(100)	0(0.00)	0(0.00)	0(0.00)
Pseudomonas spp(5)	0(0.00)	0(0.00)	5(100)	0(0.00)	0(0.00)	0(0.00)
Proteus spp (3)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	0(0.00)

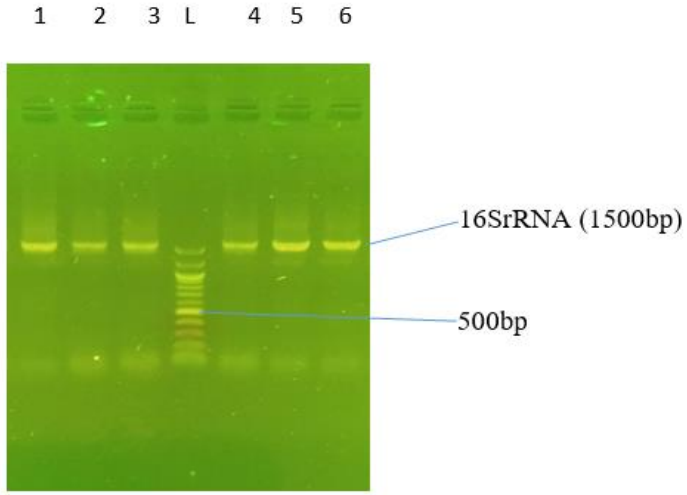

Plate 1. Agarose gel Electrophoresis sowing the Amplified 16S rRNA Fragment. Lanes 1-6 Represent the Amplified 16SrRNAbands at 1500bp while L Represents the 100bp Molecular Ladder

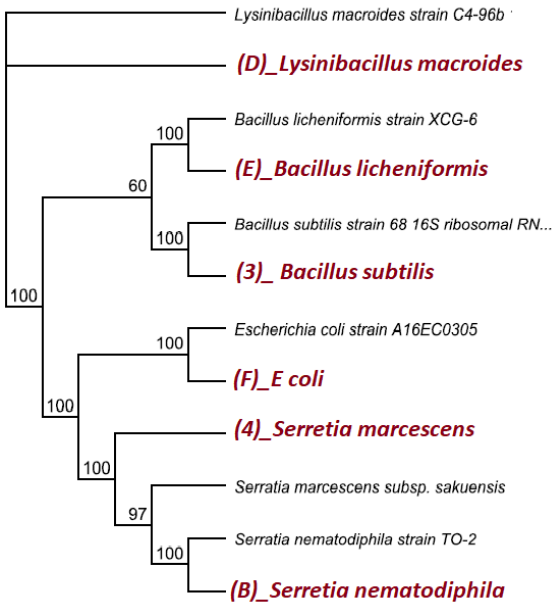

Fig. 4. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

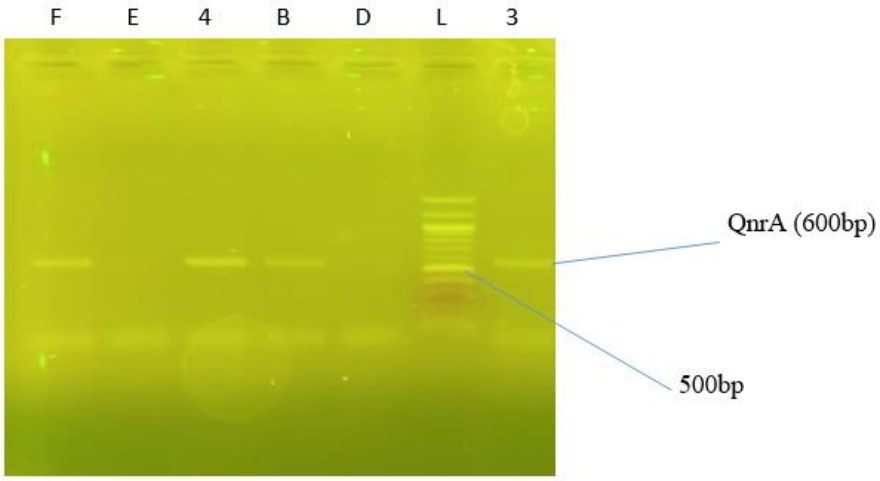

Plate 2. Agarose Gel Electrophoresis showing the QnrA Bands. Lane F, 4, B, and 3 represent the QnrA Gene Bands at 600bp while L represents the 100bp Molecular Ladder

The agarose gel electrophoresis as shown in the plate of the amplified 16S rRNA gene of the most resistant bacterial isolates before sequencing. Lanes 1 to 6 represent the 16S rRNA gene bands (1500bp) while lane L represents the 100bp molecular ladder.

Plate 2 displays the agarose gel electrophoresis showing the amplified QnrA gene bands of the isolates at 600bp. Lane L represents the 100bp molecular ladder while lane F, 4, B, and 3shows the isolates amplified QnrA gene bands at 600bp.

3.3 Phylogenetic Tree/Evolutionary Distance of Isolates

The evolutionary distance between the bacterial isolates from this study and the accession numbers of their closest relatives on the phylogenetic tree are revealed on Fig. 4.

4. DISCUSSION

4.1 Bacterial Population in Soil Samples

“The increasing prevalence of antibiotic-resistant bacteria is a global threat to public health. Agricultural use of antibiotics is believed to contribute to the spread of antibiotic resistance, but the mechanisms by which many agricultural practices influence resistance remain obscure. A route of direct human exposure to bacteria found in soil. Nutritionists currently endorse what mothers have forever told their children, that vegetables are an indispensable component of a healthy diet” [16].

The total heterotrophic bacterial counts were highin the vegetable farms ($7.30\pm3.54x10^6$cfu/g) compared to control with $5.53\pm3.18x10^6$Cfu/g. There was a significant difference ($p\leq0.05$) in the total heterotrophic bacterial counts between the different vegetable farms sampled. *Staphylococcal*, *Salmonella-Shigella* and Coliform counts were high and the counts varied in the three (3) different vegetable farms. During month two (2), The total heterotrophic bacterial counts were high($4.69\pm1.69x10^6$cfu/g) compared to control, $6.47\pm0.11x10^6$Cfu/g. There was no significant difference ($p\leq0.05$) in the total heterotrophic bacterial count between the different vegetable farms sampled. The *Staphylococcal*, Coliform, Faecal coliform and *Salmonella-Shigella* counts were also high with varying counts across the different vegetable farms. There was a significant difference ($p\geq0.05$) in total coliformand *Salmonella-Shigella* count. The high coliform counts could have resulted from fecal materials from domestic animals, humans, and wildlife that contain enteric bacteria contributing to the bacterial content of the soil and animal manures, wastewater, or waste treatment residues. In the composite soil, total heterotrophic bacterial counts were between$1.81\pm0.67x10^6$ and $2.83\pm0.05x10^6$cfu/g across the farms. There was a significant difference ($p\leq0.05$) in the total heterotrophic bacterial count between the different vegetable farms sampled. This agrees with the work of Romain *et al*. [17] who reported the high number of microorganisms on vegetable soils in London. The farms also had varying *Staphylococcal*, coliform, faecal coliform and *Salmonella-Shigella*

counts. This is probably due to inadequate environmental hygiene standards, pathogens carried in raw manure that is used as a fertilizer on the soil as well as contamination by manure-borne pathogenic microorganisms together with the indigenous microorganisms [18].

The total heterotrophic bacterial counts for only the vegetables from the farms revealed that the counts were slightly lower than that of the soils $3.55\pm0.07x10^5$ to $16.40\pm2.82x10^5$cfu/g for the different farms. There was a significant difference at ($p\leq0.05$) in the total heterotrophic bacterial counts between the different vegetable farms sampled. *Staphylococcal*, coliform, faecal coliform and *Salmonella-Shigella* counts were also lower but significant in the various vegetables farm. These vegetables represent leafy vegetables (waterleaf) and make contact with the soil and surface presentation to key environmental factors: sun, rain, and wind which also contain microorganisms [19]. The faecal materials used as fertilizer contributed to the high amount of coliforms because the vegetables are grown in soil fertilized with dairy or swine manure at agronomic rates of application. The high amount of coliform seen in this work agrees with the work of Yang *et al*. [20] which revealed the presence of resistant enteric bacteria in manure-fertilized vegetables. The seeds are sowed directly into freshly manured soil and antibiotic-resistant bacteria naturally found in soil that may find their way onto harvested vegetables [17].

4.2 Prevalence of the Bacterial Isolates

A total of forty-six (46) bacterial isolates were identified as *Escherichia coli*, *Enterobacter* spp, *Shigella* spp, *Salmonella* spp, *Pseudomonas* spp *Proteus* spp, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Serratia marcesens*, *Serratianemato diphila*. The occurrence of *Proteus*spp, *Salmonella* spp, *Staphylococccus* spp and *Bacillus* spp in the soil indicates that soil is one of the biggest reservoirs of microbial diversity, yet the processes that define the community dynamics are not fully understood [21]. Similarly, Susan and Sameer [22] isolated bacterial species from the genus *Bacillus, Pseudomonas, Proteus* and *Escherichia* in their study of bacterial species in soil. *Bacillus*spp (45.45%) had the highest occurrence in composite soil, and occurred most in the vegetables from the different farms. The occurrence of *Bacillus* sppin all the soils sampled and also as the most frequently occurring bacteria is an indication of the dominant habitation of soils by bacterial species specifically *Bacillus* [23,24]. This study has also shown that the soil can be a reservoir of bacteria such as *Staphylococcus* [25]. The occurrence of *Staphylococcus* and *Escherichia coli* having the highest occurrence could also be attributed to such factors as contamination between normal skin (hands, fingers, faces) flora, nasal discharge, soil, faecal matter used on the farm in form of manure as well as its ubiquitous distribution in the environment [25].

4.3 Susceptibility Pattern of the Bacterial Isolates

“The use of antibiotics in animal production and the abundance of antibiotic-resistant bacteria and plasmids carrying antibiotic resistance determinants in manure have engendered concern that recycling of manure onto agricultural land used for crop production can disseminate resistance to crops destined for animal or human consumption” [26]. Antibiotic sensitivity test as interpreted using the Clinical Laboratory Standard Institute Guideline [7] revealed that all the bacterialspecies are uniquely resistant to most antibiotics as all the isolates had varying susceptibility to the antibiotics tested. Susceptibility pattern of *Bacillus* spp showed that they were susceptible to Cloxacillin, Ofloxacin and Cefuroxime (100%) and resistant to Ceftazidime, and Augmentin (100%)> Erythromycin (9.1%) and this could be attributed to high level of awareness among residents on the use of antibiotics [27] which agrees with the work of Williams *et al*., [28]. The sensitivity observed for *Bacillus spp.* in this study is in agreement with results from the studies of antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates by Fiedler *et al.* [29].Susceptibility of *Staphylococcus* spp indicated that a greater number of *Staphylococcus* spp were susceptible to Gentamicin and Ofloxacin (100%) but they were resistant to Ceftazidime, Augmentin, Ceftriaxone, Cefuroxime, Erythromycin and Cloxacillin (100%) and the resistance of *Staphylococcus aureus* in this study to several antibiotics of the increasing growing burden of antibiotic resistance by *Staphylococcus aureus*as discussed by Guo *et al.* (2020) in their review of the Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. The susceptibility pattern of *Enterobacter* spp indicated that a greater number of the *Enterobacter* spp were susceptible to Ofloxacin, Gentamicin, Ciprofloxacin and Ofloxacin (100%) and resistant

to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%).
The susceptibility pattern of *Salmonella* spp indicated that *Salmonella* spp were susceptible to Ofloxacin, Gentamicin, Ciprofloxacin and Nitrofurantoin (100%), and revealing resistance to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%) and in a study by Patil and Mule, [30], 251 *Salmonella* isolates were sensitive to Cefixime which is not in agreement with results from this study in which all *Salmonella* isolates were resistant to Cefixime. Susceptibility pattern of *Escherichia coli* revealed that *Escherichia coli* were susceptible to Ofloxacin, Gentamicin, Ciprofloxacin, Nitrofurantoin (100%) more resistant to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%) and this can be as a result of the enzyme β-lactamases and this is in agreement with the work of Ogbonna *et al*., [31]. The susceptibility pattern of *Shigella* spps howed that they were more susceptible to Ofloxacin, Gentamicin, Ciprofloxacin and Nitrofurantoin (100%) but resistant to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%) and *Pseudomonas* spp showed that they were more susceptible to Ofloxacin, Gentamicin and Ciprofloxacin (100%) and resistant to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%). The results of the susceptibility pattern of *Proteus*sppshowed that they were more susceptible to Ofloxacin, Gentamicin and Ciprofloxacin (100%), Nitrofurantoin (66.7%) and resistant to Cefuroxime, Cefixime, Ceftazidime and Augmentin (100%). It was observed that the bacterial isolates were more susceptible to Ofloxacin, Gentamicin belonging to aminoglycosides group is not surprising because it is known to work against most gram negative bacteria, by binding to their ribosomes and inhibiting protein synthesis [32]. This is in agreement with the work of Zhang *et al*. [33] where the organisms were susceptible to the antibiotics such as ofloxacin, gentamicin and nitrofurantoin.

The high resistance of the bacterial isolates to the beta-lactam antibiotics such as Ceftazidime, Cefixime, Augmentin and Cefuroxime as observed in this study can possibly be due to the extreme use of these antibiotics and the acquisition of resistant genes such as bla_{CTX-M}, bla_{SHV}, QnrA and bla_{TEM} [34]. The abundance of resistance genes has been reported to be highly enriched in animal manures and Zhang *et al*. [33] described that cattle manure application increased the abundance of antimicrobial resistant genes in plant root while poultry manure application increased antimicrobial resistant genes in rhizosphere, root endophyte and phyllosphere. Thus, the continuous increase or high persistence of antimicrobial resistant genes on vegetable farm environments may pose potential threats to human health and the ecological environment and this agrees with this current research.

The Multiple Antibiotic Resistance Index of bacterial isolates which revealed that 100% of all thebacterial isolateshad multidrug resistance index greater than 0.2 and indicated a high risk source of contamination where antibiotics are frequently used [35,9].

4.4 Molecular Identification and Detection of Resistant Gene

The result of the obtained 16SrRNA sequence of the isolate produced during the mega blast search were very similar to the sequences from the non-redundant nucleotide NCBI database produced an exact similarity. The 16S rRNA of the isolates D, E, 3, F, 4 and B showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16SrRNA of the isolates D, E,3, F, 4 and B within the *Lysinibacillus* spp, *Serratia*spp, *Bacillus* spp, *Escherichia* spp which revealed a closely relatedness to *Lysini bacillusmacroides*, *Bacillus licheniformis*, *Bacillus subtilis*, *Escherichia coli*, *Serratia marcescens* and *Serratianemato diphila.* These six (6) isolates were the most resistant isolates to the antibiotics tested. Molecular screening was conducted for the detection of QnrA gene and the six (6) resistant bacterialisolates were subjected to the analysis which revealed that four (4) (*Serratia marcescens*, *Serratia nematodiphila, Escherichia coli* and *Bacillus subtilis*) out of six (6) isolates screened had the QnrA gene present in their genome. Quinolones are a class of synthetic and broad-spectrum antibacterial agents that interfere with bacterial DNA gyrase (bacterial topoisomerase II) and topoisomerase IV, preventing the supercoiling of DNA, and ultimately promoting DNA strand breakage [36]. In addition to their use in human medicine, quinolones are extensively utilized as therapeutics and prophylactic additives to prevent the occurrence of disease in livestock and fish farms and resistance to quinolone

in bacterial isolate scan occur by either chromosomal mutation in DNA gyrase genes or acquisition of transferable plasmid-mediated quinolone resistance (PMQR) genes [37]. Generally, plasmid-mediated resistance is a rising concern and can be transferred among various bacterial species and stimulate their transfer into other pathogenic species through horizontal gene transfer (HGT) [38,69-72].

The agarose gel electrophoresis showing the amplified QnrA gene bands of the isolates at 600bp. Lane F, 4, B, and 3shows the isolates amplified QnrA gene bands at 600bp and represent a major gene that confer resistance to the antibiotics. This fluoroquinolones resistant genes have been widely known to be responsible for the resistance in most gram negative and positive bacterial isolates and have their abundance in animal manures used on the farms. Animal manure (Poultry droppings) is an important reservoir of antibiotic-resistant bacteria, antibiotic-resistance genes (collectively known as the “resistome”), antibiotic-resistant bacteria are also abundant in manure from animals with no history of antibiotic treatment, indicating the natural presence of bacteria intrinsically resistant to antibiotics in animal gastrointestinal tracts [39,64-68]. Antibiotic-resistance genes from the soil resistome can enter the food chain via contaminated crops such as the vegetables and have potential consequences on human health if transferred to human pathogens [39].

5. CONCLUSION AND RECOMMENDA-TIONS

5.1 Conclusion

This research was focused on the characterization of antibiotic resistant bacteria from soil and vegetables which serves as a reservoir for antibiotic resistant organisms due to the application of animal manures to improve crop yield. The high bacterial counts and prevalence of bacteria is a threat to public health. The aim was achieved by revealing the presence of antibiotic resistant bacteria from the soil and vegetables fertilized with poultry droppings. This study showed that these soils as well as the vegetables serve as reservoirs for the antibiotic resistant bacteria due to the increase in antibiotic resistance among the bacteria which is of great public health concern. To further strengthen the indiscriminate use of antibiotics both in the health and agricultural sector as major means through which bacterial resistance to antibiotics is spread. Ofloxacin and Gentamicin can be possible drugs of choice for the treatment of infection from these resistant bacteria and QnrA genes are responsible for confirming resistance in this bacteria as obtained in this study.

5.2 Recommendations

The following suggestions are given as a result of the findings from the study:

i. The use of antibiotic indiscriminately for agricultural purposes should be discouraged through campaigns.
ii. Campaigns should be adopted that is projected towards educating the general public on the danger of the indiscriminate use of antibiotics to check the proliferation of antibiotic resistant strains.
iii. Enhanced sanitary conditions are strongly recommended for the farms environment
iv. Government and non-governmental agencies should provide funding for morestudy into the molecular components of antibiotic resistance.
v. It is urgent and necessary to explore optimal fermentation processes to improve the removal efficiency of antibiotic resistant genes and pathogens in animal manure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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