Molecular Characterization and Antibiotic Resistant Bacteria from Soils in Rivers State University Environment

ABSTRACT

Antibiotic resistance has become a subject of global concern to health systems all over the world and the shedding of antibiotic resistant bacteria from humans and animals into the soil is a threat to public health. This research was carried out to molecularly characterize and assess antibiotic resistant bacteria from soil in Rivers State University. Bacteria from soil in four locations; waste dump site, fish farm, poultry farm and home soil were enumerated and identified using standard microbiological techniques such as serial dilution, plating and incubation, isolation, biochemical testing and molecular identification. Sensitivity testing on the bacterial isolates was done using Kirby-Bauer Disk diffusion method. These resistant isolates were also subjected to molecular testing to ascertain the presence of resistant genes. The results revealed mean total Heterotrophic Bacterial Counts (THBC) for 32 samples ranged from $1.05\pm0.72 \times 10^7$ to $3.63\pm2.58 \times 10^7$ CFU/g in fish farm and waste dump samples respectively. Total Coliform Counts (TCC) ranged from $3.65\pm2.91 \times 10^5$ to $6.92\pm4.41 \times 10^5$ CFU/g in Fish farm and Waste dump samples while Total Staphylococcal count ranged from $3.80\pm1.71 \times 10^4$ to $11.46\pm9.21 \times 10^4$ CFU/g in Fish farm and Waste dump samples respectively. Total Pseudomonad Count (TPC) ranged from $0.09\pm0.09 \times 10^3$ to $2.47\pm1.67 \times 10^3$ CFU/g in Home soil and Fish farm samples. There was a difference ($p\leq0.05$) in total heterotrophic bacterial, Staphylococcal and Pseudomonad counts, but no difference ($p\geq0.05$) in total coliform. Seventy-nine (79) bacterial isolates were identified in this study belonging to the following genera; *Bacillus* spp, *Staphylococcus* spp., *Micrococcus* spp, *Pseudomonas* spp, *Serratia* spp, *Proteus* spp, *Klebsiella* spp, *Salmonella* spp and *Escherichia* spp with Staphylococcus (58.83%) having the highest occurrence and Pseudomonas (11.11%) had the least occurrence from the samples. Antibiotic sensitivity revealed that most isolates were resistant to many antibiotics tested with the highest resistance were observed for Cefuroxime, Ceftazidime and Cefixime (100%) for the gram negative organisms and also Cefuroxime, Ceftazidime and Cloxacillin for gram positive bacteria. However, all bacteria showed sensitivity to gentamicin. Multiple Antibiotic resistance (MAR) index for all bacteria were above 0.2. Resistant bacterial isolates were identified molecularly as *S. aureus, S. sciuri, M. luteus, B. cereus, B. subtilis, P. aeruginosa, S. mercescens, P. vulgaris, E. coli* and *Salmonella typhimurium. Pseudomonas aeruginosa, Serratia mercescens, Proteus vulgaris* and *E. coli* had *CTX-M* gene present in their genome while *Staphylococcus aureus, Staphylococcus sciuri, Micrococcus luteus, Bacillus cereus* and *Bacillus subtilis* had the *mecA* gene present in their genome. This study has highlighted the rise in antibiotic resistance in bacteria from soil, hence, there is need to checkmate indiscriminate use of antibiotics in agriculture.

Keywords: Molecular Characterization, Antibiotic Resistance, Soil and Bacteria

Introduction

Soil is a critical component of the planetary health system. It plays a fundamental role in human health and well-being, primarily because most food is derived from soil-plants and represents the major pathway for the delivery of essential nutrients, such as nitrogen, phosphorus and trace elements, to humans (Beavington, 2000; Steffan *et al.*, 2018)

Antimicrobial resistance is recognized as one of the biggest threats to global public health and food security, which has received increasing attention over the past years. There are hundreds of thousands human deaths each year worldwide attributable to antimicrobial-resistant infections, and scientists predicted this number will reach 10 million in 2050 when the antimicrobial-resistant infection will become a bigger killer than cancer (O'neill, 2014)

The antimicrobial resistome has the significant relevance to human health since it is imminent, highly mobile, and has the capability of causing failures in the antibiotic treatment for infectious

diseases in human. There is a variety of routes of antimicrobial resistome transmission from soil to human including direct contact, food chain, and water/air environment as a transfer stop, consequently posing a threat to public health (Zhu *et al.,* 2019). Also for instance, antimicrobial resistance in soils can be absorbed by plants and subsequently migrate into the food chain. Meanwhile, soil resistome could also spread to water bodies by naturally surface run-off and could be aerosolized into the air, facilitating further transport of antimicrobial resistome with soil origins to human (Zhu *et al.,* 2019). Antibiotic resistance genes (ARGs) (the gene possessed by microorganisms responsible for the exhibition of antibiotic resistance traits) as a new environmental contaminant of global concern have posed serious threat to human health and resulted in great societal and economic cost worldwide (Zhu *et al.,* 2019).

Soil is a heterogeneous habitat and represents a broad spectrum of different ecological niches. Soil contains a large genetic diversity at small spatial scale, favoring exchange of genetic materials by means of horizontal gene transfer (HGT) that will contribute to Antibiotic resistance genes dissemination between bacteria and eventually acquisition by pathogen genomes, therefore threatening antibiotic therapies (Zhu *et al.,* 2019).

New antibiotic-resistant bacteria are shed from humans and animals into the environment and have become recognized as an important environmental contaminant (Forsberg *et al.,* 2012). Human activity also exposes soils to pollutants, such as antibiotics, heavy metals or disinfectants, that are themselves selective agents. These effects indicate that previously susceptible soil microbes can more readily acquire antibiotic resistance via mutation or lateral gene transfer from co-polluting resistant bacteria (Peterson and Kaur, 2018). Hence, this research is carried out to molecularly characterize antibiotic resistant bacteria from soil in Rivers State University Environment.

MATERIALS AND METHODS

Description of study Area

The study was carried out in four (4) different locations in Rivers State University, Rivers State, Nigeria; waste dump site (4.7998891 E 6.696575 N), fish farm (4.7952212 E 6.9787408 N), poultry farm (4.7998991 E 6.976885 N) and Home residence (4.7974881 E 6.976786 N) in Port Harcourt Local Government Area.

Sample Collection

A total of thirty-two (32) soil samples were collected with a sterile soil auger from the four (4) different locations under aseptic conditions from Rivers State University environment. The samples were put in a sterile black polyene bags and labelled properly, put into an ice-chest and transported to the Department of Microbiology Laboratory Rivers State University for bacteriological analyses.

Microbiological Analysis

Sample Preparation

Ten grams (10g) of the soil samples was aseptically dispensed into a beaker containing 90ml of the diluent. The beaker was gently and repeatedly shaken as it facilitates the detachment of the adhered bacteria from the soil particles as much as possible into the solution (Cheesbrough, 2005)

Bacteria Enumeration and Preservation

A serial tenfold dilution was carried out from dilutions 10^{-1} to 10^{-6}. Aliquot (0.1ml) from appropriate dilutions (10^{-5}, 10^{-3}, 10^{-2} and 10^{-1}) was spread plated in duplicates onto Nutrient Agar, MacConkey Agar, Mannitol salt agar and Centrimide agar. The plates were incubated at 37ºC for 24 hours. The colonies formed on the plates were counted and described morphologically. The colonies formed on Mannitol Salt agar were used for the enumeration of the population of *Staphylococcal* count and MacConkey for other coliforms, Centrimide agar for *Pseudomonad* counts and colonies formed on Nutrient Agar was used to estimate the total heterotrophic bacterial counts (THBC). Representative discreet colonies were purified by sub-culturing on freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hours to obtain pure culture (Taylor, 2008). The pure cultures were stored in 10% (v/v) glycerol suspension at -4^{o}C as a cryo-preservative agent.

Isolation and Identification of the Bacterial Isolates

Representative bacterial colonies were isolated based on their colonial/morphological characteristics such as the size, margin, surface, color, elevation, texture and transparency and identified through conducting series of biochemical tests such as Oxidase, Catalase, Coagulase,

Citrate Utilization, Methyl red, Indole, Voges Proskauer and sugar fermentation tests to confirm the identity of the test bacteria (Cheesbrough, 2005) (Aditi *et al*., 2017).

Antibiotic Susceptibility Testing

The antibacterial susceptibility profiles of the bacterial isolates were carried out using Kirby Bauer disk diffusion method to some antibiotics using sterile Mueller-Hinton agar. The 0.5 McFarland turbidity standards containing $x10^8$ cells was used for the standardization of the bacterial isolates. A sterile swab was deepened into the bacterial suspension and streaked over the surface of the agar plates, rotating the agar plate 60º each time to ensure even distribution of the inoculum. The plates were left to air dry for 3–5 min. Antibiotics disk impregnated with Cloxacillin (5µg), Erythromycin (5µg), Gentamicin (10µg), Ofloxacin (5µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Cefixime (5µg) and Augmentin (30µg) were aseptically placed on the surface of the inoculated agar plate containing the bacteria suspension with sterile forceps. Each disk was pressed down to make contact with the agar. The plates were then incubated for 24 hours at 33 to 35ºC in an inverted position. The zones of inhibition were measured in millimeter (mm) using a meter rule and compared to (CLSI, 2017)

Molecular Studies

DNA Extraction and Quantification

Boiling method was used for the extraction process as described by Bell *et al*. (1998). Pure culture of the bacterial isolate was put in Luria-Bertani (LB) Broth and incubated at 37oC. Zero point five millilitre (0.5ml) of the broth culture of the bacterial isolates in Luria Bertani (LB) was put into properly labeled Eppendorf tubes and filling to mark with normal saline and was centrifuged at 14000rpm for 3 minutes and the supernatant was decanted leaving the DNA at the base. This process was repeated 3 times. The cells were re-suspended in 500ul of normal saline and heated at 95^{o}C for 20 min. The heated bacterial suspension was cooled on ice (About 10minutes) 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 down-stream reactions (Bell *et al*., 1998). The extracted DNA was quantified by using the Nanodrop 1000 Spectrophotometer as described by Olsen and Marrow (2012).

Amplification of 16S rRNA and bla$_{CTX-M}$ and *MecA* Gene

The 16S rRNA Amplification was carried out using an ABI 9700 Applied Biosystems, Thermal Cycler, as described by Srinivasan *et al*. (2015). The 16s rRNA region of the rRNA gene of the bacterial isolates were amplified using the forward primer; 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and Reverse primer; 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers and bla_{CTX-M} and *MecA* primers were used on ABI 9700 Applied Bio-systems thermal cycler at a final volume of 40 µL for 35 cycles. The PCR mix includes: (Taq polymerase, DNTPs, MgCl2), the primers at a concentration of 0.5µM and the extracted DNA as template, Buffer 1X and water. 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 130V for 30 minutes and visualized on a blue light trans-illuminator for a 1500bpamplicons (Srinivasan *et al*., 2015)

DNA Sequencing

Sequencing of the amplified product was carried out using the Big-Dye Terminator kit on a 3510 ABI sequencer. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBig Dye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows; 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4minutes (Srinivasan *et al*., 2015).

Phylogenetic Analysis

Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN prior to the edition of the obtained sequences using the bioinformatics algorithm Trace edit. MAFFT were used to align these sequences. The evolutionary history was inferred using the Neighbor Joining method in MEGA 6.0 (Saitou and Nei, 1987). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

Determination of Multiple Antibiotic Resistance Index (MAR)

Multiple antibiotic resistance was done as described by Osunduya *et al*., (2013). Multiple antibiotic resistance (MAR) index was ascertained by using the formula MAR = a/b, where 'a' stands for the number of antibiotic to which the test isolates depicted resistance and 'b' stands for the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Krumperman, 1985).

Data Analysis

Statistical analysis was carried out on data obtained in the study. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for significance and means separation between the soil samples respectively. This was done using a computer-based Programme-SPSS version 25. Results were represented in tables and charts.

RESULTS

The bacterial population obtained from the various soil samples are shown in Table 1. The Total Heterotrophic Bacterial Counts (THBC) for 32 samples ranged from $1.05\pm0.72 \times 10^7$ to $3.63\pm2.58 \times 10^7$ CFU/g in fish farm and waste dump samples respectively. Total Coliform Counts (TCC) ranged from $3.65\pm2.91 \times 10^5$ to $6.92\pm4.41 \times 10^5$ CFU/g in Fish farm and Waste dump samples while Total Staphylococcal count ranged from $3.80\pm1.71 \times 10^4$ to $11.46\pm9.21 \times 10^4$ CFU/g in Fish farm and Waste dump samples respectively. Total Pseudomonad Count (TPC) ranged from $0.09\pm0.09 \times 10^3$ to $2.47\pm1.67 \times 10^3$ CFU/g in in Home soil and Fish farm samples. There was a significant difference ($p\leq0.05$) in total heterotrophic bacterial, Staphylococcal and Pseudomonad counts, but no significant difference ($p\geq0.05$) in total coliform counts.

The percentage occurrences of the various bacteria in the various samples are shown on Fig. 1. *Bacillus* spp and *Staphylococcus* spp occurred in all the locations sampled with their highest occurrence in the fish farm samples. *Micrococcus* spp occurred in the fish farm (66.67%). *Serratia* spp occurred in waste dump (66.67%) with no occurrences in other samples. *Proteus* had 50% occurrence in both fish farm and waste dump samples. *Klebsiella* spp occurred highest in fish farm samples (40%), *E. coli* and *Salmonella* each with 50% of their total occurrence in fish farm samples. *Pseudomonas* occurred in all samples with its highest occurrence in fish farm samples. All identified bacteria genera from this study occurred in the fish farm samples except *Serratia* spp.

Table 1: Microbial Population of soils from Rivers State University Environment

Sample Type	THB x10^7 Cfu/g	TCC x10^5 Cfu/g	TSC x10^4 Cfu/g	TPC X10^3 Cfu/g
FF	1.05±0.72^{a}	3.65±2.91^{a}	3.80±1.71^{a}	2.47±1.67^{b}
HS	2.31±1.29^{abc}	4.28±3.05^{a}	5.64±3.95^{ab}	0.09±0.09^{ab}
PF	2.46±1.85^{abc}	4.08±3.47^{a}	4.85±2.52^{a}	1.43±0.07^{ab}
WD	3.63±2.58^{abc}	6.92±4.41^{a}	11.46±9.21^{ab}	1.64±0.35^{ab}
P=value	0.046	0.902	0.231	0.224

KEY: **FF**-Fish farm, **WD**-Waste Dump, **P**-Poultry, **HS**-Home soil, THBC (Total Heterotrophic Bacteria count), TCC (Total Coliform Count), TSC (Total Staphylococcal counts), TPC (Total *Pseudomonas* counts) *Mean with the same superscript along the columns are not significantly different (p≥0.05)

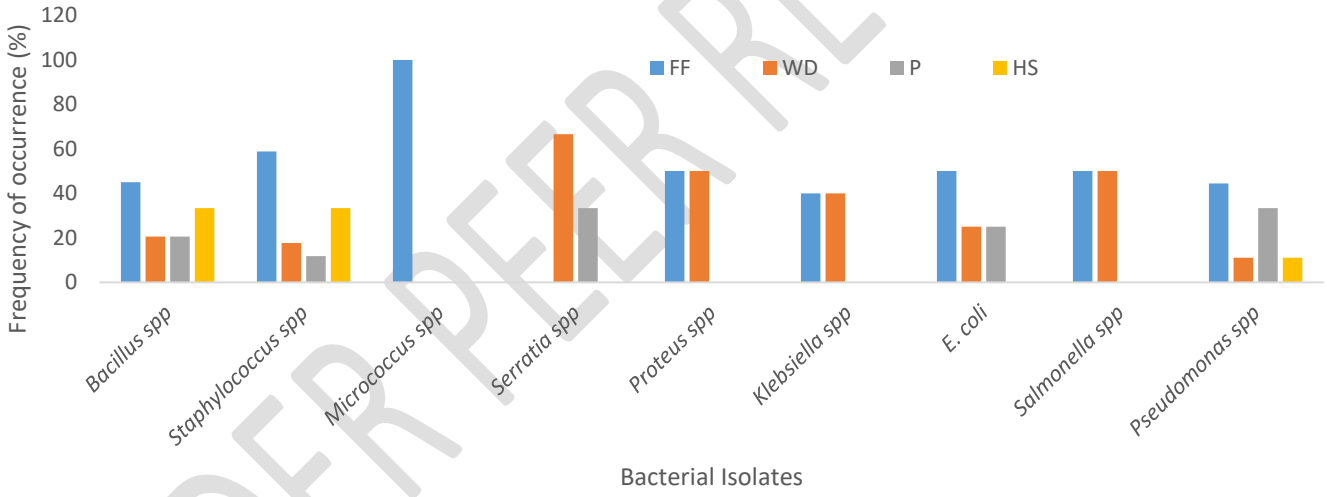
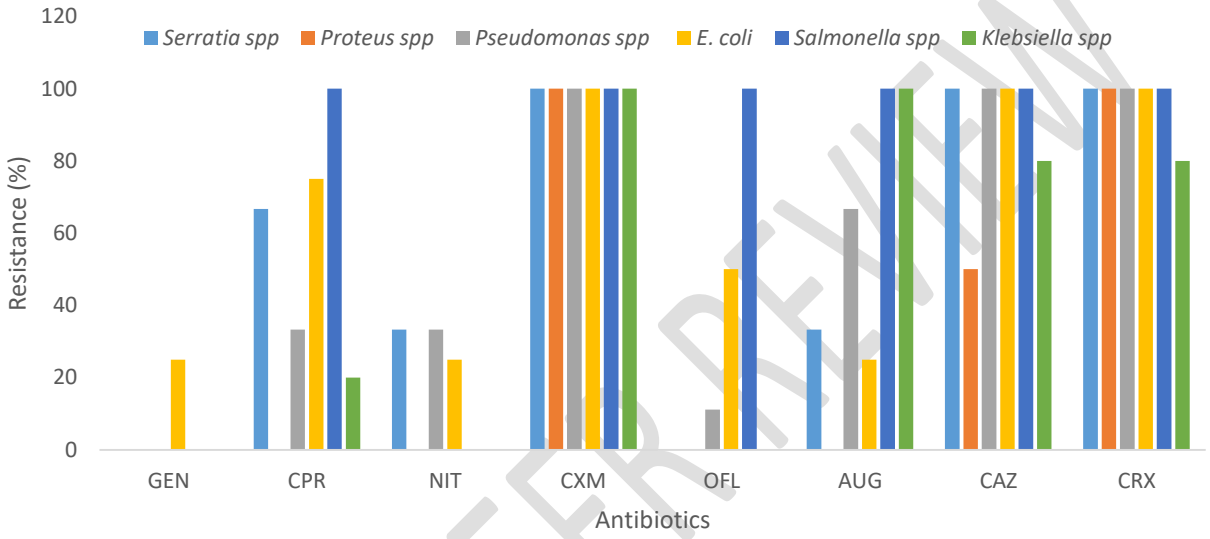


Fig. 1: Percentage Occurrence of bacteria in all the soil Samples
KEY: FF-Fish farm, WD-Waste Dump, P-Poultry, HS-Home soil

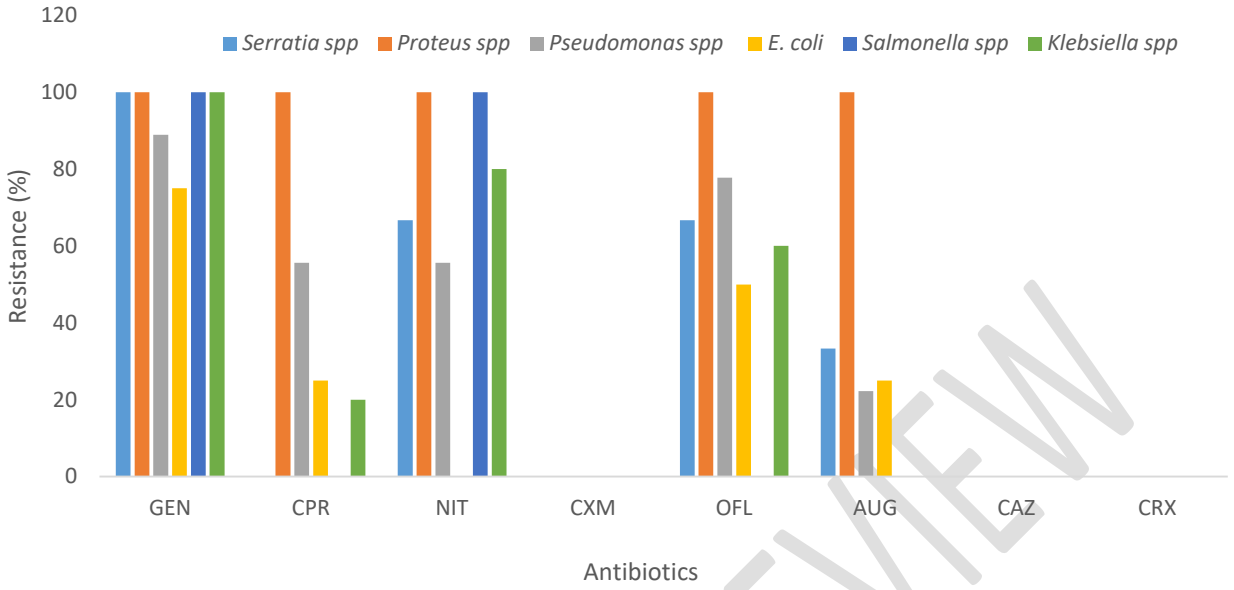
The result of the susceptibility pattern of the bacterial isolates as shown in figure 2-5 revealed that *Serratia* spp, *Pseudomonas* spp, *Staphylococcus* spp and *Micrococcus* spp were most susceptible to gentamicin (100%) and Ofloxacin (77.8%) and resistant to Cefixime, Ceftazidime and Cefuroxime (100%) while *Proteus* spp was susceptible to all antibiotics except for cefuroxime, Cefixime and Ceftaxidime in which it showed 100, 100 and 50% resistance respectively. *Salmonella* spp. was sensitivity to Gentamicin (100%) and Nitrofurantoin (100%) but resistant to all other antibiotics tested. *Klebsiella* spp. was susceptible to Gentamicin (100%),

Nitrofurantoin (80%) and Ofloxacin (60%) but showed greatest resistance to Cefixime (100%) and Augmentin (100%). *Bacillus* spp was most susceptible to Ofloxacin (97.1%) followed closely by Gentamicin (88.2%) but very resistant to Cefuroxime (100%), ceftazidime (100%) and cefixime (100%). The Multiple Antibiotic Resistance (MAR) Indices of Bacterial species isolated from soil samples are shown on Table 2 indicated that all (100%) the bacterial isolates had a MAR index greater than 0.2.



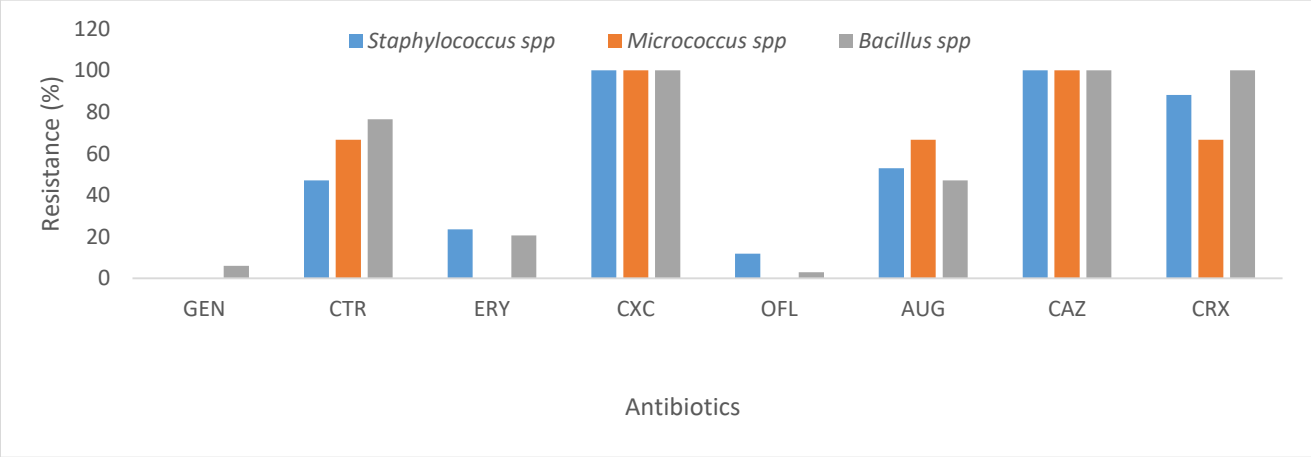
KEY: (GEN) Gentamycin, (CPR) Ciprofloxacin, (NIT) Nitrofurantoin, (CXM) Cefixime, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Fig. 2: Resistant pattern of Gram-negative bacteria to different antibiotics



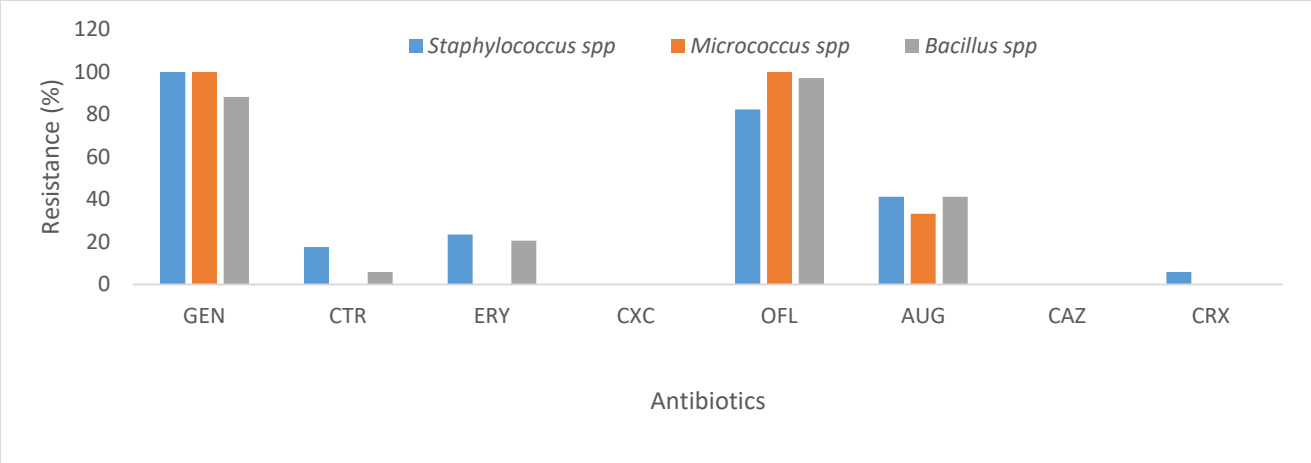
KEY: (GEN) Gentamycin, (CPR) Ciprofloxacin, (NIT) Nitrofurantoin, (CXM) Cefixime, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Fig. 3: Sensitivity Pattern of Gram-negative bacteria to different antibiotics



KEY: (GEN) Gentamycin, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) cloxacilin, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Fig. 4: Resistant Pattern of Gram-Positive bacteria to different antibiotics



KEY: (GEN) Gentamycin, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) cloxacilin, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Fig. 5: Sensitivity pattern of Gram-Positive bacteria to different antibiotics

Table 2: Multiple Antibiotic Resistance Index

MAR Index	*Serratia* spp n(%)	*Proteus* spp n(%)	*Pseudomonas* spp n(%)	*E. coli* n(%)	*Salmonella* spp n(%)	*Klebsiella* spp n(%)	*Staphylococcus* spp n(%)	*Micrococcus* spp n(%)	*Bacillus* spp n(%)
0.3	0(0.00)	1(50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(5.88)	0(0.00)	0(0.00)
0.4	0(0.00)	1(50)	4(44.44)	0(0.00)	0(0.00)	2(40)	2(11.76)	1(33.33)	5(14.71)
0.5	2(66.67)	0(0.00)	2(22.22)	1(25)	0(0.00)	2(40)	8(47.06)	1(33.33)	15(44.12)
0.6	1(33.33)	0(0.00)	2(22.22)	2(50)	0(0.00)	1(20)	4(23.53)	1(33.33)	8(23.53)
0.7	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(11.76)	0(0.00)	0(0.00)
0.8	0(0.00)	0(0.00)	0(0.00)	1(25)	2(100)	0(0.00)	0(0.00)	0(0.00)	4(11.76)
0.9	0(0.00)	0(0.00)	1(11.12)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(5.88)
Total	**3(100)**	**2(100)**	**9(100)**	**4(100)**	**2(100)**	**5(100)**	**17(100)**	**3(100)**	**34(100)**

The agarose gel electrophoresis of the amplified 16S rRNA gene of the most resistant bacterial isolates as shown on plate 1. Lanes 1 to 10 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 *CTX-M* gene bands of the isolates at 200bp. Lane L represents the 50bp

molecular ladder while lane 6 to 10 shows the isolates amplified *CTX-M* gene bands at 200bp. The amplified *mecA* gene of the 10 most resistant bacterial isolates to antibiotics is shown on Plate 3. Lane L represents the 100bp molecular ladder, while Lane 1 to 5 shows the *mecA* band at 500bp. This shows that 5 out of the 10 isolates screened for *mecA* gene had the gene present in their genetic material. 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 6.

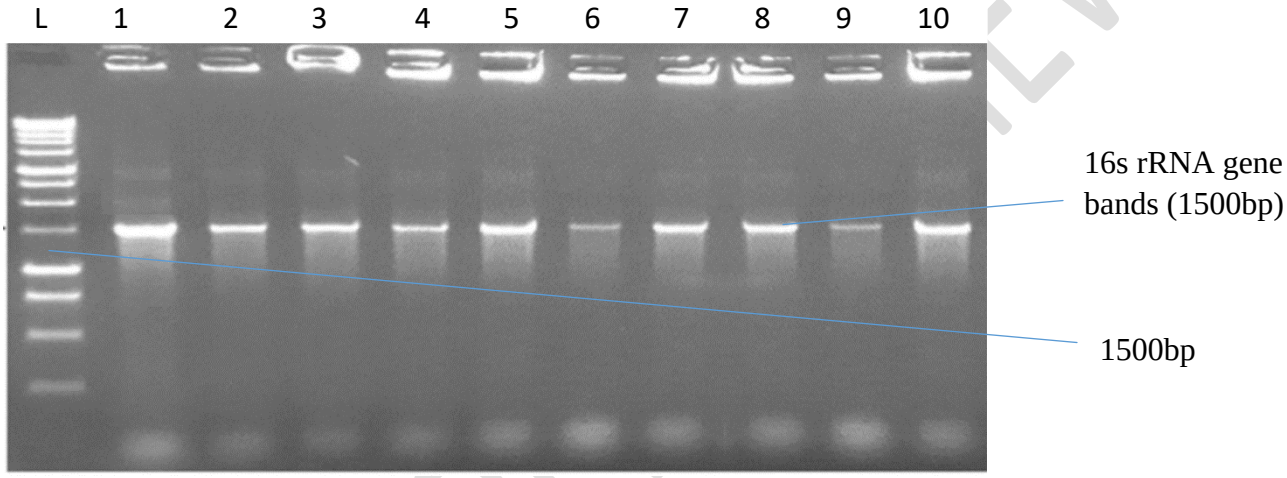


Plate 1: Agarose Gel Electrophoresis Showing the Amplified 16srRNA Gene Bands at 1500bp

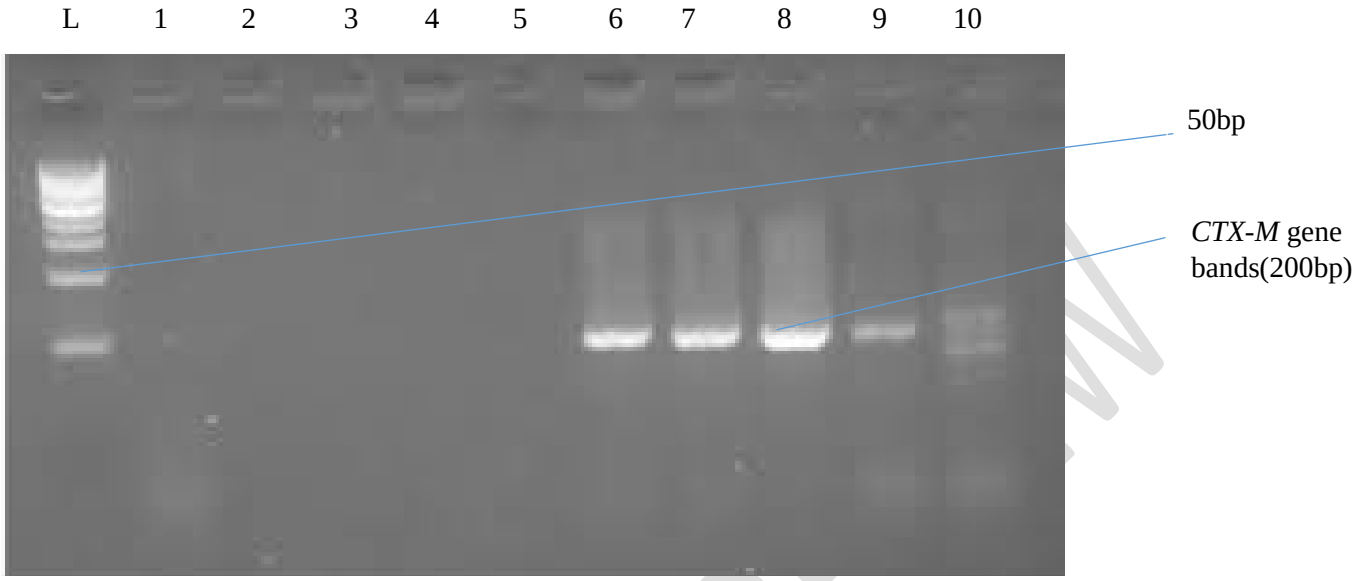


Plate 2: Agarose gel electrophoresis showing the amplified *CTX-M* gene bands of the isolates at 200bp. Lane L represents the 50bp molecular ladder.

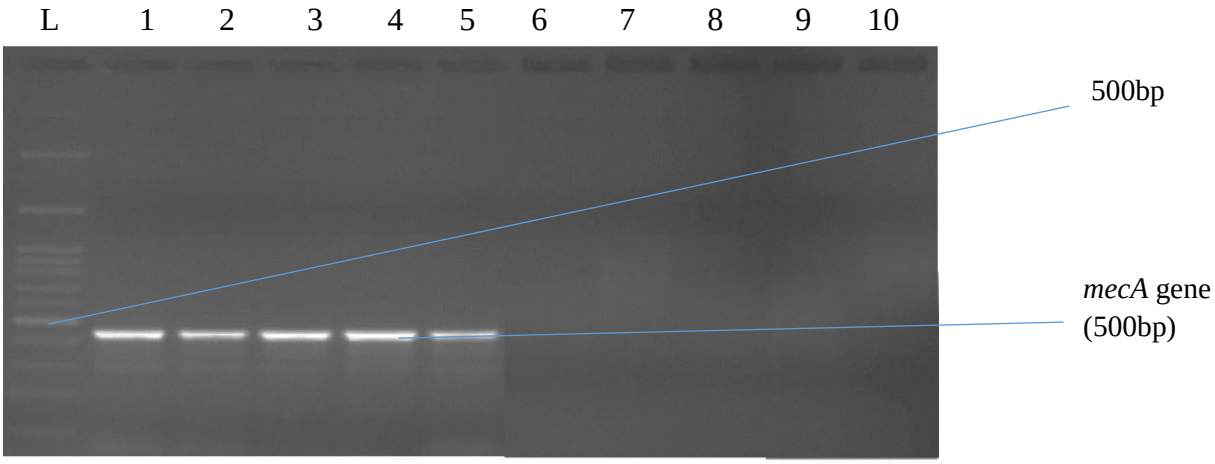


Plate 3: Agarose Gel Electrophoresis Showing the Amplified *mecA* Gene Bands of some isolates at 500bp

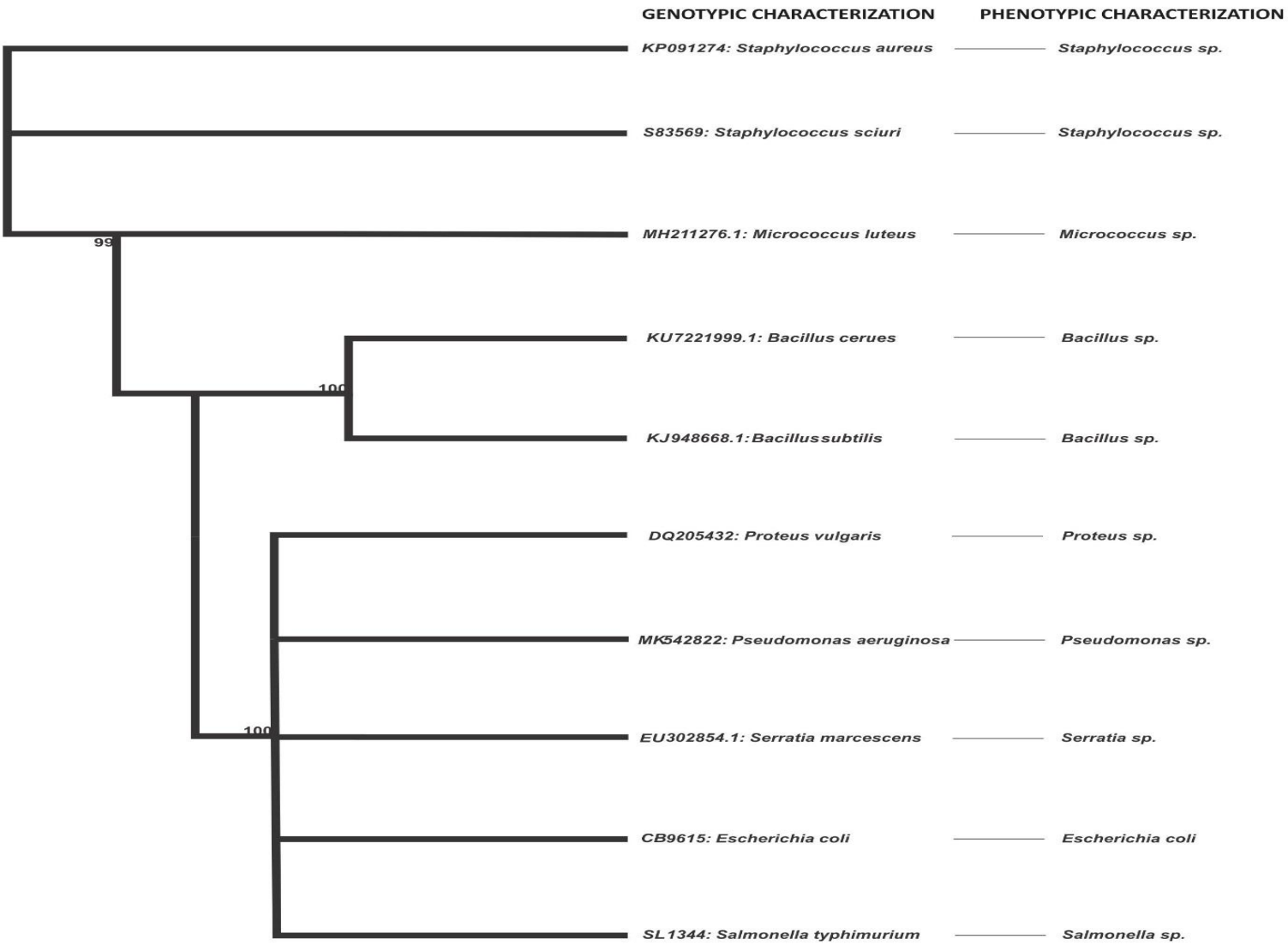


Figure 6: Phylogenetic Tree showing evolutionary distance between bacterial Isolates

DISCUSSION

Soil contain large amount of pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas* spp, *Bacillus* spp etc. which is known to cause severe infections to humans. Soil is a reservoir of antimicrobial resistance and can act as a source of resistant determinants that can spread to human pathogens and human activity also exposes soils to pollutants, such as antibiotics (Yang *et al.*, 2019)

The total heterotrophic bacteria, coliform and Staphylococcal count were high in waste dump site ($3.63\pm2.58 \times10^7$; $6.92\pm4.41 \times10^5$; $11.46\pm9.21 \times10^4$ CFU/g) in this study (Table 1) and is comparable to those btained in a similar study by Williams and Hakam (2016) who obtained high counts of total heterotrophic bacterial count ranging from 2.4×10^7 to 1.2×10^8 CFU/g from a waste dump site in Port Harcourt metropolis, Nigeria. Total Pseudomonad count was high in fish farm sample ($2.47\pm1.67 \times10^3$ CFU/g) which might be from the feeds used as well as the storage water (Armalyte *et al*., 2019). The presence of a variety of bacteria such as *Bacillus* spp., *Staphylococcus* spp, *Salmonella* spp etc. in the soils show that soil is one of the biggest reservoirs of bacterial diversity (Armalyte *et al*., 2019). The soil samples from the fish farm environs had a greater variety of the bacterial species as seen in the frequency of occurrence of bacterial isolates with all bacterial isolates from this study occurring in these soils except *Serratia* spp (Fig. 1) The occurrence of *Bacillus spp* in all the soils sampled and also as the most frequently occurring bacteria is an indication of the dominant habitation of soils by bacterial species in the genus *Bacillus* (Yadav *et al*., 2015; Garbeva *et al*., 2003). The prevalence of *Escherichia coli* in waste dump, poultry and fish farm soils in this study can be attributed to the prevailing presence of faecal contamination for poultry and fish farms as well as other waste matter around these soils. Gentamicin was the most effective antibiotic on *Serratia* spp making it the antibiotic with the highest efficacy of *Serratia* species isolates. *Serratia* spp showed marked resistance to Cefixime, Ceftazidime and Cefuroxime with all (100%). *Serratia* sensitive to Gentamicin as evidenced in this study is similar to findings by Şimşek (2019). *Serratia marcescens* isolates were most sensitive to Gentamicin with (99.4%) showing sensitivity to the antibiotic. Gentamicin, an aminoglycoside, is known to be effective against most Gram negative bacteria, including *Serratia* species, by attaching to their ribosomes and blocking protein synthesis hence their effectiveness is not unusual (Vakulenko and Mobashery, 2003). *Proteus* spp was susceptible to all antibiotics (gentamycin, ciprofloxacin, Nitrofurantoin, Ofloxacin, and Augmentin) except for cefuroxime and cefixime in which *Proteus* spp showed high resistance. The sensitivity of *Pseudomonas* spp to gentamicin and Ofloxacin in the present study is in agreement with the results of antibiogram of *Pseudomonas* spp. from the study by Akani *et al* (2019). The resistance of *Pseudomonas* spp. to ceftazidime also corroborates with findings of Yayan *et al.* (2015) in their study of antibiotic Resistance of *Pseudomonas aeruginosa.* Garba *et al*. (2012) found that *Pseudomonas* had a high sensitivity to Ofloxacin and Gentamicin while

simultaneously having a high resistance to Amoxicilin/Clavulanic acid (Augmentin) in their investigation. *P. aeruginosa* resistance is a serious public health problem, especially because it is a leading source of nosocomial infections in hospitals (Akani *et al*., 2019). The sensitivity pattern of *E. coli* and *Salmonella* spp to antibiotics in this study showed that isolates were mostly resistant to Cefixime, Ceftazidime and Cefuroxime with all isolates (100%) resistant to these antibiotics. The sensitivity of *E. coli* to ciprofloxacin are not in agreement with the findings from this study in which isolates showed resistance. In a similar study by Ali Shah *et al* (2020), the lowest sensitivity amongst *Salmonella* isolates tested was to ciprofloxacin (3.7%). This is also in agreement with the results of this study. *Klebsiella* spp isolates were sensitive to Gentamicin (100%), Nitrofurantoin (80%) and ofloxacin (60%) but showed resistance to Cefixime and Augmentin (100%), Ceftazidime and Cefuroxime (80%). *Staphylococcus* spp were most sensitive to gentamicin (100%), followed closely by Ofloxacin (82.4%); *Staphylococcus* isolates were resistant to cloxacilin (100%), ceftazidime (100%). The sensitivity of *Staphylococcus* isolates to Gentamicin and ofloxacin in this study is in agreement with results from similar studies by Nwankwo and Nasiru (2011) in which *Staphylococcus aureus* susceptibility to Ofloxacin was 76.6% and Gentamicin 73.4%. *Micrococcus* spp in this study showed 100% sensitivity to gentamicin and ofloxacin and 100% resistance to Cloxacillin and Ceftazidime. *Bacillus* spp in this study showed highest resistance (100%) to cloxacillin, ceftazidime and cefuroxime while isolates were sensitive to gentamycin (88.2%) and ofloxacin (97.1%) (Fig.2-5) which is in agreement with results from the studies of antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates by Fiedler *et al.* (2019) and the resistance demonstrated by *Bacillus cereus* might also be as a result of their ability to form spores (Forsberg *et al.*, 2012). Hundred percent (%) of the bacterial isolates had a MAR index ≥ 0.2 (Table 2). It's crucial to understand that MAR index values more than 0.2 indicate a high-risk source of contamination where antibiotics are often administered (Osunduya *et al*., 2013; Davis and Brown, 2016). The obtained 16S rRNA sequence from the isolate produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the bacterial isolates 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 showed a high percentage similarity to other species at 99% and 100% (Fig. 6). The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 within the *Staphylococcus* spp, *Micrococcus*

spp, *Bacillus* spp, *Proteus spp, Pseudomonas* spp, *Serratia* spp, *Escherichia* spp and *Salmonella* spp revealed a closely relatedness to *Staphylococcus aureus, Staphylococcus sciuri*, *Micrococcus luteus, Bacillus cereus*, *Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa*, *Serratia marcesens*, *Escherichia coli* and *Salmonella typhimurium*. Ten (10) bacterial isolates were the most resistant isolates to antibiotics tested. Resistant gene analysis for the detection of *CTX-M* gene in the ten (10) resistant bacterial isolates revealed that four (4) out of ten isolates screened had the *CTX-M* gene present in their genome (Plate 2 and 3). *CTX-M* and *mecA* gene is largely responsible for imparting resistance to the third/fourth generation of the cephalosporin group of antibiotics some of which were used in the study and its overproduction can even further increase the ability of isolates to resist these antibiotics completely. Literature reveals that the continued presence of cephalosporins tend to induce the over production of Beta-lactamase enzyme coded by the *CTX-M* gene and other genes and *CTX-M* gene was largely detected in this study (Bush, 2018). Presence of this gene in large quantities aided by some other intrinsic factors such as efflux pump action, reduced permeability of outer membrane, all increases the ability of bacterial isolates to resist multiple antibiotics (Dreier and Roggerone, 2015).

Conclusion and Recommendation

There was a high bacterial load, subsequent prevalence and resistant bacteria observed in this study which is a clear indication that the soil serves as a reservoir for these resistant bacteria. The current study has highlighted the alarming rise in antibiotic resistance among bacteria. The risks of resistance of bacteria to antibiotics are very high considering the MAR index values obtained from the study. The study has revealed a consistent increase in antibiotic resistance in bacteria contributed through some genes acquisition. Campaigns against the indiscriminate use of antibiotics in poultry, fish farming and other agricultural purposes is highly advised.

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