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Antibiotic Resistance Profiles of Bacterial Isolates from Soil Samples within and around Fountain University, Osogbo, Nigeria

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Abstract

Antibiotic resistance is an increasing global public health concern that extends beyond clinical settings to environmental reservoirs, including soil. This study examined soil samples from different sites within and around Fountain University to identify bacterial isolates and assessed their resistance patterns. Standard microbiological techniques (serial dilution, culture on Nutrient Agar, Gram staining, catalase, oxidase, indole, MR-VP, citrate utilization, and sugar fermentation tests) were used for characterization. Antibiotic susceptibility was evaluated using the disk diffusion method on Mueller-Hinton agar. The identified bacterial isolates *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Serratia marcescens*, and *Alcaligenes faecalis*, showing varying resistance profiles. Some isolates exhibited multidrug resistance, indicating soil as a potential source of resistance gene transfer to clinical pathogens. The findings highlight the importance of environmental surveillance, effective waste management, and responsible antibiotic use. These measures are essential for reducing the environmental spread of antibiotic resistance and supporting a One Health approach to antimicrobial resistance control.

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1.0 Introduction

Soil biodiversity, as defined by the Convention on Biological Diversity, refers to the variety of life forms within the soil, including the diversity of species, genes, and ecosystems (Anthony *et al.*, 2023). Soil microorganisms, which include bacteria, fungi, archaea, viruses, protozoa, and microscopic algae, play a critical role in maintaining soil health and fertility (Islam *et al.*, 2020). The loss of soil microbial diversity can have profound implications for ecosystem services such as nutrient cycling and disease resistance, which are essential for crop growth and health (Zhao *et al.*, 2024).

Current research has extensively documented factors such as soil pH, moisture, temperature, and land management practices as critical determinants of microbial community structure. Soil plays a major role as a source of nutrients in the environment; it serves as a shelter for plants and other species, and works as a large bioreactor, where pollutant decomposition as well as nutrient alteration occurs. Over the years, soil bacteria have become increasingly antibiotic resistant, despite more stringent antibiotic-use regulations in medicine and agriculture (Kumar *et al.*, 2019).

Soil serves as one of the richest microbial reservoirs on earth, containing diverse communities of bacteria, archaea, fungi, viruses, and protozoa. These microorganisms are central to critical ecosystem functions such as organic matter decomposition, nutrient cycling, and the maintenance of soil structure and fertility (Tripathi *et al.*, 2018). They also engage in symbiotic relationships with plants, facilitating nutrient uptake and enhancing resilience to stress. Recent studies emphasize the soil microbiome's importance in sustainable agriculture. Beneficial soil microbes aid in nitrogen fixation, phosphorus solubilization, and the production of plant growth-promoting substances (Banerjee *et al.*, 2019).

Soil serves as a major natural reservoir of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), functioning as a continuous source of resistance traits that predate human antibiotic use (Cycoń *et al.*, 2019). With escalating use of antibiotics in human medicine, animal farming, and agriculture, there has been increased environmental contamination through wastewater, manure application, and crop irrigation, driving the spread and enrichment of ARGs in soil ecosystems (Xiao *et al.*, 2023).

Studies conducted between 2019 and 2025 illustrate that soils receiving antibiotic-rich manures, or those irrigated with reclaimed water, show elevated abundances of ARGs especially genes conferring resistance to sulfonamides, tetracyclines, aminoglycosides, and macrolides. This increase is often accompanied by a greater presence of mobile genetic elements facilitating horizontal gene transfer (HGT) (Lau *et al.*, 2020). Meanwhile, urban and agricultural soil environments have been identified as hotspots for ARG dissemination, with anthropogenic pollutants including antibiotics, heavy metals, and non-antibiotic pharmaceuticals acting as co-selective pressures that enhance gene exchange among soil microbes (Gentile *et al.*, 2024).

Beyond its environmental relevance, antibiotic resistance in soil carries direct public-health significance: soil functions as a reservoir of resistance genes that can be transmitted to human pathogens through horizontal gene transfer, contributing to the emergence of multidrug-resistant strains that reduce therapeutic options and increase morbidity (Larsson and Flach, 2022). Agricultural soils in particular show elevated levels of antibiotic-resistant bacteria, and where produce is grown in contaminated soil or irrigated with untreated water, resistant organisms and resistance genes can enter the food chain, raising food-safety concerns (Ifedinezi *et al.*, 2024).

Resistance in soil bacteria arises through several converging mechanisms, including enzymatic drug inactivation (e.g., β -lactamases), multidrug efflux

pumps, alteration of antibiotic target sites, and reduced membrane permeability, all of which can act synergistically to confer high-level resistance (Belay *et al.*, 2024; Hasan *et al.*, 2021). These traits are mobilized and disseminated within and beyond the soil resistome largely through horizontal gene transfer.

Both natural and anthropogenic factors drive the accumulation of resistance determinants in soil. Antibiotic-producing Actinobacteria carry intrinsic self-resistance genes that form part of the natural soil resistome (Mukhopadhyay *et al.*, 2024), while anthropogenic inputs particularly livestock manure, with reported ARG concentrations of 10^6 – 10^{11} copies per gram, and industrial or municipal wastewater substantially amplify resistance gene loads in agricultural and urban soils (He *et al.*, 2020; Han *et al.*, 2022).

Antibiotic resistance is therefore a growing global crisis in which environmental reservoirs complicate control efforts. Agricultural and urban practices such as the application of manure, sewage sludge, and pharmaceutical waste introduce antibiotics and ARGs into soil, generating selective pressures that enrich resistant bacterial communities; polluted soils have been reported to harbour resistance genes spanning as many as fifteen antibiotic classes, often co-associated with heavy-metal resistance genes (Hung *et al.*, 2022; Salam, 2020). Understanding resistance patterns among soil-dwelling bacteria is therefore essential for the early detection of emerging threats and for guiding responsible antibiotic use and soil-management practices.

The study aimed to isolate and characterize soil bacteria and assess their antibiotic resistance, focusing on bacterial identification, total bacterial load, and characterization of selected isolates using biochemical and molecular techniques.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Soil samples (2–5 cm depth) were randomly collected from various locations in Fountain University, farmland, Osogbo, and Africa Bridge, Osogbo for the isolation and screening of soil-dwelling bacteria to characterize the organisms.

Media Preparation and Isolation

Nutrient agar, citrate agar, and starch agar were prepared to facilitate the growth, isolation, and identification of bacteria. The soil sample (1 g) was subjected to serial dilution, and 0.1 mL of the 10^{-5} diluent of each sample was inoculated on sterile agar plates using the pour-plate and spread-plate techniques, and incubated at 30 °C for 24 h. Colonies were sub-cultured and stored on Nutrient Agar slants

in the refrigerator at 4 °C, with sub-culturing repeated at two-week intervals to maintain viability.

2.2 Morphological and Biochemical Identification

Isolates were examined for colonial and morphological characteristics and subjected to Gram staining following 24 h of incubation. Gram-stained smears were heat-fixed, stained with crystal violet, treated with Gram's iodine, decolourized with 95 % alcohol, counterstained with safranin, and examined under oil immersion; Gram-negative cells appeared pink and Gram-positive cells appeared purple. Biochemical characterization included the catalase test (bubbling with 6 % hydrogen peroxide), the oxidase test (blue-purple colour development with tetramethyl-p-phenylenediamine reagent), the citrate utilization test (colour change on Simmons' citrate agar after 24 h at 37 °C), and the Methyl Red–Voges-Proskauer (MR–VP) test. Starch hydrolysis was assessed by flooding starch-agar cultures with Gram's iodine and observing clear zones of hydrolysis around growth. Sugar fermentation was evaluated in phenol-red broths containing maltose, sucrose, lactose, fructose, or glucose with inverted Durham tubes; a colour change from red to yellow indicated acid production, while gas bubbles in the Durham tube indicated gas production, following 24–48 h incubation at 37 °C.

2.3 Antimicrobial Sensitivity Test

Colonies from an overnight bacterial culture were aseptically inoculated into sterile nutrient broth and incubated for 24 h. Following incubation, a sterile cotton swab was immersed in the broth culture and used to uniformly inoculate the surface of Mueller–Hinton agar plates by streaking in four directions, with an additional sweep along the edges to ensure even distribution. Antibiotic discs were subsequently placed on the agar surface using sterile forceps, ensuring adequate spacing to avoid overlapping inhibition zones. Each disc was gently pressed to achieve full contact with the agar. The plates were then inverted and incubated at 35 °C for 18 h.

2.4 Molecular Characterization

One representative isolate (Sample H), selected on the basis of its broad-spectrum antibiotic sensitivity, was subjected to 16S rRNA gene sequencing at Inqaba Biotechnical Industries, Ibadan, Nigeria for confirmation of species identity. The amplified

sequence was analysed by BLAST against the GenBank nucleotide database.

3.0 RESULTS

3.1 Total Viable Count

Total viable count was carried out to determine the number of live microorganisms present in the samples. After incubation, visible colonies were counted and recorded as colony-forming units per millilitre (CFU/mL) as shown in Table 1.

3.2 Morphological Characterisation

Morphological characterization involves examining the physical appearance of bacterial colonies grown on culture media to aid in preliminary identification. In this study, isolates were assessed based on colony size, shape, colour, margin (edge), elevation, and opacity. These features help distinguish between different bacterial species, as each typically exhibits a unique growth pattern (Table 2).

3.3 Gram Staining

Following air-drying, the prepared slide was examined microscopically using the oil-immersion objective. All isolates showed positive growth on Nutrient Agar (NA), indicating the presence of culturable bacteria, including potential lactic acid bacteria. Isolates that grew on NA generally exhibited diverse morphology, as this medium supports a wide range of bacterial types, and primarily showed Gram-positive rods and cocci. The observations obtained from the microscopic analysis are summarized in Table 3.

3.4 Biochemical Tests

To identify and characterize the bacterial isolates, a series of biochemical and carbohydrate fermentation tests were conducted. The biochemical tests assessed specific enzymatic activities and metabolic capabilities of the organisms, including Methyl Red (MR), Voges-Proskauer (VP), indole production, citrate utilization, urease activity, catalase, oxidase, and hydrolysis of macromolecules such as starch, casein, and gelatin as are shown in Table 4a. In addition, sugar fermentation tests were performed using maltose, sucrose, lactose, fructose, and glucose to determine the ability of the isolates to produce acid and/or gas from these carbohydrates; the results are summarized in Table 4b

Table 1: Total Viable Count and Identified Bacterial Isolates Recovered from the Different Soil Samples

Sample Colony Forming Units (CFU/mL)	
S1	1.89×10^{-6}
S2	1.4×10^{-5}
S3	9.0×10^{-4}
S4	1.3×10^{-6}
S5	1.0×10^{-5}
S6	7.0×10^{-5}
S7	7.0×10^{-4}
S8	1.53×10^{-6}
S9	5.0×10^{-5}
S10	3.0×10^{-5}
S11	9.0×10^{-5}
S12	4.0×10^{-5}

Table 2: Cultural and Cellular Morphological Characteristics of Bacterial Isolates Recovered from the Different Soil Samples

Isolate	Colour Shape	Elevation
<i>Staphylococcus aureus</i>	Yellow Filamentous Flat	
<i>Staphylococcus epidermidis</i>	Yellow Filamentous Flat	
<i>Enterococcus faecalis</i>	Yellow Filamentous Flat	
<i>Staphylococcus aureus</i>	Yellow Filamentous Flat	

Isolate	Colour Shape	Elevation
<i>Serratia marcescens</i>	Yellow Filamentous Flat	
<i>Staphylococcus saprophyticus</i>	Yellow Filamentous Flat	
<i>Serratia marcescens</i>	Yellow Filamentous Flat	
<i>Alcaligenes faecalis</i>	Yellow Filamentous Flat	

Table 3: Gram Reaction of Identified Bacterial Isolates Recovered from the Different Soil Samples

Isolate	Gram Reaction	Shape
<i>Staphylococcus aureus</i>	Positive	Cocci
<i>Staphylococcus epidermidis</i>	Positive	Cocci
<i>Enterococcus faecalis</i>	Positive	Cocci
<i>Staphylococcus aureus</i>	Positive	Cocci
<i>Serratia marcescens</i>	Negative	Rod
<i>Staphylococcus saprophyticus</i>	Positive	Cocci
<i>Serratia marcescens</i>	Positive	Rod
<i>Alcaligenes faecalis</i>	Negative	Rod

Table 4a: Biochemical Test Characteristics of Identified Bacterial Isolates Recovered from the Different Soil Samples

Note: MR = Methyl Red; VP = Voges–Proskauer

Isolate	MR	VP	Indo le	Cata lase	Oxid ase	h Hydr	Citrat e Utiliz ation
<i>Staphylococcus aureus</i>	Negative	Negative	Positive	Positive	Positive	Negative	Positive
<i>Staphylococcus epidermidis</i>	Negative	Negative	Negative	Negative	Positive	Negative	Negative
<i>Enterococcus faecalis</i>	Negative	Negative	Positive	Negative	Negative	Negative	Positive
<i>Staphylococcus aureus</i>	Positive	Positive	Negative	Positive	Negative	Negative	Positive
<i>Serratia marcescens</i>	Positive	Positive	Negative	Positive	Positive	Negative	Positive
<i>Staphylococcus saprophyticus</i>	Positive	Positive	Negative	Negative	Positive	Negative	Positive
<i>Serratia marcescens</i>	Positive	Positive	Negative	Positive	Negative	Negative	Positive
<i>Alcaligenes faecalis</i>	Positive	Positive	Positive	Negative	Negative	Negative	Positive

Table 4b. Sugar Fermentation Test Results of Identified Bacterial Isolates Recovered from the Different Soil Samples

Isolate	Maltose		Sucrose		Lactose		Fructose
	A	G	A	G	A	G	
<i>Staphylococcus aureus</i>	+	-	-	-	+	-	-
<i>Staphylococcus epidermidis</i>	-	+	-	+	-	+	-
<i>Enterococcus faecalis</i>	-	-	-	+	+	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	-
<i>Serratia marcescens</i>	+	-	-	+	+	+	-
<i>Staphylococcus saprophyticus</i>	-	-	-	-	+	-	-
<i>Serratia marcescens</i>	-	+	-	+	-	+	-
<i>Alcaligenes faecalis</i>	+	-	+	-	+	-	+

Note: A = Acid Production; G = Gas Production; (+) = positive reaction; (-) = negative reaction

3.5 Antibiotic Susceptibility Profiles of Identified Bacterial Isolates Recovered from Different Soil Samples Based on Zones of Inhibition (mm)

Antimicrobial susceptibility testing was carried out to evaluate the response of the bacterial isolates to various antibiotics. The zones of inhibition were measured and recorded to determine the effectiveness of each antibiotic against the isolates as are presented in Table 5. Most isolates were susceptible, indicating the

antibiotic is likely to be effective in treating infections caused by these bacteria; a few were resistant, indicating the antibiotic is unlikely to be effective; and very few were moderate, signifying that higher doses of antibiotic would be needed. Susceptible zones measured ≥ 20 mm, resistant zones measured 0–4 mm, and moderate (intermediate) zones measured 10–12 mm.

3.6 Molecular Characterization and Identification of One Bacteria Isolate Recovered from the Different Soil Samples

Molecular characterization was carried out on one of the bacterial isolates using 16S rRNA gene sequencing to confirm its identity. The amplified

sequence was subjected to BLAST analysis, and the result showed a 100% similarity with an *Alcaligenes faecalis* strain (Accession No. KF475853.1), with a sequence length of 1200 base pairs and 100% query coverage. The E-value of 0.0 indicates a highly significant match, confirming the precise identity of the isolate.

Table 5: Antibiotic Susceptibility Profiles of Identified Bacterial Isolates Recovered from Different Soil Samples Based on Zones of Inhibition (mm)

Test organism	CPX	S	SXT	E	PEF	CN	APX	Z	AM	R
<i>Staphylococcus aureus</i>	20 (S)	0 (R)	12 (I)	0 (R)	11 (R)	0 (R)	0 (R)	0 (R)	0 (R)	12 (I)
<i>Staphylococcus epidermidis</i>	20 (S)	20 (S)	20 (S)	20 (S)	20 (S)	14 (I)	0 (R)	6 (I)	4 (I)	10 (I)
<i>Enterococcus faecalis</i>	0 (R)	20 (S)	20 (S)	20 (S)	20 (S)	13 (I)	0 (R)	0 (R)	0 (R)	13 (I)
<i>Staphylococcus aureus</i>	20 (S)	20 (S)	20 (S)	20 (S)	20 (S)	16 (I)	0 (R)	0 (R)	0 (R)	20 (S)
<i>Serratia marcescens</i>	0 (R)	20 (S)	20 (S)	0 (R)	0 (R)	6 (R)	16 (S)	20 (S)	0 (R)	0 (R)
<i>Staphylococcus saprophyticus</i>	20 (S)	20 (S)	0 (R)	11 (I)	20 (S)	0 (R)	0 (R)	0 (R)	0 (R)	13 (I)
<i>Serratia marcescens</i>	20 (S)	14 (I)	0 (R)	13 (I)	20 (S)	11 (I)	0 (R)	0 (R)	0 (R)	20 (S)
<i>Alcaligenes faecalis</i>	20 (S)	20 (S)	20 (S)	13 (I)	0 (R)	20 (S)	20 (S)	20 (S)	16 (S)	20 (S)

S = Susceptible (≥ 20 mm); I = Intermediate (10–12 mm); R = Resistant (0–4 mm).

Antibiotic discs used: CPX – Ciprofloxacin; S – (Streptomycin); SXT – (Cotrimoxazole); E – Erythromycin; PEF – Pefloxacin; CN – Gentamycin; APX – Amplicox; Z – Zinnacef; AM – Amoxicillin; R – Rocephin.

Table 6: Presumptive Identification of Bacterial Isolates Based on Morphological and Biochemical Characteristics

Isolate	Suspected organism
A	<i>Staphylococcus aureus</i>
B	<i>Staphylococcus epidermidis</i>
C	<i>Enterococcus faecalis</i>
D	<i>Staphylococcus aureus</i>
E	<i>Serratia marcescens</i>
F	<i>Staphylococcus saprophyticus</i>
G	<i>Serratia marcescens</i>
H	<i>Alcaligenes faecalis</i>

Table 7. Molecular Characterization — Sequence Prediction Report

S/N	Sample ID	Organism	Sequence Length (bp)	% Identity	Accession No. (BLAST hit)	E-value	Query Coverage (%)
1	H	<i>Alcaligenes faecalis</i>	1200	97.00%	KF475853.1	0.0	100%

DISCUSSION

This study investigated the antibiotic resistance profiles of bacteria isolated from various soil samples collected within and around Fountain University, Osogbo, Nigeria. The findings highlight the microbial diversity of soil ecosystems and emphasize the underscore the growing concern regarding environmental antibiotic resistance. The total viable count (TVC) confirmed the presence of culturable bacteria in all samples, indicating rich microbial populations. Notably, the sample from Adegunwa boys' hostel exhibited the highest microbial load compared to those from Love Garden and Girls' Hostel (F.K. Lawal). This suggests a higher microbial density, possibly due to greater organic matter, human activity, or moisture content in that area. High microbial density can drive intense microbial competition, which in turn promotes antibiotic production and facilitates the emergence and transfer of antibiotic resistance genes (ARGs).

Gram staining of the isolates revealed that the majority were Gram-positive cocci and rods, consistent with commonly encountered soil bacteria such as *Bacillus* spp. and Firmicutes. These organisms are known for their resilience in diverse environments and often possess intrinsic resistance mechanisms. The biochemical characterization of isolates using catalase, oxidase, MR-VP, indole, citrate, starch hydrolysis, and sugar fermentation tests

enabled accurate species-level identification.

In particular, isolate H (*Alcaligenes faecalis*) drew special attention due to its broad-spectrum sensitivity to nearly all antibiotics tested, which contrasted sharply with the multidrug resistance observed in several other isolates. Because of this exceptional sensitivity, the isolate was selected for molecular characterization to confirm its identity and better understand its genetic background.

The molecular identification results further supported the biochemical characterization of the isolate. The isolate was subjected to 16S rRNA gene sequencing at Inqaba Biotechnical Industries, Ibadan, and the sequence analysis revealed a 97% identity match with 100% query coverage to *Alcaligenes faecalis* strain (Accession No. KF475853.1), with an E-value of 0.0. These findings provide strong molecular evidence supporting the identification of the isolate as *Alcaligenes faecalis*.

Alcaligenes faecalis is a Gram-negative, rod-shaped, motile, and aerobic bacterium commonly found in soil, water, and wastewater environments. It plays a role in nitrogen cycling through processes such as denitrification and can survive in diverse, even polluted, habitats due to its metabolic versatility. Although generally considered non-pathogenic, it has increasingly been reported as an opportunistic pathogen, particularly in immunocompromised patients, where it can cause infections such

as bacteraemia, urinary tract infections, and wound infections (Choi *et al.*, 2020). Environmental strains are often susceptible to antibiotics, but clinical isolates are showing growing resistance.

CONCLUSION

This study confirmed the presence of diverse bacteria in soil, including both beneficial and potentially harmful antibiotic-resistant species. Organisms such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Serratia marcescens* were among the suspected organisms identified, with some showing multidrug resistance. These findings highlight soil as a reservoir for resistance genes, posing risks to public health. Continuous monitoring and better management of antibiotic use are crucial to controlling environmental antimicrobial resistance.

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