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Antibiotic Resistance Patterns in Soil-Dwelling Bacteria from Selected Parts of Tanzania Highlight Soil as a Reservoir of Antimicrobial Resistance

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Abstract

Antimicrobial resistance (AMR) is a growing global health challenge, with soil ecosystems increasingly recognized as important reservoirs of antibiotic-resistant bacteria and resistance determinants within the One Health framework.This study highlights soil as a reservoir of AMR in Tanzania. From 13 soil samples across six districts, 29 bacterial isolates representing nine genera were identified, mainly *Bacillus*, *Pseudomonas*, and *Enterobacter*. Gram-negative bacteria showed broader resistance than Gram-positive species, with high resistance to β-lactams (especially amoxicillin-clavulanic acid and ceftriaxone) but lower resistance to meropenem and gentamicin. Multidrug resistance occurred in 6.9% of isolates, confined to Gram-negative strains. Resistance patterns varied by site, reflecting community composition rather than geographic hotspots. These findings underscore the role of soil ecosystems in harboring resistant bacteria shaped by natural and human influences, emphasizing the need to integrate environmental surveillance into Tanzania's One Health AMR monitoring framework.

1. INTRODUCTION

The emergence of antimicrobial resistance (AMR) represents a critical global health and economic challenge. In 2019, an estimated 5 million deaths were associated with bacterial AMR worldwide and 1.3 million deaths directly attributable to resistant infections, placing AMR among the leading causes of death globally. Without proper action, this trend is projected to rise up to 10 million by 2050 (Butler et al., 2022; O`Neill, 2016; Murray et al., 2022). The burden is disproportionately high in low- and middle-income countries (LMICs), particularly in the Sub-Saharan African region, where, in the same year, approximately 1.05 million deaths were associated with AMR (Totaro et al., 2025).

Beyond mortality, AMR imposes a substantial economic burden through prolonged hospitalizations, increased use of costly second and third-line therapies, and reduced productivity (Promise et al., 2024). Without effective intervention, AMR is projected to cause significant global GDP losses by 2050, with the greatest impact expected in resource-limited settings (By & Neill, 2016).

The rapid global rise of AMR has prompted the World Health Organization (WHO) to call for urgent discovery and development of new antibiotics, particularly against multidrug-resistant (MDR) pathogens that threaten modern healthcare (OMS, 2024). Through its updated Bacterial Priority Pathogens List (BPPL) 2024, WHO identified critical bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, resistant *Enterobacterales*, and drug-resistant *Mycobacterium tuberculosis* as urgent priorities for research and treatment development (OMS, 2024). Despite the need, the antibiotic discovery pipeline has stagnated for decades, with many current candidates representing modifications of existing scaffolds rather than entirely new classes, while resistance continues to spread globally (Hutchings et al., 2019). Consequently, WHO and researchers advocate greater investment in natural product bioprospecting, genome mining, and sustainable innovation incentives to revitalize antibiotic development (MacNair et al., 2023).

Soil-dwelling bacteria constitute a vast reservoir of natural antibiotics and antibiotic resistance genes (ARGs), as over 70% of clinically relevant antibiotics originate from soil-dwelling bacteria (Bizuye et al., 2013; Khanna et al., 2011). The ability of soil bacteria to produce antibiotics is intrinsically linked to the concurrent evolution of resistance mechanisms. This is particularly true, as anthropogenic pressures from agricultural antibiotic use, manure application, pharmaceutical waste, and hospital effluents can enrich soil ecosystems with resistant populations, thereby facilitating the horizontal transfer of ARGs to other human and animal pathogens (Nesme & Simonet, 2015; Sassi et al., 2025).

In Tanzania, AMR surveillance efforts have largely concentrated on clinical and veterinary isolates, with limited investigation of antibiotic-resistant bacteria from soil ecosystems. This is despite the growing recognition of AMR as a priority within the One Health framework (Mudenda et al., 2023). Hence, environmental bacterial reservoirs of resistance remain insufficiently explored in many LMICs.

Since soil represents both a major environmental resistome and an important reservoir of antibiotic-producing microorganisms, characterization of antibiotic susceptibility patterns among soil bacteria can provide valuable insights for antibiotic discovery programs. Resistance profiling may help identify bacteria with intrinsic self-resistance mechanisms often associated with antibiotic biosynthesis pathways, thereby supporting prioritization of promising isolates for downstream natural product investigations (Allen et al., 2010; Hopwood, 2007).

The antibiotic panel used in this study comprised natural product-derived antibiotics, semi-synthetic derivatives, and fully synthetic antimicrobial agents. Natural product-derived antibiotics included erythromycin, gentamicin, and vancomycin, which originate from environmental microorganisms (Hutchings et al., 2019). Semi-synthetic antibiotics included β-lactam derivatives such as amoxicillin-clavulanic acid, cefepime, ceftriaxone, meropenem, and piperacillin-tazobactam, which were chemically modified from naturally occurring antibiotic scaffolds (Katz and Baltz, 2016). In contrast, ciprofloxacin and trimethoprim-sulfamethoxazole are synthetic antimicrobial agents with no direct natural environmental analogues (Larsson and Flach, 2022). This distinction is important in interpreting resistance patterns among soil bacteria because resistance to natural product-derived antibiotics may reflect ancient ecological competition and intrinsic environmental resistomes, whereas resistance to synthetic antibiotics may indicate anthropogenic selective pressures associated with clinical use, agricultural activities, and environmental contamination (Dcosta et al., 2011; Larsson and Flach, 2022).

Therefore, this study aimed to isolate cultivable soil-dwelling bacteria with antibacterial potential and determine their antibiotic susceptibility patterns from selected organic-rich and seawater-logged environments across Tanzania.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

Ethanol (Carlo Ebra Reagents S.A.S, France), VITEK® MS-CHCA (α-cyano-4-hydroxycinnamic acid) matrix (BioMérieux S.A., Marcy-l'Étoile, France), Muller-Hinton Agar (Neogen, UK), Muller Hinton Broth (HI MEDIA, India), R2A Agar (Chaitanya Agro Biotech Pvt. Ltd., India), Lysogeny Broth (LB) (Neogen, UK), Bacteriological Agar (Neogen, UK), semi-permeable membrane filters 0.22 µm (Membrane Solutions, China), pure soil bacterial isolates, and Quality control strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213).

2.2 List of Antibiotic Discs

Amoxicillin-clavulanic acid (AMC) 20/10 µg, Cefepime (CPM) 30 µg, Ceftriaxone (CRO) 30 µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (ETM) 15 µg, Gentamicin (GEN) 10 µg, Meropenem (MER) 10 µg, Piperacillin-tazobactam (PPT) 30/60 µg, Trimethoprim-sulfamethoxazole (T/S) 1.25/23.75 µg, and Vancomycin (VAN) 30 µg (HI MEDIA, India).

2.3 Key Instruments and Apparatus

Biosafety cabinet (BIOBASE Group, China), Autoclave (BIOBASE Group, China), Incubator (BIOBASE Group, China), Spectrophotometer (MRC Ltd, Israel), Cuvettes (Hellma GmbH & Co. KG, Germany), Ultrasonic cleaner (CASO MEDICAL, Germany), Compound microscope (ASKANIA RME 5, Germany), MALDI-TOF VITEK® MS (BioMérieux S.A., Marcy-l'Étoile, France), Neubauer counting chamber (HBG, Germany), Metallic core borer (52 mm diameter, 38.5 cm long), Isolation Chips (3D printed in house), Soil collection containers (Eco Consumer Products Ltd., Tanzania) and Vernier calliper (Mitutoyo, Japan).

2.4 Study sites and Soil samples collection

A total of 13 soil samples were collected from 6 different districts across Tanzania (Fig. 1**)** and the sites were selected based on organic matter content (3.5% OC), moderate pH (6.6–7.3) as guided by Tanzania Agriculture Research Institute (TARI) soil maps and salinity of the soils as indicated in Table 1. Briefly, before soil sample collection from study sites, the soil surface was cleaned by gently removing debris such as dry leaves, grasses, stones, and other organic materials to minimize contamination and ensure that the collected samples represented the actual underlying soil microbial community. Thereafter, a soil sample of about two-thirds of a 500 mL sterile plastic container was collected at 15 cm depth from a soil surface by using a sterile metallic core borer. The collected soil samples were transported to PharmRx Discovery Lab at Muhimbili University of Health and Allied Sciences (MUHAS) within 24 hours of collection, stored in a refrigerator at 4° C and processed within 72 hours.

Table 1
Distribution of soil samples collected from different districts in Tanzania and their characteristics

District	Region	No. of sites	Nature of sampling site	Soil nature
Kisarawe	Coastal	4	Natural forest reserve	High organic matter content and moderate pH
Arumeru	Arusha	2	Agroforestry and vegetative slope of Mt. Meru	
Rungwe	Mbeya	1	Water-rich natural landscape with nearby agricultural activities	
Mlele	Katavi	1	Wetlands during rainy season with herbivore herds	
Mtwara Dc	Mtwara	1	Near residents with agricultural activities	
Kinondoni	Dar-es-salaam	4	Periodically sea-water logged areas	High salinity

Figure 1A map of Tanzania showing all soil samples collection sites

2.5 Soil bacteria isolation

Soil bacteria were isolated using the isolation chip (iChip) technology as described in our previous work (Hidat et al., 2026). Briefly, 1 g of soil was suspended in 10 mL sterile phosphate-buffered saline (PBS) in a 15 mL sterile Falcon tube. The suspension was vigorously shaken and subjected to ultrasonication at 40 kHz for 40 seconds to detach bacterial cells from soil particles. The suspension was allowed to settle for at least 10 minutes, after which the supernatant was aseptically transferred into a sterile test tube and served as the bacterial stock suspension. Two ten-fold serial dilutions (10^{-3}–10^{-4}) were prepared in PBS to obtain an appropriate working concentration. The estimation of viable bacteria cells was previously described (Hidat et al., 2026). Briefly, 100 µL of the working suspension was mixed with 100 µL of safranin and incubated for 15 min. Approximately 10 µL of the above suspensions was loaded onto a hemocytometer, and bacterial cells were enumerated microscopically at 1000× magnification using standard counting procedures.

The obtained values informed further dilution of the respective suspension to an estimated final concentration of 10^3 CFU/mL upon mixing with 20 mL molten LB agar (45° C). This concentration was selected to ensure approximately 1 bacterial cell per through-hole of the iChip (Hidat et al., 2026; Nichols et al., 2010). The sterilized central iChip plate was thereafter pressed into the inoculated molten agar to load the agar-cell mixture into the through-holes. Upon solidification, excess agar was scraped off from the sides using a spatula, yielding discrete agar plugs within the through-holes. The central plate was then sandwiched between two semi-permeable membranes and the outer plates using metallic fasteners. The assembled iChips were re-embedded into their corresponding native soil samples within a 1 L plastic container covered with gauze mesh and incubated at ambient temperature (27–30°C) for 14 days (Alessi et al., 2018; Berdy et al., 2017; Hidat et al., 2026). Periodic irrigation with sterile-distilled water was performed every 48 hours to ensure adequate moisture and promote the diffusion of soil-derived nutrients.

After incubation, iChip devices were retrieved, cleaned to remove excess soil, and aseptically disassembled. Individual agar plugs were carefully extracted and transferred onto agar plates containing R2A agar enriched with lyophilized water-soluble extracts from the respective soil sample. The extracts were prepared by autoclaving 500 g of soil in 500 mL of distilled water, as described in previous studies with modifications (Chaudhary et al., n.d.; George et al., 2011). The plates were incubated at 30°C for 48–72 hours until visible colony development was observed, generating successfully domesticated soil bacteria.

2.6 Identification of soil bacterial Isolates by MALDI-TOF VITEK® MS

The pure bacterial cultures were obtained by sub-culturing isolates on LB agar and incubated at 37° C for 18 hours to ensure optimal growth and purity. The isolates were identified using Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) with the VITEK® MS system, following the manufacturer's instructions and established protocols (Dubois et al., 2012). A single well-isolated colony was selected and directly smeared as a thin layer onto a designated spot on a VITEK® MS target slide using a VITEK PICKME™ Pen. The smear was overlaid with 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution and air-dried at room temperature (Bizzini et al., 2011).

The prepared target slides were then loaded into the VITEK® MS instrument, and spectra were acquired in positive linear ion mode over a mass range of approximately 2,000–20,000 Da. The generated spectra were automatically analyzed using the VITEK® MS reference database, and identification was assigned by comparison with validated reference spectra. Identification confidence was interpreted based on the VITEK® MS confidence values, with high-confidence results accepted at the species or genus level. Isolates with low-confidence scores or no identification were suggested for further identification through Next-Generation sequencing (Rychert, 2019).

2.7 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the identified bacterial isolates was tested using Kirby-Bauer disc diffusion assays with modifications and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Matuschek et al., 2014).

2.7.1 *Inoculum Preparation*

The bacterial inoculum was prepared by using an overnight culture of a well-isolated colony from an agar plate, picked with a sterile wire loop, into 2 mL LB broth in a sterile test tube, and incubated in a shaking incubator at 37° C and 200 RPM overnight. Fresh cultures were thereafter prepared by drawing 200 µL of the overnight culture into 20 mL of LB broth and incubating for 5–6 hours under the same conditions. The bacterial inoculum was standardized to 10^8 CFU/mL in 1 mL of Muller-Hinton Broth (MHB) by determining their respective OD_{600} values and using a Newmann's correlation curve.

2.7.2 Inoculation of agar plates and application of antibiotic discs

About 100 µL of the standardized inoculum was transferred onto an MHA agar plate and evenly distributed using a glass spreader. The plates were allowed to air dry for 5 minutes under sterile conditions. Antibiotic discs were thereafter applied firmly using a sterile tweezer within 15 minutes of inoculation. The choice of an antibiotic for the given isolate was guided by the EUCAST recommendations from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 16.0, 2026. https://www.eucast.org."

The plates were subsequently incubated at 35° C for 18 hours, after which the Zones of Inhibition (ZoI) were measured in triplicate to the nearest millimeter using a vernier caliper, followed by calculation of the mean. Based on the EUCAST reference breakpoints, the susceptibility was interpreted and recorded as S (Susceptible), I (Susceptible, increased exposure), or R (Resistant).

2.7.3 Antibiotic Susceptibility Testing of Quality Control Strains

Quality control strains were included to ensure the accuracy and reliability of the antibiotic susceptibility testing procedure. The reference strains used were *Escherichia coli* ATCC 25922 for Enterobacterales, *Pseudomonas aeruginosa* ATCC 27853 for *Pseudomonas* species and *Acinetobacter* species, and *Staphylococcus aureus* ATCC 29213 for *Bacillus* species. Antibiotic susceptibility testing of the quality control strains was performed alongside environmental isolates.

3. RESULTS

3.1 Identities of Soil Bacterial Isolates

A total of 29 bacterial isolates representing 9 genera were identified including 8 *Pseudomonas*, 7 *Bacillus*, 5 *Enterobacter*, 3 *Citrobacter*, 2 *Acinetobacter*, 1 *Cytobacillus*, 1 *Lysinibacillus*, 1 *Klebsiella*, and 1 *Delftia* (Table 2). MALDI-TOF MS analysis identified most bacterial isolates to the species level with confidence above 99%, while a few were identified with species-level ambiguity, yielding equal-probability (50/50%) matches for closely related taxa.

Table 2
MALDI-TOF MS-based identification of soil bacterial isolates and corresponding confidence levels

Isolate ID	Sampling site	Bacterial isolate	Family	Identity %confidence
CK01-A	Kisarawe	*Bacillus cereus*	*Bacillaceae*	99.9
CK01-B	Kisarawe	*Bacillus thuringiensis*	*Bacillaceae*	99.9
CK03-A	Kisarawe	*Enterobacter roggenkampii*	*Enterobacteriaceae*	99.9
CK03-D	Kisarawe	*Citrobacter braakii*	*Enterobacteriaceae*	99.9
CK03-I	Kisarawe	*Citrobacter freundii*	*Enterobacteriaceae*	99.9
CK04-2	Kisarawe	*Enterobacter kobei*	*Enterobacteriaceae*	99.9
CK04-B	Kisarawe	*Enterobacter asburiae*	*Enterobacteriaceae*	99.9
CK04-5	Kisarawe	*Klebsiella oxytoca*	*Enterobacteriaceae*	99.9
CK05-B	Kisarawe	*Pseudomonas oleovorans/mendocina*	*Pseudomonadaceae*	50/50
CK05-F	Kisarawe	*Citrobacter youngae*	*Enterobacteriaceae*	99.9
AA01-A	Arumeru	*Pseudomonas fluorescens*	*Pseudomonadaceae*	99.0
AA01-B	Arumeru	*Enterobacter asburiae/cloacae*	*Enterobacteriaceae*	50/50
AA01-C	Arumeru	*Pseudomonas fluorescens/chloraphis*	*Pseudomonadaceae*	50/50
AA01-H	Arumeru	*Acinetobacter schindleri*	*Moraxellaceae*	99.9
AA02-C	Arumeru	*Pseudomonas putida*	*Pseudomonadaceae*	99.9
DK01-C	Kinondoni	*Bacillus licheniformis*	*Bacillaceae*	99.9
DK02-A	Kinondoni	*Cytobacillus firmus*	*Bacillaceae*	99.9
DK03-C	Kinondoni	*Bacillus subtilis*	*Bacillaceae*	99.9
DK07-B	Kinondoni	*Bacillus amyloliquifaciens*	*Bacillaceae*	99.9
NM03-A	Mtwara DC	*Pseudomonas mendocina/oleovorans*	*Pseudomonadaceae*	50/50
NM03-G	Mtwara DC	*Pseudomonas oleovorans*	*Pseudomonadaceae*	99.9
NM03-I	Mtwara DC	*Pseudomonas mendocina*	*Pseudomonadaceae*	99.9
NM03-K	Mtwara DC	*Lysibacillus fusiformis*	*Caryophanaceae*	99.8
NM03-L	Mtwara DC	*Delftia acidovorans*	*Comamonadaceae*	99.9
KM01-D	Mlele	*Bacillus flexus*	*Bacillaceae*	99.9
KM01-E	Mlele	*Pseudomonas aeruginosa*	*Pseudomonadaceae*	99.9
KM01-F	Mlele	*Bacillus firmus*	*Bacillaceae*	99.9
MR02-A	Rungwe	*Enterobacter hormaechei*	*Enterobacteriaceae*	99.9
KM01-B	Mlele	*Acinetobacter calcoaceticus*	*Moraxellaceae*	99.9

3.2 Antibiotic Susceptibility Profiles of Soil Isolates

Gram-negative bacteria, especially members of the *Enterobacteriaceae* and *Pseudomonadaceae*, exhibited broader resistance spectra than Gram-positive *Bacillus* species, which remained largely susceptible, with only occasional resistance to erythromycin and vancomycin. At the genus level, *Enterobacter* and *Citrobacter* isolates consistently showed resistance to amoxicillin-clavulanic acid and ceftriaxone, while *Pseudomonas* species displayed variable susceptibility, including notable resistance to ciprofloxacin and cefepime. Additionally, *Delftia acidovorans* exhibited a multi-drug resistance phenotype, and meropenem resistance was uniquely observed in *Bacillus* thuringiensis as shown in (Table 3).

Table 3
Zones of inhibition (mm) of soil bacterial isolates against selected antibiotics.

Bacteria isolate		Antibiotic ZoI (EUCAST breakpoint) (mm)								
Genus	**Species**	**AMC**	**ETM**	**CRO**	**CPM**	**CIP**	**GEN**	**MER**	**PPT**	**T/S**
Pseudomonas	*mendocina/oleovorans*				26.5(21)I	16.5(26)R		22.8(18)I	28.2(18)I	
	oleovorans				19.4(21)R	10.5(26)R		19.6(18)I	25.4(18)I	
	mendocina				21.3(21)I	10.4(26)R		20.8(18)I	27.0(18)I	
	fluorescens				25.4(21)I	28.5(26)I		23.1(18)I	24.2(18)I	
	putida				27.3(21)I	25.3(26)R		25.2(18)S	26.8(18)I	
	fluorescens/chloraphis				20.7(21)R	23.1(26)R		18.9(18)I	26.1(18)I	
	aeruginosa				32.1(21)I	35.2(26)I		40.4(18)S	30.4(18)I	
Enterobacter	*roggenkampii*	7.8(19)R		23.7(24)R		31.3(22)S	22.2(17)	27.4(16)S		
	hormaechei	11.3(19)R		18.4(24)R		29.9(22)S	21.3(17)S	27.7(16)S		
	kobei	11.4(19)R		26.4(24)I		35.8(22)S	21.1(17)S	30.5(16)S		
	asburiae	10.1(19)R		18.1(24)R		25.4(22)S	21.2(17)S	27.3(16)S		
	asburiae/cloacae	6.0(19)R		16.2(24)R		27.6(22)S	22.2(17)S	27.4(16)S		
Citrobacter	*youngae*	12.6(19)R		23.1(24)R		23.5(22)I	19.5(17)S	26.1(16)S		
	braakii	10.4(19)R		23.0(24)R		19.0(22)R	19.7(17)S	26.4(16)S		
	freundii	14.3(19)R		18.1(24)R		25.4(22)S	21.2(17)S	27.3(16)S		
Bacillus	*cereus*		22.3(24)R			24.7(23)I		27.5(25)S		
	thuringiensis		24.1(24)S			26.6(23)I		12.0(25)R		
	flexus		19.3(24)R			22.7(23)R		28.1(25)S		
	firmus		23.4(24)R			29.9(23)I		41.1(25)S		
	subtilis					27.5(23)I		34.0(25)S		
	amyloliqufaciens					30.5(23)I		30.0(25)S		
	licheniformis					31.5(23)I		32.8(25)S		
Cytobacillus	*firmus*					34.5(23)I		34.5(25)S		
Lysinibacillus	*fusiformis*		17.1(24)R			10.9(23)R		27.2(25)S		
Klebsiella	*oxytoca*	8.8(19)R		23.6(24)R		26.8(22)S	21.4(17)S	26.5(16)S		
Acinetobacter	*calcoaceticus*					34.0(21)I	32.0(17)S	38.8(15)S		20.6
	schindleri					23.2(21)I	21.0(17)S	24.0(15)S		6.0(
Delftia	*acidovorans*	15.7(19)R		24.6(24)I		6.0(21)R	27.2(17)S	26.5(15)S		
Escherichia	*coli ATCC 25922*	23.0(18)S				13.1(29)R	12.1(19)R	18.8(28)R	24.6(21)S	14.4
Pseudomonas	*aeruginosa ATCC 27853*				21.2(25)R	25.3(25)S	20.9(17)S	24.3(27)R	23.9(23)S	
Staphylococcus	*aureus ATCC 29213*	25.1(19)S	25.1(23)S			22.0(21)S	25.7(19)S			32.5

Note: Antibiotic abbreviations are as follows; Amoxicillin-clavulanic acid (AMC), Erythromycin (ETM), Ceftriaxone (CRO), Cefepime (CPM), Ciprofloxacin (CIP (GEN), Meropenem (MER), Piperacillin-tazobactam (PPT), Trimethoprim-sulfamethoxazole (T/S) and Vancomycin (VAN).

_ Means not tested and blue coded are Gram positive bacteria isolates, while red coded isolates are Gram negative bacteria isolates.

Antibiotic susceptibility profiles of soil bacterial isolates demonstrated distinct and non-random resistance patterns structured by both antibiotic class and bacterial taxonomy. High levels of resistance were observed against β-lactam antibiotics, particularly amoxicillin-clavulanic acid and ceftriaxone, whereas comparatively low resistance was noted for meropenem and gentamicin, indicating preserved activity of last-line agents **(Fig. 2).**

Figure 2 Heatmap showing antibiotic resistance patterns among different bacterial strains isolated from soil samples. The colour scale represents the percentage of resistant strains within one genus, ranging from 0% (green) to 100% (red).

Analysis of resistance patterns across sampling sites did not reveal a uniform geographic hotspot of multidrug resistance. However, site-specific resistance signatures were evident. Isolates from Kisarawe-Coastal sites were predominantly composed of *Enterobacteriaceae* and consistently exhibited resistance to

β-lactam antibiotics, particularly amoxicillin-clavulanic acid and ceftriaxone. In contrast, Mtwara DC sites, characterized by a higher prevalence of *Pseudomonas* species, showed recurrent ciprofloxacin resistance, suggesting enrichment for fluoroquinolone resistance in these environments. Sites such as Kinondoni-Dar es Salaam and Mlele-Katavi, which are largely dominated by *Bacillus* species, displayed comparatively lower resistance levels, with most isolates remaining susceptible to most tested antibiotics. Overall, the observed geographic variation in resistance patterns appears to be largely driven by differences in bacterial community composition rather than by a uniform increase in resistance burden across locations.

3.3 Multidrug Resistance (MDR) Profiles

Multidrug resistance (MDR), defined as resistance to at least one antibiotic in three or more classes, was observed in 2 out of 29 isolates (6.9%). MDR was exclusively detected among Gram-negative bacteria, specifically in *Citrobacter braakii* (Table 2**)** and *Delftia acidovorans (Fig. 3*), which exhibited resistance to β-lactams and fluoroquinolones, with additional resistance to glycopeptides in the later. No MDR phenotypes were observed among Gram-positive isolates. Several isolates, particularly within *Pseudomonas, Enterobacter*, and *Klebsiella*, demonstrated resistance to two antibiotic classes.

Figure 3 Percentage resistance of soil bacterial family to selected antibiotics (VAN, MER, CIP, AMC, CRO, CPM, and T/S). Bars represent the proportion of resistant isolates within each bacterial group against antibiotics indicated by bars.

4. DISCUSSION

4.1 Identities of Soil Bacterial Isolates

The presented study reveals a diverse soil communities present in the evaluated samples of which 29 strains across 9 genera were isolated, reflecting the ecological heterogeneity of soil environments. The predominance of *Bacillus*, *Pseudomonas*, and *Enterobacter* species aligns with previous reports highlighting these taxa as dominant and adaptable components of the soil microbiota due to their metabolic flexibility and ecological resilience (Janssen, 2006; Torsvik & Øvreås, 2002).

The successful identification of isolates using MALDI-TOF MS with high confidence levels (> 99%) demonstrates the robustness of this technique for environmental bacterial characterization. However, instances of species-level ambiguity, particularly among closely related taxa such as *Pseudomonas* spp. and *Enterobacter* spp., reflect known limitations of MALDI-TOF databases in resolving phylogenetically similar environmental strains (Singhal et al., 2015). This suggests that complementary molecular approaches, such as 16S rRNA or whole genome sequencing, may be necessary to achieve higher taxonomic resolution (Rychert, 2019).

4.2 Antibiotic Susceptibility Profiles of Soil Isolates

The antibiotic susceptibility profiles observed in this study revealed structured and non-random resistance patterns, with pronounced resistance to β-lactam antibiotics such as amoxicillin-clavulanic acid and ceftriaxone. This trend is consistent with the widespread distribution of β-lactamase genes in environmental bacteria, which have evolved over time due to natural exposure to β-lactam-producing microorganisms (Allen et al., 2010). These resistance patterns may partly reflect naturally evolved defence mechanisms within soil microbial communities, as many environmental microorganisms have historically coexisted and competed with antibiotic-producing organisms (Dcosta et al., 2011; Hutchings et al., 2019).

Soil ecosystems are widely recognized as reservoirs of ARGs, forming part of the environmental resistome (Dcosta et al., 2011). While resistance in these environments is partly intrinsic and naturally occurring, it is increasingly shaped by anthropogenic activities such as agricultural runoff, improper pharmaceutical disposal, and urban contamination (Kümmerer, 2009).

In contrast, the relatively low resistance observed against meropenem and gentamicin may reflect their more restricted use, higher cost, and limited accessibility in community settings compared with commonly prescribed first-line antibiotics (Chapuma et al., 2025). Meropenem is a carbapenem classified within the WHO Watch group and is generally reserved for severe or multidrug-resistant infections because it is one of the few remaining effective options against extended-spectrum β-lactamase (ESBL)-producing Gram-negative pathogens and other difficult-to-treat bacteria (WHO., 2022). Additionally, carbapenems are expensive antibacterial agents that require hospital administration, further limiting their routine use in outpatient settings (Zaidah et al., 2017). These factors likely contribute to the preserved activity of meropenem among environmental isolates. Nevertheless, the detection of isolates with intermediate susceptibility remains concerning, as it may signal early emergence of carbapenem resistance genes in the environment, underscoring the need for continuous AMR surveillance and antimicrobial stewardship (Takkonelli Evelina et al., 2018).

4.3 Gram-Positive versus Gram-Negative Resistance Patterns

A clear distinction in resistance profiles between Gram-negative and Gram-positive bacteria was observed, with Gram-negative isolates exhibiting broader resistance spectra. This can be attributed to intrinsic structural and functional characteristics, including the presence of an outer membrane, efflux pump systems, and a high propensity for horizontal gene transfer (Blair et al., 2015).

Members of the *Enterobacteriaceae*, particularly *Enterobacter* and *Citrobacter*, demonstrated consistent resistance to β-lactam antibiotics, likely due to the production of extended-spectrum β-lactamases (ESBLs), which are widely reported in both clinical and environmental isolates (Paterson & Bonomo, 2005). Similarly, *Pseudomonas* species displayed variable resistance patterns, reflecting their genomic flexibility and intrinsic resistance mechanisms linked to environmental adaptability (Lister et al., 2009).

In contrast, Gram-positive bacteria, particularly *Bacillus* species, exhibited relatively low resistance levels. This observation is consistent with their ecological strategy, which relies more on physiological resilience such as sporulation rather than acquisition of resistance genes (Cycoń et al., 2019). However, the

detection of meropenem resistance in *Bacillus thuringiensis* represents an atypical finding, suggesting potential emergence of novel resistance traits that warrant further molecular investigation.

4.4 Site-Specific Resistance Patterns

The absence of a uniform geographic hotspot for antibiotic resistance indicates that resistance distribution is not solely dependent on location but is strongly influenced by microbial community composition and environmental characteristics. Site-specific resistance patterns observed in this study reflect the interplay between ecological conditions and microbial diversity.

For instance, Kisarawe (Coastal region), characterized by high organic matter content and moderate pH, supported a predominance of Enterobacteriaceae, which exhibited consistent resistance to β-lactam antibiotics. High nutrient availability in such environments promotes microbial proliferation and increases opportunities for horizontal gene transfer, facilitating the spread of resistance genes (Graham et al., 2016).

In contrast, Mtwara DC sites, located near residential areas with agricultural activities and dominated by *Pseudomonas* species, showed recurrent resistance to ciprofloxacin. Because ciprofloxacin is a synthetic antimicrobial agent with no natural environmental analogue, the observed resistance may suggest anthropogenic selective pressures associated with human activities and agricultural practices in the surrounding environment (Larsson & Flach, 2022). This observation may also reflect the adaptive capacity of *Pseudomonas spp*., which are known for their metabolic versatility, biodegradation potential, and intrinsic resistance mechanisms that enhance survival under environmental stress conditions (Martinez, 2009).

Sites such as Kinondoni and Mlele, dominated by *Bacillus* species, exhibited lower resistance levels, suggesting that community composition plays a more critical role than geographic proximity in shaping resistance patterns. These findings support previous studies demonstrating that environmental resistomes are structured by ecological and anthropogenic factors rather than spatial distribution alone (Berendonk et al., 2015).

4.5 Multidrug Resistance (MDR) Profiles

The relatively low prevalence of multidrug resistance (6.9%) indicates that while resistance determinants are present in the studied soils, they have not yet fully converged into widespread MDR phenotypes. The exclusive occurrence of MDR among Gram-negative bacteria, specifically *Citrobacter braakii* and *Delftia acidovorans*, is consistent with their known role as reservoirs and vectors of resistance genes due to their genomic plasticity and ability to acquire mobile genetic elements (Rice, 2008).

The identification of MDR in *Delftia acidovorans*, a typically environmental organism, is particularly noteworthy, as it highlights the increasing recognition of environmental bacteria as carriers of clinically relevant resistance traits. Additionally, the presence of isolates resistant to two antibiotic classes suggests a gradual accumulation of resistance determinants, which may eventually lead to MDR under sustained selective pressure. These findings emphasize that environmental reservoirs act as early warning systems for emerging antimicrobial resistance, underscoring the importance of proactive monitoring (Martinez, 2009).

Interestingly, the resistance profiles observed among environmental soil isolates in this study share similarities with resistance trends previously reported among clinical human pathogens in Tanzania. Several hospital-based studies have documented high resistance rates to β-lactam antibiotics, particularly ceftriaxone and amoxicillin-clavulanic acid, among clinical isolates of *Escherichia coli*, Klebsiella *pneumoniae*, and *Pseudomonas aeruginosa* (Moremi et al., 2016; Mshana et al., 2009). In addition, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae have been increasingly reported in Tanzanian healthcare settings (Manyahi et al., 2017; Moremi et al., 2021; Ndugulile et al., 2005). However, compared to the relatively low MDR observed in the present study, appears to be substantially more widespread among clinical isolates, where ESBL-producing and MDR Enterobacteriaceae are frequently encountered (Kibwana et al., 2020; Manyahi et al., 2017). The comparatively lower MDR burden observed among the soil isolates may suggest that environmental reservoirs currently represent an earlier stage in the resistance dissemination pathway, although the presence of emerging resistance traits highlights the potential role of environmental bacteria in the transmission and persistence of antimicrobial resistance within the One Health framework.

Notably, the detection of opportunistic pathogens, including *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, and *Enterobacter hormaechei*, supports the concept that soil functions as a reservoir of clinically relevant microorganisms. This finding reinforces the ecological link between environmental and clinical niches, where soil serves as a potential source of opportunistic infections and resistance determinants (Berg et al., 2005).

4.6 Implications for Public Health and Environmental Surveillance

The findings of this study reinforce the role of soil as a critical reservoir of antibiotic resistance and opportunistic pathogens, highlighting its importance within the global antimicrobial resistance network. The coexistence of diverse bacterial taxa with varying resistance profiles underscores the complexity of environmental resistomes and their potential impact on public health.

The observed variability in resistance patterns across sites further supports the need for a context-specific approach to antimicrobial resistance surveillance. These findings align with the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health (Robinson et al., 2016).

In regions experiencing rapid urbanization and agricultural intensification, such as Tanzania, environmental surveillance should be integrated into national antimicrobial resistance strategies. Soil ecosystems, in particular, should be considered not only as reservoirs but also as early indicators of emerging resistance trends, providing opportunities for timely intervention.

5. CONCLUSION

This study elucidates the diversity and antibiotic susceptibility profiles of soil-dwelling bacteria across diverse ecological settings in Tanzania. The data reveal that soil is not merely a passive reservoir of microbial life, but an active environmental resistome shaped by distinct ecological and anthropogenic pressures. Notably, resistance patterns are structured rather than random; while isolates exhibit high resistance to β-lactam antibiotics, susceptibility to critical, last-resort agents like meropenem and gentamicin remains intact. Gram-negative bacteria demonstrate broader resistance profiles than Gram-positive isolates, though multidrug resistance remains in its early stages of emergence. Crucially, site-specific variations in resistance are driven primarily by shifts in microbial community composition rather than geographic distance. The presence of opportunistic pathogens and emerging resistance traits in these environmental isolates underscores the role of soil in the persistence and dissemination of AMR. Ultimately, these findings underscore the urgent necessity of integrating environmental ecosystems into AMR surveillance frameworks using a comprehensive One Health approach. Future efforts employing molecular characterization and whole-genome sequencing are vital to decode the specific resistance genes and mobile genetic elements driving these dynamics.

Declarations

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Competing Interests

None declared.

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Author Contributions

Sabina D. Hidat: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Joan Masatu:** Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Nelson E. Masota:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization. **Rogers Mwakalukwa:** Validation, Supervision, Project administration, Formal analysis. **Chantal Bader:** Writing – review & editing and Validation. **Joseph Sempombe:** Writing – review & editing, Supervision, Resources, Methodology. **Doreen Mloka:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology.

Data Availability

All data supporting the findings of this study are available within the paper

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Figures

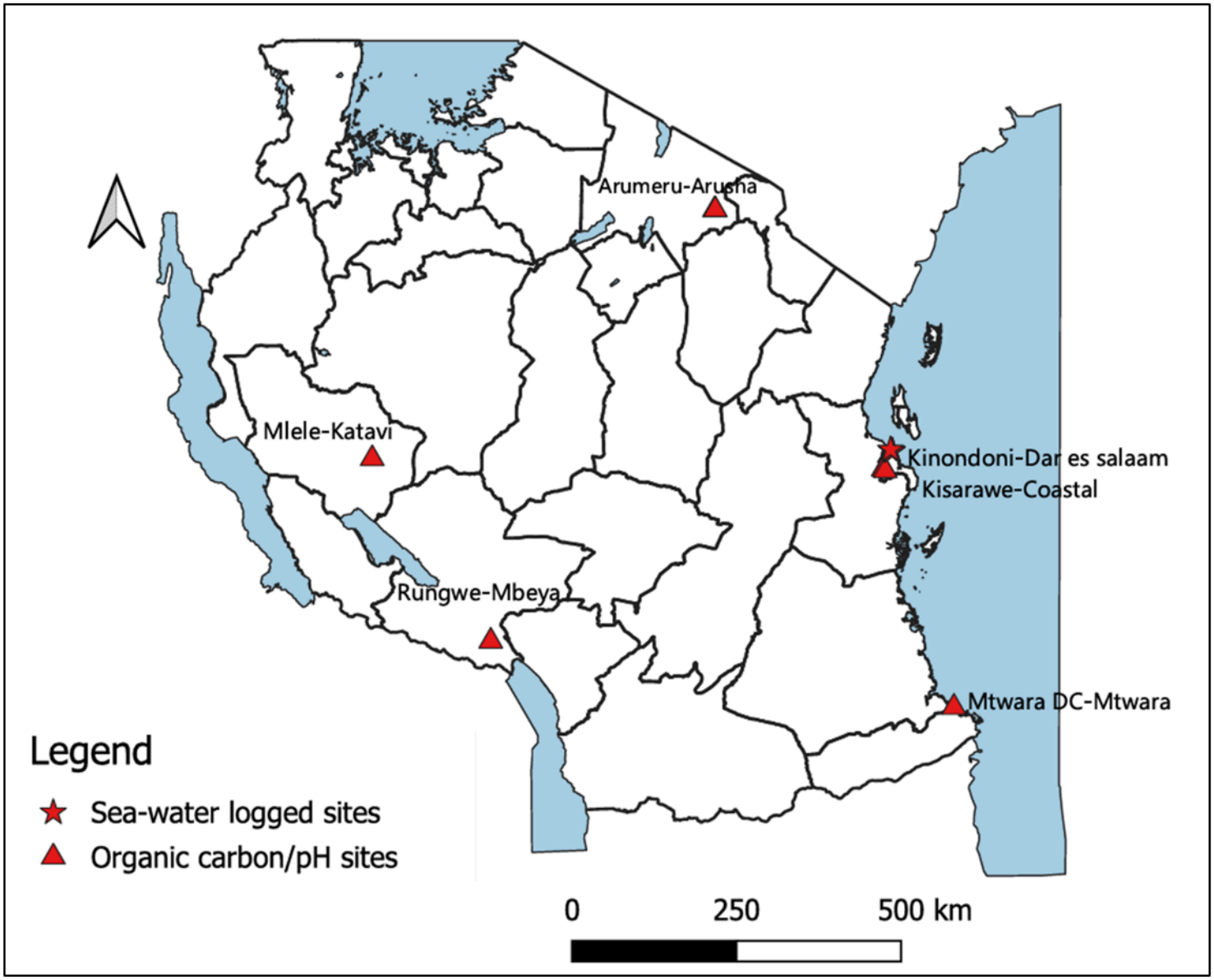


Figure 1

A map of Tanzania showing all soil samples collection sites

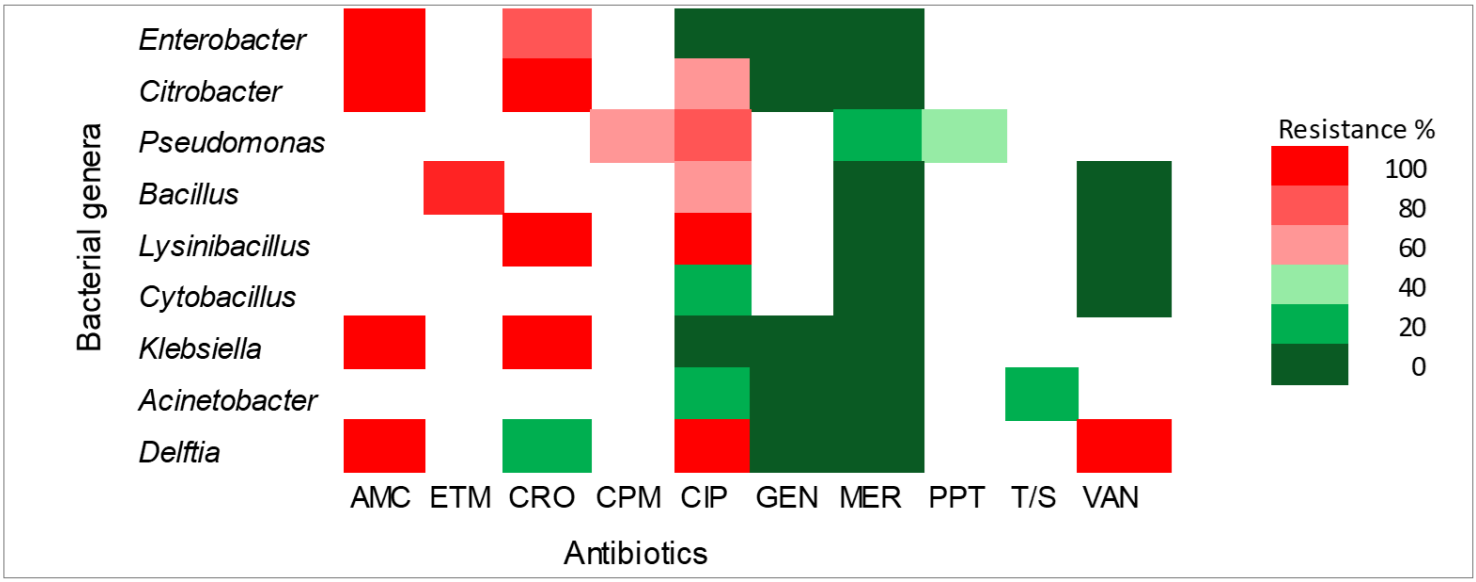


Figure 2

Heatmap showing antibiotic resistance patterns among different bacterial strains isolated from soil samples. The colour scale represents the percentage of resistant strains within one genus, ranging from 0% (green) to 100% (red).

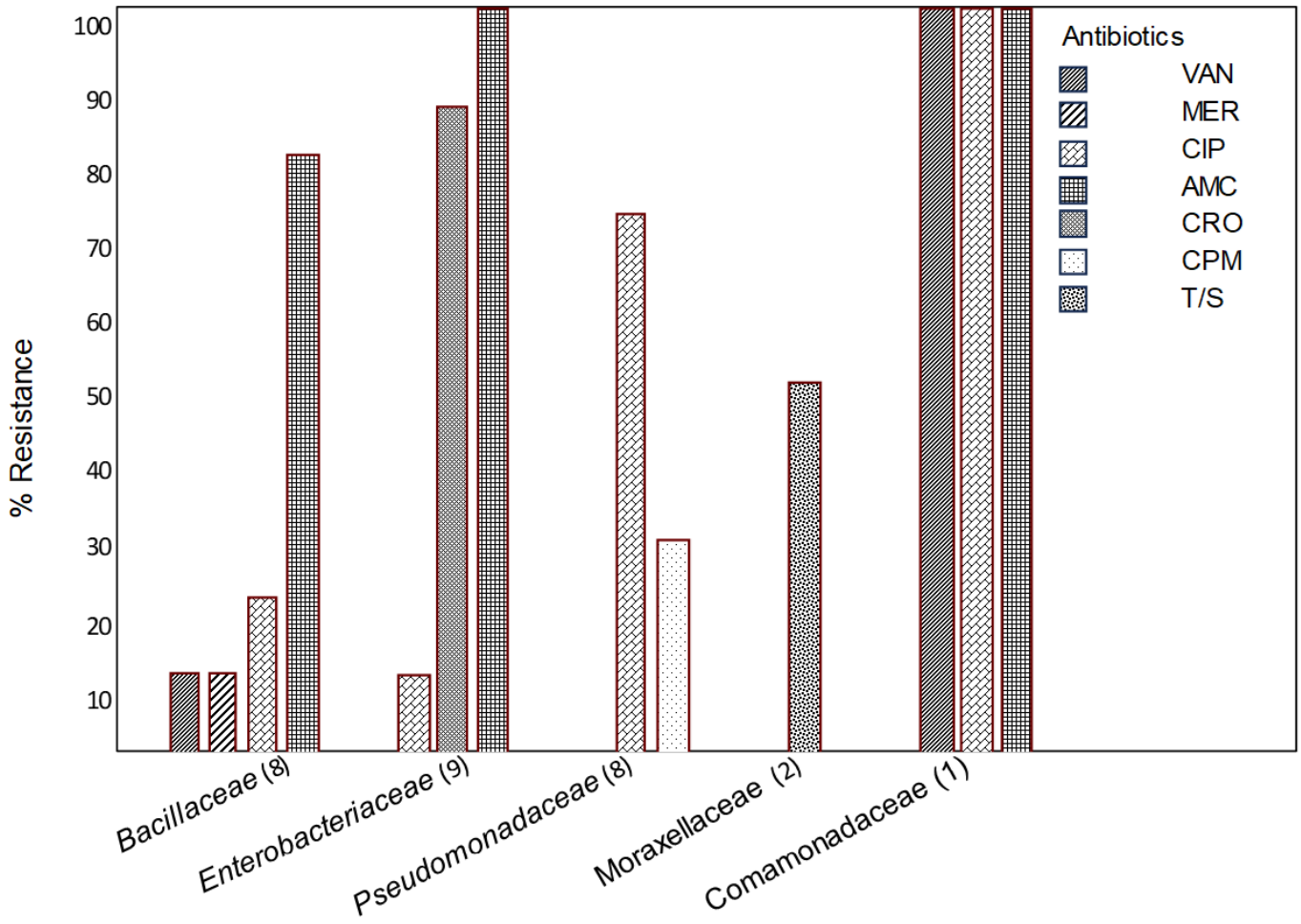


Figure 3

Percentage resistance of soil bacterial family to selected antibiotics (VAN, MER, CIP, AMC, CRO, CPM, and T/S). Bars represent the proportion of resistant isolates within each bacterial group against antibiotics indicated by bars.