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Antibiotic Susceptibility Profile of Soil-Borne Microorganisms Isolated from Selected Dump-sites in Ogbomoso, Oyo State

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Abstract
Due to the lack of a leachate collection mechanism at most dump-sites waste is known to be a source of soil pathogens. This study aimed to detect microorganisms in dump-site soils coupled with testing the susceptibility of the detected microorganisms to selected antibiotics Soil samples were collected from five separate dump-sites in Ogbomoso, Oyo State, Nigeria. From the soil samples that were collected, eight species of bacteria and eight species of fungi were isolated. The bacterial and fungal derived from the collected soil samples were tested for antibiotic susceptibility using the conventional disc diffusion method. Results obtained indicate that the microbial loads varied between 1.7 and 4.8 x 10^5 CFU/g for fungal isolates while it varied from 1.0 to 8.0 x 10^5 CFU/g for bacterial population. Fungal isolates;Alternaria alternata, Candida albicans, Rhodotorula minuta, Fusariun oxysporum, Aspergillus flavus, Aspergillus tamarii, Fusarium solani and Penicillium digitatum were detected while bacterial isolates; Bacillus subtilis, Bacillus cereus, Staphylococcus epidermis, Clostridium sp., Acetobacter sp., Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were equally detected from the dump-sites' soil samples. The bacterial species tested were completely resistant to cefuroxime, but completely susceptible to gentamicin and ofloxacin. At varying doses, the fungal isolates demonstrated resistance to and susceptibility to griseofulvin, itraconazole, and ketoconazole.Based on the results of this study, antibiotics such as gentamicin and oflaxacin should be considered first line of defense against infections caused by both soil-borne Gram-positive and Gram-negative bacteria

Keywords: Refuse, Dump-site, Soils, Microorganisms, Antibiotic resistance.

INTRODUCTION
In Nigeria, as well as in many other developing nations, both urban and rural areas are plagued by the presence of waste, such as garbage, plastics, bottles, disposable cups, discarded tires, and even human and livestock excrement. Many dump-sites especially in low- and middle-income countries, lack proper infrastructure and resources for effective waste management, leading to uncontrolled dumping and environmental degradation (Mor and Ravindra, 2023). These wastes are visually unappealing, create unsightly views, and emit unpleasant odors, particularly when their organic components are decomposed by putrefying bacteria (Gadallah, 2016). Microbial communities in dump-sites contribute to the degradation and transformation of organic matter through processes such as aerobic and anaerobic decomposition, fermentation, and methanogenesis (El-Saadony *et al.,* 2023). However, the

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ecological functions of dump-sites microorganisms can be disrupted by anthropogenic activities, such as the deposition of toxic chemicals or the introduction of non-native species, leading to alterations in microbial community structure and function (Prakash and Verma, 2022). Microorganisms thrive in environments where waste materials are dumped without consideration, endangering human health (Saha *et al*., 2021). Soil-borne microorganisms play a crucial role in ecosystem dynamics, participating in nutrient cycling, decomposition, and maintaining soil fertility (Deng, 2023).

Dump-sites pose a significant environmental and public health challenges due to the accumulation of organic matter, plastics, metals, and other potentially hazardous materials (Chavan *et al.,* 2022). These dump-sites serve as a habitat for disease-carrying organisms and other bothersome creatures that can transmit illnesses like typhoid, infantile diarrhea, and cholera to both humans and animals (Muteeb *et al.,* 2023). Waste materials pose a threat to soil quality, health, water, and the entire ecosystem (Bhat *et al.,*2022). Landfills offer a plentiful supply of microorganisms, the majority of which are disease-causing (Nieder *et al.,* 2018). Bacteria and fungi among other microorganisms, quickly colonize wastes because they feed on its components and multiply swiftly; some of these microbes are even dangerous to humans and can harbor diverse antibiotic resistance genes (Mondal and Palit, 2019). Understanding the antibiotic susceptibility profile of soil-borne microorganisms in such settings is vital for assessing the potential risks they pose and devising effective management strategies. Therefore, this study aimed to characterize the antibiotic susceptibility profile of soil-borne microorganisms isolated from selected dump-sites in Ogbomoso. Oyo State, Nigeria.

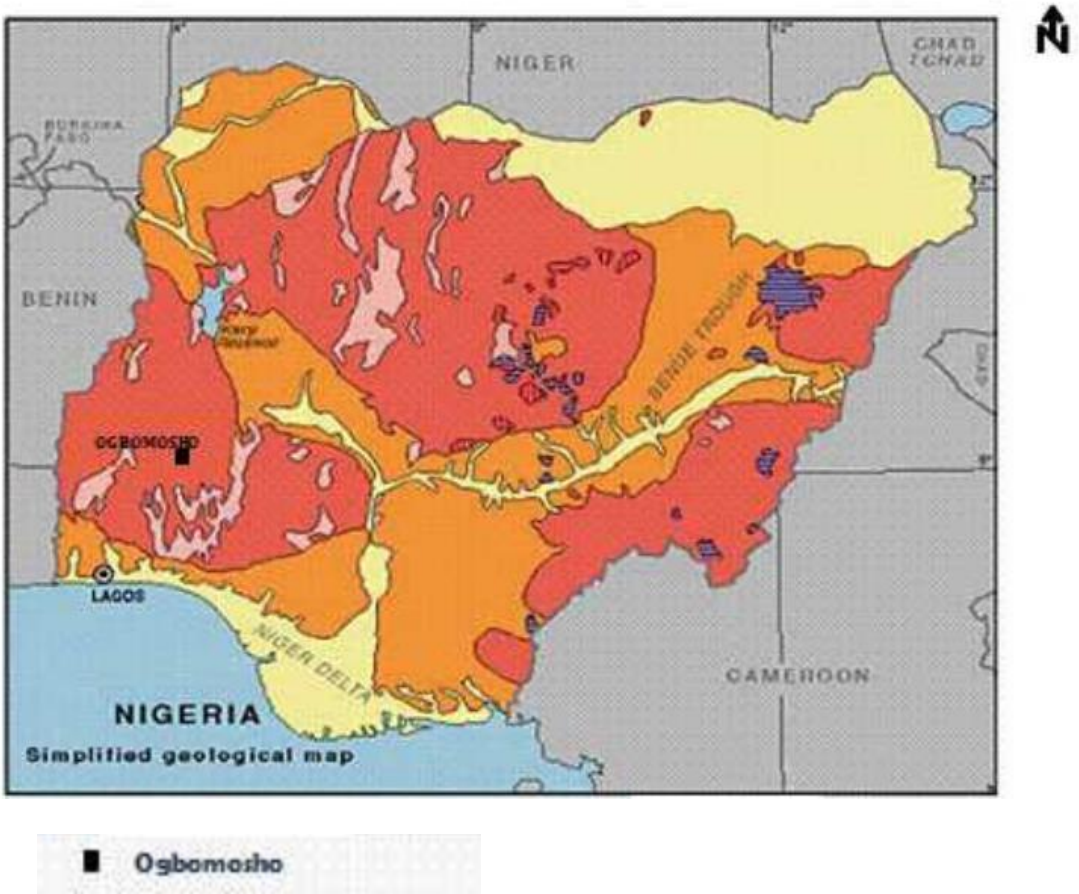

Figure 1: Geological Map of Nigeria showing the location of Ogbomoso

MATERIALS AND METHODS

Sample Collection

The study area adopted for soil collection encompassed five separate waste dump-sites in Ogbomoso, Oyo State, southwestern Nigeria, spanning from latitude 8°06'70" to 8°06'98.7" north and from longitude 4°14'28.2"E to 4°14'56.9"E. were. Soil samples from the chosen dump-sites were collected using sterilized soil auger at 10cm depth into well labelled sterile universal bottles. The universal bottles containing the soils were immediately transferred to the laboratory for analysis.

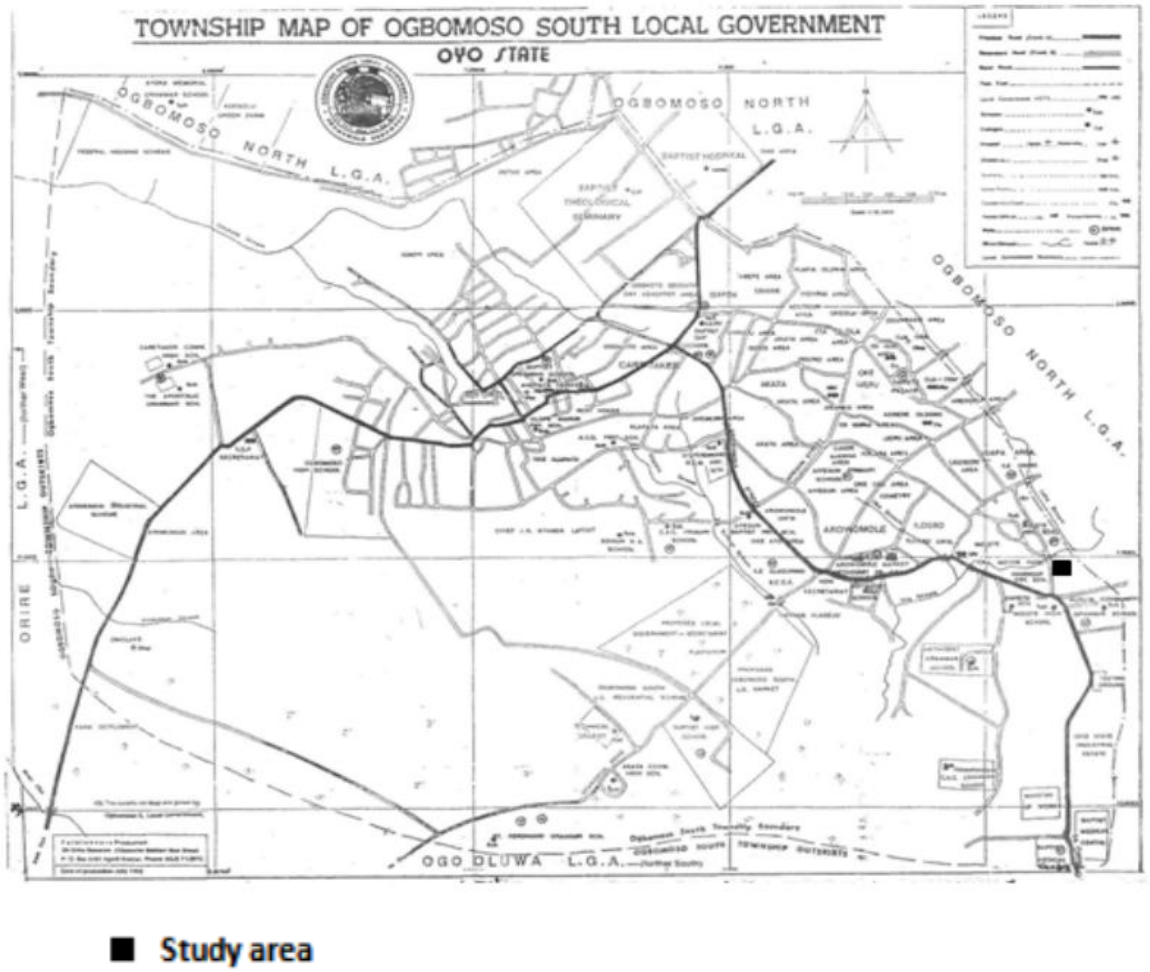

Figure 2: Map of Ogbomoso showing the location of the study area

Sterilization of Media and Materials

The media were prepared in accordance with the manufacturers'specifications and subsequently sterilized at a temperature of 121˚C for a duration of 15 minutes using an autoclave. The isolation of bacteria was performed using MacConkey agar and Nutrient Agar, whereas Fungi were isolated using Potato Dextrose Agar. The glasswares utilized were sterilized by subjecting it to a temperature of 180 ˚C in a hot air oven for a duration of 6 hours.

Isolation and Enumeration of Microorganisms

Bacteria

Soil samples collected were serially diluted and then aseptically placed onto freshly produced Nutrient agar and MacConkey agar plates using the pour plate method. The plates that were treated with the inoculum were placed in an incubator set at a temperature of 37 ˚C for a duration of 24 hours. Following this incubation period, the plates were inspected for the

presence of any growth. The discrete colonies that formed were transferred to new culture plates and placed in an incubator at a temperature of 37 ˚C for a duration of 24 hours (Ogodo *et al.,* 2022). (Plate 1).

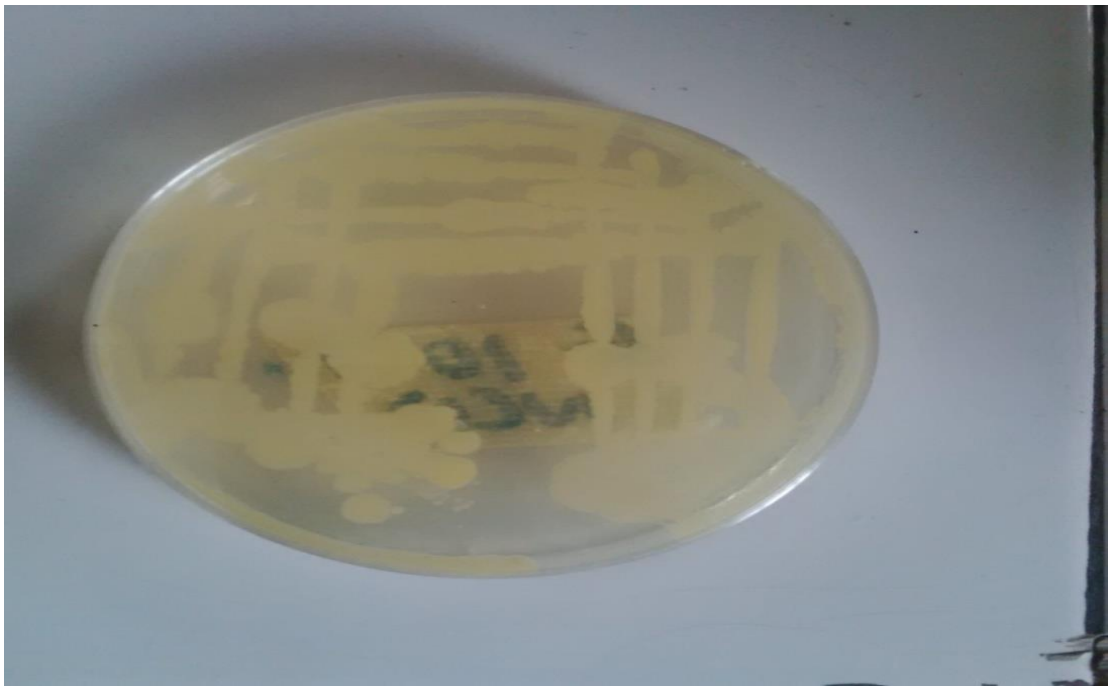

Plate1: Bacterial growth on nutrient agar medium

Fungi

One gram of soil was added into the tube containing 9mL of sterile distilled water to obtain 1/10 (stock solution) and a series of 1/100, 1/1000, 1/10,000, and 1/100,000 dilutions was prepared by adding 1mL of solution to 9 ml of sterile distilled water respectively (Waksman & Fred., 1922). One mL suspension from each dilution was transferred onto Potato Dextrose Agar (PDA) (Johnston & Booth, 1983) media. The fungal culture was raised by using spread plate technique, 0.1ml of diluted sample was plated in a sterile petri plates, containing Potato Dextrose Agar. 1% streptomycin solution was added to the medium before pouring into petri plates for preventing bacterial growth. The plates were incubated at 37 ˚C for 48 hours. After incubation colonies were examined under the microscope for ascertaining the identity of fungi with the help of lactophenol staining (Rebecca *et al* 2012). The isolated fungi were purified using streak plate technique on Potato Dextrose Agar (PDA) medium, ensuring morphological homogeneity. Purity confirmation was conducted through microscopic examination at 400X magnification. Subsequently, cultures were sub-cultured on PDA slants, grown for 5 days and stored at 4°C as stock cultures (Saini *et al.,* 2016).

Characterization of the Isolates

The isolated bacteria were characterized using morphological and biochemical methods, following the guidelines outlined in Bergy's Manual of Bacteriology (Dadaşoğlu and Kotan, 2017). The fungi were identified on the basis of their colony and morphological characteristics. The morphological characteristics evaluated included colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, and pigment production. The characteristics were compared with the standard description of, 'A

manual of Soil Fungi', by Gilman, (1957), 'Industrial Mycology' by Onions *et al*. (1981) and 'Compendium of Soil Fungi' by Domsch and Gams (1980).

Antibiotic Susceptibility Testing

The experiment was conducted using the disc diffusion method (Krishnan *et al.,* 2019) following the instructions provided by CLSI (2018) on Muller –Hinton agar after normalization of broth to 0.5 McFarland standard to test all the isolates against a panel of fourteen distinct antibiotics. The antibiotics utilized include ceftazidime, cefuroxime, gentamicin, ceftriaxone, cloxacillin, augmentin, ofloxacin, erythromycin, ciprofloxacin, augmentin, nitrofurantoin, penicillin, cefixime, and novobiocin (Oxoid, UK). Three concentrations of antifungal stock solution were prepared for fungi. Sterile perforated filter papers were then dipped into each stock solution using a sterile forceps. Antifungal agents of three different concentrations (Griseofulvin, Itraconazole, and Ketoconazole) were determined for each isolate. The papers were incubated at room temperature for 48 hours (Plate 2).

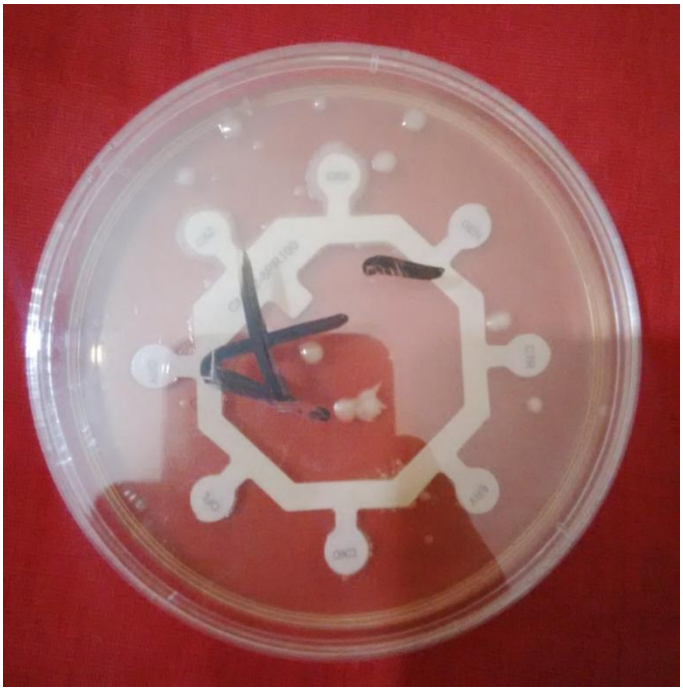

Plate 2: Antibiotics susceptibility testing

RESULTS

The total bacterial and fungal counts from the different soil samples are shown in Table 1.

Table 1: Microbial loads of microorganisms isolated from different soil samples

Sample	Bacteria (x 10^5CFU g^{-1}) Nutrient Agar	Bacteria (x 10^5CFU g^{-1}) MacConkey Agar	Fungi(x 10^5CFU g^{-1}) Potato Dextrose Agar
A	1.4	8.0	1.7
B	1.0	2.1	3.6
C	8.0	5.0	4.2
D	2.8	2.4	4.8
E	5.0	8.0	2.5

Key: CFU = Colony Forming Unit; SFU: Spore Forming Unit

Frequency of the Isolated Microorganisms from the Soil Samples

From all the soil samples, eight (8) bacterial isolates and (8) fungal isolates were identified. *Escherichia coli* and *Pseudomonas aeruginosa* had the lowest (6.25%) frequency among the bacteria isolates, while *Bacillus subtilis* had the highest prevalence (25%), followed by *Bacillus cereus, Clostridium* spp., *Staphylococcus epidermis*, *Acetobacter* spp., and *Staphylococcus aureus* (12.50%). Of all the fungi isolates, *Alternaria alternata, Fusarium oxysporum, Aspergillus flavus,* and *Rhodotorula minuta* had the highest prevalence (18.18%), while *Candida albilcans, Aspergillius tamari, Fusarium solani,* and *Penicillium digitatum* had the lowest percentage occurrence (9.09%)

The results of the antibiotic susceptibility tests are shown in Table 2.

Table 2a: Antimicrobial susceptibility tests for Gram-positive bacteria isolates

Bacteria	GEN	ERY	OFL	CRX	CXC	CAZ	AUG	CTR	NV	P	CEP
Bacillus subtilis	36(S)	22(S)	37(S)	R	18(S)	R	15(S)	20(S)	R	20(S)	35(S)
Bacillus cereus	19(S)	12(S)	24(S)	R	R	R	14(S)	R	R	R	24(S)
S. epidermis	23(S)	32(S)	29(S)	R	R	R	R	R	24(S)	R	R
S. aureus	17(S)	R	29(S)	R	R	26(S)	R	28(S)	R	R	30(S)
% Resistance	0	25	0	100	75	75	50	50	75	75	25

Key: GEN = Gentamicin; ERY = Erytromycin; OFL= Ofloxacin; CRX = Cefuroxime; CXC = Cloxacillin; CAZ = Ceftazidine;
AUG=Augmentin; CTR = Ceftriaxone; NV=Novobiocin, P=Penicillin; CEP = Cephalosporin;
Percentage;S=Sensitive; I=Intermediate;R=Resistance

Table 2b: Antimicrobial susceptibility tests for Gram-negative bacterial isolates

Bacteria	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CIP	CRO	ERY	NV	P
Clostridium spp	R	R	14(S)	R	32(S)	R	R	20(S)	R	R	R	R
Acetobacter spp	R	R	22(S)	R	42(S)	R	40(S)	40(S)	40(S)	40(S)	40(S)	30(S)
P. aeruginosa	R	R	16(S)	R	21(S)	R	R	28(S)	23(S)	17(S)	13(S)	R
E. coli	R	R	16(S)	R	21(S)	R	R	28(S)	23(S)	17(S)	13(S)	R
% Resistance	100	100	0	100	0	75	50	0	75	50	50	50

Key: CAZ=Ceftazidine; CRX=Cefuroxime; GEN=Gentamicin; CXM=Cefixime; OFL= Ofloxacin; AUG=Augmentin;
NIT=Nitrofurantoin; CIP=Ciprofloxacin; CRO=Ceftraxone; ERY=Erytromycin; NV=Novobiocin; P=Penicillin; %=Percentage;S=Sensitive; I=Intermediate;R=Resistance

The results of fungal sensitivity tests are shown in Tables 3a and 3b

Table 3a: Fungal sensitivity test using Griseofulvin

Fungi	400 mg	500 mg	750 mg	1000 mg
Alternaria alternata	12(S)	12.8(S)	13.5(S)	14.8(S)
Candida albicans	R	R	R	R
Rhodotorulla minuta	R	R	R	R
Fusarium oxysporum	14.6(S)	15.5(S)	20.4(S)	25(S)
Aspergillus flavus	15(S)	16(S)	18.2(S)	24.4(S)
Aspergillus tamarii	15.5(S)	16.2(S)	17.8(S)	23(S)
Fusarium solani	11.3	12.4	14.2	18.3
Penicillium digitatum	R	R	R	R
% Resistance	37.5	37.5	37.5	37.5

Key: % = Percentage, S= Sensitive, I= Intermediate, R= Resistance

Table 3b: Fungal sensitivity test using Itraconazole

Fungi	75 mg	100 mg	200 mg	500 mg
Alternaria alternata	10(S)	11.5(S)	14.2(S)	18.5(S)
Candida albicans	11(S)	12(S)	18(S)	25.5(S)
Rhodotorulla minuta	3.5(S)	15.1(S)	17.3(S)	22.1(S)
Fusarium oxysporum	R	R	R	R
Aspergillus flavus	R	R	R	R
Aspergillus tamarii	9.8 (S)	12(S)	14.8(S)	18(S)
Fusarium solani	R	R	R	R
Penicillium digitatum	R	11(S)	11.7(S)	13.4(S)
% Resistance	50	37.5	37.5	37.5

Key: % = Percentage, S= Sensitive, I= Intermediate, R= Resistance

Table 3c: Fungal sensitivity test using Ketoconazole

Fungi	100 mg	200 mg	300 mg	500 mg
Alternaria alternata	11(S)	13.5(S)	15.2(S)	17.2(S)
Candida albicans	15(S)	19.2(S)	23.7(S)	42(S)
Rhodotorulla minuta	17.5(S)	18.4(S)	19.5(S)	22.4(S)
Fusarium oxysporum	18.2(S)	10.8(S)	11.4(S)	12.5(S)
Aspergillus flavus	R	R	R	R
Aspergillus tamarii	R	R	R	R
Fusarium solani	R	11.2 (S)	13.5 (S)	15(S)
Penicillium digitatum	11.3 (S)	12.4(S)	12.9(S)	14.8(S)
% Resistance	37.5	25	25	25

Key: % = Percentage, S= Sensitive, I= Intermediate, R= Resistance

DISCUSSION

The majority of the microorganisms detected in this study are soil-dwelling species that collaborate to develop symbiotic relationships in the soil (O'Callaghan *et al.,* 2022).They make up a significant amount of the nutrients in the soil, which are constantly transferred into the plant growth cycles by the system (Leghari*et al.*,2016). The report of Egbenyah *et al.* (2021), which identified *Bacillus cereus, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis* and *Pseudomonas aeruginosa* from dump-sites'soils, is consistent with all of the bacteria reported in this study. Also a study by Bassey *et al.* (2021) identified bacterial taxa belonging to genera such as *Bacillus, Pseudomonas,Enterobacter,* and *Clostridium* as predominant in dump-site soils and this agrees with bacteria identified in this study.*Bacillus subtilis* had the highest prevalence of occurrence in this study and is a common soil bacterium known for its ubiquity and ability to thrive in various environments, including dump-sites. This observation conforms with that of Zhang *et al.* (2019), although, the predominant microorganisms in dump-sites can vary depending on factors such as location, waste composition, and environmental conditions (Selvarajan *et al.,* 2021). The presence of *Aspergillus flavus* and *Aspergillus tamari* isolated from dump-sites in this study conforms with the report of Massomo (2020) who detected that *Aspergillus* species are predominant in soil from dump-sites. Additionally, every fungal species identified in this study concurs with the findings of Olaniran *et al.* (2022), with the possible exception of *Penicillium digitatum.* Findings from the study revealed the presence of soil-borne pathogens with resistance to commonly used antibiotics, raising concerns about the spread of antibiotic resistance beyond clinical settings. Soil -borne microorganisms may exhibit varying degrees of susceptibility to antibiotics, with some strains being susceptible while others may develop resistance mechanisms (Peterson and Kaur, 2018). In this study, Gentamicin, ciprofloxacin were susceptible to all bacterial isolates detected in all the soil samples assayed. This is in

concordance with the study of Odum *et al.* (2020) who reported that ciprofloxacin and gentamycin were effective against enteric bacterial isolates from dump-sites soils. However, their susceptibility to soil-borne microorganisms can vary depending on factors such as microbial species, environmental conditions and antibiotic concentrations (Lazcano *et al.,* 2021). The distribution of antibiotic resistance among the isolates in this study is similar to the study of Edet *et al.* (2023) in which the majority of the isolates were highly resistant to selected classes of antibiotics used such as cefixime, ceftazidime, cloxacilin and cefuroxime. This study showed that *Pseudomonas aeruginosa* and *Escherichia coli* are highly resistant to cefuroxime. This finding agrees with the reports of Chang*et al*. (2016) and also reported that the origin of this resistance can be traced to the faecal constituent of the wastes produced by people or animals that have been treated indiscriminately with various antibiotics and also to antibiotics production naturally by soil microorganisms. The high-level resistance of bacteria isolated from dump-sites to cefuroxime and cefixime may be due to inappropriate or overuse use of these antibiotics (Sampson *et al.,* 2022). Griseofulvin, itraconazole and ketoconazole used against the fungal isolates are not very effective. Griseofulvin and itraconazole used against fungal isolates from dump-sites in this study were not effective. Hovwever, ketoconazole proved effective against majority of the fungal isolates in this study. Their effectiveness against fungal isolates may be based on factors such as the specific fungal species, their resistance mechanisms, and the environmental conditions in the dump-sites (Mahajan *et al.,* 2017).

CONCLUSION AND RECOMMENDATION

This study demonstrated the continued efficacy of gentamicin and ofloxacin in treating infections caused by both Gram-positive and Gram-negative bacteria. Therefore, these antibiotics should be regarded as the preferred choice for combating such diseases. Given that ketoconazole continues to demonstrate efficacy against fungal infections, it should be considered the preferred medicine for treating such infections.The study provided a convincing demonstration of the diverse range of microorganisms present in soil found in dump-sites in the study area, as well as their microbial loads and vulnerability to infection. Hence, it is imperative to investigate the ecological interactions of these microbes and their advantageous utility, rather than merely considering them as a nuisance to the environment, given that certain isolated organisms have economic values. Enhancements should be implemented on the current antibacterial agents due to the presence of resistant and intermediate microorganisms.

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