

MICROBIOLOGICAL ANALYSIS OF A MUNICIPAL DUMPSITE AND IT'S ENVIRON IN LAGOS STATE, NIGERIA

Abstract

Indiscriminate waste discharge and improper management of waste disposal facilities remains Nigeria's biggest environmental concerns as it poses substantial risk to both public health and ecosystem. This study explored how waste dumpsite affects the environment and public health. Soil, leachate, borehole water and air samples collected from a dumpsite situated in Kosefe Local Government in Lagos State, Nigeria and its environs were microbiologically analyzed. Microbial isolation and enumeration utilized Nutrient, Eosin Methylene Blue, Salmonella Shigella agar and MacConkey agar. Bacterial characterization was done phenotypically using morphological and biochemical techniques. Antibiotic susceptibility employed Kirby-Bauer disk diffusion. Soil had the highest bacteria count (3.0×10^7 CFU/g), followed by leachate (1.48×10^7 CFU/ml), and borehole water (5.2×10^6 CFU/ml), while air had the least bacterial count (4.0×10^6 CFU/m³). Thirty two bacterial species belonging to seven genera were isolated and identified. *Bacillus* spp. occurred in all samples asides water, while *Salmonella* spp. and *Escherichia coli* were found in leachate and borehole water. Antibiotic resistance profile revealed that all the isolates were susceptible to gentamicin and ofloxacin while 38% were resistant to amoxicillin and 56% of the isolates were resistant to ceftriaxone. Individuals living in close proximity to dumpsites are at high risk of infections caused by bacterial pathogens. This calls for an urgent regulatory system on proper waste disposal in that area.

Keywords: Phenotypic characterization, microorganisms, resistant profile, dumpsite,

Introduction

Worldwide and in Nigeria, a wide range of wastes are continuously produced (Kehinde *et al.*, 2020). Food wastes, agricultural wastes, human wastes (faeces and urine), and medical wastes are common forms of trash found in home dumpsites. Some of these wastes pose a threat to people's health. When improperly sited, waste treatment and disposal facilities have been shown to have the potential to endanger the health of nearby communities (Tomita *et al.*, 2020). When waste is deposited on land, microorganisms flourish and use the waste's components as food sources, which leads to the breakdown of complex wastes (Williams and Hakam, 2016). These dumpsites frequently have repulsive smells and are places where people can easily get infected.

Pathogenic bacteria and fungi are frequently found occurring in waste dumps (Nair, 2021). The use of dumpsite for defecation as well as for animal sheds, increases the spread of bacteria that are resistant to antibiotics. Also, improper drug disposal, both used and unused, encourages the growth of drug-resistant microbial species in the environment, thereby increasing the risk to human health (Borquaye *et al.*, 2019). Pathogenic microorganisms found in soil can potentially result in air pollution as dust particles are lifted into the atmosphere by air currents. Additionally, they can contaminate groundwater via leachate originating from the disposal sites. These scenarios are plausible due to the recognized role of atmospheric transport in the dispersion of microbes and the transmission of diseases. (Pepper *et al.*, 2015). Consequently, these pathogens can enter the human body through inadequate hygiene practices, injuries sustained within disposal site environs, or by being inhaled. Additionally, the elevated presence of rodents and other typical disease carriers can facilitate the dissemination of these pathogens. (Waturu *et al.*, 2017).

The widespread emergence of multidrug resistance within microbial populations has evolved into a significant worldwide challenge. (Mwaikono *et al.*, 2015). These microorganisms have developed strategies to circumvent the impacts of numerous currently employed antibiotics. Research has highlighted that domestic landfill sites host elevated quantities of microbes that are resistant to multiple drugs (Idahosa *et al.*, 2017; Mwaikono *et al.*, 2015). The antibiotic susceptibility pattern and the virulence of bacteria identified in municipal waste disposal sites, and its effects on individuals working at waste sites in Benin City was observed. It was found that all the isolates obtained exhibited multiple resistance pattern to antibiotics. (Sitotaw *et al.*, 2021). Additionally, they found that some of these isolates possessed pathogenic characteristics. This high prevalence can be attributed to the indiscriminate disposal of medical wastes, human carriers, and livestock defecation, resulting in the introduction of resistant strains. Through horizontal gene transfer, other susceptible strains in the environment may then pick up resistance genes (Borquaye *et al.*, 2019). The likelihood of infection with antibiotic-resistant pathogens among human populations is increased by the high diversity of antimicrobial-resistant bacteria in dumpsites as well as the frequent animal and human interactions on waste disposal sites (Nyandjou *et al.*, 2019).

The demographic most vulnerable to the public health risks linked to household landfill sites are waste-pickers, often referred to as scavengers, who reside and labor in unsanitary environments. These individuals are commonly observed visiting dumpsites to collect recyclable items such as plastics, bottles, metals, and clothing. This activity escalates the potential for infections due to breathing in polluted aerosols, sustaining cuts from sharp objects, and contracting wound infections from pathogens present in the soil. (Jutta and Sayed, 2017). An additional concern involves the utilization of reused packaging materials sourced from landfill sites to distribute food products in markets. Furthermore, majority of municipal landfills in Nigeria are situated relatively close to residential areas.

This transforms these waste disposal sites into matters of environmental concern, impacting not only scavengers exposed to the health risks linked with such sites but also the inhabitants of urban communities. (Navarro Ferronato and Vincenzo Torretta, 2019).

Materials and Methods

Study Area

The Mile 12 dumpsite popularly called “Gangere”, located behind the popular Mile 12 International market within the confines of the Kosofe local government area in Lagos State, Southwest Nigeria, was used for this study. Bottles, plastics, domestic garbage, various trash, and decomposing vegetables from the market are accumulated in heaps, while cart pushers dash back and forth as they do brisk business. Mile 12 international market has been in existence for the past 40 years and has become one that houses different perishable foodstuffs. Several people build their homes in the residential community situated close to the dumpsite, while others carry out their daily businesses activities like cooking, where they make different delicacies to sell. Kosofe Local Government lies between latitude 6.561638N and longitude 3.384247E, covers a total area of 81 square kilometers, with an average temperature of 27°C. Figure 1 is the map of Lagos State showing Kosofe Local Government and Mile 12 town. Figure 2 (a, b, and c), shows sections of dumpsite at Mile 12, Kosofe LGA, Figure 3 shows section of the dumpsite occupied by residents, Figure 4 shows the leachate section of the dumpsite, while Figure 5 (a and b) shows boreholes A and B located in houses in the vicinity of the dumpsite.

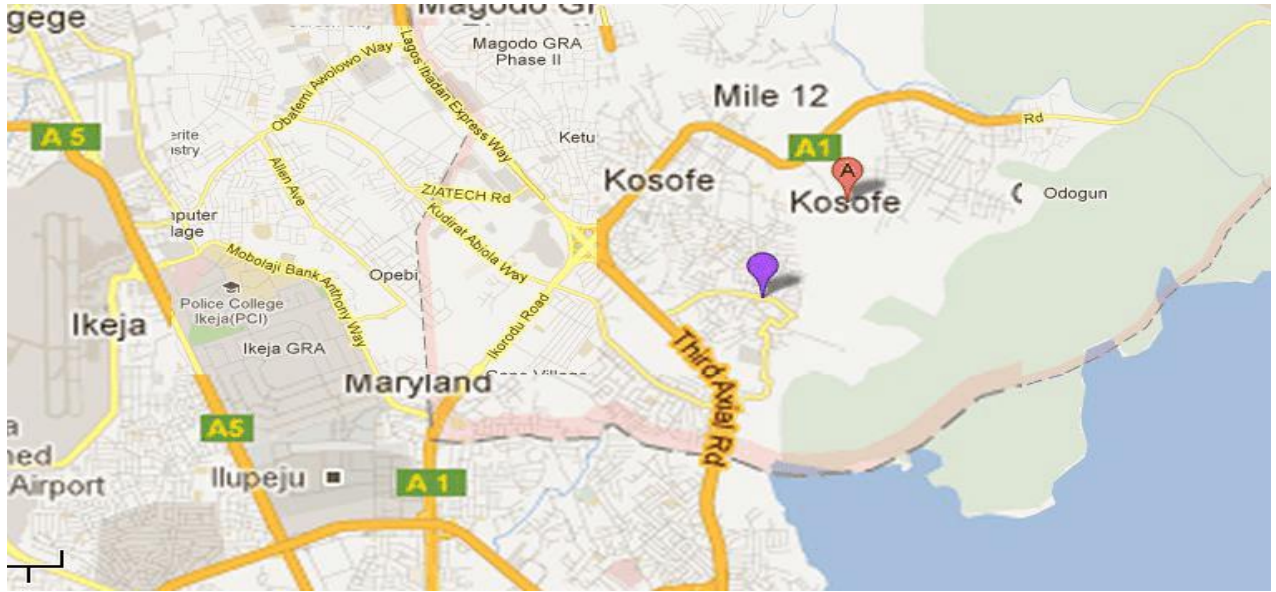


Figure1: Map of Lagos State showing Kosofe Local Government Area.



(2a)



(2b)



(2c)

Figure 2: Sections of Dumpsite at Mile 12, Kosofe LGA, Lagos State



Figure 3: Residential areas close to the Dumpsite at Mile 12, Kosofe LGA, Lagos State



Figure 4: Leachate collection section of the Dumpsite at Mile 12, Kosofe LGA, Lagos State



(5a)



(5b)

Figure 5: Boreholes at residential areas close to the dumpsite at Mile 12, Kosofe LGA, Lagos State

Collection of Samples

The samples which include soil, leachate and air were aseptically collected at strategic locations on the dumpsite, while borehole water and soil (control) were collected at residences close to the dumpsite. Soil and leachate samples were collected in sterile containers. Samples of surface soil were randomly retrieved from three distinct locations at a depth of 0-15cm from the top layer using an auger once surface debris has been cleared. The composite sample was thereafter obtained by mixing all samples thoroughly. The leachate was collected directly into the sterile containers, and water were collected aseptically from

two (2) different boreholes around the dumpsite. Air was sampled by exposing nutrient agar plates in duplicates at a distance of 50m away from dumpsite for 10 minutes during the busy hours of the day. The leachate and water samples were placed on ice-packs. All samples were transported immediately to the laboratory for analysis. Samples when not analyzed immediately, were stored at 4°C before analyses were conducted.

Analysis of bacterial population and Identification of Isolates

Enumeration of bacterial populations of the soil, borehole water, and leachate samples collected from the dumpsite was done utilizing the pour plate technique. Serial dilution of the soil, leachate and water samples were done up to 10^{-10} with the first dilution of the leachate and water from boreholes made by mixing 1ml with 9ml of sterile distilled water. For the soil samples, 1gram of each soil sample was dispense into 9ml of sterile distilled water in a 15ml screw capped test tube, and mixed thoroughly to form the stock solution, thereafter, 1ml each from 10^{-6} dilutions were poured into sterilized Nutrient agar, MacConkey agar, Salmonella Shigella agar, and Eosin Methylene Blue agar respectively. The plates including the air samples were incubated aerobically at 37°C for 24 hrs. After the incubation period, the bacteria colonies on the plates were counted using a colony counter. Bacterial counts were expressed as colony forming units per milliliter (CFU/ml) for water and leachate samples, colony forming units per gram (CFU/g) for soil samples and colony forming units per square meter (CFU/m³) for air sample. Further sub culturing was done until morphological distinct and pure colonies were obtained. Bacterial isolates were characterized to genus level based on morphological, cellular and biochemical characteristics as described by the Bergey's manual of systematic bacteriology (1984)

Test for Antibiotic Sensitivity

The susceptibility of the bacterial isolates to four antibiotics was tested using the Kirby-Bauer disc diffusion method. Sensitivity discs impregnated with following antibiotics; Ofloxacin (5ug), Ceftriaxone (30µg), Gentamicin (10µg), and Amoxicillin (30µg) were used. The isolates were subcultured on nutrient agar at a temperature of 37°C for a duration of 24 hours. The resulting colonies were suspended in 4 ml of sterile normal saline until reaching a turbidity level equivalent to the 0.5 MacFarland standards. 0.1 ml of each bacterial suspension was then individually inoculated onto prepared Mueller-Hinton agar, and using sterile swabs, a uniform bacterial layer was created after which respective antibiotic discs were placed on the plates and left undisturbed for a 15minute period to facilitate initial diffusion, followed by aerobic incubation at a temperature of 37°C for 24 hours. (Okpo *et al.*, 2017). The measurement of the zones of inhibition around each disc was measured and interpreted using the Clinical Laboratory Standard guidelines (CLSI, 2012). The isolates responses to the antibiotics were categorized as susceptible, intermediate, or resistant.

Results

The heterotrophic bacterial counts in soil, water, leachate, and air samples collected from within and around the waste dumpsite is presented in Table 1. The highest bacteria count of 3.0×10^7 CFU/g was obtained in the soil sample, followed by the leachate sample with 1.48×10^7 cfu/ml, water from borehole B with 1.26×10^7 CFU/ml and borehole water A with 5.2×10^6 CFU/ml respectively. The least bacterial counts of 4.0×10^6 CFU/m³ was obtained from the air sample.

A total of 32 isolates were isolated from the dumpsites and its environment in this study. The isolated bacteria belong to six genera namely; *Bacillus*, *Escherichia coli*, *Pseudomonas* spp, *Streptococcus* spp, *Salmonella* spp, *Shigella* spp and *Providencia* spp. Presented in Table 2

and 3 are the phenotypic characteristics of the bacterial isolates that were obtained from the dumpsite and its environs,. Figure 6 shows the percentage frequency of occurrence of bacterial isolates. The most isolated bacteria is *Bacillus* spp. (28%) followed by *Escherichia coli* (25%), *Pseudomonas* spp. (13%), *Streptococcus* spp. (13%), *Salmonella* spp. (9%), *Shigella* spp. (9%) and *Providencia* spp (3%).

The resistance profile of the isolates to the antibiotics examined in this study is presented in Fig 7. All bacterial isolates were susceptible to gentamicin and ofloxacin, 38% of the isolates were resistant to amoxicillin and 56% of the isolates were resistant to ceftriaxone.

DISCUSSION

Waste management is a serious problem in Nigeria. While municipal solid waste disposal sites exist throughout the states, these locations suffer from inadequate management. The nation's failure to effectively handle its waste has resulted in escalated leachate impact and pollution, posing significant environmental and human health apprehensions. Dumpsites have the potential to contaminate both underground and surface water sources through seepage and leaching. This is a significant challenge since landfill sites are known to harbor antibiotic-resistant bacteria and genes, which could contribute to the problem of antibiotic resistance (Wang *et al.*, 2018). The results obtained in this study, suggests a high degree of fecal contamination, based on the probable species of organisms obtained which was found to be most occurring in leachate sample. The least contamination was seen in the air sample. Similar studies have been conducted in various parts of the world, and the results have varied depending on the sample source and location. Alao *et al.* (2012), reported total bacterial counts ranging from 2.2×10^6 to 3.8×10^7 cfu/g in soil samples, which is consistent with the high count obtained in the current study. Johnson and Kuehn, (2002) also reported that total

bacterial counts in leachate samples from dumpsites ranged from 2.5×10^6 to 1.1×10^7 cfu/ml, which is also in alignment with the discoveries of this study.

Seven genera of bacterial isolates which includes; *Providencia* spp., *Bacillus* spp., *Salmonella* spp., *Escherichia coli*, *Shigella* spp., *Pseudomonas* spp. and *Streptococcus* sp. was reported in the current study. A study conducted by Williams and Hakam, (2016) in Port-Harcourt metropolis, Nigeria, stated the identification of *Bacillus* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus*, and *Streptococcus* sp. respectively. Previous research by Oviasogie and Agbonlahor, (2013) in Benin City, Nigeria, follows a similar trend. Similar bacteria species has been isolated from dumpsites in the south-west regions of Nigeria by other researchers, such as Achudume *et al.* (2007), Adekanle *et al.* (2014), Odeyemi, (2012), and Sulaimon *et al.* (2016). Mwaikono *et al.* (2015), indicated in their report the high abundance of these bacterial presence within municipal dumpsites in Tanzania. Regular encounters between humans and animals on dumpsites may be the reason for the high occurrence of *Escherichia coli*, which can potentially transmit faecal enteric pathogens to the soil. The presence of *Bacillus* spp., indicates a serious health risk because they are responsible for a number of common diseases and their spores can contaminate food, leading to food poisoning. The results obtained in the current study, suggest that the dumpsite harbours diverse bacteria species that could pose public health risk.

The identification and characterization of microorganisms present in soil samples are crucial in understanding their ecological roles and potential applications in biotechnology. In a similar study by (Ahmed *et al.*, 2019), eleven bacterial isolates were obtained from soil samples collected from a municipal waste dumpsite in Nigeria. The isolates were identified as *Bacillus* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. based on their morphological and biochemical characteristics. (Mueen *et al.*, 2020), also reported that *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp., and *Staphylococcus* spp in soil

samples collected from a dumpsite in Pakistan. The results obtained in this study are consistent with those reported in previous studies, indicating the presence of *Bacillus* spp. and *Pseudomonas* spp. in soil samples from dumpsites. However, the presence of *Streptococcus* sp. and *Providencia* spp. in the dumpsite soil is worthy of note as it may indicate potential health risks associated with exposure to such microorganisms.

The occurrence of gram-negative bacteria in dumpsite environments have also been reported in similar studies conducted by various researchers. (Sabir *et al.*, 2019), found that the majority of bacteria isolated from dumpsite leachate were gram-negative rods, including *Escherichia coli* and *Pseudomonas aeruginosa*. Similarly, (Ye *et al.*, 2020), reported that the most dominant bacterial phylum in dumpsite leachate was *Proteobacteria*, which includes gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. In terms of bacterial characteristics, the ability to produce catalase and utilize glucose is commonly found in aerobic bacteria, which are known to thrive in dumpsite environments (Breijyeh *et al.*, 2020). The variable results in indole production, citrate utilization, and oxidase activity among the isolates may suggest the presence of different species within the same bacterial genera, which is not uncommon in complex environments such as landfills.

Various microorganisms including the pathogenic bacteria, have the potentials of contaminating water sources that are in close proximity to dumpsites and this poses a threat to human health. Research have been carried previously to investigate the microbial quality of borehole water in these areas, and the results obtained shows the presence of various pathogenic bacteria, including *Escherichia coli*, *Salmonella* spp., and other gram-negative rods (Ayandele *et al.*, 2020). Results obtained in this current study, on samples of borehole water retrieved from around the dumpsite environs follows a similar trend. The occurrence and presence of these pathogenic bacteria species in these samples can easily be detected

through their growth and morphology presented on various microbiological selective media, as well as through gram staining, catalase test, glucose test, and citrate test respectively.

Air pollution from dumpsites has been found to have a significant impact on the microbial communities in the surrounding air. One study reported the isolation of six bacterial strains from air samples collected in a landfill site, with the predominant isolates identified as *Bacillus* spp. and *Staphylococcus* spp. (Sulaiman *et al.*, 2016). Another study identified *Bacillus* spp. as the most abundant bacterial genus in air samples collected from a dumpsite in India (Kumar *et al.*, 2015). In a similar project report, two isolates from air samples collected in the vicinity of a dumpsite were identified as *Bacillus* spp. based on their morphology and biochemical characteristics. Both isolates were catalase positive and were able to utilize glucose and sucrose, but not lactose. They were also both able to utilize CO₂ and negative for H₂S production, which is consistent with the characteristics of *Bacillus* spp. One of the isolates, LA2, was positive for the oxidase test, further supporting its identification as *Bacillus* spp. Findings from the current investigation supports these reports from previous researchers on the dominance of *Bacillus* spp. in air samples in dumpsite environments.

The notable resistance demonstrated by the isolates against antibiotics raises concerns for public health. The fact that these bacteria exhibited resistance to commonly prescribed antibiotics for treating typical bacterial infections underscores the importance of dumpsites as potential sources for acquiring antibiotic-resistant strains and the emergence of novel forms of diseases. Several studies have reported antibiotic resistance of bacteria isolated from dumpsite soils. Adekanle *et al.* (2014), indicated that isolation of antibiotic-resistant bacteria from dumpsites in southwest Nigeria, and Odeyemi, (2012), reported high levels of antibiotic resistance among bacteria isolated from dumpsite soils in Ogun state, Nigeria. Sulaimon *et al.*, (2016), also reports the isolation of antibiotic-resistant bacteria from dumpsites in Lagos state, Nigeria. Among these, every gram-positive isolate showed resistance to cefuroxime and

ampicillin/cloxacillin, while 75% displayed resistance to cotrimoxazole and streptomycin. Furthermore, a study conducted in Tanzania by Mwaikono *et al.* (2015), showed high levels of antibiotic resistance among bacterial isolates from municipal dumpsites. According to the study findings, ciprofloxacin and gentamycin demonstrated effectiveness against enteric bacterial isolates sourced from dumpsites. Additionally, the report highlighted that out of the 80 isolates obtained, 56% (45 isolates) exhibited resistance to a minimum of two antibiotics. Some isolates obtained in the current study, were resistant to Amoxicillin and Ceftriaxone of the four tested antibiotics which makes it align with report by (Mwaikono *et al.*, 2015). Nonetheless, this study diverges from the perspective of (Oviasogie and Agbonlahor, 2013), who asserted the effectiveness of fluoroquinolones against dumpsite isolates. In contrast, the current research documented substantial resistance to ciprofloxacin and pefloxacin. This has a high tendency of occurrence due to gene mutation which may lead to the acquisition of antibiotic resistance genes by these bacteria species. These findings suggest the potential dissemination of enduring and challenging-to-treat bacterial infections within the local community. Furthermore, the improper reuse of food packaging materials (such as bottles) collected by scavengers from dumpsites presents an additional pathway for these isolates to be transmitted to human hosts.

Conclusion

The high bacterial counts and the presence of potentially harmful organisms in the soil and leachate samples in this study is an indication that the dumpsite poses a significant public health risk to people living in the environment. To prevent such risks, a proper design plan for constructing and allocating waste sites should be adopted, placing them away from sensitive areas like residential areas, markets, and rivers. This would regulate waste management practices and reduce the adverse consequences of leachate on the environment and the well-being of the public.

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Table 1: Total heterotrophic bacterial counts of samples around the dump site

S/N	Sample Code	Mean Bacterial count
1	LS	3.0×10^7 CFU/g
2	LC	9.8×10^6 CFU/g
3	LL	1.48×10^7 CFU/ml
4	LW _A	5.2×10^6 CFU/ml
5	LW _B	1.26×10^7 CFU/ml
6	LA	4.0×10^6 CFU/m ³

Key: CFU = Colony Forming Unit; LS- Soil sample obtained from dumpsite; LC- Soil sample obtained far from dumpsite; LL- Leachate collected from dumpsite; LW_A- Water collected from Borehole A; LW_B- Water collected from Borehole b; LA- Air sample collected from dumpsite.

Table 2: Characterization of bacterial colonies obtained from the dumpsite soil and soil control.

Sample source	Rep. isolates	Morphological examinations		Biochemical examinations									Suspected organisms
		Colony characteristics on Agar plates	Gram staining	CA	CO	HS	LT	SU	GU	CL	IN	OX	
Dumpsite soil	1	Circular, rough, creamy on NA	Positive rods	+	+	-	-	-	+	+	-	-	<i>Bacillus</i> spp.
	2	Irregular, rough green pigment on NA	Negative rods	+	+	-	-	-	+	+	-	+	<i>Pseudomonas aeruginosa</i>
	3	Circular, rough, creamy on NA	Positive rods in chains	+	+	-	-	-	+	+	-	+	<i>Bacillus subtilis</i>
Soil control	1	Circular, mucoid , grayish-white on NA	Positive cocci	-	-	-	+	+	+	+	-	-	<i>Streptococcus</i> spp.
	2	Irregular, rough green pigment on NA	Negative rods	+	-	+	-	-	+	+	-	+	<i>Pseudomonas aeruginosa</i>
	3	Irregular, rough green pigment on NA	Negative rods	+	-	+	-	-	+	+	-	-	<i>Pseudomonas</i> spp.
	4	Flat, convex, colorless on MAC	Negative rods	+	+	-	+	+	+	+	+	-	<i>Providencia</i> spp.
	5	Circular, rough, creamy on NA	Positive rods	+	+	+	+	+	+	-	-	-	<i>Bacillus</i> spp.

NA: Nutrient agar. CA: Catalase test. CO: CO₂ test. HS: Hydrogen sulfide test. LT: Lactose test. SU: Sucrose test. GU: Glucose test. IN: Indole test. OX: Oxidase test. +: Positive result. -: Negative result.

Table 3: Characterization of bacterial colonies obtained from leachate, borehole water and air.

Sample source	Rep. isolates	Morphological examinations		Biochemical examinations									Suspected organisms
		Colony characteristics on Agar plates	Gram staining	CA	CO	HS	LT	SU	GU	CL	IN	OX	
Leachate	1	Circular, rough, creamy on NA	Positive rods	+	+	+	+	+	+	-	-	-	<i>Bacillus</i> spp.
	2	Irregular, smooth, colorless with a black center on SSA	Negative rods	+	+	+	+	+	+	-	-	-	<i>Salmonella</i> spp.
	3	Circular, glistening, metallic green sheen on EMB	Negative rods	+	+	-	+	+	+	-	+	-	<i>Esherichia coli</i>
	4	Irregular, smooth, colorless colonies	Negative rod	+	-	+	+	-	+	+	+	-	<i>Shigella</i> spp.
	5	Irregular, rough, green pigment on NA	Negative rod	+	+	+	+	+	+	+	-	+	<i>Pseudomonas aeroginosa</i>
Borehole water	1	Circular, smooth, grayish-white on NA	Negative cocci	-	+	-	+	+	+	-	+	+	<i>Streptococcus</i> sp.
	2	Circular, glistening, metallic green sheen on EMB	Negative rods	+	+	-	+	+	+	-	+	-	<i>Esherichia coli</i>
	3	Irregular, smooth, colorless with a black center on SSA	Negative rods	-	+	-	+	+	+	-	-	-	<i>Salmonella</i> spp.
Air	1	Circular, rough, creamy on NA	Positive rods	+	+	+	+	+	+	-	-	-	<i>Bacillus</i> spp.

NA: Nutrient agar. MAC: Mac conkey agar. CA: Catalase test. CO: CO₂ test. HS: Hydrogen sulfide test. LT: Lactose test. SU: Sucrose test. GU: Glucose test. IN: Indole test. OX: Oxidase test. +: Positive result. -: Negative result.

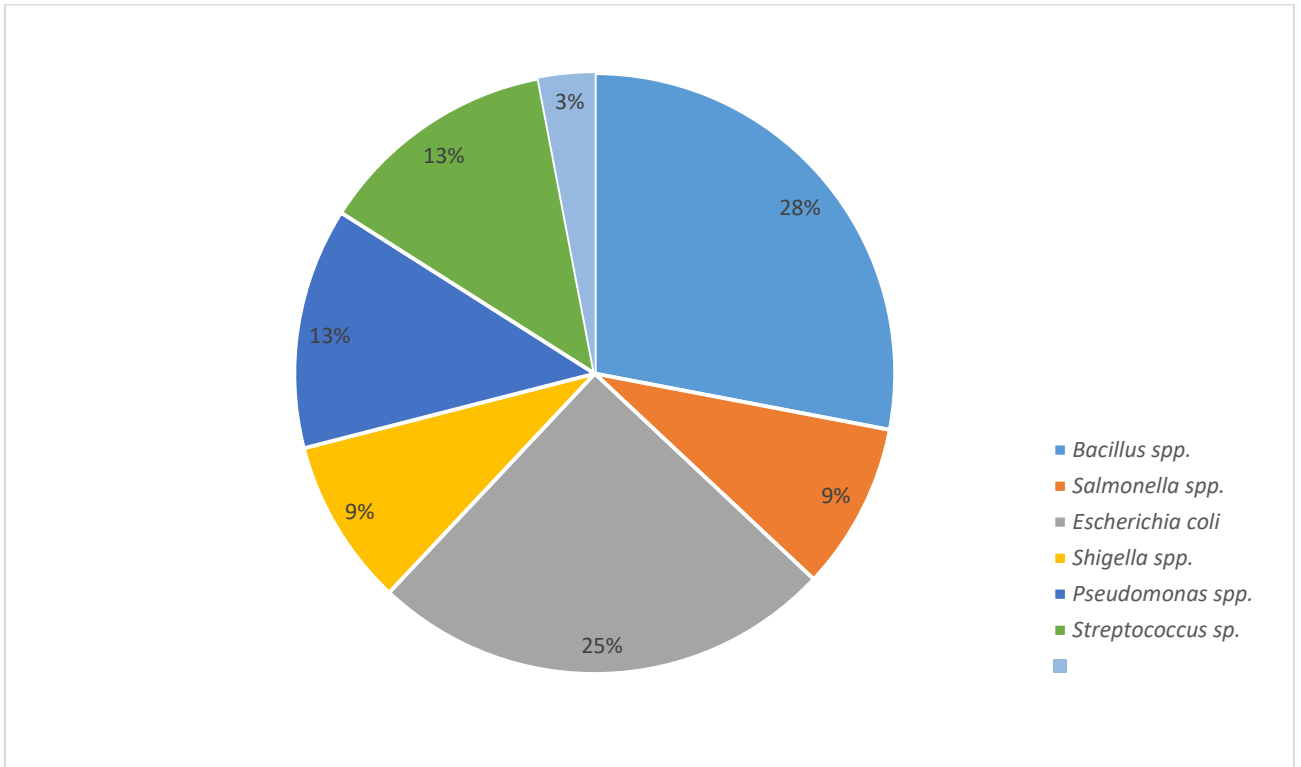


Figure 6. Percentage frequency of occurrence of bacterial genera isolated from all the samples

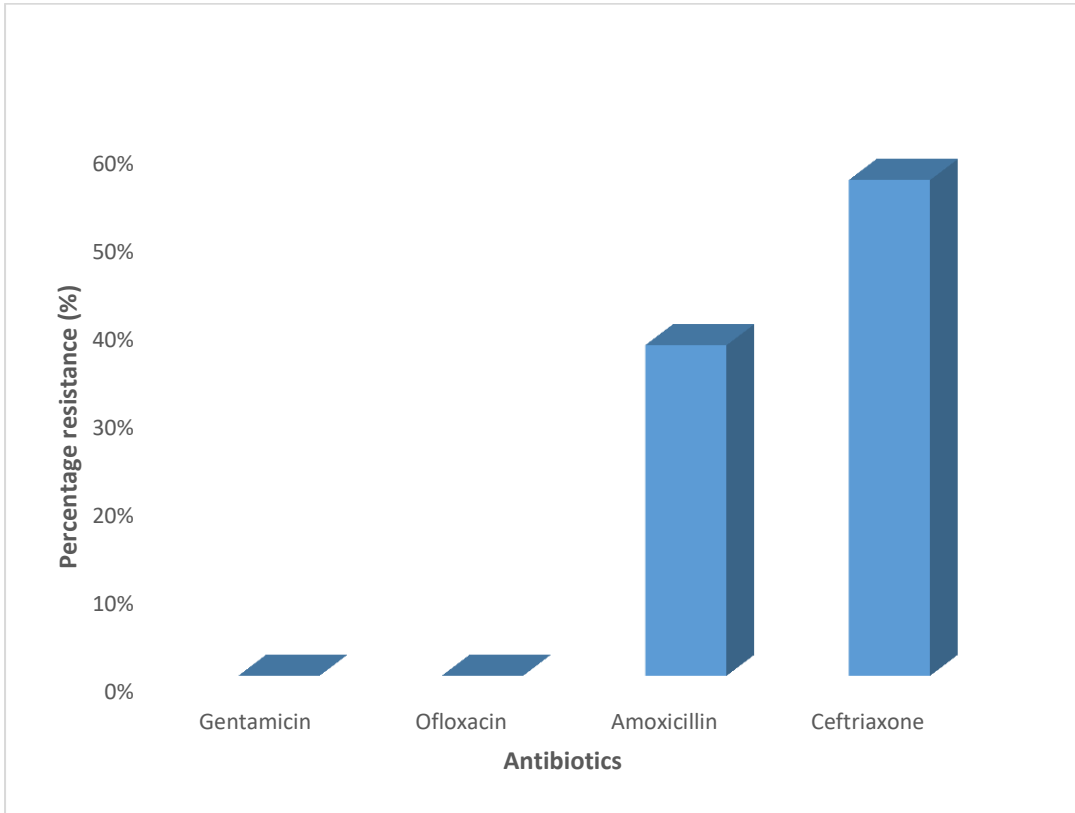


Fig 7. Antibiotic resistance pattern of isolated bacteria to test antibiotics