

Bacterial Community and Antibiotic Resistance of Bacteria of a Municipal Solid Waste Dumpsite Soil, Leachate and Surrounding Borehole Water

OMOKARO OBIRE & SAMUEL AZUBUIKE DOLLAH
Rivers State University, Port Harcourt, Nigeria

Abstract

In Nigeria commingled wastes are transported in open trucks to open dumpsites often located near residential areas. This constitutes a serious health hazard. The bacterial community and antibiotic sensitivity of bacteria of a municipal solid waste dumpsite soil, leachate and surrounding borehole water were investigated fortnightly over a period of 12 months using standard techniques. The objectives were to; determine the antibiotic sensitivity of bacteria isolated from waste dumpsite soil and leachate; provide a sound scientific basis for advising and encouraging the proper disposal of municipal solid waste. Result of viable heterotrophic bacterial count ranged from 4.9×10^6 to 1.93×10^7 CFU/g for dumpsite soil, 5.2×10^5 to 1.01×10^6 CFU/g for control soil, 9.0×10^6 to 1.29×10^7 CFU/ml for leachate and 9.5×10^1 to 1.7×10^2 CFU/ml for borehole water. Analysis of Variance revealed significant difference between the samples and the period of study at $p \leq 0.05$. Bacteria isolated were *Bacillus cereus*, *Corynebacterium xerosis*, *E. coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescense*, *Staphylococcus aureus* and *Streptococcus pyogenes*. A dominant, abundant, frequent, occasional and rare (DAFOR) analysis of the bacteria showed that *Pseudomonas* species and *M. luteus* were dominant at the dumpsite soil. All the bacteria were resistant to ampicillin but susceptible to gentamycin and ciprofloxacin. *Pseudomonas* species and *S. aureus* were the most resistant (66.67%) to the antibiotics while *C. xerosis* and *K. pneumoniae* were the least resistant (22.22%) being resistant to only two antibiotics. This showed that all the isolated bacteria exhibited multiple drug resistance. Bacteria isolated from the dumpsite soil and leachate were more resistant than those of control soil and borehole water. The dumpsite investigated was found capable of encouraging the proliferation of pathogenic microorganisms which possess multiple drug resistance. A combination therapy may be the only way to effectively eradicate the diseases caused by these organisms –a major challenge to healthcare workers.

Keywords: Waste dumpsite soil, leachate, borehole water, bacteria, antibiotic resistance.

Introduction

Wastes are substances, solutions, mixtures or articles for which no direct use is envisaged but which are transported for reprocessing, dumping, elimination by incineration or other methods of disposal (Chuku and Anuchi, 2016). In time past, wastes and their disposal did not pose a significant problem, for the population was small, the amount of land available for the assimilation of wastes was large and people ate directly from nature so that processing and packaging were little or non-existent (Chukwuemeka *et al.*, 2012).

Due to dense human and animal population as well as urbanization and non-advanced pattern of consumption in developing countries, the amount of waste generated is often beyond the local ecosystems biodegradative threshold and ecological imbalances have occurred where the natural assimilative capacity has been exceeded (Yao *et al.*, 2016). The inability of most administrations to manage the wastes has resulted in serious environmental pollution and epidemic outbreaks of diseases (Amoah and Kosoe, 2014). Municipal solid waste disposal is an enormous concern in developing countries across the world, as poverty, population growth, and high urbanization rates combine with ineffectual and underfunded governments, prevent efficient management of waste (UNEP, 2002). Solid waste problems are not new. It was not until the 19th century that public health control measure became a vital consideration to public officials, who began to realize that food waste has to be collected and disposed off in a sanitary manner to control rodents and flies, the vectors of diseases (WHO, 2016).

For example, the government of Nigeria has promulgated a fair collection of Federal, State and Local Government legislation on waste management yet; the public is largely ignorant of these laws (Sambo, 2016; Uchendu, 2016). And neither the Federal Government, nor State or Local Government possesses the expertise, equipment and technology to enforce the requirements of these laws. Because of these problems, solid waste as handled in Nigeria constitutes hazardous waste and liability for improper handling of waste has not been strictly enforced (Ziraba *et al.*, 2016).

In spite of these laws, states have allowed local contractors to adopt the most convenient method of waste evacuation. The use of open, unhygienically located dumps and the burning of refuse is a common practice in Nigerian cities (Okwesili *et al.*, 2016). Public waste containers are seen at various points in our cities which retain waste for days and sometimes weeks before they are cleared. Recently, these waste receptacles have disappeared and wastes are dumped on the roadside, forming embarrassing mountainous heaps.

It is therefore clear that the government and the public continue to underplay the need for discipline in waste management as is evidenced by the crude approach.

In developing country like Nigeria, because of ignorance and poor attitude towards the environment, wastes are not systematically collected and disposed off. The wastes are a mix of different materials such as domestic wastes, clinical/pathological wastes from health institutions and patent medicine stores as well as agricultural and industrial wastes. These are often dumped unsorted into creeks, riv-

ers, gutters, ditches, unprotected borrow pits or simply in an open unused piece of land (Obire *et al.*, 2002).

When waste is dumped, degradation sets in and it undergoes a number of biological, physical and chemical changes which in the presence of moisture, or rain results in a solution of high concentration of both chemical and biological substances referred to as leachate (Obire and Aguda, 2002). The decomposition of the wastes and production of leachate are usually accompanied by pungent odours due to the release of some gases. Ecological phenomena such as soil, water and air pollution have been attributed to leachates from improperly managed solid wastes for instance; leachate is known to have contaminated soil, surface and ground water resources (Murtaza *et al.*, 2017).

Aside from environmental degradation and loss of aesthetics, the waste dumpsite can be a source of pathogenic microorganisms some of which can be resistant to commonly used antibiotics. This could dangerously threaten the health of humans and animals having direct and indirect contact with the dumpsite and its products. The aim of this investigation was therefore to determine the bacterial diversity of a municipal solid waste dump soil, leachate and surrounding borehole water; and the possible presence of antibiotic resistant organisms with a view to providing a source of information for the government and general public on the potential impact of the present solid waste disposal method on humans and environmental health.

Materials and Methods

Description of the Study Area

The study area is a borrow pit used by the Rivers State Environmental Sanitation Authority for the dumping of unsorted, commingled municipal solid waste from all around the Port Harcourt Metropolis. It is located at Shell location road off Rmuolumeni Road in Port Harcourt. It lies within the geographic coordinates of P_1 : N $04^{\circ} 49' 42.26''$, E $006^{\circ} 58' 29.7''$; P_2 : N $04^{\circ} 49' 30.1''$, E $006^{\circ} 58' 25.7''$; P_3 : N $04^{\circ} 49' 30.7''$, E $006^{\circ} 58' 23.9''$; P_4 : N $04^{\circ} 49' 40.1''$ E $006^{\circ} 58' 24.7''$. It has with area of about 37,100 sq. meters and experiences a mainly tropical climate with annual rainfall of about 2,554.4mm (NMA, PH, 2007).

The site was a borrow pit from where clay was obtained for the construction of the Rumuolumeni- Iwofe Road. It was an open dumpsite about 100meters from the Rumuolumeni- Iwofe Road along Shell location Road. Between 2002 and 2007 and at the time of this study, this dumpsite received most of the municipal solid waste matter from the Port Harcourt metropolis and samples collected from it were therefore representative of the municipal solid waste stream from the city dumped by public and private waste management operators. Segregation and treatment does not occur for the different kind of waste except for scavengers who collect waste materials for recycling.

This dumpsite does not have any kind of infrastructure for protection against the contamination provoked by the leachate produced. The waste dumpsite is situated in an area of approximately 37,100m². There are residential houses sur-

rounding the dumpsite many of which are within close vicinity. Some commercial establishments and a private hospital are located about 15 meters away the dumpsite.

Experimental Design

The random sampling technique was used. In this method, samples were regularly taken randomly at the site from Geographical Positioning System (GPS) designated stations. Ten sampling points were randomly distributed in the dumpsite using an "Etrex V" GPS with World Geodetic System (WGS) 84 geographical settings.

Collection of soil samples, leachate and borehole water for analysis.

Soil samples from the municipal solid waste dumpsite and reference soil (control) were collected for the isolation of bacteria and to determine their frequencies. The control soil samples also collected from a fallow piece of land about 500m away from the dumpsite. A control was included to check for differences from the dumpsite soil. Leachate samples from the dumpsite and Borehole water samples in the residential areas around the dumpsite were also collected as to isolate the bacterial species and to determine their frequencies and analyse for potability of the borehole water.

Soil samples were collected from the 10 randomly designated points at the dumpsite from the control station. Using a soil auger, which ensures that sample were taken to exactly the same depth on each occasion from the zone of microbial activity. Surface soil (0-20cm) was taken from the 10 points into 3% acid alcohol sanitized plastic basin and a composite sample was taken from that bulk using a sanitized plastic spoon.

The soil samples are put into sterile Ziploc antifreeze polyethylene bags and sent to the laboratory for analysis. Two packs of the same soil sample were taken for physicochemical and microbiological analysis.

Leachate samples were collected at the same stations where the dumpsite soil samples were collected. Where this was not possible, the leachate closest to the dumpsite soil sampling station was collected. Acid-alcohol sanitized plastic spoon was used to collect the leachate samples into acid-alcohol sanitized plastic basin. After collecting, the leachate in the basin was mixed and sub samples were put into sterile Ziploc antifreeze bags and immediately sent to the laboratory for physicochemical and microbiological analyses.

Three borehole water sources located near the dumpsite were sampled and used to check for possible vertical or horizontal flow of the leachate into groundwater sources as well as the presence of coliforms. The water samples were collected using sterile 1 litre plastic containers. The taps were turned open, allowed water to run to waste for sometime before the collection into containers for microbiological analysis.

For all the sampling, simper-care nitrile L8-9 examination hand gloves were worn to avoid contamination of the samples by the body flora. All soil and leachate samples for microbiological analysis were stored in the fridge at 4⁰C and analysed within 48hrs.

Sample collection for this study lasted 12 calender months, from May 2006 to April, 2007, which included two seasons in Nigeria (wet and dry seasons). Soil

and Leachate samples were collected fortnightly while borehole water samples were collected monthly (on the first fortnight of each month that soil and leachate samples were collected).

Microbiological Analysis of Soil, Leachate and Borehole Water

Internationally accepted analytical methods and procedures were adopted in this study. Blanks, sterility control plates and duplicates were also analysed. Aseptic technique was used in all microbiological analysis. All glass wares such as pipettes, petri dishes, beakers etc were wrapped in aluminium foil and sterilized at 200°C for 1 hour in Carbolite PF 60 oven. Unless otherwise stated, all media, physiological saline (diluent), membrane filtration apparatus and Whatman No 1 filter paper (carefully wrapped in aluminium foil) were sterilized in the Schoeller- Bleckmann-steels (SBS 20) sterilizer and Tuttnauer 13200EN autoclave – steam sterilizer at 121°C and 15psi pressure for 15 minutes. Work bench top, and inoculating loop were sanitized with 3% acid alcohol. All used media in Petri dishes and test tubes were decontaminated in an autoclave before discharging into the environment.

The following media were used for bacterial estimation; Nutrient agar, MacConkey Agar, Slanetz and Bartley Medium, Eosin Methylene Blue Agar, Sensitest media and Peptone water. Diluent Used for Inoculum Preparation was 0.85% (W/V) physiological saline

Inoculum Preparation and Inoculum Size Enumeration

The soil was homogenized and 1.0g was aseptically transferred using a flame sterilized steel spatula, into a sterile test tube containing 9.0ml of the diluents. 1ml of water or leachate was aseptically transferred into a sterile test tube containing 9.0ml of diluents. These gave 10⁻¹ dilution.

For appropriate dilution 0.1ml was aseptically removed with a sterile pipette and used to inoculate (spread plate) triplicate sets of the media used for cultivation and enumeration of organisms. 30-300 colonies that developed after incubating were counted, except for the coliforms of which any number that grew was counted. The colonies were expressed as colony forming unit per gram or ml using the standard formula.

Isolation and enumeration of total aerobic heterotrophic bacteria

Nutrient agar was used for the culturing, isolating and enumeration of total aerobic heterotrophic bacteria. Soil bacteria were estimated by the soil dilution spread plate method in which serial dilution of the soil sample in sterile physiological saline were plated on nutrient agar plates, incubated at 37°C for 24 hours after which total aerobic heterotrophic bacterial counts were recorded. Discrete colonies were picked and sub cultured onto nutrient agar plates for subsequent preparation of axenic, stock and starter cultures for characterization and identification tests.

Axenic and Stock Cultures

Bacterial colonies picked based on morphological (shape, size etc) and cultural (colour, pigmentation) characteristics were sub cultured into 10ml of sterile peptone water and incubated at 37⁰C for 24 hours. Then a loopful of each culture was streaked onto nutrient agar plates and incubated as above. Discrete colonies were picked and Gram stained. Those that were distinctly Gram positive or Gram negative were then used to prepare stock cultures by streaking again onto nutrient agar slopes and incubated at 37⁰C for 24 hours. Then, the cultures (slants) were stored in the refrigerator at 4⁰C. Isolates from the cultures were inoculated into sterile peptone water and incubated at 37⁰C for 24 hours. These cultures served as the starter cultures used for various tests.

Identification of Bacterial Isolates

The pure isolates were identified to the species level on the basis of their cultural, morphological and physiological characteristics in accordance with schemes and methods described by Barrow and Feltham (1993). Microscopic examination of isolates was carried out using the oil immersion objective (oil plan 100/1.25 DIN) of Gillett + Sibert (GS) series 20 microscope. Attached to the microscope is a Ricoh XR/KR – 10M 35mm Single Lens Reflex (SLR) camera which was used to take slides photographs. Gram staining reaction was performed to distinguish between Gram positive and Gram negative bacteria. Other tests performed were: Motility test, Catalase test, Oxidase test, Citrate utilization test, MR indicates methyl red test while VP indicates Voges-Proskaur test and Methyl red Voges – Proskaur (MRVP) test.

From the results obtained from the above tests, the identification of isolates was concluded with reference to Bergey's Manual of Determinative Bacteriology by Holt *et al.*, (1994).

Antibiotic Sensitivity Test

The disc diffusion method of Kirby and Bauer (WHO, 1987) was used for this study to check the bacterial sensitivity to commonly used antibiotics.

Sensitest agar was prepared from a dehydrated base according to the manufactures (Lab M) recommendation. The medium was cooled to 40-50⁰C and poured into the plates, set on a level surface, to a depth of approximately 4mm. when the agar has hardened, the plates were dried for 30 minutes at 35⁰C by placing them in the upright position in the incubator with the lids titled.

Nutrient broth prepared according to the manufactures recommendation, was distributed in 5ml quantities and sterilized by autoclaving. Then antibiotic disc which contained gentamycin (10µg), ampicin (10µg), ciprofloxacin (10µg), erythro-mycin (10µg), tetracycline (30µg), amoxicillin (30µg), ampiclox (30µg), streptomycin (30µg), chloramphenicol (30µg) and previously stored in the refrigerator were brought out and left at room temperature for one hour to allow the temperature to equilibriate. This procedure reduces the amount of condensation that occurs when warm air reaches the cold container.

Single antibiotics discs were placed on the inoculated plates using a pair of sterile forceps spaced evenly approximately 15mm from the edge of the plate. For convenience, a template was used to place the discs uniformly. Each disc was gently pressed down to ensure even contact with the medium and inoculum. The plates were incubated at 35⁰C within 30 minutes of their preparation for 18hrs. No plate was stacked on the other. At the end of the incubation period, the diameter of each zone (including the diameter of the disc) was measured and recorded in mm. Measurements were made with a ruler on the undersurface of the plate without opening the lid. The results were then interpreted according to the critical diameter (zone size interpretive chart) of NCCLS (1984) as cited in WHO/LAB (1987).

Determination of Total Coliform for borehole water

Total coliform in borehole water samples was determined by membrane filtration in accordance with ASTM D 5392-93.

Membrane filtration method was performed by setting up the membrane filtration apparatus comprising of Erlenmeyer flask, vacuum pump and porous support. With the aid of a sterile forceps, sterile absorbent pad was placed in sterile Petri dish and saturated with sterile MacConkey broth (a selective medium). Sterile membrane filter (0.45µm) was placed on porous support using sterilized forceps.

After filtration, the membrane filter was placed in the Petri dish on the pad with the grid side up. This was carefully done in such a way that no air bubble was trapped between the pad and the filter. All plates including duplicates were incubated in inverted position at 37⁰C for 24-48 hours (Shirley and Bissonnette, 1991). After incubation, yellow colonies were counted as the number of total coliform bacteria while colonies of other type were not counted. Counts were reported as colony forming units per 100ml (CFU/100ml) of borehole water sample analyzed.

Results

The monthly mean of bacterial count in the control soil, dumpsite soil, and leachate is presented in Figures 1 while that of the borehole water is presented in figures 2. Bacterial counts for the control soil, dumpsite soil, leachate and water ranged from 5.2×10⁵ to 1.03×10⁶ CFU/g, 4.9×10⁶ to 1.9×10⁷ CFU/g, 9.0×10⁶ to 1.5×10⁷ CFU/ml and from 9.5×10¹ to 1.7×10² CFU/ml respectively.

Results for coliform count showed that no coliform was isolated from the borehole water during the period of study except for the month of November when two *E. coli* were isolated.

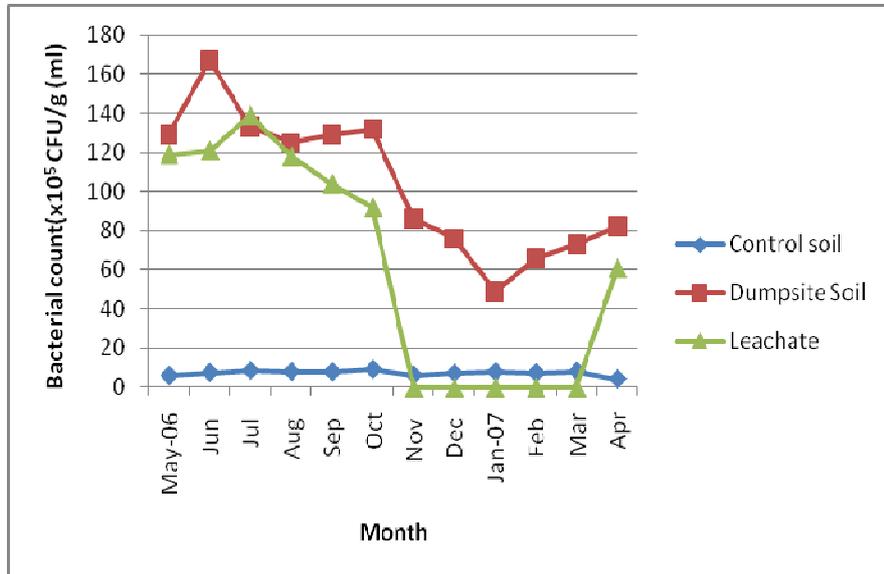


Fig. 1: Mean of heterotrophic bacterial count of control soil, dumpsite soil and leachate

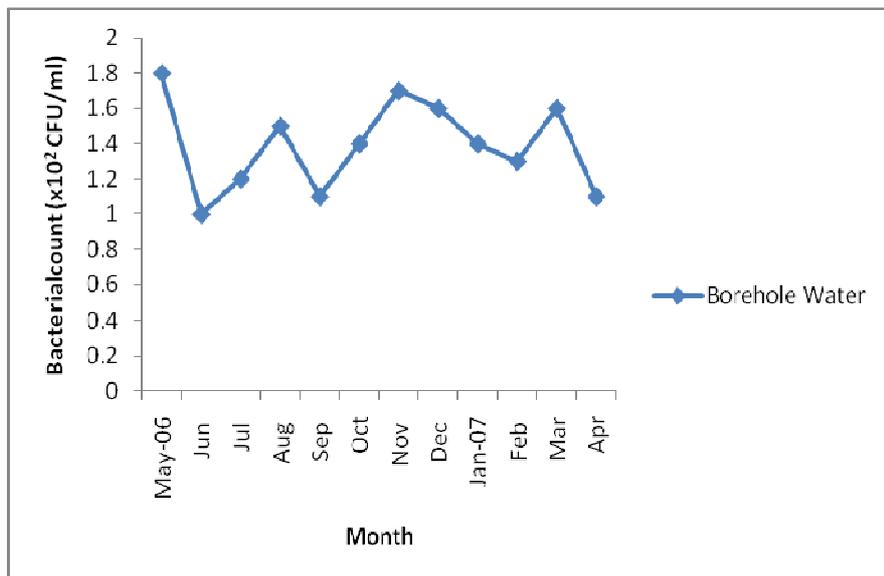


Fig. 2: Mean of heterotrophic bacterial count of borehole water

The frequency of occurrence (%) of the bacterial isolates in the various samples during the 12 months of investigation is as shown in the Figure 3 below. The bacteria isolated from the dumpsite soil and leachate were *Escherichia coli*,

Pseudomonas aeruginosa, *Pseudomonas fluorescense*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Bacillus cereus*, and *Proteus vulgaris* while *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Bacillus cereus* and *Corynebacterium xerosis* were isolated from the control soil. Bacteria isolated from the borehole water were *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Proteus vulgaris*.

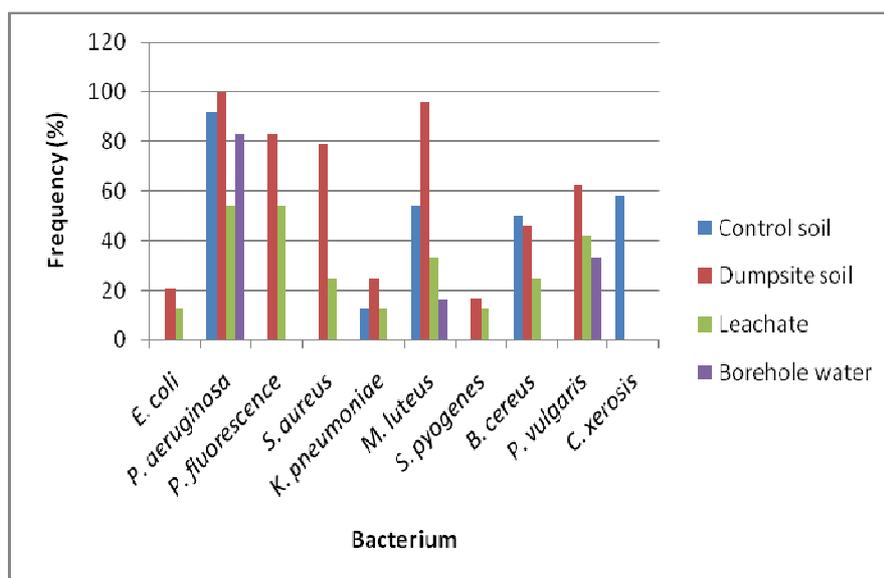


Fig. 3: Frequency of bacteria in soils, leachate and borehole water

A dominant, abundant, frequent, occasional and rare (DAFOR) analysis of the bacterial species isolated showed that *P. aeruginosa*, *P. fluorescense*, and *M. luteus* were dominant at the dumpsite soil, *S. aureus* and *P. vulgaris* were abundant while *B. cereus* was occasional. *P. aeruginosa* was dominant in the control soil, *M. luteus*, *B. cereus* and *C. xerosis* were frequent while *K. pneumoniae* was rare. *M. luteus* was dominant in the leachate while *P. aeruginosa* and *P. fluorescense* were frequent. *P. aeruginosa* was dominant in the borehole water while *M. luteus* was rare.

The resistance of bacterial isolates to the tested antibiotics is shown in Figure 4. All the isolates were resistant to Ampicillin. On the other hand, none of the organisms was resistant to Gentamycin and Ciprofloxacin.

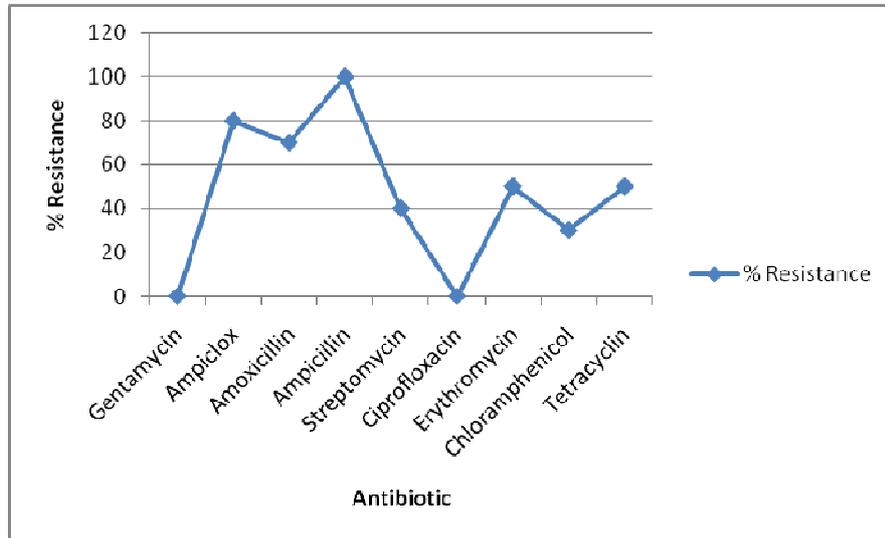


Fig. 4: Resistance of bacterial isolates to the tested antibiotics

The sensitivity (%) of bacteria isolates from waste dump soil to tested antibiotics is shown in Figure 5. While the number of resistant isolates to the number of antibiotics is shown in Figure 6.

P. aeruginosa, *P. fluorescence* and *S. aureus* were the most resistant (66.67%) to the antibiotics; being resistant to six (6) of the nine (9) antibiotics used. On the other hand, *C. xerosis* and *K. pneumoniae* were the least resistant (22.22%) being resistant to only two (2) of the antibiotics used. This showed that all the isolated bacteria exhibited multiple drug resistance (Figure 6).

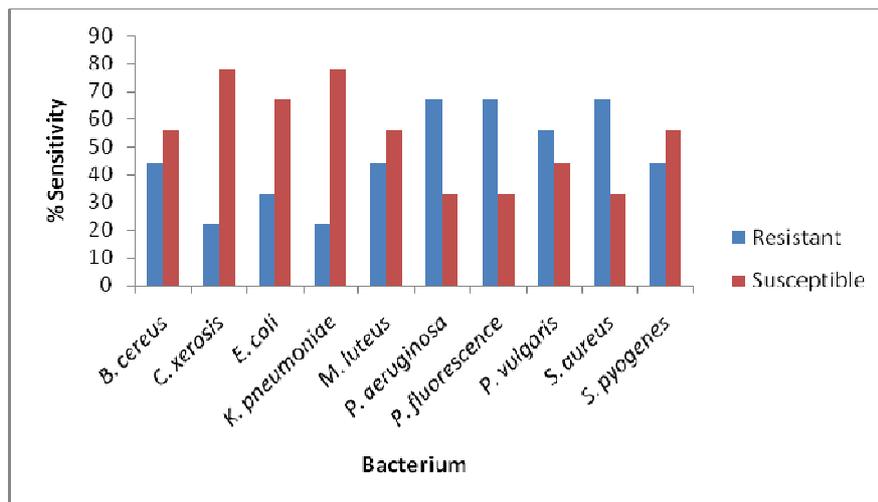


Fig. 5: Sensitivity of bacteria from waste dump soil to tested antibiotics

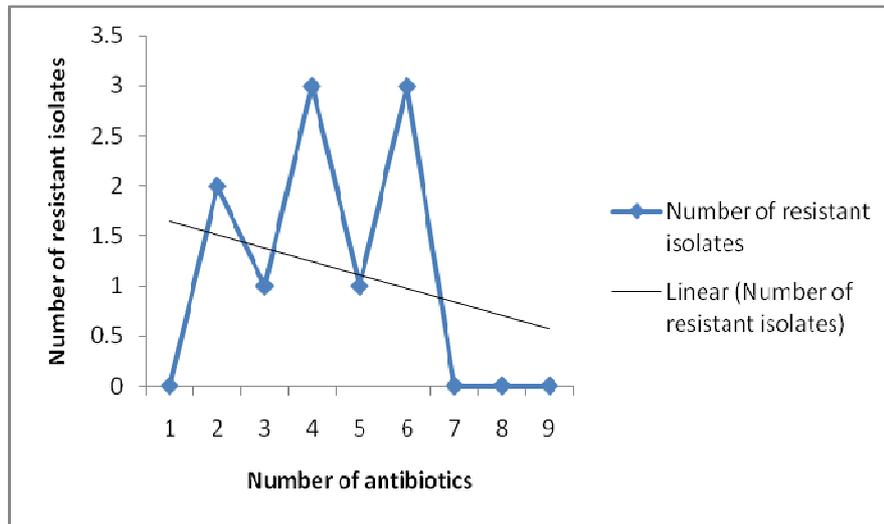


Fig. 6: Resistance of isolates to number of antibiotics

Discussion

The present investigation has revealed the population (count) and types of bacteria and the antibiotic susceptibility of bacteria of a municipal waste dump soil and its associated leachate and those of nearby borehole water.

The mean values of bacterial counts for the dumpsite soil were higher than for the leachate. This may be because the organic wastes were actually mixed with the soil and so more of the wastes were associated with the soil than with the leachate. The dilution effect of rain may also affect the concentration of organic matter in the leachate thereby encouraging the presence and growth of more bacterial species in the soil-waste mixture. Furthermore, the soil matrix may have provided a substratum for attachment of more bacterial cells than the leachate. The control soil count was a “degree” less than that of the dumpsite soil and leachate. This was not unexpected since the dumpsite soil and leachate had more nutrients which supported the growth and proliferation of more bacteria.

The mean values of bacterial count for the dumpsite soil though in agreement with those reported from similar environment by Obire *et al.* (2002), the bacterial counts for the leachate reported in this present study were numerically slightly higher than those reported by Obire and Aguda (2002). The present study showed slightly higher bacterial count than those reported by Obire *et al.* (2002). It is possible that the high salinity of the mangrove environment surrounding their study site may have influenced the lower count.

The mean values of bacterial count of the borehole water sample were far lower than those of the waste dump soil and leachate and control soil. The same pattern of higher values was recorded for the waste dump soil and leachate during the wet season and lower values during the dry season was maintained for the bacterial

counts. Peak values for bacterial count were observed in June for dumpsite soil, October for control soil and July for leachate. The results obtained in this study agreed with those reported by Obire *et al.*, (2002) that high bacteria counts were recorded during the peak of the rainy (wet) season.

The bacterial counts in the borehole water near the municipal solid waste dumpsite showed little variability and slight high and low consistency throughout the study period. Counts too were generally low. It appeared therefore, that the season does not have a major influence on the bacteria counts of the borehole water. This may be attributed to the fact that the surrounding soil has a clayey texture which impeded the easy leaching of material to the groundwater shielding it from the several impacts of the waste dump and the many vicissitudes of environmental and seasonal factors. Ukpong and Okon (2013) and Palamuleni and Akoth (2015) also reported similar findings.

At 95% confidence limit, a two-factor ANOVA for bacterial count revealed that there were significant differences between the bacterial counts during the sampling period. Also, there were significant interactions between the bacterial count and the period. This is shown in the F-calculated values being very much greater than the critical values.

This constant and high significance may have been due to the nature of the study site. It was a site with abundant nutrient sources allowing various microbial species to grow and proliferate especially at certain times when more organic wastes are dumped than at such times when the municipal waste stream would comprise more of difficult-to-degrade materials.

The bacterial species isolated in the course of this study were *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescense*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Bacillus cereus*, *Proteus vulgaris* and *Corynebacterium xerosis*. The DAFOR analysis on the isolation/occurrence of bacterial species in the different samples revealed that, *P. aeruginosa*, *P. fluorescense* and *M. luteus* to be the dominant species in the dumpsite soil. *P. aeruginosa* was dominant in the control soil and water. *S. aureus* was abundant in the dump soil. *P. aeruginosa* and *P. fluorescense* occurred frequently in leachate while *M. luteus* and *B. cereus* occurred frequently in the control soil. Other organisms occurred either occasionally or rarely. Interestingly, *E. coli* and *K. pneumoniae* had lower frequencies.

All the organisms isolated in this work have been reported in previous studies to be associated with waste materials (Obire and Aguda, 2002; Obire *et al.*, 2002). Most of the bacterial genera isolated in this study have been reported by other workers as potential pathogens (Chesborough, 1985). In addition to the general ubiquity of microorganisms and their metabolic versatility, the presence of these potential pathogens reported in this investigation may be attributed in part to the disposal of raw human faecal discharges and other human wastes at the dumpsite (Obire *et al.*, 2002). The presence of these pathogenic forms in open dumpsite in towns and cities is a major health and environmental threat and a cause for concern considering that leachate from such dumpsite often form part of the storm water in cities.

The bacteria isolated from the borehole water include *Pseudomonas aeruginosa* and *Micrococcus luteus*. Some of these bacterial species have been reported to be capable of growth in potable water even in the absence of coliform organisms

and can be pathogenic (Pandey *et al.*, 2014). Coliforms were not detected or isolated in the borehole water sample except in November when two *Escherichia coli* were isolated.

Generally, the borehole water met the WHO standard for microbiological water quality. However, the presence of these other organisms in the water is a cause for concern since some could become pathogenic when the immunity of persons drinking such water becomes lowered or compromised. The presence of *E. coli* in the water samples in November 2006 is particularly disturbing. However, because the water had no coliforms except in November, it is possible that this contamination is not a serious problem and could not have resulted from underground contamination by leachate.

The occurrence of similar organisms such as *Pseudomonas aeruginosa* and *Micrococcus luteus* in the dumpsite soil, control soil, leachate and water may be more than a coincidence. It has been shown that in some situations, biological contaminants can travel long distances underground without appreciable attenuation by aquifer material. However, that these organisms are also regularly found in the control soil may suggest that they are the normal flora of the dumpsite neighborhood.

A consideration of the economic microbiology of these organisms will also be interesting. The positive activities of these organisms in waste management can be properly harnessed to accelerate the bioconversion of waste into compost/organic fertilizer for use in gardening, agriculture and horticulture (Obire *et al.*, 2002).

The antibiotic sensitivity response of isolated organisms indicated that none of the isolates was resistant to gentamycin and ciprofloxacin. The efficacy of these two antibiotics to most bacterial species has been reported. They have been considered as being the most effective antibiotics against Gram positive and Gram negative infections respectively. However, sensitivity response to these two drugs was higher among the Gram negative than the Gram positive bacteria. Ibiebele and Sokari (1989) had reported that all the organisms isolated from well water were susceptible to gentamycin. However, Sokari and Kigigha (1996) reported resistance to gentamycin and sensitivity to ciprofloxacin by bacterial species isolated from medicine bottles in Port Harcourt. Thus, generally, gentamycin and ciprofloxacin are effective against most bacterial species isolated in this present study. This effectiveness may be due to the fact that the drugs though common are not used frequently probably because they are less known, may be more expensive, or because of their nephrotoxic side effects (Fair and Tor, 2014).

All the Gram negative bacteria were resistant to erythromycin but the Gram positive bacteria had intermediate response. This common response among the two groups of bacteria may be a reflection of a common property shared such as the nature of their cell wall or by-pass mechanism exhibited by most Gram negative organisms (Silhavy *et al.*, 2010). Isolates were generally resistant to the penicillins (Ampiclox, Amoxicillin and Ampicillin). In fact, in almost all cases where isolates exhibited multiple or simultaneous resistance, the antibiotics included the penicillins, chloramphenicol and tetracycline. It is possible that the organisms have been regularly exposed to these antibiotics considering the self medication culture in Nigeria (Obire *et al.*, 2009). Thus, a selection pressure may have ensured the survival and acquisition of resistance factors against these antibiotics (Fair and Tor, 2014). This

trend is disturbing considering the fact that these antibiotics are among those commonly used in Nigeria.

Generally, *S. aureus*, the *Pseudomonas* species and *P. vulgaris* were more resistant to all the antibiotics tested while *M. luteus*, *S. pyogenes*, *B. cereus*, *C. xerosis*, *E. coli* and *K. pneumoniae* were more sensitive to all the antibiotics; although there was no antibiotic to which all the isolates were sensitive or resistant. However, resistance was more among the Gram negative than the Gram positive organisms. This observation is supported by Drawz and Bonomo (2010). This may be as a result of the production of beta lactamase elaborated by Gram negative bacteria or the nature of their cell wall. *S. aureus* also produces beta lactamase and this may account for its resistance to the antibiotics (Drawz and Bonomo, 2010).

Conclusion

The bacterial isolates in this study exhibited multiple resistance to several antibiotics. Occurrence of multiple resistant bacterial strains in polluted site such as a MSW dumpsite is in agreement with the observations of Alam and Deng (2015). This level of resistance would be attributable at least in part, to the uncontrolled use of antibiotics and the self medication practice which is very common in Nigeria (Obire *et al.*, 2009) as well as the extent of pollution at such dumpsite and the composition of the wastes. These factors may combine to provide an intense selection pressure in favour of organisms that possess genes coding for drug resistance. Munita and Arias (2016) pointed out the importance of bacteria acting as a reservoir of plasmids coding for antibiotic resistance. The ingestion (or inhalation) of such resistant bacteria by humans could lead to a transfer of drug resistance to the recipient gut flora and/or to susceptible pathogens by cross infection (Bottone, 2010). The existence of multiple resistant bacteria at the MSW dumpsite studied therefore constitutes a public health hazard. It is of great concern in healthcare as only a combination of antibiotics may be useful in combating medical situations involving such multiple resistant strains. Good waste management practice is important to reverse this trend in addition to avoiding self medication and drug abuse.

Correspondence

Professor Omokaro Obire
Department of Microbiology
Rivers State University
P.M.B 5080, Port Harcourt, Nigeria
E-mail: omokaro515@yahoo.com

References

- Amoah, S.T. and Kosoe E. A. (2014). Solid waste management in urban areas of Ghana: Issues and experiences from Wa. *Journal of Environment Pollution and Human Health*. 2 (5): 110-117.
- ASTM (1999). Water and environmental technology. *Annual book of ASTM standards*. American Society for Testing and Materials, Philadelphia Section 11:01-05.
- Barrow. G. L. and Feltham, R. K. A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edition. Cambridge, UK: Cambridge University Press. P.331.
- Bottone E. J. (2010). *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev*. 23(2): 382 - 398.
- Cheesbrough, M (1985). *Medical Laboratory Manual for Tropical Countries*. London. 2: 58-204
- Chuku U. J and Anuchi S. O. (2016). Impact of abattoir wastes on the physicochemical properties of soils within Port Harcourt Metropolis. *The International Journal of Engineering and Science*. 5 (6): 17 – 21.
- Chukwuemeka, E. E. O., Osisioma, B. C., Onwuka, Ebele and Ugwu, Joy. (2012). The challenges of waste management of Nigeria sustainable development: A study of Enugu State. *International Journal of Research Studies in Management*. 1 (2): 79 – 87.
- Drawz S. M and Bonomo R. A. (2010). Three decades of β -Lactamase inhibitors. *Clin Microbiol Rev*. 23(1): 160–201.
- Fair, R. J. and Tor Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. 6: 25–64.
- Holt J. G, Kreig, N. R., Sneath, P. H. A., Staley J.T. and Williams, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*. 9th Edn. Williams and Wilkins.
- Ibibebe, D. D. and Sokari T. G. (1989). Occurrence of drug resistant bacteria in communal well water around Port Harcourt. Nigeria. *Epidemiol Inf*. 103: 193-202.
- Munita J. M and Arias C. A. (2016). Mechanisms of antibiotic resistance. *Microbiol Spectr*. 4(2): 1 – 37.
- Murtaza G, Habib R, Shan A, Sardar K, Rasool F and Javeed T. (2017). Municipal solid waste and its relation with ground water contamination in Multan, Pakistan. *International Journal of Applied research*. 3(4): 434 – 441.

Nigeria Metrological Agency (NMA). (2007). Department of Metrological Service, Federal Ministry of Aviation, Federal Secretariat, Port Harcourt.

Obire, O and Aguda, M (2002). Bacterial Community of Leachate from a waste dump and on adjacent stream. *J. Appl. Sci. Environ. Mgt.* 6(2): 71-75.

Obire. O., Gbarawin. D and Puteti R. R. (2009). Antibiotic resistance in *E. coli* isolated from patients. *Drug Invention Today.* 1(2): 140 – 145.

Obire, O, Nwaubeta, O and Adué S. B. N. (2002). Microbial Community of a Waste-dumpsite. *J. Appl. Sci. Environ. Management.* 6(1): 78-83.

Alam O and Deng T. (2015). Environmental and public health risks associated with antibiotic resistance genes (ARGs) spread in environment: A comprehensive review. *International Journal of Scientific Research in Science and Technology.* 1(4): 128 – 139.

Okwesili J, Ndukwe C and Nwuzor C. I. (2016). Urban solid waste management and environmental sustainability in Abakaliki urban Nigeria. *European Scientific Journal.* 12 (23): 155 – 183.

Palamuleni L and Akoth M. (2015). Physicochemical and microbiological analysis of selected borehole water in Mahikeng, South Africa. *Int J Environ Res Public Health.* 12(8): 8619–8630.

Pandey P. K, Kass P. H, Soupir M. L, Biswas S and Singh V. P. (2014). Contamination of water resources by pathogenic bacteria. *AMB Express.* 4: 51 – 66.

Shirley. J. J. and Bissonnette, G. K. (1991). Detection and identification of ground-water bacteria capable of escaping entrapment on 0.45- μ m-pore-size membrane filters. *Applied and Environmental Microbiology.* 5(8): 2251-2254.

Silhavy T. J, Kahne D, and Walker S. (2010). The bacterial cell envelope. *Cold Spring Harb Perspect Biol.* 2(5): 1 – 16.

Sokari, T. G. and Kigigha L.T. (1996). Characteristics of Bacteria from medicine Dispensing Bottles sold in Port Harcourt, Nigeria. *Discov. Innov.* 10(3/4): 50-54.

Ukpong E. C and Okon B. B. (2013). Comparative analysis of public and private borehole water supply sources in Uruan Local Government Area of Akwa Ibom State. *International Journal of Applied Science and Technology.* 3(1): 76 – 91.

United Nation Environment Programme. (2002). Africa Environment Outlook. Past, Present and Future perspective. Available <http://www.unep.org/neo/210.htm>. [11/23/07]

WHO. (1987). *Guidelines for Antimicrobial Susceptibility Testing*. WHO/LAB/87.1:21-38.

WHO (2016). International health regulation. *Handbook. Vector surveillance and control at Ports, airports and ground crossings*. 92pp.

Yao X, Wang Z and Zhang H. (2016). Dynamic changes of the ecological footprint and its component analysis response to land use in Wuhan, china. *Sustainability*. 8 (329): 1 – 14.

Ziraba A. K, Haregu T. N and Mberu B. (2016). A review and framework for understanding the potential impact of poor solid waste management on health in developing countries. *Archives of Public Health*. 74: 55 – 65.