Environmental Distribution and Antibiotic Resistance Patterns of Bacterial Isolates from Open Drainage Systems in Port Harcourt, Southern Nigeria

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Abstract: Bacterial isolates from wastewater and sediments of the Ntanwogba open drainage system in Port Harcourt city was tested for their susceptibility to antibiotics. Wastewater samples were collected twice a week for a period of six months from February through July using standard analytical methods. Results obtained showed that the sediment samples had *Escherichia coli* with the highest percentage of occurrence of 23.8%, followed by *Klebsiella pneumoniae* and *Proteus mirabilis* M18 with 19% each, while *Burkholderia multivorans* and *Pseudomonas fluoresceins* had the least occurrence of 4.8% each. Wastewater samples showed that *Escherichia coli* also had the highest percentage occurrence of 21.2%, followed by *Enterobacter asburiae* and *Plesiomonas shigelloides* with 15.2% each, while *Pseudomonas fluoresceins* had the least occurrence of 6.1%. Antibiotic sensitivity of strains was conducted using the disc diffusion method. The antibiotic sensitivity test carried out on the bacterial isolates showed 100% resistance to Augmentin, Ceftazidime, Cefuroxime, Ceftriaxone, Cloxacillin and Cefi.xime. However, 2.08% were susceptible to Ciprofloxacin, 33.3% to Erythromycin, 77.8% to Gentamicin, 87.5% to Nitrofurantoin and 96.3% to Ofloxacin. *Bacillus ginsengisoli* was the most resistant, but sensitive to only Ofloxacin, *Burkholderia multivorans* was the most susceptible bacterial isolate and was susceptible to Ciprofloxacin, Gentamicin, Nitrofurantoin, Ofloxacin. This was followed by *Enterobacter asburiae* that was sensitive to Gentamicin, Nitrofurantoin, Ofloxacin. While *Escherichia coli, Klebsiella pneumoniae, Plesiomonas shigelloides, Proteus mirabilis* Ml 8, *Proteus mirabilis* Ml 9, *Pseudomonas fluoresceins* and *Pseudomonas nitroreducens* were all sensitive to Nitrofurantoin and Ofloxacin. The high level of resistance to antimicrobial agents recorded in this study shows that the wastewater effluents and the receiving water bodies could pose a potential health risk to the surrounding communities who depend on these water sources for various domestic activities. Therefore, proper waste water management is fundamental for maintaining public health and protecting the quality of the environment.

Keywords: Open drains, pathogenic bacteria, antibiotic resistance, wastewater, sediments

Introduction

Drainage system is an interconnecting network of watercourses for carrying off excess water. It is a crucial part of living in a city or urban area, as it lessens storm water by carrying runoffs away to nearest watercourses or ground water. According to Alom and Khan (2014), costal inhabitants including Nigeria have encountered grave problems as a result of poor drainage systems. The lack of adequate waste collection and disposal system results in residents dumping their refuse into drainage systems thereby causing poor sanitation as it leads to the blockages of drains. Runoffs from these blocked drains pick up significant amount of debris, heavy metals, litters, nutrients, oil, organic matter, sediment and bacteria especially during torrential rain falls and then forms stagnant pool that provides breeding sites for diseases like typhoid, infantile diarrhoea, malaria, yellow fever, schistosomiasis and cholera in humans and animals (Odeyemi, 2012), and causes pollution and stench, thus defacing aesthetic value of the environment (Ogbonna *et al*., 2007).

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Open ground excreta defecation and grey water from kitchens and washing cloth flow through drainage canals that are connected to rivers and streams (Mazhindu *et al*., 2010). This river is used as sources of washing cloths and bathing for the waterfront communities who live near and around to the river. Improper management of household wastewater adversely affects the environment by unpleasant or offensive odour and diseases like typhoid, diarrhoea, common cold and asthma. It also affects the quality of the environment through reduction in the aesthetic value of the city due to unpleasant odour by the stagnant pool of the wastewater on the open drainage channels. Such open channels provide a rich source of microorganisms most of which are pathogenic (Odeyemi *et al*., 2011, Odeyemi, 2012). This is because most of the rodents and vector insects are attracted to filthy dumps which serve as shelter and food source (Ogbonna and Erheriene, 2017). Reports show that bacteria such as *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis* and *Pseudomonas aeruginosa* are commonly found in such wastewater and sediment samples of such drainage systems which may subsequently cause contamination of water resources which serves as a source of water supplies to the immediate community (Ogbonna and Erheriene, 2017: Nrior *et al*., 2017a).

According to Finch *et al.*, (2003) and Kummerer (2004) antimicrobial agents plays major roles in reducing the burden of infectious diseases that may affect the public. However, the impact of these antibiotics is negated by the emergence of resistance and its rapid spread. When a microorganism is able to withstandthe effects of an antimicrobial drug, it is considered to be resistant. Inappropriate and incoherent use of antibiotics is the predominant factor that promotes the spread of resistance (Ndihokubwayo *et al.*, 2013). Most discharges of untreated wastewater from houses, industries and hospitals release concentrated forms of infectious agents and antibiotic resistant microbes which possess resistant genes (Davidson, 1999; Abah and Ohimain, 2010). The aim of this study therefore was to reveal the antibiotic resistance patterns of bacterial isolates from open drainage systems in Port Harcourt metropolis.

Materials and Methods

Description of Area of Study

The Ntanwogba creek is located on the western flank of Port Harcourt city of Rivers State, Nigeria. The stream lies between latitude 40 50'' and 50 00''N, and longitude 60 55'' and 70 00''E. According to Gobo *et al* (2008), the climate of the area is that of tropical equatorial latitude with rainfall occurring almost all year round. As one of the coastal states of Nigeria, it has one of the highest average rainfalls. The flat estuarine terrain and impermeable alluvial soil make drainage difficult. The Ntanwogba creek is a black water stream with its water source which runs through Orazi forest of Rumueme town across Abacha Road, Cherubim Road, Olu-Obasanjo Road, Okija Road and Afam Street (D/line), and meanders through the densely populated city of Port Harcourt into the Upper Bonny Estuary.

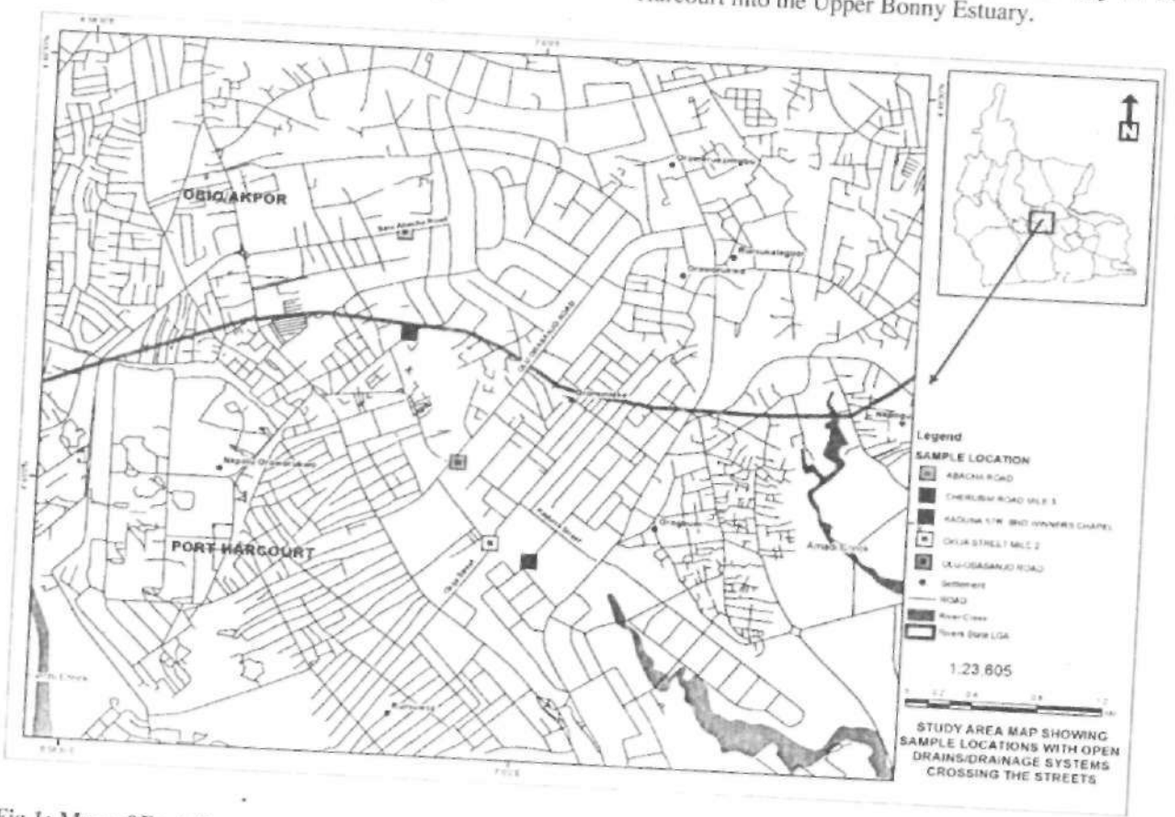

Fig 1: Map of Port Harcourt showing sampling stations along the Ntanwogba creek

Collection of Samples

Wastewater samples were collected from open drains along the Ntanwogba creek with sterilized plastic bottles collected from the Department of Microbiology Laboratory of the Rivers State University. Each sample bottle was rinsed with the appropriate sample before the final collection according to the standard methods (APHA, 2012). To collect the water sample, base of the sterilized sample bottle was held with one hand, plunged about 30cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow (APHA, 2012). The container was filled with wastewater samples from different locations starting from the upstream (Afam /Kaduna street behind the Winners chapel) to the downstream (at Abacha road, all sites in Port Harcourt, Rivers State Nigeria) leaving a gap of about 2cm and then covered. Sediment samples for analysis were also collected along the same water course. To collect the sediment samples,

the bottles were opened and held with the left hand while using the right hand with a plastic scooper to scoop the sediment sample. The sample bottles were filled with sediment sample and covered immediately. After collection, the samples were immediately labelled and transported in a cooler packed with ice blocks for analysis. Sample collection was carried out twice a week for a period of six months from the month of February to July

Microbiological Analyses

Serial Dilution

Ten-fold Serial dilutions of the samples were made according to the methods described by Ogbonna and Idam (2007) and Oliveira *et al.* (2016). One millilitre each of the waste water samples obtained from the open drainage channels was separately added to 9 ml of 0.1% peptone water diluent to give a 10^{-1} dilution. After thorough shaking, further 10-fold (v/v) dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluent to a range of 10^{-3} dilutions.

Inoculation and Incubation

One millilitre of appropriate ten-fold serial dilution was inoculated onto appropriate surfaces of dried Nutrient agar, Blood agar, CLED agar, MacConkey agar and Salmonella-Shiegella agar platesin triplicates using the pour plate and spread plate methods. Colonies which grew on the plates were counted and recorded as colony forming unit (CFU) of the sample (Lateef *et al.*, 2005; Guo *et al.*, 2013; Hussain *et al.*, 2013; Nrior *et al.*, 2017a). Inoculated plates were incubated at 37°C for 24 hours.

Maintenance of Pure Culture

Bacterial isolates grown on Nutrient agar, Blood agar, Cystine lactose electrolyte deficient agar, MacConkey agar and Salmonella-Shigella agar were purified by repeated sub-culture onto nutrient agar media. Pure cultures were inoculated on Nutrient agar slants and incubated at 37°C for 24 hours, and then preserved in the refrigerator at about 4°C for further tests.

Antibiotic Sensitivity Test

Bacterial isolates were tested for their sensitivity and resistance to antibiotics by means of the disc diffusion method (CLSI, 2000). They were investigated using Gram positive discs by Abtek Biological Ltd containing the following antibiotics: Ceftazidime (CAZ) 30µg; Cefuroxime (CRX) 30µg; Gentamicin (GEN) 10µg; Ceftriaxone (CTR) 30µg; Erythromycin (ERY) 5µg; Cloxacillin (CXC) 5µg; Ofloxacin (OFL) 5µg and Augmentin (AUG) 30µg. Gram negative discs (Abtek Biological Ltd) containing the following antibiotics: Nitrofurantoin (NIT) 300µg; Ciprofloxacin (CPR) 5µg; Ceftazidime (CAZ) 30µg; Cefuroxime (CRX) 30µg; Gentamicin (GEN) 10µg; Cefixime (CXM) 5µg; Ofloxacin (OFL) 5µg and Augmentin (AUG) 30µg.

The commercial antibiotic discs were placed on nutrient agar plates previously seeded with 18-24 hours broth culture of the test organisms using sterile cotton swab stick. The plates were incubated at 37°C for 48 hours, after which zones of inhibition were examined and interpreted accordingly. Earlier, the potencies of all the antibiotics used in the study were confirmed using susceptible *Escherichia coli* strains obtained from Department of Microbiology Laboratory, Rivers State University, Port Harcourt, Nigeria.

DNA Extraction and Quantification

For identification, the chromosomal DNA of each isolate was extracted using a ZR fungal/bacterial DNA mini preparation extraction kit supplied by *Inqaba* South Africa according to the manufacturer's instructions.

Lysis of cells

One thousand microlitres of 24 hours old luria bertani culture containing bacterial isolates were introduced into ZR bashingbead lysis tubes and centrifuged at 14000xg for 2 minutes to concentrate the cells. The supernatant was decanted and the procedure repeated. A heavy growth of bacterial isolates was suspended in 200µl of isotonic buffer into ZR Bashing Bead lysis tubes, 750µl of lysis solution was added to the tube. The tubes were secured in a bead beater (Gene disruptor) fitted with a 2ml tube holder assembly and processed at maximum speed for 8 minutes. The ZR bashingbead lysis tube was centrifuged at 10000xg for 1 minute (Frostegard *et al.*, 1999; Kresk and Wellington, 1999).

Removal of contaminants

Using a collection tube 400µl of supernatant was transferred to Zymo-Spin IV filter and centrifuged at 7000xg for 1 minute. In the collection tube 1200µl of ZR fungal/bacterial DNA binding buffer was added to the filtrate bringing the final volume to 1600µl, 800µl was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10000xg for 1 minute, from the collection tube the flow through was discarded. The remaining 800µl was transferred to the same Zymo-Spin IIC column and spun. In a new collection tube 200µl of the DNA Pre-wash buffer was added to the Zymo-Spin IIC column and spun at 10000xg for 1 minute (Miller *et al.*, 1999).

Recovery of DNA

To elute the DNA, the Zymo-Spin IIC column was transferred to a clean 1.5µl centrifuge tube. 100µl of DNA elution buffer was added to the column matrix and centrifuged at 10000xg for 30 seconds. Using Nanodrop 1000 spectrophotometer the extracted DNA

was quantified and the ultra-pure DNA was stored at -20°C for other downstream reaction.

PCR Amplification of 16S Ribosomal RNA

The 16S ribosomal RNA (rRNA) of the bacterial isolates was amplified using the polymerase chain reaction (PCR) technique in which two universal primers: forward primer 27F' (5´-AGAGTTTGATCMTGGCTCAG-3´) and reverse primer 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used incorporated into the PCR mix. The mix included: the x2 dream taq master mix (taq polymerase, DNTPs, MgCl) supplied by Inqaba, South Africa, the primers at a concentration of 0.4M and the extracted DNA as template. The following PCR conditions were used: Initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 30 seconds in 35 cycles and final extension at 72°C for 5 minutes. The PCR product was confirmed by electrophoresis on 1% agarose gel at 120V for 15 minutes and visualized on UV trans-illuminator.

Sequencing was done at Inqaba Biotechnological, Pretoria South Africa using the Big Dye terminator kit on a 3510 ABI sequencer. With the bioinformatics algorithm trace edit the obtained sequences were edited and similar sequences were downloaded from the National Centre for Biotechnology Information (NCBI) database using BLASTN.

Results

Prevalence of Isolates from Sediment at the various Stations

Using molecular identification method, a total of ten (10) bacterial species were identified from the different sampling stations. The bacterial species were identified as *Bacillus ginsengisoli* strain A1Cr, *Proteus mirabilis M18, Klebsiella pneumoniae* strain DSM 30104, *Burkolderia multivorans* strain AUO, *Plesiomonas shigelloides* strain 187-907R, *Pseudomonas fluorescens* strain PF1, *Esherichia coli, Enterobacter asburiae* strain TYP8, *Proteus mirabilis M19, Pseudomonas nitroreducens* strain LBQSKN1 as. shown in (Table 1). The results show that the microorganisms isolated from the open drains had a percentage rate of 13% and 15% for *Bacillus ginsengisoli* respectively for wastewater and sediments and *Proteus mirabilis*had 12% and 11%while *Pseudomonas fluorescens* 10% and 12% from wastewater and sediments etc. respectively (Figs 1 and 2). Generally most of the microorganisms were isolated more from the wastewater samples.

Table 1: Distribution of Bacterial Isolates from all stations

Organisms	Abacha Road		Afam Street		Cherubim Road		Okija Road		Olu-Obasanjo Road	
	Sediment	Wastewater	Sediment	Wastewater	Sediment	Wastewater	Sediment	Wastewater	Sediment	Wastewater
Bacillus ginsengisoli	24 (14%)	19 (17%)	24 (11%)	18 (14%)	29 (14%)	20 (15%)	27 (12%)	20 (14%)	30 (14%)	21 (16%)
Proteus mirabilis M18	18 (11%)	13 (12%)	23 (11%)	11 (9%)	22 (10%)	16 (12%)	29 (13%)	17 (12%)	29 (13%)	14 (10%)
Klebsiella pneumoniae	11 (7%)	6 (6%)	17 (8%)	11 (9%)	9 (4%)	6 (5%)	14 (6%)	7 (5%)	15 (7%)	9 (7%)
Burkolderia multivorans	8 (5%)	2 (2%)	9 (4%)	3 (2%)	8 (4%)	3 (2%)	11 (5%)	5 (3%)	9 (4%)	4 (3%)
Plesiomonas shigelloides	19 (11%)	13 (12%)	23 (11%)	16 (13%)	24 (11%)	17 (13%)	25 (11%)	21 (14%)	23 (10%)	11 (8%)
Pseudomonas fluorescens	14 (8%)	10 (9%)	21 (11%)	16 (12%)	23 (11%)	16 (12%)	23 (10%)	18 (12%)	21 (10%)	17 (13%)
Esherichia coli	19 (11%)	14 (13%)	22 (11%)	12 (9%)	25 (12%)	12 (9%)	27 (12%)	11 (8%)	23 (10%)	12 (9%)
Enterobacter asburiae	16 (10%)	8 (7%)	22 (10%)	10 (8%)	16 (8%)	9 (7%)	11 (5%)	8 (5%)	15 (7%)	11 (8%)
Proteus mirabilis M19	20 (12%)	13 (12%)	25 (12%)	12 (9%)	27 (13%)	13 (10%)	33 (15%)	19 (13%)	28 (13%)	17 (13%)
Pseudomonas nitroreducens	19 (11%)	11 (10%)	24 (11%)	19 (15%)	28 (13%)	18 (14%)	25 (11%)	21 (14%)	25 (11%)	19 (14%)

Table 2: AntibioticResistance Profile of Bacterial Strains from the open drains

Antimicrobial Agents	*Bacillus ginsengisoli* A1Cr	*Burkholderia multivorans* AUO	*Escherichia coli*	*Klebsiella pneumoniae* DSM 30104	*Proteus mirabilis M18*	*Pseudomonas fluorescens* PF1	*Pseudomonas nitroreducens* LBQSKN1
Augmentin (AUG)	R	R	R	R	R	R	R
Ceftazidime (CAZ)	R	R	R	R	R	R	R
Ciprofloxacin (CPR)	0	S	I	I	I	I	I
Cefuroxime (CRX)	R	R	R	R	R	R	R
Ceftriaxone (CTR)	R	0	0	0	0	0	0
Cloxacillin (CXC)	R	0	0	0	0	0	0
Cefixime (CXM)	0	R	R	R	R	R	R
Erythromycin (ERY)	R	0	0	0	0	0	0
Gentamicin (GEN)	R	I	R	R	I	R	R
Nitrofurantoin (NIT)	0	S	S	S	S	S	S
Ofloxacin (OFL)	S	S	S	S	S	S	S
% Resistance	63.6%	36.4%	45.5%	45.5%	36.4%	45.5%	45.5%

Table 3: Antimicrobial Susceptibility Testing of the Isolates showing zones of inhibition

Antimicrobial Agents	*Bacillus ginsengisoli* A1Cr	*Burkholderia multivorans* AUO	*Escherichia coli*	*Klebsiella pneumoniae* DSM 30104	*Proteus mirabilis M18*	*Pseudomonas fluorescens* PF1	*Pseudomonas nitroreducens* LBQSKN1
Augmentin (AUG)	0	0	0	0	0	0	0
Ceftazidime (CAZ)	0	0	0	0	0	0	0
Ciprofloxacin (CPR)	-	32	25	27	25	29.5	25
Cefuroxime (CRX)	0	0	0	0	0	0	0
Ceftriaxone (CTR)	0	-	-	-	-	-	-
Cloxacillin (CXC)	0	-	-	-	-	-	-
Cefixime (CXM)	-	0	0	0	0	0	0
Erythromycin (ERY)	14.5	-	-	-	-	-	-
Gentamicin (GEN)	13.5	18.1	11	11.3	14.5	9	6.5
Nitrofurantoin (NIT)	-	25	17.5	22	21.5	22	22
Ofloxacin (OFL)	32	30	22	17	18.5	22	24

Table 2 shows the antimicrobial susceptibility pattern of the isolates using disc diffusion test. The results showed that the isolates were resistant to were 100% resistant to Augmentin, Ceftazidime, Cefuroxime, Cefixime, Ceftriaxone and Cloxacillin and this was demonstrated by their zones of inhibition while Nitrofurantoin and Ofloxacin were susceptible to the isolates. Oxfloxacin and Ciprofloxacin was highly susceptible to *Bacillus ginsengisoli* and with a diameter of zones of inhibition of 32 mm each while *Burkolderia multivorans and Pseudomonas fluorescens* were all susceptible to Oxfloxacin and Ciprofloxacin with a diameter of zones of inhibition of 32 and 29.5 mm respectively while *Pseudomonas nitroreducens* showed least resistance activity to Gentamycin with a diameter of zones of inhibition of 6.5 mm each (Table 3).

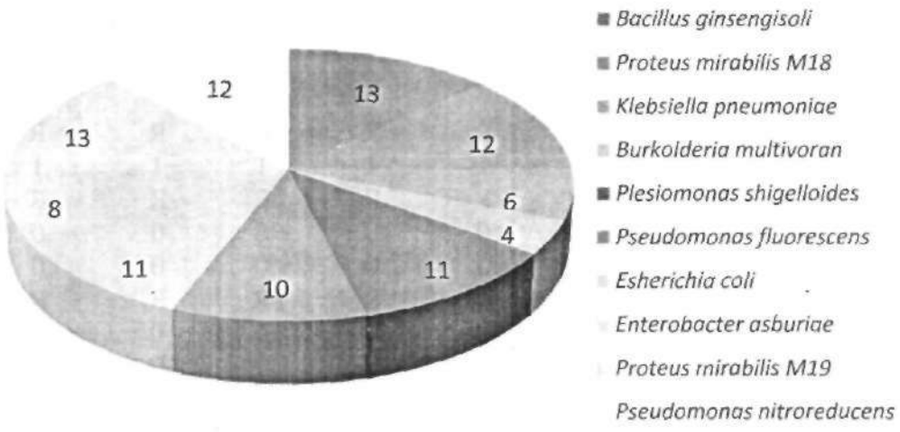

Fig 1: Frequency of Occurrence in Wastewater for all stations (%)

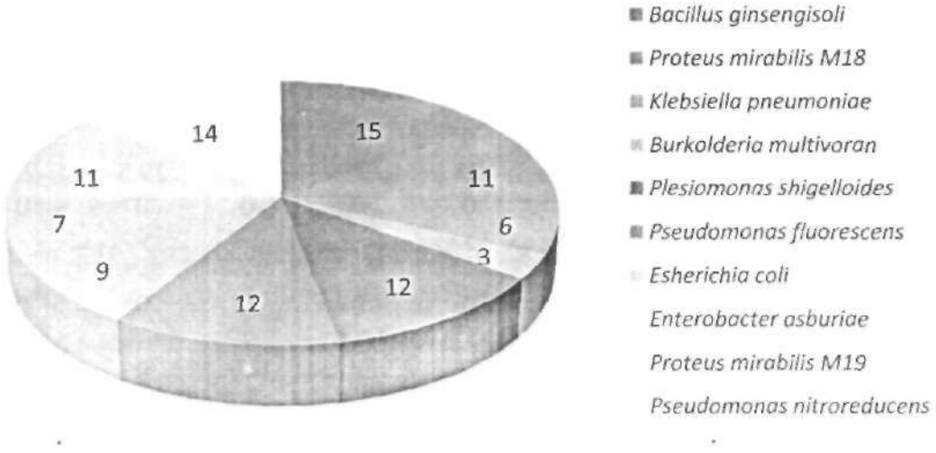

Fig 2: Frequency of Occurrence in Sediments for all stations (%)

The in this study could represent a potential public health risk.

Discussion

The results obtained in this study correlates with that of Adieze *et al* (2015) who observed high prevalence of antibiotic-resistant bacteria with various degrees of resistance to antibiotics in similar environments. The results show that the bacteria obtained from wastewater are higher than those obtained from sediment. The bacteria isolated from wastewater were identified as *Bacillus ginsengisoli, Burkholderia multivorans, Enterobacter asburiae, Escherichia coli, Klebsiella pneumoniae, Plesiomonas shigelloides, Proteus mirabilis* M19 and *Pseudomonas fluorescens*. Aside from runoffs from rainfall, other

sources are waste generated from various anthropogenic activities which are channelled directly into the tributaries of the river through open drainage channels. This act could introduce enteric pathogens such as *Escherichia coli* and excess nutrients into the river, resulting to eutrophication (Odeyemi, 1991; Adeyemo *et al.,* 2002). The consequences of such anthropogenic pollution during various socio economic activities can lead to the transmission of diseases by water borne pathogens, eutrophication of water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (Amisu *et al.*, 2003; Nafarnda *et al.*, 2012). There isa high number of bacteria from the family Enterobacteriaceae and a considerable number of coliform bacteria which indicates that these open drainage systems are hazardous and constitutes serious public health risk (Odeyemi, 2012). Out of the microorganisms isolated, *Escherichia coli* had the highest frequency of occurrence; this may be attributed to their ability to withstand competition from other indigenous microorganisms with higher growth rates (Lewis *et al.*, 2002).

Bacterial isolates from both wastewater and sediments from the open drainage system tested for their susceptibility to antibiotics were resistant to Augmentin, Ceftazidime, Cefuroxime, Cefixime, Ceftriaxone and Cloxacillin. Consequently, these antimicrobial agents are perhaps ineffective as the drug of choice in the treatment of infections caused by these organisms (Kummerer, 2004). The resistance observed here may be due to acquisition of resistance genes through specific proteins and efflux pumps (Prescott *et al.*, 2005). Also exposure to environmental pollutants and changes in nutrient composition can lead to selective pressure that favours antibiotic resistance in certain organisms (Furuya and Lowry, 2006; Ram *et al.*, 2008). A high level of resistance has been observed with members of the family Enterbacteriaceae which has further increased the incidence of pathogenic strains of bacteria with acquired antibiotics resistance. Ajayi and Akonai, (2003) reported that this is traceable to the faecal constituent of the wastes produced by humans or animals that have been treated indiscriminately with various antibiotics. In developing countries, where drugs are available to the public, self-administration or abuse of antibiotic by patients can cause increase in the prevalence of drug resistant strains (Lateef, 2004).

Conclusion

From the results obtained in this study, antibiotics resistant bacteria are widespread as nearly all the isolated microorganisms from the open drains were resistant to most of the antibiotics for which they were tested for. This may be due to either the intrinsic resistance of many microorganisms to antibiotics or acquired resistance of the organisms enabled by the transfer of resistance of drug resistance. The resistance to antibiotics by the bacterial isolates is very common and draws attention to the global challenges of antibiotic resistance to public health. In order to enhance the quality of the open drainage systems, the waste management authorities must conscientiously prevail on residents and those involved in various socioeconomic activities around drainage channels to check indiscriminate disposal of excreta and other waste products and improve on environmental sanitation. Improper management or discharge of wastewater from industries, factories and many households into open drainage channels may adversely affect the aesthetics of the environment through unpleasant or offensive odours and consequently cause diseases like typhoid, diarrhoea, common cold and asthma. Also, government should improve from time to time the inadequate sanitation infrastructure like drainage canals and wastewater treatment plants to contain flash floods that can contribute to health problems. Therefore increasing public understanding and awareness onopen drainages and wastewater management is critical (Idris-Nada *et al.*, 2013)

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