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RESEARCH ARTICLE

Class 1 Integron, Sulfonamide and Florfenicol Resistance Genes in Bacteria from Three Unsanitary Landfills, Ibadan, Nigeria

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ABSTRACT

Objective: Municipal solid waste (MSW) landfills recently emerged as potential reservoirs of antibiotic resistance. However, little is known about the potentials of unsanitary landfill sites which are common in developing countries as reservoir of antibiotic resistance. In this study, we investigated the antibiotic resistance pattern and presence of selected resistance genes in bacteria from three MSW landfills in Ibadan, southwestern Nigeria.

Methodology: Fifty six antibiotic- and metal-resistant bacteria isolated from leachate and leachate-contaminated surface and groundwater collected from three MSW landfills in Ibadan, Southwestern Nigeria, were searched for sulfonamides, florfenicol and beta-lactams resistance genes and integrons by PCR.

Results: None of the 27 β-lactamase genes tested was detected in the isolates. *sul1*, *sul2* and *floR* were however detected in 12 (21.4%), 2 (3.6%) and 12 (21.4%) bacteria species identified as *Alcaligenes faecalis*, *Advenella kashmirensis*, *Brevundimonas spp.* and *Ralstonia pickettii*. The 12 bacteria carrying *sul1*/*floR* gene combination were also positive for class 1 integron. Analysis of the gene cassettes in the variable regions of the class 1 integrons (>1.5kb) of the 12 intl1-positive isolates revealed the presence of *aadB* and unknown genes. The deduced amino acid sequence of the unknown portion of the gene cassettes shared 42-65% identity with sequences of Class D β-lactamase and OXA-2-like protein in the GenBank.

Conclusions: Findings of this study suggest that the landfill ecosystem is a potential site for the evolution of novel resistance genes and are hence important reservoir of antibiotic resistant bacteria. *J Microbiol Infect Dis 2019; 9(1): 34-42.*

Keywords: *Landfill leachate, antibiotic resistance, sul genes, floR, aadB, class D β-lactamase*

INTRODUCTION

Antibiotic resistance in bacteria is a major threat to human health worldwide [1] and environmental bacteria are increasingly being recognized as important reservoir of clinically relevant antibiotic resistance genes (ARG) [2]. There are increasing concerns about the impact of anthropogenic pollution on environmental reservoirs of antibiotic resistance [2,3] which may increase the possibility of recruiting antibiotic resistance genes into clinical bacteria species from environmental reservoirs of resistance [4]. As a result, attention is turning to potential reservoirs of resistance outside the clinic [5]. Presently, there are concerns that heavy metals, which are one of the commonest environmental pollutants may co-select for antibiotic resistance in environmental bacteria [6,7]. Co-selection may occur when genes for antibiotic and metal resistance are located in the same cell in a process called co-resistance or when a single resistance mechanism confers resistance to metal and antibiotics in a process called cross-resistance [8]. Metals enter the environment from industrial activities, agriculture, aquaculture, wastewater and municipal solid waste (MSW) usually dumped in landfills. Landfills represent an important source of metals input into the environment in many developing countries like Nigeria, where solid waste management is often a serious challenge.

Nigeria, with a population of about 160 million people generate about 0.58 kg of solid waste/person/day [9] which are commonly

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disposed in unsanitary landfills usually operated at standards below recommendations for public health safety. The wastes are not sorted before disposal, and mixtures of domestic, industrial, agricultural and hospital wastes are commonly co-disposed in the same landfill site. The landfills are also used by surrounding populations for open defecation and discharge of human and animal fecal waste containing high concentrations of pathogenic and potentially pathogenic bacteria species. These conditions make them potential hotspots for the development, proliferation and spread of antibiotic resistance. Once developed, resistance can spread from landfills into human populations through direct contact with the landfill or indirectly via leachate contamination of surrounding soil and aquatic ecosystems, especially where the resistance traits are associated with mobile genetic elements. This risk is particularly high for countries like Nigeria where landfill sites are often reclaimed for residential purposes and people living in close proximity to landfill sites use leachate-contaminated surface and groundwater sources for domestic purposes. There is also a growing population of scavengers who regularly visits urban landfill sites to collect recyclable materials as a means of livelihood.

Some recent studies have reported the incidence of antibiotic resistance in bacteria isolated from landfill leachate in Nigeria [10] and Thailand [11]. Similarly, Wang et al [12], Wu et al [13] and Song et al [14] reported the detection of ARGs in the metagenomic DNA fraction of leachate and refuse from landfill sites in China. Some of these studies have reported a positive correlation between the presence of metals and occurrence of antibiotic resistance genes in the leachate emanating from the investigated landfills pointing to possible co-selection pressure in the landfill ecosystem [15,16]. While these studies contributed immensely to establishing landfills as important reservoir of antibiotic resistance, they did not provide information on the taxonomic identity of the bacteria host and/or the genetic context of detected ARG. Further, few of the studies investigated the possible spread of ARB and ARGs into surrounding aquatic ecosystems via leachate contamination.

In this study, bacteria isolated from leachates and leachate-contaminated surface and ground water collected from, or within the vicinity of three unsanitary MSW landfills in Ibadan, southwestern Nigeria were tested for susceptibility to seven antibiotics and for tolerance to Zinc (Zn), Copper (Cu) and Lead (Pb). The isolated bacteria strains were searched for 31 genes conferring resistance to sulfonamides (n=3), beta-lactams (n=27) and florfenicol (n=1) as well as Class 1, 2 and 3 integrons. Our overall aim is to investigate the potentials of unsanitary landfills as reservoir of antibiotic resistance that can spread to the human population.

METHODS

Study area

Ibadan (7°23′47″N 3°55′0″E) the capital of Oyo State, Nigeria, is the third largest city by population in Nigeria with a population of 3.2 Million inhabitants. As at the time of this study, the city has three functioning unsanitary landfill sites namely Ajakanga, Aba-eku and Awotan established in 1996, 1997 and 1998, and covering approximately 10, 10 and 25 hectare^2 respectively. The sites are operated by the Ibadan Waste Management Authority. Ajakanga (07°18' 43"N 03°50' 27"E; 165 m above sea level) is in Oluyole Local Government area of Oyo State while Awotan (07°27' 49"N 03°50'56"E; 237m above sea level), also known as Apete dumpsite, is located in Iddo Local Government Area. Aba-eku (07°19'28"N 03°59'17"E; 162 m above sea level), also known as Afofunra, is located at Ona Ara Local government area. Aba-eku dumpsite was scheduled for upgrading to a sanitary landfill in 1998 but the upgrading process is yet to commence as at the time of this study. There is no provision for the collection and treatment of leachate in any of the three dumpsites and the leachate generated from decomposing wastes run freely into surrounding aquatic and soil ecosystems.

Sample collection

Composited samples of leachate (n=15) were collected from the leachate stream flowing from the three landfills (Ajakanga=AJL, Awotan=AWL, Aba eku=ABL) into sterile 500 ml glass sampling bottles every two weeks from June through

August 2014. Similarly, leachate-contaminated surface water (n=10) was collected upstream (UPS=50 m) and downstream (DWS=250 m) of the leachate-contaminated stream at Aba-eku, and groundwater samples (n=25) were collected from 2 hand-dug wells at Aba-Eku (W1 and W2, n=10) and three hand-dug wells in Awotan (W3, W4 and W5, n=15). All the sampled wells are within residential houses located about 200 – 500 m from the landfills and serve as sources of domestic water supply for the inhabitants of the buildings. Sample collection from wells was limited to residential houses where access was granted for the purpose of sample collection.

Sample processing

Physicochemical analysis

The temperature, pH, total dissolved solids (TDS), electrical conductivity (EC) (Eutech PCSTestr 35 Multi Parameter tester, Eutech-Thermo Scientific, Singapore) and dissolved oxygen (DO) (DO Meter, PDO-520 Model, Lutron Electronics, Taiwan), as well as the concentrations of Cu, Zn, Pb, Cd and Cr were determined as described [17]. The metals were selected for analysis either because they are frequently detected in leachates or have been linked with occurrence of antibiotic resistance in the environment [7]. Samples for metal analysis were digested in 6N HNO_3 before the concentrations of each metal was determined by Atomic Absorption Spectrophotometry (AAS) (ZEEnit 650P, AnalytikJena, Jena, Germany). Values obtained were compared to standards regulating the discharge of effluents [18] and drinking water quality [19] in Nigeria.

Bacteria isolation and determination of total heterotrophic bacteria count (THBC)

Bacteria were isolated without metal or antibiotic selection on Mueller Hinton Agar (MHA) plates by the surface spread method. Dilutions (10^{-5}) of leachate and water samples were spread on the surface of MHA plates incubated at 35 ^{0}C overnight for determination of the THBC of the samples and isolation of bacteria. One representative of each morphologically distinct colonies growing on the plates were selected, purified on fresh agar plates and stored in glycerol broth (15%) at -15 ^{0}C. All bacterial isolates were subjected to gram staining and biochemical characterization tests. Isolates showing negative gram reactions were selected for further studies of antibiotic resistance and metal tolerance.

Antibiotic susceptibility and metal tolerance testing

Antibiotic susceptibility

Isolated bacteria were tested for susceptibility to ertapenem (ETP, 10 µg), cefpodoxime (CPD, 30 µg), ciprofloxacin (CIP, 10 µg), gentamicin (CN, 10 µg), tetracycline (TET, 30 µg), florfenicol (FFC, 30 µg) and sulfamethoxazole/ Trimethoprim (SXT, 25 µg) (OXOID^{TM}) by disc diffusion as described by the Clinical and Laboratory Standards Institute [20]. Zones of growth inhibition around each disc after overnight incubation at 35 ^{0}C were measured and interpreted by the zone diameter interpretive standards of the CLSI [20].

Test of metal tolerance

Tests of metal tolerance were carried out by spot inoculation on metal-supplemented MHA plates as described by Ünaldi Coral et al [21]. Filter sterilized salts of the metals (ZnCl, PbCl and $CuSO_4$) were added to cooled MHA at the following graded concentrations (µg/ml): 100, 200, 400, 600, 800, 1000, 1,100. The plates were incubated for 48 hrs. at 35 ^{0}C. The minimum concentration of metals to inhibit bacterial growth after 48 hours was recorded as the minimum inhibitory concentration (MIC).

Statistical analysis

Pearson's correlation analysis was performed to establish relationships between resistance to heavy metals and antibiotics among the bacteria isolates at 0.05 and 0.01 levels of significance. Data analysis was performed using SPSS 19.0 software (SPSS Inc, Chicago IL).

Ethical approval

No special ethical or regulatory approval is needed to carry out the research reported in this study.

PCR detection of antibiotic resistance genes and integrons

Total genomic DNA was extracted from the test bacteria by boiling lysis [22] and the lysates used in PCR to detect antibiotic resistance genes in isolates displaying phenotypic

resistance to SXT, FFC and the beta-lactams (CPD and ETP) with primers targeting *sul1*, *sul2* and sul3 [23], *floR* [24], the ESBLs *blaTEM, blaSHV, blaCTX-M, blaGES, blaVEB* and *blaPER*; and carbapenemases *blaVIM, blaNDM, blaKPC, blaIMP, blaSPM, blaAIM* and *blaDIM* [25], oxacillinases *blaOXA-1, blaOXA-2, blaOXA-10,* bla_{OXA-23}*, blaOXA-24,* bla_{OXA-48}*, blaOXA-51* and *blaOXA-58* and *ampC blaCMY-1, blaCMY-2, blaACC, blaACT, blaDHA* and *blaFOX* [26] as well as Class 1, 2 and 3 integrons [27]. The variable regions of class 1 integrons and the promoters were amplified with previously described primers and protocols [27-29]. All PCR reactions were carried out in 25 µl volumes with DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific). Selected amplicons of each detected gene (1.2% agarose gels) were sequenced (Macrogen Inc, Amsterdam Netherlands). The taxonomic identities of all isolates positive for at least one of the resistance genes tested were confirmed by amplification and sequencing of the 16S rRNA genes.

RESULTS

Leachate and leachate-contaminated surface and groundwater characteristics

The chemical and microbiological characteristics of the leachate and leachate-contaminated surface and groundwater samples are shown in Table 1. The temperature, pH, TDS and EC were lowest in W3 while the lowest value of DO was measure in AJL. All the metals tested were detected in the leachates and water samples. The level of Cu ranged from 0.03 (W1) to 0.27 mg/l (AWL), Zn from 0.39 (UPS) to 0.76 mg/l (AWL), Pb from 0.12 (W3) to 0.47 mg/l (ABL), Cd from 0.03 (W3) to 0.21 mg/l (AWL) and Cr from 0.17 (W4) to 0.57 mg/l (ABL). The pH of the leachates and surface water are within recommended standards by NESREA [18], while all the well water samples have pH below recommended values for drinking water [19]. Similarly, all the leachate samples as well as water samples from wells W1, W2, and W4 have TDS above the maximum recommended for discharged effluent and drinking water. The DO in two samples of leachate (AJL and ABL) are below the minimum (4.0 mg/l) recommended by NESREA [18]. The NESREA regulation did not specify any standard for EC, however, all the well water samples have values of EC lower than that set for drinking water by SON [19]. The concentrations of Cu, Zn, Pb and Cd in all the leachate samples and Cr concentration in ABL and AWL, as well as the concentrations of Pb, Cd and Cr in all the wells are all above recommended standard for discharged effluents and drinking water in Nigeria.

Bacteria isolates and their susceptibility to antibiotics and heavy metals

Forty six (46) out of 56 (82%) Gram negative bacteria selected for tests of antibiotic resistance and metal tolerance showed varying degree of co-resistance to metal and antibiotics. These were identified as members of the *Phylum Proteobacteria* namely: *Ralstonia* (12), *Alcaligenes* (11), *Pseudomonas* (9), *Enterobacter* (3), *Stenotrophomonas* (3), *Proteus* (2), *Citrobacter* (2), *Erwinia* (1) *Advenella* (1) *Brevundimonas* (1) and *Raoultella* (1). Results of susceptibility testing showed that 34 (74%) of the isolates were resistant to CPD while 23 (50%), 21 (45.6%), 19 (41%), 18 (39%), 6 (13%) and 6 (13%) respectively showed resistance to SXT, ETP, FFC, TET, CN and CIP. The metal MIC among these isolates raged between 200 µg/mL and ≥1100 µg/mL (Table 2). Overall, 34 isolates (74%) showed resistance to more than one antibiotics with multiple antibiotic resistance (MAR) index ranging between 0.29 and 1.0. One isolate, *A. faecalis* AP31 from AWL was resistant to all the antibiotics tested with MAR index of 1.0 and metal MICCu/Pb/Zn of 300, 1100 and 300 µg/ml respectively. Resistance to Cu and Zinc showed a positive correlation with resistance to some of the tested antibiotics ($p < 0.05$ and $p < 0.01$). Cu resistance correlated positively with resistance to ertapenem (0.813) and florfenicol (0.855) while Zn resistance correlated positively with resistance to ciprofloxacin (0.678), gentamicin (0.899) and tetracycline (0.735).

Detection of antibiotic resistance genes and integrons

sul1, *sul2* and *floR* conferring resistance to sulfonamides and florfenicol were detected in 14 (25%) bacteria species identified by 16S rRNA gene sequencing as *A. faecalis* (n=11), *Advenella kashmirensis, Brevundimonas sp*. and *Ralstonia pickettii*. The detected *floR* shared 91-

96% sequence identity with *floR* in uncultured bacterium clone CH-0406 (KU548555.1) and *Stenotophomonas maltophilia* strain GZP-Sm1 (KM649682.1). *sul1* and *floR* were detected in 12 isolates all showing phenotypic resistance to SXT and FFC suggesting a co-selection of the two genes in the bacteria isolates. *sul2* was detected in 3 isolates with the gene occurring together with *sul1*/*floR* in one isolate and singly in two isolates identified as *A. kashmirensis* from ABL and *Brevundimonas sp.* from W5 (Table 2). Class 2 and 3 integrons were not detected in any of the isolates, but class 1 integron was detected in the 12 isolates carrying the *sul1*/*floR* gene combination.

Sequencing of the 1.5-1.7 kb amplicons obtained with primers targeting the variable regions of detected class 1 integrons showed that in all the 12 isolates, a portion of the sequences (about 600 bp) shared 90-99% sequence identity with *aadB* on the class 1 integrons of *Corynebacterium aspernum* (AJ871915.1) and *Pseudomonas aeruginosa* strains 19 (HM367614.1), 29081162 (JF742758.1), 4019-1 (KF468743.1) and P5 (JF412714.1). This gene probably formed the basis of resistance to gentamicin observed in some of the isolates. The remaining portion of the sequence showed no significant similarity with any known gene in the GenBank. Thus, to decipher the possible identities of these regions, they were used in a *BLASTX* search in the GenBank Database. In all the isolates, the translated nucleotide sequences shared 42-65% amino acid identity with Class D β-lactamase of *Synechococcus sp*. NKBG15041c (WP024546971.1), uncultured bacterium (AMP47147.1) or *Colwellia psychrerythraea* (WP033083257.1), and OXA-2-like protein of an uncultured bacterium (ALG03676.1).

To further confirm this observation, we next searched for putative ORFs in the sequences using orf-finder (https://www.ncbi.nlm.nih.gov/orffinder/) and confirmed the identities of detected ORFs by Smart*BLA*ST (https://*bla*st.ncbi.nlm.nih.gov/smart*bla*st/smart*Bl a*st.cgi). Two ORFs were identified in two isolates from Awotan leachate (*A. faecalis* AP12) and well (*A. faecalis* APW412) showing significant alignment with beta-lactamases while only one of such ORF was identified for the remaining ten isolates. ORF3 (AP12, 55aa) and ORF8 (APW312, 38aa) as well as the single ORF identified in the sequences of the remaining 10 isolates (55-86aa) shared 58-66% sequence identities with class D β-lactamase of *Synechocystis sp.* PCC6803 (WP070097971.1), and 36-50% identities with class D carbapenem-hydrolyzing β-lactamase *Bla*A of *Shewanella oneidensis* MR-1 (NP716468.1). ORF8 (AP12, 52aa) and ORF2 (APW312, 55aa) shared 50% and 67% identities with class D β-lactamase of *Synechocystis sp*. PCC6803 (WP070097971.1). Interestingly, eleven and one of the isolates gave positive amplification signals with primers targeting bla_{OXA-48} and bla_{OXA-23} respectively. bla_{OXA-48} and bla_{OXA-23} are carbapenem hydrolyzing Class D β-lactamase. However, sequencing of the amplicons showed they were non-specific products with the deduced amino acid sequence of the $_{OXA-23}$ detected in *C. ferundii* AB18 sharing 78% identity with an MFS transporter protein of *Caulobacter sp.* Root 656 with accession number WP057182697.1. In addition, seven of the eleven isolates showed resistance to ETP suggesting a possible role for the ORFs in carbapenem resistance. However, further experiments would be required to confirm the role of the genes in carbapenem resistance. The gene cassettes in all the isolates are under the control of weak promoters PcW which was associated with inactive second promoters P2 in two isolates from ABL and AWL respectively (Table 2). Weak promoters (PcW) usually have strong excision activity [29] which can promote the excision and rapid dissemination of the gene cassettes carried on this integron to other bacteria of the landfill ecosystems.

Table 1. Physicochemical and microbiological characteristics of leachates and leachate-contaminated surface and groundwater samples.

Sampling Points			Physicochemical parameters					Heavy metals (mg/l)					Mean [c] THBC (cfu/ml) x10[8]
Sites	**Coordinates**	**Elevation (m)**	**Temp (ºC)**	**pH**	**TDS (mg/l)**	**EC (µs/cm)**	**DO (mg/l)**	**Cu**	**Zn**	**Pb**	**Cd**	**Cr**	
AJL	N07°18'43" E03°50'27"	165	29.6	6.85	2200	3120	2.7	0.04	0.44	0.46	0.18	0.15	2.72±1.3
ABL	N07°19'28" E03°59'17"	162	29.5	7.15	3080	4530	3.3	0.31	0.66	0.47	0.19	0.57	2.08±0.74
AWL	N07°27'49" E03°50'56"	237	26.0	7.65	1670	2350	16.9	0.27	0.76	0.41	0.21	0.53	1.63±0.4
UPS	N07°19'33" E03°59'22"	149	29.7	6.95	1380	937	8.5	0.22	0.39	0.27	0.17	0.51	2.25±1.0
DWS	N07°19'32" E03°59'27"	147	29.1	6.92	366	517	8.6	0.07	0.49	0.23	0.08	0.24	4.67±1.6
W1[a]	N07°19'31" E03°59'22"	151	30.5	5.98	647	924	8.8	0.03	0.54	0.16	0.06	0.21	2.19±1.8
W2[a]	N07°19'29" E03°59'19"	156	29.6	5.97	685	977	8.9	0.05	0.65	0.19	0.17	0.26	2.80±1.8
W3[b]	N07°27'40" E03°50'60"	218	24.5	4.98	352	497	8.0	0.12	0.45	0.12	0.03	0.25	2.38±0.6
W4[b]	N07°27'42" E03°50'52"	220	27.3	5.22	681	960	72.5	0.14	0.42	0.22	0.07	0.17	2.0±1.2
W5[b]	N07°27'42" E03°50'51'	218	27.1	5.75	462	651	77.5	0.15	0.53	0.20	0.12	0.18	2.88±1.1

AJL=Ajakanga Leachate, ABL=Aba eku Leachate, AWL=Awotan Leachate, UPS=river water collected upstream of landfill site, DWS=river water collected downstream of landfill site, W1-5=Wells1, 2, 3, 4, 5, TDS=total dissolved solids, EC=Electrical conductivity, DO=dissolved oxygen, THBC=total heterotrophic bacteria count, a=W1-2 are located at Aba Eku Land fill site, b=W3-5 are located at Awotan Landfill site, c=THBC is a mean of values determined for all sampling times for each sample

Table 2. Antibiotics and metal resistance profiles and antibiotic resistance genes detected in the Bacterial isolates from the landfill sites (continued in the next site).

Isolates	Source	MAR Index	Phenotypic pattern of Antibiotic resistance	Metal MIC (µg/ml)			Detected Resistance Genes	Promoter Type
				Cu	Zn	Pb		
Advenella kashmirensis AB17b	ABL	0.14	SXT	800	1100	1100	*sul2*	
Brevundimonas sp. $APW_5$10	W5	0.14	SXT	300	700	1100	*sul2*	
Ralstonia pickettii AJ21	AJL	0.14	FFC	600	700	1100		
R. solanacearum AP29	AWL	0.14	TET	300	600	1100		
R. pickettii $APW_5$1	W5	0.14	TET	500	500	1100		
R. pickettii AP3	AWL	0.14	CPD	600	500	1100		
Pseudomonas taetrolens AP25	AWL	0.14	CPD	300	200	1100		
P. alcaligenes AB24	ABL	0.14	CPD	600	300	1100		
P. alcaligenes AB13	ABL	0.14	TET	500	600	1100		
P. mendocina AP5	AWL	0.14	CPD	600	1100	1100		
R. solanacearum AB10	ABL	0.14	TET	600	900	1100		
Stenotrphomonas maltophilia AP23	AWL	0.14	CPD	300	1100	1100		
Citrobacter freundii AB18	ABL	0.29	ETP, CN	600	900	1100		

R. solanacearum AB6	ABL	0.29	SXT, TET	500	500	1100		
Erwinia mallotivora AJ17	AJL	0.29	CPD, CIP	500	300	1100		
P. jinjuensis UP20	UPS	0.29	ETP, CPD	300	200	1100		
Enterobacter cloacae ABW_2 19	W2	0.29	SXT, TET	500	300	1100		
P. fluorescens AP6	AWL	0.29	CPD, TET	500	1100	1100		
P. taetrolens AP21	AWL	0.29	ETP, CPD	500	600	1100		
Alcaligenes faecalis AP12	AWL	0.29	CPD, SXT	900	900	1100	*floR, IntI1/sul1*	PcW
A. faecalis AJ11	AJL	0.43	CPD, FFC, SXT	600	600	1100	*floR, IntI1/sul1*	PcW
A. faecalis UP22	UPS	0.43	CPD, FFC, SXT	600	1100	1100	*floR, IntI1/sul1*	PcW
A. faecalis DS13	DWS	0.43	CPD, FFC, SXT	800	1100	1100	*floR, IntI1/sul1*	PcW
A. faecalis ABW_1 16	W1	0.43	ETP, CPD, SXT	600	300	1100	*floR, IntI1/sul1*	PcW
Ent. aerogenes AP12	AWL	0.43	ETP, CPD, FFC	600	600	1100		
S. maltophilia DS16	DWS	0.43	ETP, CPD, FFC	300	600	1100		
S. maltophilia $APW_3$1	W3	0.43	CPD, SXT, TET	300	700	1100		
R. solanacearum UP19	UPS	0.43	ETP, CPD, FFC	400	300	1100		
R. solanacearum AB8	ABL	0.43	CPD, TET, CN	600	500	1100		
Proteus vulgaris AB9	ABL	0.43	CPD, TET, CN	500	500	1100		
R. pickettii $ABW_1$6	W1	0.43	FFC, SXT, CN	500	300	1100		
Roultella planticola DS12	DWS	0.43	ETP, CPD, FFC	1100	1100	1100		
A. faecalis DS18	DWS	0.57	ETP, CPD, FFC, SXT	300	300	1100	*floR, IntI1/sul1*	PcW
Ent. cloacae $APW_3$3	W3	0.57	ETP, CPD, FFC, SXT	900	300	1100		
P. stutzeri UP13	UPS	0.57	ETP, CPD, SXT, TET	600	1100	1100		
R. pickettii $ABW_1$17	W1	0.57	ETP, CPD, FFC, SXT	600	300	1100		
C. freundii AB14	ABL	0.57	ETP, CPD, FFC, CIP	500	500	1100		
R. pickettii AB17	ABL	0.71	ETP, CPD, FFC, SXT, TET	800	1100	1100	*floR, IntI1/sul1*	PcW-P2
A. faecalis $ABW_1$22	W1	0.71	ETP, CPD, FFC, SXT, CIP	200	600	1100	*floR, IntI1/sul1*	PcW
A. faecalis $APW_3$16	W3	0.71	ETP, CPD, FFC, SXT, TET	500	1100	1100	*floR, IntI1/sul1*	PcW
P. vulgaris $APW_3$14	W3	0.71	ETP, CPD, FFC, SXT, TET	700	1100	1100		
R. pickettii UP15	UPS	0.71	ETP, CPD, SXT, TET, CN	700	1100	1100		
A. faecalis $ABW_2$16	W2	0.86	ETP, CPD, FFC, SXT, CIP, TET	600	300	1100	*floR, IntI1/sul1*	PcW
A. faecalis $APW_4$12	W4	0.86	ETP, CPD, FFC, SXT, CIP, TET	600	200	1100	*floR, sul2, IntI1/sul1*	PcW
A. faecalis AP31	AWL	1.00	ETP, CPD, FFC, SXT, CIP, TET, CN	300	1100	300	*floR, IntI1/sul1*	PcW-P2

MIC=Minimum Inhibitory concentration, ABL=Aba-eku leachate, W1 and W2=Wells at Aba-eku landfill, UPS=Water sample collected upstream, DWS=Water samples collected downstream at Aba-eku, AWL=Awotan leachate, W3, W4, W5=Wells at Awotan landfill, AJL=Ajakanga leachate, ETP=Ertapenem, CPD=Cefpodoxime, FFC=Florfenicol, SXT=Sulphamethoxazole/Trimethoprim, CIP=Ciprofloxacin; TET=Tetracycline, CN=Gentamicin; Zn=Zinc, Pb=lead, Cu=Copper

DISCUSSION

Recent studies have raised concerns on the important role played by MSW landfills as reservoir of antibiotic resistance. However, only few of these studies emanated from sub-Saharan Africa where MSW landfills are the commonest method of solid waste management. In this study we detected *sul1*, *sul2*, *floR* as well as class 1 integron carrying *aadB* as a gene cassette in bacteria isolated from leachates and leachate contaminated water from three unsanitary MSW landfills in Ibadan, Nigeria. The bacteria were identified as members of the *Phylum Proteobacteria* and have previously been reported in landfill leachates in different parts of the world [30-32]. This is worrisome as some of the bacteria species such as *Alcaligenes*, *Pseudomonas*, *Stenotrophomonas* and *Raoultella* are important opportunistic pathogens and reservoir of novel antibiotic resistance genes and their isolation in household wells used for drinking and other

domestic purposes by the surrounding population of the waste dumpsites is a risk to public health. None of the tested resistance genes was detected in 32 isolates (69.6%) despite the presence of phenotypic resistance to various antibiotics suggesting that the observed resistance was mediated by novel resistance genes or by non-specific resistance mechanisms such as reduced outer membrane permeability or multidrug resistance efflux pumps [33].

sul1/*floR*/intl1 occurred together in 12 bacteria isolated from leachates (ABL, AWL, AJL) well water (W1, W2, W3, W4) and stream water (UPS, DWS) collected from the three sampling sites suggesting a cross contamination of the surrounding surface and groundwater by ARBs from the landfill sites and a possible co-occurrence of these genes in the isolates. Further, as the three sampling sites do not share ecological connectivity, it is likely the co-occurrence of these genes is linked to bacteria taxonomic identity rather than conditions predominating in the landfill ecosystem. Usually, *floR*-carrying bacteria exhibit high levels of simultaneous resistance to other antibiotics with resistance to potentiated sulfonamides among the most frequent [34,35]. In conclusion, we detected *sul1*, *sul2* and *floR* in 14 bacteria species isolated from three MSW landfills and surrounding aquatic ecosystem in Ibadan, southwestern Nigeria. We could not detect any of the 27 β-lactams resistance genes tested for in PCR despite the presence of phenotypic resistance to β-lactams, but an unknown portion of the variable region of class 1 integrons found in 12 isolates carrying the *sul1*/*floR* combination show significant alignment with class D β-lactamases suggesting that the landfill ecosystem may be a reservoir of previously unknown ARGs which can potentially be transmitted from this environmental reservoir to the human population. Further experiments would however be needed to confirm this as well as investigate potential transmission of these antibiotic resistant bacteria into the human population. It is also important to put measures in place to properly manage leachate from landfill sites for public health safety.

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