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THE BURDEN, ANTIBIOGRAM AND PATHOGENICITY OF BACTERIA FOUND IN MUNICIPAL WASTE DUMPSITES AND ON WASTE SITE WORKERS IN BENIN CITY.

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ABSTRACT

Municipal wastes may harbour microbial pathogens but the quantum and disease-causing capacities of such organisms are rarely investigated. This study sought to establish the burden, antibiogram and pathogenicity of bacteria in 4 selected waste dumpsites and those found on hands and clothings of the respective waste dumpsite workers in Benin City. Swabs from hands and clothings of waste site workers and specimens of top soils and leacheates from waste dumpsites were obtained at monthly intervals for 12 months from 4 sites in Benin City (University of Benin, Costain Road, Government Reservation Area, and Benin Bye-pass). The samples were cultured on Blood, MacConkey and Griseofulvin nutrient agar plates and incubated aerobically and anaerobically. Heterotrophic viable bacteria counts were determined for soil and leacheate specimens. The predominant bacterial isolates from the cultured plates were phenotypically characterized using standard methods. Antimicrobial disc susceptibility tests and pathogenicity tests were also carried out on the predominant isolates. Eleven Bacteria Genera were isolated from topsoils and leacheates. They were in the following order of predominance: Bacillus sp (18.20%) Staphylococcus sp (13.93%), Escherichia coli (12.72%), Proteus sp (12.12%), Streptococcus sp (12.12%), Klebsiella sp (9.70%), Pseudomonas sp (7.90%), Citrobacter sp (5.45%), Bacteroides sp (3.03%), Clostridium sp (2.42%), Serratia sp (2.42%). Bacterial isolates from the waste workers were essentially similar to those isolated from the dumpsites although the latter were more heavily colonized. The highest bacterial burden was recorded from the top soil samples at Benin Bye-pass dumpsite with a mean viable count of 2.0 x106+ 0.88 x106cfu/gm while the least was from the University of Benin dumpsite with mean count of 0.7x106+0.56x106cfu/gm.The antibiograms of the isolates revealed that the organisms were most susceptible to the fluroquinolones especially Ciprofloxacin (96.6%) and Pefloxacin (94.6%). They were least susceptible to Amoxacillin (51.7%) and Erythromycin (55.8%). All bacteria species isolated showed multiple drug resistance patterns with Serratia marcescens and Pseudomonas aeruginosa each exhibiting higher levels of multidrug resistance. A number of the isolates exhibited pathogenicity traits. Whereas 27.6% of them were positive for virulence gene, 24.3% were invasive while 17.2% were diarrheagenic. We conclude that municipal waste dumpsites and those working at such sites bear heavy burdens of potentially pathogenic multidrug resistant bacteria which may constitute major public health hazards, not only to the immediate communities but also to the families of such site workers. The need to educate these waste workers on the use of appropriate protective materials and about the risks and hazards associated with their job are emphasized. Proper disposal and recycling of these wastes ought to be mandatory.

INTRODUCTION

Solid, semi-solid, liquid or gaseous wastes are generated on daily basis in cities all over the world and Nigeria is no exception. The management of such wastes usually vary according to the country's level of development and the seriousness the authorities attach to the exercise. In most African cities and towns, wastes are usually heaped in huge quantities around the streets, forming ugly mountains which obstruct traffic and pose serious health hazard to humans[1]. This has remained a common practice especially at market places in many Nigerian cities[2,3].

In a bid to sanitize our communities and cities, some Nigerian authorities identified specific locations as demarcated waste dumpsites. Some of these sites are on the outskirts of the cities, while others are however too close to residential areas, farms, river sides and creeks. At these sites, the wastes are usually deposited on top soils. Here the organic components decompose and are biodegraded with the help of contaminating microbes and other indigenous soil/environmental organisms.

It is common site in Nigeria to see waste scavengers and free range animals defecating at dumpsites. These activities lead to further contamination of the underlying soils and leacheates. The latter is said to be formed when rain run-off infiltrates through solid wastes and dissolves or suspends its constituents, resulting in a usually contaminated liquid known as leacheate. Others opined that greater concentrations of leacheates result from aerobic and anaerobic microbial decomposition of wastes in combination with the waste derived constituents[4,5]. The main danger of such contaminated leacheates is the possibility of their direct discharge to surface water or seepage to underground water which may be the sources of potable water for surrounding communities[6,7].

Obire and Aguda[5] earlier found a high burden of bacteria from leacheates and their surrounding stream and reported the presence of such pathogens as Vibrio, Shigella, Nieseria, Klebsiella, Escherichia coli, Pseudomonas aeruginosa, etc. They did not, however, determine the pathogenicity of their isolates and did not investigate the possible public health risks on the waste workers at these leacheate sites. The current study therefore sought not only to establish the bacteria burden, but also to determine the antibiograms of such isolates from topsoils and leacheates from urban dumpsites and the hands and clothings of the site workers. The work further sought to investigate the disease-causing capabilities of the isolated organisms in order to evaluate the possible risks and public health significance of municipal waste dumps and associated activities of the site workers in Benin City.

MATERIALS AND METHODS

Sample Collection:

Specimens were collected at monthly intervals between January 2010 and December 2010. Topsoil and leacheate samples were obtained on each visit for a total of 12 visits from 4 waste dumpsites in Benin metropolis at University of Benin (A), Costain Road (B), Government Reservation Area (C) and Benin Bye-pass (D), Swab specimens were also collected from the

KEYWORD: Waste dumpsites, site workers, potentially pathogenic multidrug resistant bacteria.

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hands and clothings of the above site workers at each visit, using sterile swabs wet with peptone water broth. All samples were immediately transported to Lahor Research Laboratories for microbiological investigations. They were kept at 4°C pre-analysis.

Ethical Considerations:
We informed all the waste workers at every site about our project and assured them that the outcome of the project could encourage the Environmental Agencies, who usually engage them, to provide them with adequate protective materials for their job. They all gave us their consents before commencement of the project.

Cultural Methods:
Media for cultivation of bacteria, unless otherwise stated, were dehydrated media (Oxoid, England) and were prepared according to the manufacturer's instructions. One gram each of the soil samples were first suspended in 2ml of nutrient broth before plating out onto MacConkey, Nutrient and Blood agar plates and incubated aerobically and anaerobically at 37°C for 24-48 hours. Hands and clothing swabs were inoculated directly onto the same type of media as above and were similarly incubated. The nutrient agar employed contained anti-fungal agent griseofulvin (Glaxo Smith Nigeria, Plc, Lagos), incorporated at a concentration of 300mg per 250ml of molten agar to inhibit the growth of contaminating fungi. Bacteria isolates were phenotypically characterized according to Cowan and Steel[8].

Bacteria viable counts on soils and leacheate samples:
Total heterotrophic viable bacterial counts were carried out on each soil and leacheate samples employing the 10-fold serial log dilution method and utilizing griseofulvin nutrient agar (GNA) as the selective culture medium. Briefly, 0.1ml each of 10-fold log dilutions of soils and leacheates (from 10^{-1} to 10^{-9}) were dispensed onto GNA medium in triplicates. These were spread out all over the plates with sterile wire loop and incubated at 37°C for 24 hours. Bacteria colonies were then counted from the triplicate plates and the mean counts determined per sample. The final (overall) mean viable counts were calculated after 12 samples each had been analyzed from each site for soils and leacheates. The final means were expressed in colony forming units (cfu) $\pm$ S.D. per gm of soil or per ml of leacheate as appropriate.

Antimicrobial Susceptibility Testing of Isolates:
Disc-diffusion susceptibility testing was performed as earlier described[9]. Briefly, overnight broth cultures of the isolates were seeded uniformly onto sensitivity test agar (Oxoid) plates by inoculating 0.1ml of the diluted (MacFarland No 1 turbidity tube) suspension over the entire surface of the agar plate. Commercially procured antibiotic discs (Abtek sensitivity discs, Abtek Biological Ltd, Liverpool) were asceptically placed on the seeded plates and incubated at 37°C for 24 hours. The 13 antimicrobial agents used were Streptomycin (10µg), Tetracyclin(30µg), Septrin(30µg), Chloramphenicol(30µg), Gentamycin(10µg), Erythromycin(15µg), Ampiclox(30µg), Amoxacillin(30µg), Parfloxacin(30µg), Ofloxacin(30µg), Ciprofloxacin(30µg), Citriazone(30µg) and Sparfloxacin(30µg). The diameters of the inhibition zones observed after incubation were recorded and compared with those of reference control organisms held at the Lahor Research Laboratories (Escherichia coli ATCC 25922, Staph.aureus ATCC25923 and Ps.aeruginosa ATCC 27853).

Experimental Animals for Pathogenicity Assays:
Three animal models were used for experimental pathogenicity test; the rabbit ileal loop assay for detecting heat labile (LT) enterotoxin producing diarhoeagenic isolates, infant or sucking mouse assay for detecting heat stable (ST) enterotoxin producing diarrhoeagenic isolates and the guinea pig eye (Sereny) test for detecting invasive isolates. The experimental animals, adult white albino rabbits, adult guinea pigs and infant mice (1-4 days old) were obtained from the animal house at the College of Medicine, Ambrose Alli University, Ekpoma

Guinea Pig Eye Test For Invasiveness (Sereny test):
This was carried out, on all isolates as previously described[10,11]. Essentially, one eye of the guinea pig was inoculated with one drop of a tick emulsion of the isolate while the second eye was left uninoculated to serve as control. They were left in their cages, served with water and feed and observed for 7 days for development of keratoconjunctivitis (redness/discharged from the eye). Positive symptoms within seven days in any of the animal was recorded as positive Sereny test, which is an indicator of invasiveness.

Preparation of Toxigenic Extract:
Isolates were inoculated into Tryptose Soya Broth (Oxiod, U.K) and incubated in a 37^{o}C shaker water bath for 48 hours. The broth culture was then centrifuged at 15,000 r.p.m using a refrigerated eppendolf centrifuge. The supernatant constituted the toxigenic extract[11]. All test isolates and control bacteria were similarly treated.

Rabbit Ileal Loop Assay for Heat Labile (LT) Enterotoxin Detection:
This was carried out as earlier described by Guerrant and his colleagues[12] and Agbonlahor[11]. Essentially, the adult albino white rabbits were anaesthetized with sodium barbiturate The animal was then asceptically opened up and the intestine removed and ligated at 5cm intervals starting from the ileo-ceacal region. At least 12 ligations were carried out for each animal (10 for test isolates and the last two for positive and negative control). One ml each of the toxigenic extract from the test isolates and LT positive control were inoculated unto the ligated segments. The 12th segment served as a negative control and was inoculated with 1ml of sterile saline. The inoculated loops were put back into the animal and the opening was sutured up. The animals were left over night in their cages and those that survived the surgery were reopened the following day after killing the animal with barbiturate. The loops were inspected for accumulation of fluids which indicated positive heat labile (LT) enterotoxin production.

Infant Mouse Assay for Heat Stable (ST) Enterotoxin Detection:
This assay was carried out as earlier described[11,13]. Briefly, duplicate 1-4 days old suckling mice were inoculated with 0.1ml of toxigenic extract from test isolates and ST positive control organism onto the milk filled stomach of the mice. The animal was left on the bench for four hours after inoculation. The inoculated mice were then killed with chloroform. The abdomens were opened up and their intestines of each mouse were weighed. The remaining body weights of the animals were also determined. The ratio of the intestinal weight to the remaining body weight of 0.085 or more was regarded as heat stable (ST) enterotoxin production.

Virulence Gene PCR:
We procured all our PCR reagents used in this study from Inqaba Biotechnology Industries, Hartsfield, South Africa. The

virulence gene primer SPV-C-1 had a sequence (5^1 to 3^1) of ACTCCTTGCACAACAAATGCGGA with nucleotide position 553 573 and a reverse primer SPV-C-2 with sequence (5^1 to 3^1) of TGTCTCTGCATTTCGCCATCA with nucleotide position 999 977. Their expected amplicon size (bp) was 447 and were expected to target the SPVC gene.

DNA Extraction from Bacteria:
DNA was extracted from the isolates as follows: The isolates were purified on nutrient agar plates (Oxoid, U.K) and pellets were harvested ascetically and suspended in 180µl of buffer ATL. To this suspention, 20µl of proteinance K was added if the isolate was Gram negative. However, pellets of Gram positive isolates were pre-treated with 180µl of the Lysis buffer (ATL) containing 200ng/m lysostaphin; 20mM Tris Hel; pH 8:0; 2Mm EDTA; 1.2% Triton and incubated at 37°C for 30mins before proteinase K treatment. The mixture was then voltexed and incubated at 56°C for 3 hours, with occasional voltexing in between. The tube was briefly centrifuged to remove drops from side of the tube. Then 200µl of buffer A1 was added, pulse-voltexed for 15secs and incubated at 70°C for 10mins and briefly centrifuged. To the mixture, 200µl of ethanol (96-100%) was added and pulse-voltexed for 15secs and centrifuged briefly.

The Spin column (supernatant) was transferred into a 2ml collection tube and centrifuged at 8000rpm for 1min. To this, 500µl of buffer AW1 was added and centrifuged at 800rmp for 1min. The spin column was then transferred into another clean 2ml collection tube to which 500µl of buffer AW2 was added and centrifuged at 400rpm for 3mins. The spin column was similarly pipetted into a fresh 2ml collection tube and finally centrifuge at 800rpm for 1min. The product constituted the extracted double stranded DNA of the isolate.

The PCR Master Mix:
Into an eppendolf tube, 20µl each of the forward and reverse primers were added and kept on ice. To this mixture, 15µl of DNA polymerase, 5µl of Deoxynucleic acid triphosphate (DNTPs) and 20µl of buffer 2B (sodium acetate and acetic acid). The mixture was voltexed for 1 min. Then 10µl of co-factor (magnesium Chloride) was added to the mixture. This constituted the DNA master mix.

The Polymerase Chain Reaction:
The extracted DNA from each isolate was pipetted in 10µl amounts into tubes and 10µl of the master mix was added to each and voltexed to ensure thorough mixing of the components and then briefly centrifuged. The tubes were immediately loaded into the PCR madime (MJ Research PTC 200 Peltier Thermal Cycler-Biodirects, US) which was programmed with the following conditions: denaturation at 95°C for 5mins, then 40 cycles of denaturation at 95°C for 45secs, annealing at 56°C for 45secs and extension at 72°C for 1min.

Electrophoresis and visualization of Gel:
Alliquots of 15µl each of the DNA emplification products and DNA molecular size marker (ladder or standard, 100-1500bp) were loaded onto the 2% agarose gel stained with ethidium bromide and ran at 90V for 2 hours to ensure adequate separation. The products were then visualized on a 302mm UV ultra-illuminator (Alpha-imager). Photographs of the separated bands in agarose gels were taken with the in-built camera on the Alpha-imager.

RESULTS

The four dumpsites were heavily contaminated with at least genera of bacteria with Bacillus species (18.20%) as the least (table 1). The waste workers at the various sites were equally contaminated with the same genera of bacteria with the exception of anaerobic bacteria (Bacteroides and Clostridium sp). The Isolates were evenly distributed in the soil and leacheate samples of the dumpsites (table 2). Isolates from the hands were generally fewer than those from the clothings of the site workers.

Table 3 shows that 15 bacteria spp that were identified among the 11 bacteria genera isolated. While Escherichia coli (9.5%) Bacillus subtilis (6.8%), Enterococcus. feacalis (6.8%) and Ps. aeruginosa (6.8%) predominated from the soils and leacheates, Bacillus subtilis (6.8%), Staph. epidemidis (5.4%), Escherichia coli (4.8%) and Staph. aureus (3.4%) predominated among isolates from hands and clothings of site workers.

The mean heterotrophic viable counts revealed that the heaviest bacteria burden in the soils ($2.00 \times 10^6 \pm 0.88 \times 106$ cfu/gm) and leacheates ($1.92 \times 10^6 \pm 0.73 \times 10^6$ cfu/ml) were at the Benin Bye-pass dumpsite while the lowest burden was recorded at the University of Benin site (table 4).

Whereas the isolates were most susceptible to Ciprofloxacin (96.6%), Pefloxacin (94.6%), Gentamycin (91.8%) and Ofloxacin (91.2%), they were least susceptible to Amoxacillin (51.7%) and Erythromycin (55.6%). They all however displayed multiple drug resistance patterns, especially the Serratia marcescens and Ps.aeruginosa isolates (table 5).

A large number of the bacteria exhibited pathogenicity traits as shown in table 6. Whereas 27.6% were positive for virulence gene, 24.3% were invasive by the sereny test while 17.2% were enterotoxigenic by the rabbit ileal loop test and the suckling mouse assays which are indicators of diarrhoegenicity of the isolates

Plate 1 shows the product of virulence PCR on amplified DNA extracts from some of the isolates. Lanes marked L were the 100-1500bp molecular markers or standards while lanes 1-6 were extracts from 6 of the isolates. While Escherichia coli strains on Lanes 1-4 showed bands of about 447bp in size, thus indicating the presence of a virulent gene in them, others in lanes 5 and 6 were negative.

Table 1: Distribution of Bacteria Genera According to Dumpsite Locations and Site Workers

Isolates	Dumpsites					Waste Workers					Overall Frequency (%)
	Isolation Frequencies										
	A	B	C	D	Total	A	B	C	D	Total	
Bacillus sp	5	4	4	6	19	2	3	4	3	12	31 (18.20)
Staphylococcus sp	2	3	2	3	10	4	2	4	3	13	23 (13.93)
Escherichia coli	4	3	3	4	14	2	1	2	2	7	21 (12.72)
Proteus sp	3	2	3	5	13	2	2	1	2	7	20 (12.12)
Streptococcus sp	4	3	2	6	15	1	1	1	2	5	20 (12.12)
Klebsiella sp	3	4	1	5	13	0	1	1	1	3	16 (9.70)
Pseudomonas sp	2	5	3	2	12	0	0	0	1	1	13 (7.90)
Cirobacter sp	3	2	1	3	7	0	1	1	0	2	9 (5.45)
Bacteroides sp	1	1	1	2	5	0	0	0	0	0	5 (3.03)
Clostridium sp	1	1	1	1	4	0	0	0	0	0	4 (2.42)
Serratia sp	0	0	1	2	3	0	0	0	1	1	4 (2.42)

Dumpsite

A = University of Benin

B = Costain Road

C = Government Reservation Area

D = Benin Bye-Pass

Table 2: Distribution of Isolates in Soils, Leacheates, Hands and Clothings

Isolates	Dumpsites		Waste Workers		Overall Isolation Rates (%)
	Soil	Leacheate	Hand	Clothing	
Bacillus sp	10	9	2	10	31(18.20)
Staphylococcus sp	4	6	6	7	23(13.93)
Escherichia coli	6	8	4	3	21(12.72)
Proteus sp	7	6	2	5	20(12.12)
Streptococcus sp	8	7	1	4	20(12.12)
Klebsiella sp	5	8	0	3	16(9.70)
Pseudomonas sp	7	5	0	1	13(7.90
Citrobacter sp	4	3	0	2	9(5.45)
Bacteroides sp	2	3	0	0	5(3.03)
Clostridium sp	1	3	0	0	4(2.42)
Serratia sp	2	1	0	1	4(2.42)

Table 3: Prevalence of Bacteria Species at Dumpsites and on Site Workers.

Bacterial Species	Dumpsite Frequency (%)	Waste Workers Frequency (%)	Total Isolates
Bacillus subtilis	10 (6.8)	10 (6.8)	20
Bacillus cereus	6(4.1)	1 (0.7)	7
Staphylococcus aureus	3 (2.0)	5 (3.4)	8
Staph. epidemidis	7 (4.8)	8 (5.4)	15
Escherichia coli	14 (9.5)	7 (4.8)	21
Proteus mirabilis	7 (4.8)	4 (2.7)	11
Proteus vulgaris	4 (2.7)	3 (2.0)	7
Proteus rettgerri	2 (1.4)	Nil	2
Streptococcus pyogenes	5 (3.4)	1 (0.7)	6
Enterococcus faecalis	10 (6.8)	4 (2.7)	14
Klebsiella pneumoniae	5 (3.4)	Nil	5
Klebsiella aerogenes	4 (2.7)	3 (2.0)	7
Pseudomonas aeruginosa	10 (6.8)	1 (0.7)	11
Citrobacter freundii	7 (4.8)	2 (1.4)	9
Serratia marcescens	3 (2.0)	1 (0.7)	4

Table 4: Mean Heterotrophic Viable Bacteria Counts in Soils and Leacheates

Site	Sample	Viable Counts	
		Mean ± SD	Range
A	Soil (cfu/gm)	$0.70 \times 10^6 \pm 0.56 \times 10^6$	$1.10 \times 10^6 - 3.00 \times 10^6$
	Leacheate (cfu/nl)	$0.10 \times 10^6 \pm 0.65 \times 10^6$	$0.08 \times 10^6 - 3.00 \times 10^6$
B	Soil	$0.78 \times 10^6 \pm 0.39 \times 10^6$	$0.55 \times 10^6 - 2.40 \times 10^6$
	Leacheate	$1.29 \times 10^6 \pm 0.62 \times 10^6$	$0.30 \times 10^6 - 1.50 \times 10^6$
C	Soil	$1.54 \times 10^6 \pm 0.81 \times 10^6$	$0.40 \times 10^6 - 4.50 \times 10^6$
	Leacheate	$0.19 \times 10^6 \pm 1.26 \times 10^6$	$0.31 \times 10^6 - 2.80 \times 10^6$
D	Soil	$2.00 \times 10^6 \pm 0.88 \times 10^6$	$0.30 \times 10^6 - 7.00 \times 10^6$
	Leacheate	$1.92 \times 10^6 \pm 0.73 \times 10^6$	$0.32\ 10^6 - 6.20 \times 10^6$

Dumpsite

A = University of Benin Dumpsite

B = Costain Road Dumpsite

C = Government Reservation Area Dumpsite

D = Benin Bye-Pass Dumpsite

Table 5: Antimicrobial susceptibility Patterns of Species of Bacteria Isolated from Site Workers and Refuse Dumpsites

Isolates	Number tested	Number(%) susceptible to antimicrobial agents												
		S	TE	SXT	CHL	CN	APX	AM	PEF	OFX	CPX	CF	E	SP
Bacillus subtilis	20	15 (75)	13(65)	13(65)	15(75)	19(95)	16(80)	12(60)	20(100)	18(90)	19(95)	15(75)	14(70)	17(85)
Bacillus cereus	7	6 (85.7)	6(85.7)	5(71.4)	7(100)	7(100)	5(71.4)	5(71.4)	7(100)	6(85.7)	7(100)	6(71.4)	9(57.1)	5(71.4)
Staphylococcus aureus	8	5(62.5)	4(50.0)	7(87.5)	6 (75.0)	8(100)	7(87.5)	5(62.5)	8(100)	8(100)	8(100)	7(87.5)	5(62.5)	6(75.0)
Staph. epidemidis	15	13(86.6)	13(86.6)	11(73.3)	10(66.6)	15(100)	12(80.0)	10(66.6)	14(93.3)	14(93.3)	15(100)	12(80.0)	13(86.6)	15(100)
Escherichia coli	21	14 (66.6)	13(61.9)	15(71.4)	18(85.7)	18(85.7)	15(71.4)	15(71.4)	19(90.5)	18(85.5)	20(95.2)	18(85.7)	11(52.4)	17(80.9)
Proteus mirabilis	11	8(72.7)	0(0.0)	7(63.6)	9(81.8)	11(100)	7(63.6)	6(54.5)	11(100)	11(100)	11(100)	8(72.7)	6(54.5)	9(81.8)
Proteus vulgaris	7	4(57.1)	0(0.0)	6(85.7)	6(85.7)	7(100)	5(71.4)	5(71.4)	6(85.7)	5(71.4)	7(100)	6(85.7)	4(57.1)	9(81.8)
Proteus rettgerri	2	0(0)	0(0.0)	1(50.0)	2(100)	2(100)	1(50.0)	0(0.0)	2(100)	1(50.0)	2(100)	1(50.0)	0(0.0)	1(50.0)
Streptococcus pyogenes	6	4(66.7)	3(50.0)	5(83.3)	4(67.7)	6(100)	6(100)	6(100)	6(100)	4(66.7)	5(83.3)	4(66.7)	4(66.7)	3(500)
Enterococcus faecalis	14	10(71.4)	10(71.4)	10(71.4)	8(57.1)	13(92.8)	11(78.6)	9(64.3)	14(100)	13(92.8)	14(100)	12(92.8)	10(71.4)	11(78.6)
Klebsiella pneumoniae	5	1(20.0)	3(60.0)	1(20.0)	3(60.0)	4(80.0)	0(0.0)	0(0.0)	4(80.0)	4(80.0)	4(80.0)	3(60.0)	1(20.0)	3(60.0)
Klebsiella aerogenes	7	5(71.4)	4(57.1)	3(42.8)	4(57.1)	5(71.4)	2(28.6)	2(28.6)	7(100)	5(71.4)	6(85.7)	6(85.7)	4(57.1)	5(71.4)
Pseudomonas aeruginosa	11	4(36.4)	4(36.4)	2(18.2)	5(45.4)	8(72.7)	2(18.2)	2(18.2)	10(90.9)	9(81.8)	11(100)	10(90.9)	3(27.3)	9(81.8)
Citrobacter freundii	9	4(44.4)	4(44.4)	3(33.3)	4(44.4)	8(88.9)	3(33.3)	2(22.2)	8(88.9)	7(77.8)	9(100)	7(77.8)	3(33.3)	8(88.9)
Serratia marcescens	4	0(0)	0(0)	0(0)	0(0)	4(100)	0(0)	0(0)	3(75)	3(75)	4(100	3(75)	0(0)	2(50)
Total	**147**	**93(63.2)**	**91(61.9)**	**89(60.5)**	**101(68.7)**	**135(91.8)**	**93(63.3)**	**76(51.7)**	**139(94.6)**	**134(91.2)**	**142(96.6)**	**118(80.3)**	**82(55.8)**	**117(79.6)**

Key:

S	=	Streptomycin	PEF	=	Pefloxacin
TE	=	Tetracyclin	OFX	=	Ofloxacin
SXT	=	Septrin	CPX	=	Ciprofloxacin
CHL	=	Chloramphenicol	CF	=	Ceftriazone
APX	=	Ampiclox	E	=	Erythromycin
AM	=	Amoxacillin	SP	=	Sparfloxacin
CN	=	Gentamycin			

Table 6: Pathogenicity Assay Profiles of Bacteria Isolates

Bacterial species (number)	Animal Models			Molecular Marker
	Guinea Pig Eye Test	Rabbit Ileal Loop Assay	Infant Mouse Assay	Virulence PCR
Bacillus subtilis (20)	+ (2)	-(20)	-(20)	+(2)
Bacillus cereus (7)	+(3)	+(3)	-(7)	+(3)
Staphylococcus aureus (8)	+(4)	+(2)	-(8)	+(4)
Staph. epidemidis (15)	-(15)	-(15)	-(15)	-(15)
Escherichia coli (21)	+(3)	+(4)	+(5)	+(4)
Proteus mirabilis (11)	+(1)	+(3)	-(11)	-(11)
Proteus vulgaris (7)	+(2)	-(7)	-(7)	-(7)
Proteus rettgerri (2)	+(2)	-(2)	-(2)	+(2)
Streptococcus pyogenes (6)	+(3)	-(6)	-(6)	+(3)
Enterococcus faecalis (14)	-(14)	-(14)	-(14)	-(14)
Klebsiella pneumoniae (5)	+(5)	-(5)	-(5)	+(5)
Klebsiella aerogenes (7)	-(7)	+(2)	-(7)	+(7)
Pseudomona aeruginosa (11)	+(5)	+(3)	-(11)	+(5)
Citrobacter freundii (9)	+(7)	-(9)	+(2)	+(4)
Serratia marcescens (4)	+(4)	-(4)	+(1)	+(4)
Percentage positive	**24.3%**	**17.2%**		**27.6%**

+ = Positive
- = Negative
( ) = Number of isolates

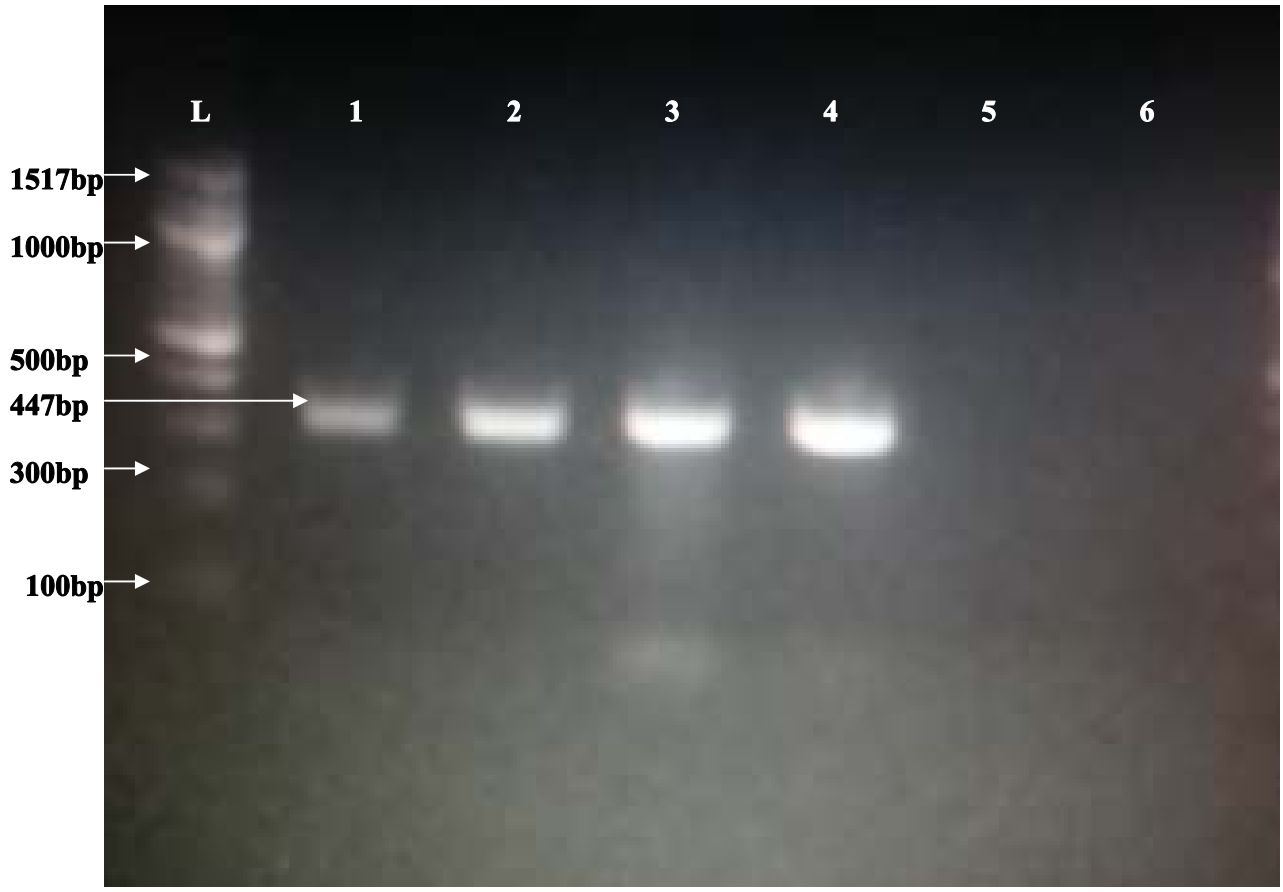

Plate 1: Virulence PCR products on DNA extracts from bacterial isolates from leacheate, topsoil at dumpsites; hand and clothing from dumpsite workers in Benin City. Note that L is a molecular marker (ladder). Lanes 1-4 show bands of molecular size 447bp indicating the presence of virulence genes in their DNA extracts. Lanes 5 and 6 were negative.

Lane 1 = *Escherichia coli* (strain EC6/TS/D) from topsoil at dumpsite D.

Lane 2 = *Escherichia coli* (strain EC2/CLT/D) from the clothing of a site worker at dumpsite D.

Lane 3 = *Escherichia coli* (strain EC5/CLT/A) from the clothing of a site worker at dumpsite A.

Lane 4 = *Escherichia coli* (strain EC1/LCH/A) from the leacheate at dumpsite A.

DISCUSSION

In Nigeria, there is paucity of information on the types of bacteria which contaminate waste dumpsite soils, leacheates or the hands and clothings of site workers. This study showed that the predominant bacteria which colonized waste dumpsites were: Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staph. epidemidis, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Proteus rettgerri, Streptococcus pyogenes, Enterococcus faecalis, Klebsiella pneumoniae, Klebsiella aerogenes, Pseudomonas aeruginosa, Citrobacter freundii and Serratia marcescens. Most of these organisms had earlier been reported by Ekundayo[14] as being associated with waste decay. The isolates obtained from the waste dumps and the site workers in this study were essentially similar. As earlier reported by Obire and Aguda[5], most of these isolates are known to be involved in the biodegradation of organic matter. Some of our isolates such as Escherichia coli, Staphylococcus aureus, Proteus sp, Pseudomonas aeruginosa, Streptococcus pyogenes, etc, are potential pathogens that have been incriminated with various human diseases. The finding of some of these organisms on the hands and clothings of the site workers is worrisome as such workers could serve as reservoirs for potential contamination of other close contacts.

The presence of these isolates in leacheates at the dumpsites is also a major public health threat as leacheates may seep into nearby surface or underground potable waters. Our worries were further compounded by the findings that 27.6% of our isolates harboured virulence gene, while 24.3% were enteroinvasive and 17.2% were capable of causing diarrhoea and that most of them were also found on the clothings and hands of the site workers. The band sizes of the Escherichia coli strains from the workers and dumps confirm their similarities (plate 1). The above findings are in agreement with those who reported that pathogenic microorganisms including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Clostridium sp, Pseudomona aeruginosa and Proteus sp are frequently carried on clothes and hands of waste collectors[15].

It is pertinent to note that some strains of Bacillus cereus, Staph. aureus, Esch. coli, Proteus mirabilis, Klebsiella aerogenes, Pseudomonas aeruginosa, Citrobacter freundii and Serratia marcescens, out of our isolates, produced either heat labile (LT) or heat stable (ST) enterotoxins or both. This indicates that these strains are capable of causing acute diarrhoea diseases. The above isolates except Bacillus cereus had earlier been shown to be enterotoxigenic[16]. The current study therefore confirms the diarrhea-causing capabilities of these organisms. Three of our Bacillus cereus strains were LT positive. Enterotoxigenicity in Bacillus sp is rarely reported. Our current finding is therefore remarkable since B. cereus has been incriminated with cases of food poisoning, especially in fried rice in Chinese restaurants that led to diarrhoea in England, Wales, Scotland, USA, Canada, Hungary and Finland[17]. Our three isolates of Bacillus cereus may therefore be among the rare strains that are enterotoxigenic and therefore are capable of causing diarrhoea.

It is also worthy of note that the heaviest bacteria burden in this study was found at the Benin Bye-pass dumpsite and that the least was at the University of Benin dumpsite. This may not be too surprising since the Benin Bye-pass location is apparently the largest dumpsite in the city where every rubbish/garbages from different parts of the town are dumped. Because of its obscured location, a number

of scavengers and animals are invariably at the site at all times sorting out their needs from the dumps. In the process, they could defecate and urinate freely at the site, thus further contaminating the site with human and animal infective wastes. On the other hand, university of Benin site is closely located to an ever busy University of Benin community. Scavengers may sort out their needs from this site but are most unlikely to openly pass their excrements at the site because of its open location. Moreover, most of the generated wastes dumped at this location are essentially office and household wastes which are likely to harbour less indigenous microbes ab initio.

The current study revealed that the isolates were generally susceptible to the fluroquinolones especially Ciprofloxacin, Pefloxacin and Ofloxacin. They were also highly sensitive to the aminoglycoside, Gentamycin. This may not be unconnected with the fact that the fluroquinolones and Gentamycin have been rarely abused in Nigeria especially as the fluroquinolones are relatively newer in our markets. On the other hand, the older antimicrobial agents tested in this study, such as Amoxicillin, Erythromycin, Septrin, Tetracyclin and Streptomycin were relatively less effective against the isolates. This may be due to long abuse of this drugs through, for example, self medication and the fact that microbes readily adopt survival strategies by mutation or otherwise especially when they are constantly exposed to sub-lethal doses of these drugs.

We wish to conclude that our waste dumpsites and the hands and clothings of the site workers bear heavy burdens of potentially pathogenic multidrug resistant bacteria which could constitute major public health hazards, not only to the immediate communities but also to the families and other contacts of the waste workers. The need to regularly educate these workers about the risk and hazard associated with their job cannot be overemphasized. Proper disposal and possible recycling of municipal wastes in our cities, as done in developed world, are advocated. Finally, all employees of Environmental Agencies in Nigeria must, as a matter of routine, be provided with the necessary protective masks, gloves and other overalls.

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REFERENCES

1. Falase A. Waste Management Practices in Nigeria. A welcome address at the opening of the Basal Convention African Regional Conference in Ibadan, Nigeria. 2004.

2. Sule R.A. Environmental Population in an Urban Centre Waste Disposal in Calabar. Third World Plan. Rev. 1982. 3: 4-7

3. Adedibu AA. Solid Waste Management and a New Environmental Edict: A case Study from Ilorin, Kwara State, Nigeria. The Environmentalist. 1986. 6: 63-68.

4. Boutler JI, Trevors, I and Boland G.T. Microbial Studies of Compost: Bacterial Identificatio and their Potential for Turf Grass Pathogen Suppression. World Journal of Microbial and Biotechn. 2002 18: 661-671.

5. Obire T and Aguda O. Bacteria Community of Leacheate. Journal of Applied Sciences and Environment Management 2002 6(2): 71-75.

6. Pavoni JL., Hear J.E., and Hagerty DL. Handbook of Solid Waste Disposal, Material and Energy Recovery Van Nestrand Reinhold Company. New York 1975. pp 78-83.

7. Miller RH. The Solid as a biological filter. In: Recycling Treated and Municipal Waste Water and Sludge Through Forest and Cropland. E.S. William and L.T. Kardos editors. 1973. The Persylvania State University Press, London.

8. Cowan ST. Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd edition, 1974. Cambridge University Press.

9. Barry AL. and Thornsberry C. Susceptibility Testing: Diffusion Test Procedures. In: Manual of Clinical microbiology 3rd Edition. Lennette EH., Balows A.G., Hausler Jr. WJ. and Truant J.P. Editors. American Society for Microbiology, Washington DC. 1980. pp. 463 – 474.

10. Sereny B. Experimental Keratoconjunctivitis Shigellosis Acta Microbiological Academiae Scientiarum Hungaricae 1957. 4: 367–376.

11. Agbonlahor DE. and Odugbemi T. Enteropathogenic, Enterotoxigenic and Enteroinvasive Escherichia coli Isolated from Acute Gastroenteritis Patients in Lagos, Nigeria. Transactions of Royal Society of Tropical Medicine and Hygiene. 1982. 76(2): 265-267.

12. Guerrant RL., Brunton LL., Schnaitman TC, Rebhun LJ., and Gilman AG. Cyclic Adenosine Monophosphate and Alteration of Chinese Hamster Ovary Cell Morphology: A Rapid Sensitive In-vitro Assays for the Enterotoxins of Vibrio Cholerae and Escherichia coli. Infection and Immunity 1974. 10: 320-327.

13. Dean AG., Ching Y., Williams RG. and Harden LB. Test for Escherichia coli Enterotoxin using Infect Mice: Application in A Study of Diarrhoea in Children in Honolulu. Journal of Infectious Diseases 1972. 125: 407-411

14. Ekundayo JA., Environmental Consequences of the Pollution of Lagos Lagoon. Bulletin of Science Association of Nigeria. 1977. 3: 290-299.

15. Nwachukwu CK. And Eleanya EU. Health Impact Assessment of Solid Waste Disposal Workers in Port Harcourt, Nigeria. Journal of Applied Science. 2007, 7(22): 3562-3566.

16. Evans N. Bacterial Toxins and Diarrhoea. Tropical Doctor. 1979. 9: 10-15.

17. Turnbull PCB. Food Poisoning with Special Reference to Salmonella: Its' Epidemiology, Pathogenesis and Control. Clinics in Gastroenterology. 1979. 8(3): 663-714.