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VERTICAL TRANSMISSION OF BACTERIAL PATHOGENS FROM MUNICIPAL WASTE DUMPS TO WASTE SITE WORKERS: ROLE OF MOLECULAR AND PHENOTYPIC PROFILING OF ISOLATES

OVIASOGIE F.E.[*1] ENABULELE O. I[1] AND AGBONLAHOR D.E[2,3.]

ABSTRACT

Having noticed some phenotypic similarities in bacteria isolates from hands or clothings of waste dump workers and those from the leacheates or topsoils of the dumpsites in Benin City, the need for further investigations in order to possibly establish a positive linkage between these bacteria strains became necessary. We therefore selected 8 pairs of phenotypically similar species from the site workers and dumpsites from 4 locations (University of Benin, Costain, Government Reservation Area and Benin Bye-pass) as our study bacterial candidates. The selected isolates (Bacillus subtilis, staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa and Serratia marcescens) were tested in pairs and were tested for their susceptibilities to 10 antimicrobial agents and their resistogram patterns were consequently determined. The isolates were also assayed for presence of heat labile or heat stable enterotoxins and for their invasiveness. They were further subjected to Multiplex PCR runs with primers for genes coding for virulence and resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracyclin. Phenotypic characterization of the bacteria isolates showed that similar species were invariably isolated from topsoils and leacheates at dumpsite and from the hands and clothings of site workers. The resistogram patterns showed that 5 out of the 8 pairs of strains tested were identical, thus suggesting possible vertical bacterial transmissions at the affected locations. Similarly, the pathogenicity assay profiles showed that 6 out of 8 paired strains gave identical results. However, results from the Multiplex PCR tests revealed that only the paired Pseudomonas aeruginosa strains appeared identical. We conclude that although there were phenotypic and molecular evidence of possible vertical transmissions of bacteria from waste dumps to the site workers, we suggest that the ultimate markers of similarity of strains may lie with genomic sequencing of bacteria that are being matched for strain similarity.

INTRODUCTION

In a presentation to the Nigerian Environmental Society, Falomo[1] posited that waste constituted a major public health nuisance and that the associated microbes comprising bacteria, viruses, fungi and parasites are potential human pathogens which could cause severe health hazards.

In Benin City, most of the wastes found at dumpsites are household and commercial wastes generated from homes and market places respectively. Others are industrial and agricultural wastes. These wastes, which are hardly separated before dumping, are often mixed with non-organic materials such as plastic wastes which are difficult to biodegrade[2]. The most predominant form of waste disposal in Benin City and in deed most of our cities in Nigeria is open-dumping. According to Medina[3], most of our wastes are left on the streets and even when collected for disposal, they are eventually dumped on open lands. Consequently, soil microbes colonize them, carrying out the degradation and transformation of degradable (organic) materials in the waste[4].

It is common to see human and animal scavengers defecating openly at our dumpsites. This further increase the microbial burden of the sites in addition to the indigenous organisms on the soil at the dumpsites. As rain run-off water percolate through such dumpsites, the resultant solution with high contents of organic and inorganic compounds, known as leacheate, is consequently heavily contaminated with these potentially dangerous micro-organisms.

From the preliminary part of our investigations, Oviasogie and Agbonlahor[5], recently, reported the isolation of 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%), Bacteroids sp (2.42%) Clostridium sp (2.42) and Serratia sp (2.42) from four different dumpsites and site workers in Benin City. They concluded that municipal waste dumpsites in Benin City and those working at such sites bore heavy burdens of potentially pathogenic multidrug resistant bacteria which could constitute major public health hazards. Their findings showed that the site workers carried some species of bacteria in their hands and clothings which were similar to those found on the topsoils or leacheates at the dumpsites where they worked. We became more curious when it was found that some strains of Escherichia coli that we isolated from topsoils, leacheates or workers[5] clothing at their Benin Bye-pass and University of Benin dumpsites harboured virulence genes as revealed by Polymerase Chain Reaction for virulence. Apart from their phenotypic similarities, the above findings also suggested molecular similarities which could be useful in establishing vertical transmission of these pathogens from the dumpsites to the site workers.

Accordingly, the aim of the current investigation was to employ a number of phenotypic and molecular profiling of selected isolates from the dumpsites and the site workers to ascertain if the pathogens isolated from the site workers were possibly from the dumpsites.

KEYWORD: *Molecular, Phenotypic, Bacteria typing, Vertical Transmission.*

Oviasogie F.E.[*1] *Enabulele O. I*[1] *and Agbonlahor D.e*[2,3]

Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Lahor Research Laboratories and Medical Centre, 121 Old Benin-Agbor Road, Ikpoba Hill, Benin City – Nigeria.

Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria.

** Author To Whom All Correspondence May Be Addressed.*

MATERIALS AND METHODS

Isolation and characterization of Bacteria Species from topsoils and leacheates at dumpsites and hands and clothings of site workers:

The procedures adopted were recently reported in a separate communication[5].
Briefly, samples of soil, leacheates, hand and clothing swabs were inoculated onto MacConkey, Blood and Griseofulvin agar plates (Oxoid, UK) and incubated aerobically and anaerobically at 37°C for 24-48 hours. Isolates were characterized as recommended by Cowan and Steel[6].

Selection of Bacteria Strains:
Eight bacteria species viz: Bacillus subtilis, Serratia mercescens, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella aerogenes, Proteus mirabilis and Escherichia coli constituted the most predominant organisms that were recently isolated from hands and clothings of site workers at waste dumpsites (soils and leacheates) at 4 locations in Benin City. This finding has been recently reported[5].

For the current work, these organisms were selected in pairs where the same bacteria species was isolated from both the environment and the site worker. Two pairs of predominant isolates were chosen from each site or location as illustrated in table 1.

Table 1: **Selected Paired Isolates from Dumps and Site Workers According to Dumpsites and Sources.**

Bacterial Species	Number Tested Per Site	Sources	Dumpsite Location			
			A	B	C	D
Bacillus subtilis	2	TS & CLT	X			
Staphylococcus aureus	2	LC & CLT		X		
Escherichia coli	2	TS & CLT			X	
Proteus mirabilis	2	LC & HD				X
Klebsiella aerogenes	2	TS & HD				X
Klebsiella pneumoniae	2	LC & CLT			X	
Pseudomonas aeruginosa	2	TS & HD		X		
Serratia marcescens	2	TS & CLT	X			

TS = Topsoil
CLT = Clothing
LC = Leacheate
HD = Hand
X = Isolation Site

A = University of Benin Dumpsite
B = Costain Dumpsite
C = Government Reservation Area Dumpsite
D = Benin Bye-pass Dumpsite

Antimicrobial Resistogram Pattern Determination:

Disc-diffusion susceptibility testing was performed as earlier described[7]. Briefly, overnight broth cultures of all 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 aseptically placed on the seeded plates and incubated at 37°C for 24 hours. The 10 antimicrobial agents used were Streptomycin (10µg), Tetracyclin(30µg), Septrin(30µg), Chloramphenicol(30µg), Gentamycin(10µg), Ampiclox(30µg), Amoxacillin(30µg), Parfloxacin(30µg), Ofloxacin(30µg) and Ciprofloxacin(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). The antimicrobial agents to which strains were resistant constituted their resistogram patterns.

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[8,9]. Essentially, one eye of the guinea pig was inoculated with one drop of a tick emulsion of the selected 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:

The selected strains were inoculated into Tryptose Soya Broth (Oxiod, U.K) and incubated in a 37°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[9]. 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[10] and Agbonlahor[9]. Essentially, the adult albino white rabbits were anaesthetized with sodium barbiturate, the animal was then aseptically 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[9,11]. 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.

Multiplex PCR on Drug Resistance and Virulence Genes:
All our PCR reagents for this study were procured from Inquaba Biotechnology Industries, Hartfield, South Africa. The Primers employed are those earlier used by Liebanal and Associates[12] (Table 2).

Table 2: Primers with their oligonucleotide sequences employed for the multiplex PCR assay.

Primer	Target Gene	Sequence (5^1 to 3^1)	Expected Amplicon Size (bp)
Pse – F Pse –R	Pse (Ampicillin)	GGCAATCACACT CGATGATGCGT GGCTAATACGGTCTAGACGAGT	156
FloR–F FloR–R	FloR (Chloramphenicol)	CTTTGGCTATACTGGCGATG GATCATTACAAG CGCGACAG	266
STR–F STR–R	Str (Streptomycin)	AGACGCTCCGCGCTATAGAAGT CGGACCTACCAAGGCAACGCT	293
SUl 1 –F SUl 1 – R	Sul 1 (Sulphonamide)	CGGATCAGACGTCGTGGATGT TCGAAGAACCGCACAATCTCGT	351
TetG – F TetG – R	TetG (Tetracyclin)	AGCAGCCTCAACCATTGCCGAT GGTGTTCCACTGAAAACGGTCCT	391
SPv – C–1 SPv – C–2	SPv C (Virulence).	ACTCCTTGCACAACCAAATGCGGA TGTCTCTGCATTTCGCCACCATCA	447

DNA Extraction from Bacteria:
For the Multiplex PCR assay, we selected a pair of isolates (from the environment and the site workers) at each location, which appeared identical using their pathogenicity assay results.

DNA was extracted from the isolates as follows: The isolates were purified on nutrient agar plates (Oxoid, U.K) and pellets were harvested aseptically and suspended in 180µl of buffer ATL. To this suspension, 20µl of proteinase 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 vortexed and incubated at 56°C for 3 hours, with occasional vortexing in between. The tube was briefly centrifuged to remove drops from side of the tube. Then 200µl of buffer A1 was added, pulse-vortexed for 15secs and incubated at 70°C for 10mins and briefly centrifuged. To the mixture, 200µl of ethanol (96-100%) was added and pulse-vortexed for 15secs and centrifuged briefly.

The Spin column 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 vortexed 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 amplification 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) all at the Lahor Research Laboratories, Benin City. Photographs of the separated bands in agarose gels were taken with the in-built camera on the Alpha-imager.

RESULTS
The predominant bacteria species isolated from the dumpsites and site workers are reflected in table 3 while the distribution of isolates according to soils, leacheates, hands and clothings are shown in table 4. Similar species were invariably present in the various specimens investigated with Bacillus sp., Staphylococcus sp. and Escherichia coli toping the list.

Table 5 shows the resistogram patterns of the paired bacteria strains tested. At location A (University of Benin), the Bacillus subtilis strains from topsoil and clothing of the site workers were similarly resistant to tetracyclin, septrin and amoxicillin, while a Serratia marcescens strains isolated from topsoil and another strain isolated from the clothings of the waste site workers were similarly resistant to streptomycin, tetracyclin, septrin, chloramphenicol, ampliclox and amoxicillin. These two pair of strains were therefore adjudged to be identical and were possibly transmitted vertically from the soils to site workers.

Similarly, identical results were recorded from the pathogenicity assays for paired strains tested at locations A and B and one pair each at sites C and D as shown in table 6. Six out of the 8 pairs of isolates appeared identical

However from the Multiplex PCR results, only the pair of Ps.aeruginosa strains tested appeared identical. For the other 3 selected pairs of isolates, genes for drug resistance and virulence were indiscriminately targeted. These pairs were therefore adjudged non-identical (table 7 and plate 1).

Plate 1 showed the Multiplex PCR Amplification Products of virulence and drug resistant genes. Lanes 3, 6 and 7 showed bands for virulence gene at 447 base pair (bp). There were also bands at lanes 1, 4, 5 and 8 for Ampicillin resistance at 156bp, Lanes 2 and 3 for Tetracyclin resistance at 351bp and Lanes 1, 4 and 5 for sulphonamide at 351bp. There were no bands for Chloramphenicol resistance at 266bp and Streptomycin at 293bp.

Table 3: **Predominant Bacteria Isolates from Dumpsites and on Site Workers**

Bacteria Species	Dumpsite Frequency (%)	Waste Workers Frequency (%)	Total Isolates
Bacillus subtilis	10 (5.9)	10 (5.9)	20(11.8)
Bacillus cereus	6(3.5)	1 (0.6)	7(4.11)
Staphylococcus aureus	3 (1.8)	5 (2.9)	8(4.7)
Staph. epidemidis	7 (4.1)	8 (4.7)	15(8.8)
Escherichia coli	14 (8.2)	7 (4.1)	21(12.3)
Proteus mirabilis	7 (4.1)	4 (2.3)	11(6.5)
Proteus vulgaris	4 (2.3)	3 (1.8)	7(4.1)
Proteus rettgerri	2 (1.2)	(0)	2(1.2)
Streptococcus pyogenes	5 (2.9)	1 (0.6)	6(3.5)
Streptococcus faecalis	10 (5.9)	4 (2.3)	14(8.2)
Klebsiella pneumoniae	5 (2.9)	3(1.8)	8(4.7)
Klebsiella aerogenes	4 (2.3)	3 (1.8)	7(4.1)
Pseudomonas aeruginosa	10 (5.9)	1 (0.6)	11(6.5)
Citrobacter freundii	7 (4.1)	2 (1.2)	9(5.3)
Serratia marcescens	3 (1.8)	1 (0.6)	4(2.3)
		Total	**170(100)**

Table 4: Distribution of Predomina nt Isolates According to Soils, Leacheates, Hands and Clothings

Bacteria Species	Dumpsite Isolation Frequency(%)		Wastes Workers Isolation Frequency (%)		Total
	Topsoils	Leacheates	Hands	Clothings	
Bacillus subtilis	6 (3.5)	4(2.3)	2(1.2)	8(4.7)	**20**
Bacillus cereus	3(1.8)	3(1.8)	0(0)	1(0.6)	**7**
Staphylococcus aureus	1(0.6)	2(1.2)	2(1.2)	3(1.8)	**8**
Staph. epidemidis	4(2.3)	3(1.8)	5(2.9)	3(1.8)	**15**
Escherichia coli	7(4.1)	7(4.1)	1(0.6)	6(3.5)	**21**
Proteus mirabilis	3(1.8)	4(2.3)	1(0.6)	3(1.8)	**11**
Proteus vulgaris	2(1.2)	2(1.2)	0(0)	3(1.8)	**7**
Proteus rettgerri	2(1.2)	0(0)	0(0)	0(0)	**2**
Streptococcus pyogenes	1(0.6)	4(2.3)	1(0.6)	0(0)	**6**
Streptococcus faecalis	4(2.3)	6(3.5)	3(1.8)	1(0.6)	**14**
Klebsiella pneumoniae	2(1.2)	3(1.8)	1(0.6)	2(1.2)	**8**
Klebsiella aerogenes	2(1.2)	2(1.2)	2(1.2)	1(0.6)	**7**
Pseudomonas aeruginosa	5(2.9)	5(2.9)	1(0.6)	0(0)	**11**
Citrobacter freundii	2(1.2)	5(2.9)	0(0)	2(1.2)	**9**
Serratia marcescens	2(1.2)	1(0.6)	0(0)	1(0.6)	**4**

Table 5: Resistograms of Selected Isolates According to Strains, Sources and Locations

Location	Bacteria Species	Strain ID	Source	Resistograms	Remark
A	*Bacillus subtilis*	Bs1	TS	– TE SXT AM	Identical
	Bacillus subtilis	Bs2	CLT	– TE SXT AM	
	Serratia marcescens	Sm1	TS	S TE SXT CHL APX AM	Identical
	Serratia marcescens	Sm2	CLT	S TE SXT CHL APX AM	
B	*Staphylococcus aureus*	Sa1	LC	S TE CHL AM	Non-identical
	Staphylococcus aureus	Sa2	CLT	S TE – AM	
	Pseudomonas aeruginosa	Pyo1	TS	S TE SXT CHL APX AM	Identical
	Pseudomonas aeruginosa	Pyo2	HD	S TE SXT CHL APX AM	
C	*Escherichia coli*	Ec1	TS	S TE SXT APX AM	Non-identical
	Escherichia coli	Ec2	CLT	– TE SXT APX AM	
	Klebsiella pneumoniae	Kp1	LC	S TE SXT CHL APX AM	Identical
	Klebsiella pneumoniae	Kp2	CLT	S TE SXT CHL APX AM	
D	*Proteus mirabilis*	Prm1	LC	S TE SXT APX AM	Non-identical
	Proteus mirabilis	Prm2	HD	S TE SXT – AM	
	Klebsiella aerogenes	Ka1	TS	– TE SXT CHL APX AM	Identical
	Klebsiella aerogenes	Ka2	HD	– TE SXT CHL APX AM	

S = Streptomycin
TE = Tetracyclin
SXT = Septrin
AM = Amoxicillin
CHL = Chloramphenicol
APX = Ampiclox

Table 6: Pathogenicity Assay Profiles of Bacteria Isolates

Location	Bacteria Isolate	Strain ID	Animal Models			Molecular Marker	Remarks
			Sereny Test	Rabbit Ileal Loop Test	Sucking Mouse Assay	Virulence PCR	
A	*Bacillus subtilis*	Bs1	-	-	-	-	Identical
	Bacillus subtilis	Bs2	-	-	-	-	
	Serratia marcescens	Sm1	+	-	+	+	Identical
	Serratia marcescens	Sm2	+	-	+	+	
B	*Staphylococcus aureus*	Sa1	+	+	-	+	identical
	Staphylococcus aureus	Sa2	+	+	-	+	
	Pseudomonas aeruginosa	Pyo1	+	+	-	+	Identical
	Pseudomonas aeruginosa	Pyo2	+	+	-	+	
C	*Escherichia coli*	Ec1	+	+	-	+	Non-identical
	Escherichia coli	Ec2	+	+	+	+	
	Klebsiella pneumoniae	Kp1	+	-	-	-	
	Klebsiella pneumoniae	Kp2	+	-	-	-	Identical
D	*Proteus mirabilis*	Prm1	+	-	-	-	identical
	Proteus mirabilis	Prm2	+	-	-	-	
	Klebsiella aerogenes	Ka1	-	+	-	+	Non-Identical
	Klebsiella aerogenes	Ka2	-	+	-	-	

\+ = Positive

\- = Negative

Table 7: Multiplex PCR Profiles for Ta rgeted Genes on DNA Extracts From Tested Bacteria Strains

Identity of Strains Tested	Target Genes					
	SpvC	**TetG**	**Pse**	**Sul1**	**STR**	**FloR**
Prm1	-	-	+	+	-	-
Prm2	-	+	-	-	-	-
Kp1	+	+	-	+	-	-
Kp2	-	+	+	+	-	-
Sm1	-	-	+	+	-	-
Sm2	-	+	+	-	-	-
Pyo1	+	-	-	-	-	-
Pyo2	-	-	-	-	-	-
Esch. coli-K12	-	-	-	-	-	-

+ = Positive Band at expected amplicon size

- = Negative Band

Spv = virulence gene

TetG = Tetracycline resistance gene

Pse = Ampicillin resistance gene

Sul1 = Sulphonomide resistance gene

STR = Streptomycin resistance gene

FloR = Chloramphenical resistance gene

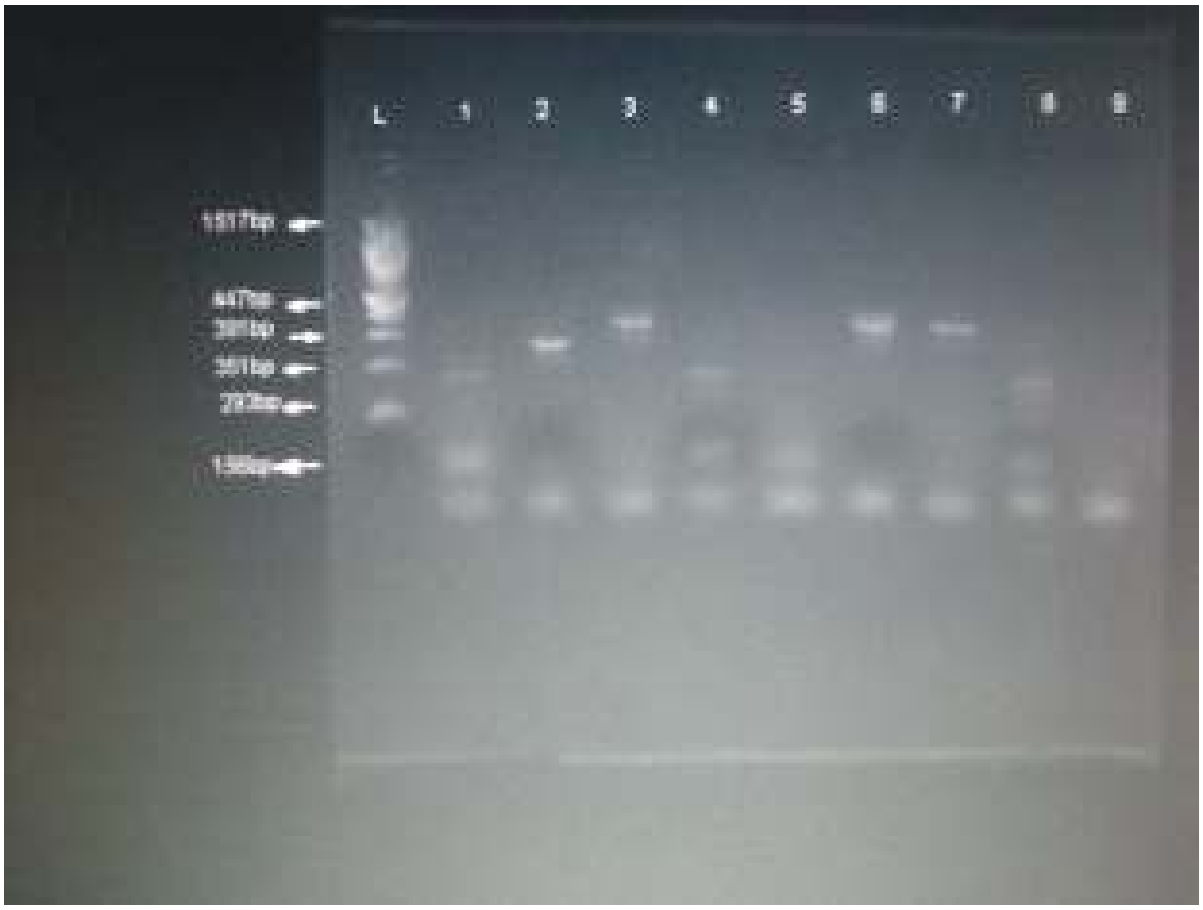

Plate 1: Multiplex PCR Amplification Products of virulence and drug resistant genes. Lanes 3, 6 and 7 showed bands for virulence gene at 447 base pair (bp). There were also bands at lanes 1, 4, 5 and 8 for Ampicillin resistance at 156bp, lanes 2 and 3 for Tetracyclin resistance at 351bp and lanes 1, 4 and 5 for sulphonamide at 351bp. There were no bands for chloramphenicol resistance gene at 266bp and Streptomycin at 293bp.

L = Ladder
Lane 1 = *Proteus mirabilis* from leacheates at location D
Lane 2 = *Proteus mirabilis* from workers' hand at location D
Lane 3 = *Klebsiella pneumoniae* from top soil at location C
Lane 4 = *Klebsiella pneumoniae* from workers' hand at location C
Lane 5 = *Serratia marcescens* from top soil at location A
Lane 6 = *Ps. aeruginosa* from top soil at location A
Lane 7 = *Ps. aeruginosa* from workers' hand at location B
Lane 8 = *Serratia marcescens* from workers' clothing at location A
Lane 9 = *Escherichia coli* K-12 control strain.

DISCUSSION

Oviasogie and Agbonlahor[5] recently found that waste dumpsites in Benin City bear a huge burden of pathogenic and multidrug resistant bacteria especially Bacillus sp, Staphylococcus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp, Streptococci, Proteus sp. and Serratia marcescens. These organisms have been frequently isolated from various clinical specimens and incriminated with human diseases[9,13-19]. A number of the strains investigated especially Serratia marcescens, Pseudomonans aeruginosa, staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis showed varying pathogenicity traits and were also multiple drug resistant from their resistogram patterns. Strains of these isolates were not only found in the solids and leacheates at the dumpsites but also on the hands and clothings of the waste dumpsite workers. This explains the degree of risk of infection these workers are constantly exposed to.

In order to be sure that the strains found in the hands and clothings of the workers were possibly acquired from the contaminating pathogens at their worksites, we compared the identities of strains of similar species selected from each dumpsite and the respective workers and found similar identities in 5 of 8 pairs with the resistogram testing while 6 out of 8 pairs gave similar results in the pathogenicity assays. These results suggested possible vertical bacterial transmissions from the environment to the site workers.

On the other hand, only the paired strains of Pseudomonas aeruginosa appeared identical in the Multiplex PCR test out of the selected isolates. This suggests that the use of this model alone may not be ideal for tracing strain identities. This finding is in agreement with the conclusions by Liebana and his Colleagues[12], where they cautioned against drawing conclusions, based on a single molecular method. In their work, they found that bacteria strains that shared one of their markers for strains identity did not necessarily belong to the same bacterial clone and concluded that a multiple typing approach would be required to enable enough discrimination in order to track strains for epidemiological investigations. Although some of the test strains exhibited multidrug resistance patterns on in-vitro testing, we were unable to target the relevant resistance genes with the molecular PCR test. The resistance noted may have been plasmid mediated or alternatively, point mutations may have occurred in strains at some of the targeted locations. Non detection of resistance genes were more pronounced in streptomycin and chloramphenicol against which all our selected strains were negative in this molecular assay.

We therefore conclude that the combined use of phenotypic and molecular assays do serve as useful markers for the determination of relationships between strains isolated from the environments and environmental workers. Although there were phenotypic and molecular evidence of vertical transmission of bacteria from waste dumps to the site workers, we suggest that the ultimate markers of similarity in bacteria strains should rest with the genomic sequencing of such paired strains.

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