VIRULENCE FACTORS AND ANTIBIOGRAM OF BACTERIAL ISOLATES FROM THE MUNICIPAL SOLID WASTE IMPACTED ENVIRON IN UYO, NIGERIA

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[1]IKPE, D. I, [2]AKINJOGUNLA, O.J., [2] ADEGOKE, A. A. AND *[2,3]ESSIEN, J. P.

[1]*Department of Science Laboratory Technology, Akwa Ibom State Polytechnic, Akwa Ibom State, Nigeria*
[2]*Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria*
[3]*International Centre for Energy and Environmental Sustainability Research, University of Uyo, Akwa Ibom State*
jomato652011@yahoo.com

ABSTRACT

Standard bacteriological and disc diffusion techniques were employed to isolate and determine the antibiotic susceptibility patterns of bacterial isolates from the Municipal Solid Waste impacted environ. The virulence factors, plasmid curing and profiles of the bacterial isolates were determined using appropriate culture media, acridine orange and gel electrophoresis, respectively. Sixteen bacterial isolates in genera *Bacillus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Micrococcus, Proteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus* and *Vibrio* were obtainedfrom the wastes, leachate and the dumpsite impacted soil, water, sediment and atmosphere. Of the 16 bacterial isolates obtained, ≤ 62.5% produced virulence factors, 62.5 % were lipase producers, 31.3% were DNAse producers, 56.3% produced haemolysins, while only *S. aureus* produced coagulase. The results showed that between 76.9% and 92.3% pathogenic bacterial isolates were resistant to Cefuroxime, Ceftriaxone and Cloxacillin, while all the isolates were resistant to Erythromycin. The resistance of these bacteria to the antibiotics was plasmid mediated with plasmid DNA size of 350 bp. The 16S rRNA gene sequencing of the antibiotic resistant strains of *Salmonella* and *Streptococcus* sp showed 99% and 100% resemblance to *S. enterica* (AC513382) and *S. sius* (NC021213.1), respectively. The dumpsites environ has multi-drug resistant pathogenic isolates which can cause various diseases that may be extremely difficult to treat.

INTRODUCTION

Waste disposal poses threat to man, animal and the environment. It has been well established that municipal solid wastes **(**MSW) which originate in the homes, institutions and commercial establishment of the community contain diverse microorganisms (Pavoni *et al.*, 1975). Apart from the toxic elements, waste and its leachate may harbour pathogenic and toxigenic microorganisms that can trigger public health problems (Donnelly *et al*., 1988). Similarly, the consumption of leachate-polluted water by the farm animals may portend some health risks. Quite a few microbial species that come from soil, water and human faecal matter have been isolated from the compost of domestic garbage or sludge (Sharma and Shah, 2005). Studies have also shown that species of *Arthrobacter*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Staphylococcus*, *Shigella, Micrococcus* and *Lactobacilli* sp are commonly associated with MSW dumpsites (Obire *et al*. 2002 and Ekpo *et al*., 2008). Mesophillic microbes such as *Bacillus* sp, some faecal coliforms, *Pseudomonas* sp, *Streptococcus* sp, *Proteus* sp, *Serratia* sp, *Streptomyces* sp, *Actinomyces* sp and *Streptosporangium* sp have been obtained during the composting of MSW (Taiwo and Oso, 2004) and strains of *Bacillus steanothermophilus*, *Thermomonospora* sp and *Clostridium thermocellum* were the thermophiles obtained from the dumpsites (Sharma and Shah, 2005). Human exposure to these microorganisms in the MSW may occur at nearly every step, starting from the generation of refuse to ultimate disposal or

by blowing debris. At dumpsites and landfills, dusts containing microorganisms could be equally spread during dumping or earth moving operations.

The rudimentary system of waste management that involves the collection and dumping of wastes in open dumpsites and also inside the ravine within the Uyo Metropolis still persists. The health and environmental challenges associated with poor waste management practices cannot be over-emphasized. However, little or no work has been done on the health implications of waste dumps on the local population of humans in Uyo metropolis. The present study was carried out to evaluate the virulence factors and antibiogram of bacterial isolates from the Municipal Solid Waste (MSW) impacted environ in Uyo, Nigeria.

MATERIALS AND METHODS

Samples Collection

Municipal solid waste (MSW) and leachate (DSL) samples were collected from the designated dumpsites at Udo Street and St. Lukes Hospital. Nearby stream water (SSW) and sediment (SDS) as well as bio-aerosol samples (BAS) from MSW impacted atmosphere were also aseptically collected during the raining and drying seasons using standard bacteriological methods.

Isolation, Identification and Assay of Virulence Factors Producing Potential of Bacteria

The pour plate technique was used for the isolation of bacteria. Bacto Nutrient Agar (BNA), MacConkey Agar (MCA), Eosine Methylene Blue Agar (EMBA), *Salmonella-Shigella* Agar (SSA), Mannitol Salt Agar (MSA) and Thiosulphate Citrate Bile Salt (TCBS) Agar were used for the isolation of heterotrophic bacteria, total coliform, faecal coliform, *Salmonella* and *Shigella*, *Staphylococcus aureus* and *Vibrio* sp, respectively (Pfeffer and Oliver, 2003; Cheesbrough, 2006). Using appropriate media, bacteria in the atmosphere of MSW impacted environ were obtained using the settle plate culture technique (sedimentation technique) as described by APHA (1998); Downes and Ito (2001). The bacterial isolates obtained were characterized to generic level using the taxonomic schemes of Cowan (1985) and Holt *et al*., (1994). The bacterial isolates were assayed for their ability to produce haemolysin, DNase, lipase and coagulase using blood agar, DNAse agar, tributyrin Agar and blood plasma, respectively (Akinjogunla and Enabulele, 2010).The susceptibility of the pathogenic bacteria to Ceftazidime (CAZ, 30µg) ; Cefuroxime (CRX, 30µg) ; Gentamicin (GEN, 10µg) ; Ceftriaxone (CTR, 30µg ; Erythromycin (ERY, 30µg) ; Cloxicillin (CXC, 5µg) ; Ofloxacin (OFL, 5µg) ; Augmentin (AUG, 30µg); Nitrofurantoin (NIT, 30µg); Ampicillin (AMP, 10µg) and Ciprofloxacin (CPR, 5µg) was determined using the Kirby-Bauer method.Highly multidrug resistant strains of *Salmonella* sp, *Staphylococcus aureus*, *Shigella* sp, *Vibrio cholerae* and *E. coli* were subjected to plasmid curing using acridine orange (Akinjogunla and Enabulele, 2010). The extraction of the isolates DNA was carried out and molecular weight of the plasmid DNA determined as described by Kraft *et al*. (1988) and Akinjogunla and Enabulele (2010). However, confirmation of species identity was carried by the 16S rRNA gene sequence analysis.

RESULTS AND DISCUSSION

Diverse species of bacteria were encountered in the dumpsite environ studied. Sixteen bacterial isolates belonging to genera *Bacillus, Enterobacter*, *Escherichia, Klebsiella, Lactobacillus, Micrococcus, Proteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus* and *Vibrio* were obtained. These isolates are typical micro-flora of water and soil (Donderski *et al*., 2005). The isolates comprise bacterial genera that are widely distributed in cultivated and uncultivated soils in the tropical and sub-tropical regions. A lot of these pathogens are responsible for most of the soil and surface water contamination as posited by Ghadiri and Rose (1992). Some of these bacteria are organic decomposers, potential plant and human pathogens (Yamada *et al.*, 2009). *S. aureus*, *E. coli*,*Salmonella* sp, *Shigella* sp, *V. cholerae* and *V. parahaemolyticus* have been reported to be commonly associated with several human ailments such as gastroenteritis, cholera, typhoid fever, dysentery and diarrhoea

(Brooks *et al*, 2004). The occurrences of the bacterial isolates in the MSW dumpsites in this study are in conformity with the reports of Obire *et al*, (2002) and Ekpo *et al*., (2008) who obtained these bacteria in MSW dumpsites. Faecal coliform such as *E. coli* were obtained in the MSW dumpsites and the faecal coliform can be harmful to the environment if discharged into rivers or waterways (USEPA, 2008). Therefore, the residents who drink from the impacted stream or farms on the impacted soil are readily exposed to these pathogenic bacteria. Yamada (2009) reported that a high number of people living around the dumping sites had illnesses related to the dermatological, gastrointestinal and respiratory diseases such as upper respiratory tract infections, chronic bronchitis, asthma, unspecified dermatitis, pruritus and illness of the skin.

Table 1: Occurrence and Distribution of Bacterial Isolates from MSW Impacted Environ

Isolates	MSW (n=6)	Dumpsites Leachate (n=6)	Soil (n=12)	Surface Water (n=6)	Sediment (n=6)	Atmosphere (n=12)
Bacilluscereus	+ (4)	+ (1)	+ (5)	-	-	-
Bacillussubtilis	+ (6)	+ (4)	+ (11)	+ (2)	+ (5)	+ (8)
Enterobacter sp	+ (2)	-	+ (2)	-	-	-
Escherichia coli	+ (5)	+ (2)	+ (12)	+ (5)	+ (6)	+ (7)
Klebsiella sp	+ (4)	+ (4)	+ (8)	+ (2)	+ (4)	+ (8)
Lactobacillus sp	+ (2)	+ (1)	-	-	-	-
Micrococcus sp	+ (4)	+ (5)	+ (7)	-	+ (3)	+ (3)
Proteus sp	-	+ (2)	-	-	-	-
Pseudomonas aeruginosa	+ (6)	+ (6)	+ (10)	+ (1)	+ (2)	+ (5)
Salmonella sp	+ (5)	+ (3)	+ (7)	+ (3)	+ (6)	+ (3)
Serratia sp	+ (4)	+ (2)	+ (4)	-	+ (3)	-
Shigella sp	+ (4)	+ (3)	+ (6)	+ (3)	+ (5)	+ (5)
Staphylococcusalbus	+ (2)	-	+ (3)	-	-	-
Staphylococcusaureus	+ (5)	+ (3)	+ (6)	+ (6)	+ (4)	+ (8)
V. cholerae	+ (4)	-	+ (3)	+ (1)	-	-
V. parahaemolyticus	+ (3)	-	+ (1)	-	-	-
Species Richness	15	12	14	8	9	8

Values in parentheses are number of isolates obtained from the samples analyzed.
+ = isolated; - = not isolated

Table 2 shows the occurrence of DNAse, haemolysin, lipase and coagulase producing bacterial isolates from the MSW dumpsites. The results show ≤ 10 (62.5%) bacterial species isolated produced one or more virulent factor(s). Of the 16 bacterial isolates obtained, 10 (62.5 %) were lipase producers. The lipase producers were *Serratia* sp, *S. aureus*, *K. pneumoniae*, *V. cholerae*, *V. haemolyticus*, *B. subtilis*, *P. aeroginosa*, *Streptococcus* sp. *B. cereus* and *Shigella* sp. Two Gram positive bacteria (*S. aureus* and *Streptococcus* sp) and three Gram negative bacteria (*V. cholerae*, *V. parahaemolyticus* and *Proteus* sp) were DNAse producers from the MSW dumpsites. Five (5) α-haemolysin (partial haemolysis) and 4 β- haemolysin (complete haemolysis) producers were isolated from the MSW dumpsites. *S. aureus* was the only coagulase positive isolate. The occurrence of α-haemolysin and lipase producing *S. aureus* in this study is in conformity with the results of Akinjogunla *et al*. (2014).

Amongst them, the *S. aureus* with 100% capability was the most virulent strain isolated, followed by *Streptococcus* sp (75%), *V. parahaemolyticus* (75%), *Shigella* sp (50%), *K. pneumoniae* (50%), *P. aeruginosa* (50%) and *V. cholerae* (50%). The *Salmonella* sp, *B. subtilis*, *Serratia* sp and *E. coli* obtained were associated with one (25%) virulence factor.

The antibiotic susceptibility results of the pathogenic bacterial isolates from MSW impacted environment are presented in Table 3. The results showed that between 10(76.9%) and 12 (92.3%) isolates were resistant to Cefuroxime, Ceftriaxone and Cloxacillin while 13 bacterial isolates were resistant to Erythromycin. The varied resistant patterns of the isolates were as

follows: *Shigella* sp was resistant to CAZ, CRX, CTR, ERY, CXC, AUG and AMP; *S. marcescens* was resistant to CAZ, CRX, CTR, ERY, CXC and AMP; *S. aureus* was resistant to CRX, ERY, CXC, AUG, AMP and CPR; *V. cholerae* wasresistant to CRX, CTR, ERY, CXC, and AMP. The resistance pattern of *V. haemolyticus* was CRX, CTR, ERY, CXC, and AMP; *K. pneumoniae* was CAZ, CRX, CTR, ERY, CXC and AMP; *E. coli* was resistant to CRX, CTR, ERY, CXC, AMP and CPR. The resistance patterns of *Proteus* spp and *B. cereus* are also shown in Table 3. *B, subtilis* was sensitive to OFL and exhibited intermediate sensitivity to GEN; *Salmonella* sp was sensitive to GEN, CTR, OFL, NIT and AMP but demonstrated intermediate sensitivity to CPR. *P. aeruginosa* was sensitive to CAZ, GEN, CTR, OFL and CPR, while *Streptococcus* sp was sensitive to GEN and OFL but exhibited intermediate susceptibility to CAZ and CPR. The resistance of *S. aureus* to most of these antibiotics is in agreement with Akinjogunla *et al.* (2014).

Table 2: Virulence Factors Producing Potential of the Bacterial Isolates

Bacterial Isolates	Virulence factors			
	Lipase	DNAse	Haemolysin	Coagulase
Shigella sp	+	-	+(α)	-
Serratia sp	+	+	-	-
S. aureus	+	+	+ (α)	+
V. cholerae	+	+	-	-
K. pneumoniae	+	-	+ (α)	-
V. parahaemolyticus	+	+	+ (β)	-
B. subtilis	+	-	-	-
Salmonella sp	-	-	+ (β)	-
Proteus sp	-	-	+ (α)	-
B. cereus	+	-	-	-
P. aeruginosa	+	-	+ (β)	-
Micrococcus sp	-	-	-	-
Streptococcus sp	+	+	+ (β)	-
Lactobacillus sp	-	-	-	-
E. coli	-	-	+ (α)	-
Enterobacter sp	-	-	-	-
Total No (%)	10 (62.5)	5 (31.3)	9 (56.3)	1 (6.3)

Key: +: Positive; -: Negative; β: Beta haemolysin; α: Alpha haemolysis

Table 3: Antibiotic Susceptibility Profile of Pathogenic Bacterial isolates from MSW Dumpsites

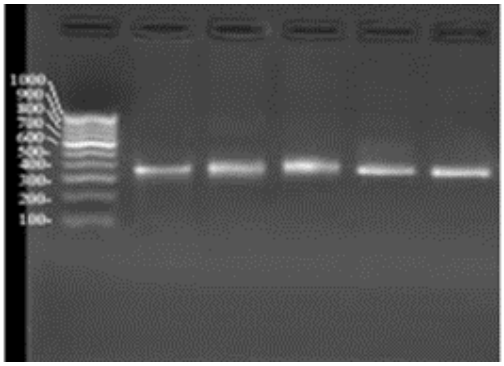
Bacterial Isolates	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	NIT	AMP	CPR
Shigella sp	R	R	S	R	R	R	S	R	S	R	S
S. marcescens	R	R	S	R	R	R	S	I	S	R	ND
S. aureus	I	R	S	S	R	R	I	R	S	R	R
V. cholerae	S	R	S	R	R	R	S	ND	ND	R	ND
K. pneumoniae	R	R	S	R	R	R	I	S	S	R	I
V. parahaemolyticus	I	R	I	R	R	R	S	ND	ND	R	I
B. subtilis	R	R	I	R	R	R	S	ND	MD	R	R
Salmonella sp	R	R	S	S	R	R	S	R	S	S	I
Proteus sp	S	R	S	R	R	S	S	R	S	R	S
B. cereus	S	S	S	R	R	S	R	R	S	R	R
P. aeruginosa	S	R	S	S	R	R	S	R	ND	ND	S
Streptococcus sp	I	R	S	R	R	R	S	ND	ND	R	I
E. coli	S	R	S	R	R	R	S	S	S	R	R

Keys: Ceftazidime (CAZ) ; Cefuroxime (CRX) ; Gentamicin (GEN) ; Ceftriaxone (CTR); Ofloxacin (OFL); Erythromycin (ERY) ; Cloxacillin (CXC) ; Augmentin (AUG);

Nitrofurantoin (NIT); Ampicillin (AMP) and Ciprofloxacin (CPR); R: resistant; S: sensitive; I : Intermediate; ND: Not determined.

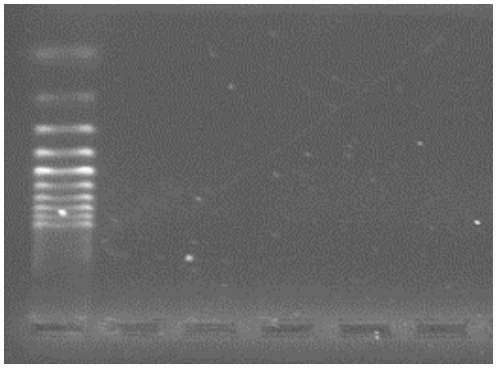
The locations of antibiotic resistant markers of *Salmonella* sp, *S. aureus, Shigella* sp, *V. cholerae* and *E. coli* from MSW dumpsites were determined using acridine orange and the plasmid profiles of these bacterial isolates are shown in Figure 1. The analysis revealed that the isolates possessed plasmids sized approximately 350 bp (Figure 1). When they were cured of these plasmids, the isolates lost their plasmids as well as their ability to resist the commercially available antibiotics tested, showing that the antibiotic resistance displayed by these isolates was plasmid mediated and can be possibly transferred to non-resistant bacteria strains.

The occurrences of plasmid mediated antibiotic resistant pathogens in this study agrees with the results of Akinjogunla and Enabulele (2010). Further confirmation of the identities of two antibiotic resistant isolates, Gram negative (*Salmonella* sp) and Gram positive (*Streptococcus* sp) were carried out and their phylogenetic relationship was analyzed. The phylogenetic analysis and molecular identification of 16s RNA sequencing revealed that the isolate (*Salmonella* sp) was closely related (99%) to known species of *Salmonella enterica* with assession number AL513382, while isolate (*Streptococcus* sp) was closely related (100% similarity) to *Streptococcus suis* with Assession number NC021213.1.



M 1 2 3 4 5

Fig 1: Plasmid Profile of Selected Bacterial Isolates from MSW Dumpsites



1 2 3 4 5 M

Fig 2: Cured Plasmid of Bacterial Isolates from MSW Dumpsites

Keys: M: Molecular Marker; 1: *E. coli*, 2: S. *aureus*, 3: *V. cholerae*, 4: *Shigella* sp, 5: *Salmonella* sp

CONCLUSION

This study has shown that dumpsites environ is laden with multi-drug resistant pathogenic bacterial isolates such as *Salmonella* sp, *Staphylococcus aureus, Shigella* sp, *Vibrio cholerae* and *E. coli* which can cause various diseases and infections that may be extremely difficult to treat.

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