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Bacterial Analysis of Soil From Waste Dumpsite

Faith Efosa Oviasogie, Christopher Uche Ajuzie, Uyiosa Glory Ighodaro

University of Benin, Benin City
Edo state

ABSTRACT

A total of 18 samples made up of six samples from hospital and 12 samples from municipal solid waste dump soil in Benin City were collected for isolation of viable aerobic bacteria. The following aerobic bacteria in decreasing order of prevalence were isolated from municipal solid waste, aerobic spore bearers (83.33%), Escherichia Coli (50.00%), Staphyloccus aureus (14.67%), Klebsiella sp (8.33%). Samples from the hospital waste yielded Pseudomonas aeruginosa, Klebsiella sp, Bacillus substilis with a prevalence of (60.00%) each, Serratia sp,Staphylococcus aureus and Escherichia Coli (20.00%) each. Antibiotic susceptibility tests on isolates were performed using standard disc diffusion method. Overall, Ciprofloxacin was the most effective agent on the isolates followed by Augmentin and Gentamycin in hospital and municipal solid waste respectively.Almost all isolates were resistant to Cloxacillin and Amoxiallin.

Keywords. Hospital solid waste, municipal solid waste, Antibiotic susceptibility tests, aerobic bacteria, Benin City.

INTRODUCTION

Benin City, which lies between longitude 5.40^0E and latitude 6.00^0N is located in the southern part of Nigeria. The ancient city is urban and has witnessed an overwhelming influx of people from the rural areas in the last few decades. This has resulted in a tremendous increase in population in the city [1].

Over 5.2 million people, which includes 4 million children die each year from waste related diseases . Waste is any substance, solution mixture or article for which no direct use is envisaged but which is transported for reprocessing, dumping, elimination by incineration or other methods of disposal. With urban industrialization, social development and population increases, solid waste production are growing rapidly, making pollution a serious problem [2].If not properly disposed and managed, the resulting environmental impart from these wastes can be disastrous [3].

Waste dumping in the metropolitan state cut across approved and non-approved dump site. Composition of the these dump site are generated from hospital, residential, commercial, market etc. After waste is generated, waste workers collect and dispose such waste along road-side, around residential area or in a government approved dumpsite. During waste collection and disposal most waste workers in developing countries hardly use protective devices. This unproductive condition may make them vulnerable to serious health problems [4].

MATERIALS AND METHODS

Sample collection

The design used in carrying out the study was random sampling which involved the transfer and culturing in an appropriate culture medium under optimum conditions for growth in the laboratory. Eighteen samples made up of six samples from hospitals and 12 samples from municipal dumpsite were collected for isolation and quantification of viable aerobic microorganisms, all from Benin City, Edo State. The samples were labeled as follows:

Municipal solid waste

1.	Constain moat municipal dumpsite	SAMPLE A
2.	Constain municipal control	SAMPLE B
3.	Uniben municipal dumpsite	SAMPLE C
4.	Uniben municipal control	SAMPLE D
5.	Isekhere moat municipal dumpsite	SAMPLE E
6.	Isekhere municipal control	SAMPLE F
7.	New Benin market municipal site	SAMPLE G
8.	New Benin market municipal control	SAMPLE H
9.	Uselu market municipal dump site	SAMPLE I
10.	Uselu market municipal control	SAMPLE J
11.	Oba market municipal dumpsite	SAMPLE K
12.	Oba market municipal control	SAMPLE L

Hospital Waste

1.	University of Benin Teaching Hospital dumpsite	SAMPLE M
2.	University of Benin Teaching Hospital control	SAMPLE N
3.	Central Hospital dumpsite	SAMPLE O
4.	Central Hospital control	SAMPLE P
5.	Uniben Health Centre dumpsite	SAMPLE Q
6.	Uniben Health Centre control	SAMPLE R

Samples were examined immediately within four hours of collection. Where immediate bacteriological examinations were not possible, samples were stored in a refrigerator at 4^0C until they were examined.

Preparation of soil samples

Soil samples were refrigerated as soon as they were collected until when needed for immediate use during which 1g of soil sample was diluted in a sterile peptone water (autoclaved at 121^0C for 15 minutes) and allowed to cool for 2-4 hours under normal room temperature to resuscitate viable microorganisms present in the soil samples. This was carried out in all soil samples using various sterile peptone water and labeled.

Enumeration, isolation, characterization and identification of viable aerobic bacteria
All isolates were identified by standard techniques as described by Cowan and Steel (1974) in the manual of identification of medical bacteria. Colonial appearance of the organisms on the media, morphological characteristics such as size, form, elevation opacity and odour. Specific biochemical test e.g catalase, coagulase, oxidase, indole production, citrate utilization, urease activity, oxidase, mannitol fermentation, methyl red and voges proskaeur were performed for the identification of the organisms.

Enumeration was done after incubation of plates at room temperature for 24hrs.

Antibiotic susceptibility tests
The disk diffusion test method by Stokes et al., (1993) was used. The following commercially prepared antibiotics was used: ampicillin (10ug), penicillin (10ug), gentamicin (10ug), tetracycline (30ug), ceftazidine (30ug), and ciprofloxacin (5ug).

An overnight broth culture of the tests isolated was suspended in sterile isotomic, 2ml of the suspension was transferred into the surface of a dried nutrient agar plate, the plate was tipped in different direction, so as to wet the whole surface excess fluid was decanted, the surface of the plate was allowed to dry for few minutes and the disk were applied on the surface. The plate were then incubated at 37^0C for 24 hours, after which the diameter of the zone of inhibition was measured.

RESULTS AND DISCUSSION

The present study was conducted to obtain an insight into the various microorganism associated with waste dump soil and their antibiotic susceptibility patterns of hospital and municipal solid waste in Benin City, Edo State.

Table I shows the prevalence of aerobic bacteria in waste dumpsites. The predominant organisms in municipal solid waste was *Bacillus* substuilis; 10 (83.33%); *Escherichia coli* 6 (50.00%); *Staphylococcus aureus* 5;5 (41.67%); *Klebsiella* sp; 3 (25.00)%, *Pseudomones aeruginosa* 2 (16.67%) and Serratia sp. 1 (8.33%) respectively. *Pseudomonas aeruginosa, klebsiella* sp and *Bacillus substilis* 3 (60.00% each) predominated in special hospital waste followed by S*erratia* sp., S*taphylococcus aureus* and *Escherichia coli*; 2 (20.00% each). One control sample from special hospital waste yielded no growth.

Table 1: Prevalence of aerobic bacteria in waste dump sites in benin city
Key: MSW – Municipal solid waste; SHW – Special hospital waste; - - culture yielded no growth

Source	No of samples	No of isolates	Percentage organisms isolated						
			Peudomona aeruginosa	Klebsiella sp	Serratia sp	Stash sp	E.coli	Bacillum substilis	Without growth
MSW	12	26	2 (16.67)	3 (25.00)	1 (8.33)	5 (41.67)	6 (50.00)	10 (83.33)	-
SHW	6	13	3 (60.00)	3 (6.00)	1 (20.00)	1 (20.00)	1 (20.00)	3 (60.00)	(20.00)
Total	18	39	5 (29.41)	6 (35.29)	2 (11.76)	6 (35.29)	7 (41.2)	13 (76.47)	1 (5.88)

Table 2: Prevalence of aerobic bacteria in municipal soil waste dumpsite in Benin City

Source	No of samples	No of isolates	Percentage organisms isolated						
			Peudomona aeruginosa	Klebsiella sp	Serratia sp	Stash sp	E.coli	Bacillus substilis	Without growth
H and G	2	2	0.00	1 (50.00)	0.00	1 (50.00)	0.00	0.00	0.00
I and J	2	4	0.00	1 (50.00)	0.00	1 (25.00)	1 (25.00)	1 (50.00)	0.00
K and L	2	4	0.00	0.00	0.00	0.00	2 (50.00)	2 (50.00)	0.00
E and F	2	4	0.00	0.00	0.00	0.00	2 (50.00)	2 (50.00)	0.00
C and D	2	6	0.00	1 (16.67)	1 (16.67)	2 (33.33)	0.00	2 (33.33)	0.00
A and B	2	6	1 (16.67)	1 (16.67)	0.00	1 (16.67)	1 (16.67)	2 (33.33)	0.00
Total	12	26	1 (5.26)	3 (15.79)	1 (5.26)	5 (26.32)	6 (31.58)	10 (52.63)	0.00

Key:

Constain moat municipal dumpsite	SAMPLE A
Constain municipal control	SAMPLE B
Uniben municipal dumpsite	SAMPLE C
Uniben municipal control	SAMPLE D
Isekhere moat municipal dumpsite	SAMPLE E
Isekhere municipal control	SAMPLE F
New Benin market municipal site	SAMPLE G
New Benin market municipal control	SAMPLE H
Uselu market municipal dump site	SAMPLE I
Uselu market municipal control	SAMPLE J
Oba market municipal dumpsite	SAMPLE K
Oba market municipal control	SAMPLE L

Table 3: Prevalence of aerobic bacteria in hospital waste dump sites in Benin City

Source	No of samples	No of isolates	Percentage organisms isolated						
			Peudomonas aeruginosa	Klebsiella sp	Serratia sp	Staph sp	E.coli	Bacillus substilis	Without growth
M and N	2	6	2 (33.33)	1 (16.67)	-	-	1 (16.67)	2 (33.33)	-
O and P	2	1	-	1 (100.00)	-	-	-	-	1 (100.00)
Q and R	2	5	1 920.00)	1 (20.00)	1 920.00)	1 (20.00)	-	1 (20.00)	-
Total	6	12	3 (25.00)	3 (25.00)	1 (8.33)	1 (8.33)	1 (8.33)	3 (25.00)	1 (8.33)

Key

University of Benin Teaching Hospital dumpsite	SAMPLE M

University of Benin Teaching Hospital control SAMPLE N
Central Hospital dumpsite SAMPLE O
Central Hospital control SAMPLE P
Uniben Health Centre dumpsite SAMPLE Q
Uniben Health Centre control SAMPLE R
Organisms yielded no growth -

Table 4: antibiotic susceptibility pattern of aerobic bacterial isolate from municipal solid waste

Organisms	No of isolate	OFI	CPX	PFX	AUG	TET	AMX	COT	GEN	NIT	CRO	CHL	ERY	CXC	PN	SXT	CEP	P
	Percentage of isolate susceptible to antibiotics																	
Pseudomonas sp	2	0.00	100.00	-	10.00	-	0.00	0.00	0.00	50.00	100.00	-	-	-	-	-	-	-
Klebsiella sp	3	100.00	66.67	-	0.00	-	-	-	100.00	0.00	-	-	-	-	0.00	100.00	10.00	-
Escherichia *coli*	6	33.33	50.00	50.00	50.00	-	-	-	-	50.00	-	-	-	-	-	-	0.00	33.33
Serratia sp	1	10.00	-	-	100.00	-	0.00	50.00	50.00	0.00	0.00	-	-	-	-	-	-	
Staphylococcus aureus	5	-	-	-	0.00	60.00	0.00	0.00	60.00	-	-	0.00	40.00	0.00	-	-	-	
Bacillus substilus	10	-	-	-	0.00	30.00	0.00	0.00	20.00	-	-	0.00	0.00	0.00	-	-	-	

TABLE 5: Antibiotic susceptibility pattern of aerobic bacterial isolate from hospital waste

Organisms	No of isolate	OFI	CPX	PFX	AUG	TET	AMX	COT	GEN	NIT	CRO	CHL	ERY	CXC	PN	SXT	CEP	P
	Percentage of isolate susceptible to antibiotics																	
Bacillus substilus	3	-	100.00	-	100.00	33.33	60.00	0.00	0.00	-	-	-	33.33	0.00	0.00	-	-	-
Pseudomonas	3	0.00	0.00	-	0.00	100.00	33.33	0.00	0.00	66.67	100.00	-	-	-	-	-	-	-
Klebsiella	3	100.00	100.00	100.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-
Escherichia	1	100.00	100.00	50.00	100.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-	-	-	-
Serratia	1	10.00	-	100.00	100.00	-	0.00	50.00	50.00	0.00	0.00	-	-	-	-	-	-	
Staphylococcus	1	-	-	-	-	100.00	0.00	0.00	60.00	-	-	100.00	100.00	100.00	-	-	-	
Total	12	41	67	58.3	37.5	43.75	58.33	0.00	12.5	4.17	16.67	25.00	16.67	8.33	0.00	-	-	-

The table reveals that 15.88% of the samples examined showed no growth. *Bacillus substilis* which was isolated from all municipal waste dump soil except sample G and H was resistant to most antibiotics used. This is in agreement with findings of [5].

Table 3: shows bacterial isolates from hospital waste dump soil. *Pseudomonas aerguginosa, klebsiella* and *Bacillus substilis* ranked highest ranging from 33.33% in samples M and V to 20.00% in sample Q and R. The next in frequency was serratia sp, *Staphylococcus aureus* and *Escherichia coli* 1 (8.33% each). *Pseudomonas aeruginosa, serratia* sp, *staphylococcus aureus Escherichia coli* and Bacillus substilis were not isolated from samples O and P.

Pseudomonas aeruginosa was the highest Gram negative organism isolated from hospital waste accounting for 25.00% overall of all the isolates (table 3). Amongst isolates from hospital waste, *Pseudomonas aeruginosa* showed higher prevalence rate hospital waste (60.00%) thin in municipal solid waste (16.67%). The high incidence of pseudomonas aeruginosa in this study is in agreement with earlier report of [6]. *Pseudomonas* is noted to survive and multiply in strange hospital environment [7]. The organism can survive in most condition even in the presence of antiseptics [5].

Table 4: Shows susceptibility profile of bacterial isolates from municipal solid waste. Ciprofloxacin and gentamycin of 25.73% and 31.48% respectively against all isolates. Cirproflocacin was highly sensitive against *Pseudomonas aeruginosa, klebsiella* sp and *Escherichia coli. Gentamycin* was highly sensitive against *Klebsiella* sp, S*erratia* and S*taphylococcus aureus.* All isolates tested against Cloxacillin, Ampicillin Chloramphenicol and Amoxillin showed resistance.

Table 5: Shows the susceptibility profile, of bacterial isolates from hospital waste. Ciprofloxacin and Augmentin were the most effective against the isolates, having efficacy of 58.33% and 43.75% respectively. All isolates tested were resistant to Cloxacillin and Amoxillin. This findings of table 4 and 5 is an agreement with [8].

Table 6: Total viable microbial counts (cfu/G/ml)

Location	Dump soil (cfu/g/ml)	Control sample	Control soil (cfu/g/ml)
A	10.0×10^5	B	9.0×10^5
C	8.0×10^5	D	5.0×10^5
E	6.0×10^5	F	4.0×10^5
G	9.0×10^5	H	8.0×10^5
I	8.0×10^5	J	6.0×10^5
K	9.0×10^5	L	7.0×10^5
M	12.0×10^5	N	6.0×10^5
O	4.0×10^5	P	-
Q	10.0×10^5	R	6.0×10^5

Table 6: Shows the total aerobic bacterial population in different location. Samples M and N had the highest bacterial population followed by sample Q and R and this is due to the fact that microorganisms find their way to the various dump sites as a result of the diagnosis, treatment of patients, prevention of infections or research on human and animal diseases [9].

CONCLUSION

The study revealed that improper management of waste from generation point to the point of disposal account for bacterial contamination of soil. Aerobic bacterial are usually more likely contaminates of soil than anaerobic bacteria, this is especially so considering the fact that the environment is aerobic.

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