South Asian Journal of Research in Microbiology

1(2): 1-11, 2018; Article no.SAJRM.40511

Microbiological and Antimicrobial Analysis of Hospital Wastewater Discharged into the Soil Environment

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VAC and TNN designed the study, performed the statistical analysis and wrote the protocol. Author VBO wrote the first draft of the manuscript. Authors VAC, VBO, HEN and TNN managed the analyses of the study. Authors VBO and BKA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2018/v1i2755
Editor(s):
(1) Osunsanmi Foluso Oluwagbemiga, Department of Biochemistry and Microbiology, University of Zululand, South Africa.
Reviewers:
(1) Narcis Barsan, University of Bacău, Romania.
(2) Mustapha Umar, Nigerian Institute of Leather and Science Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24826

Short Research Article

Received 3rd March 2018
Accepted 5th May 2018
Published 26th May 2018

ABSTRACT

This study evaluated the microbiological effects of hospital wastewater discharged into the soil environment using standard microbiological procedures. The highest total bacterial count ($8.3\pm0.5\times10^{10}$ cfu/ml) of the wastewater samples was observed in the collation point sample while the laundry wastewater sample had the least number of $5.4\pm0.5\times10^{7}$ cfu/ml. The collation point wastewater sample had the highest total coliform count ($4.1\pm0.1\times10^{8}$ cfu/ml)while the laundry wastewater sample produced the least count of $2.3\pm0.1\times10^{1}$ cfu/ml. The highest total coliform faecal count of $4.2\pm0.3\times10^{5}$ cfu/ml was observed in the collation point wastewater sample while the least count of $2.4\pm1.2\times10^{3}$ cfu/ml was seen in the laundry wastewater sample. The mortuary wastewater had the highest total fungal count of $3.1\pm0.2\times10^{5}$ cfu/ml while the least count was seen in the collation point wastewater $2.9\pm0.2\times10^{2}$ cfu/ml. The total viable numbers of the soil samples ranged from $5.0\pm0.0\times10^{8}$ cfu/g (200m away from the point of discharge) to $8.4\pm1.6\times10^{12}$ cfu/g

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(point of release) while the total coliform counts ranged from $2.3\pm0.0\times10^4$ cfu/g (200m away from the point of discharge) to $3.9\pm0.8\times10^8$ cfu/g (point of discharge). The highest total faecal count of $3.7\pm0.5\times10^4$ cfu/g was observed in the sample from the point of discharge while the least count was seen in the sample collected 200m away from point of discharge $2.3\pm0.1\times10^2$ cfu/g. Total fungal count ranged from $2.4\pm0.5\times10^7$ cfu/g (200m away from the point of discharge) to $3.4\pm0.5\times10^8$ cfu/g (point of discharge). The bacterial species isolated were *Escherichia coli, Erwinia, Serratia, Enterococcus, Staphylococcus, Streptococcus,* and *Salmonella.* Others were *Pseudomonas aeruginosa, Proteus, Neisseria, Actinomycetes, Shigella, Bacillus* and *Enterobacter* species. The fungi isolated include *Aspergillus niger, Aspergillus fumigatus, Trichophyton rubrum, Candida, Penicillium* and *Rhizopus* species. *Bacillus* spp., *Staphylococcus epidermidis, Penicillium* spp. and *Rhizopus* species were the most frequently distributed (100%), followed by *S.* species, *Enterococcus* spp., *E. coli, Pseudomonas aeruginosa, Proteus* spp. and *Candida* spp. (80%). *Salmonella* spp., *Shigella* spp., *Enterobacter* spp. and *Trichophyton rubrum* had the same rate of 60%, respectively while the least occurrence was seen in *Streptococcus* spp., *Neisseria* spp., *Actinomyces* spp. and *Aspergillus niger* with the rate of 40%, respectively. The high microbial loads of the isolates and the high densities of the coliforms indicate there is, therefore, contamination of the soil environment as a result of the discharged hospital wastewater, which could probably be hazardous to human health.

Keywords: Wastewater; soil environment; physicochemical; microbiological; human health.

1. INTRODUCTION

Wastewater is defined as any water whose quality has been adversely abused by anthropogenic influence [1]. This includes liquid waste discharged from domestic homes, industries, agricultural and commercial sectors [1]. Healthcare waste consists of both organic and inorganic substances including pathogenic microorganisms. Hospital wastes possess serious health hazards to the health workers and the public.

Hospital wastewater is wastewater generated from all activities of the hospital as medical and non-medical activities from the operating, emergency and first aid, laboratory, diagnosis, radiology, kitchen to laundry activities [2]. Hospital wastewater contains harmful pollutant, such as pathogenic microorganisms (bacteria, viruses, protozoa and helminths), residual drugs and laboratory chemicals (antibiotics, phenol, chloroform), chemical toxic (Pb), and biodegradable organic material (protein, fat, carbohydrate) [3].

There is more area of agricultural land in the world using untreated wastewater for irrigation due to lack of water [4]. Surveys of wastewater use have shown that more than 85% of the applied heavy metals are likely to accumulate in the soil, mostly at the surface [5]. Food crops such as cocoyam, cassava and tomatoes constitute an important part of the human diet since they contain carbohydrates, protein as well as vitamins, minerals and trace elements. However, in recent years their consumption is increasing gradually particularly among the urban community. This is due to increased awareness of the exposure to other culture and acquiring proper education [5].

Recently, pollution of a general environment has increasingly gathered a global interest. In this respect contamination of agricultural soils with heavy metals has always been considered a critical challenge in the set urban community [6]. Heavy metals are generally present in agricultural soils at low levels and due to their accumulation behaviour and toxicity; they have a potential hazardous effect not only on crop plants but also human health [5].

Hospital wastewaters are significant components of water, contributing to oxygen demand and nutrient loading of water bodies, promoting toxic algae blooms and leading to a destabilized aquatic ecosystem [1].

Abia State University Teaching Hospital, Aba is a referral health institution in Aba area as patients from Aba and its environs are regularly referred to this institution for proper medication. Wastewater generated from this health care institution may represent a serious health hazard and little is known about the health hazard of hospital wastes in Aba metropolis. Children, adults and animals all have the potential to come into contact with these wastes, which may pose severe health risks to them.

This study, therefore, investigated the influence of this hospital wastewater in the soil environment and assessed the microbial isolates associated with the sewage.

2. MATERIALS AND METHODS

2.1 The Study Area

The study hospital was Abia State University Teaching Hospital, Aba in South-Eastern Nigeria. The hospital is a referral health institution in the area as patients from Aba and the environs are regularly referred to the hospital for proper medication. Aba lies in the tropical rainforest region of South-Eastern Nigeria. Aba is situated at 5.11° North latitude, 7.37° East longitude and 207 meters elevation above the sea level. Aba is a big town in Nigeria, having about 897,560 inhabitants and the main trading centre in Abia State [7].

2.2 Source of Sample

The wastewater samples were collected at three consecutive times from four wastewater outlets from different units of Abia State University Teaching Hospital, Aba (laboratory wastewater, laundry wastewater, mortuary wastewater, the collation point where all the outlets meet) and the control which is the source of water for the Teaching Hospital. The five sampling points were designated A to E (A=laboratory wastewater sample, B=laundry wastewater, C=mortuary wastewater, D=collation point and E= control).

Wastewater from the collation point was discharged into a pit. Soil samples were then collected from different points in relation to distance from the discharge pit. These were the edge of the pit (P_1), 50m away from the wastewater pit (P_2), 100m away from the discharge pit (P_3) and from the site which has not been polluted by the wastewater in the hospital premises which served as the control soil sample (P_4) (500m away from the pit) [8].

2.3 Sample Collection

The samples for microbial analysis were collected in triplicates in clean, sterile containers devoid of chemical contamination. Soil samples were collected using Shiprek soil augar disinfected with cotton wool soaked in 70% ethanol at 0-15cm depth. Sterile universal bottles were used for the collection of soil samples for the microbiological analysis. The biological indices of the samples were analyzed within 2 hours of collection [8].

3. MICROBIOLOGICAL ANALYSIS

3.1 Determination of Microbial Loads of the Samples

The microbial loads of various groups of bacterial species were determined using the culture techniques involving different culture media. Five different water samples and the soil sample were analyzed for the bacterial diversity as described earlier in the sample collection. Bioloads were determined after decimal serial dilutions. Nine millilitre of distilled water were pipetted into ten test tubes prepared in duplicate and labelled 10^1 to 10^{10} for serial dilution. A volume of 1ml was taken from the stock sample and put into the 10^{-1} tube, this was mixed properly and from this $10^{-1,}$ 1 ml was transferred into the 10^{-2} tube. This was repeated, till the 10^{-9} tube using fresh 5ml pipette at each interval. 1ml was discarded from the last tube to make all equal (9ml each). Aliquots (0.2ml) from the 10^{-9} tubes were aseptically inoculated onto different culture media (agar) using the spread plate techniques. Bacterial cultures were incubated at 37°C for 24-48 hours while the fungal cultures were incubated at ambient laboratory temperature (28±2°C) for 2-5 days with daily observation.

Various culture media were used. These were Nutrient Agar for Total Heterotrophic Bacterial Count (THBC), MacConkey Agar for Total Coliform Counts (TCC), EMB Agar for Total Faecal Coliform Counts (TFCC) and Sabouraud Dextrose Agar for Total Fungal Counts (TFC). Blood agar was used to determine total potential pathogenic bacterial organisms. Counting was done using colony counter [9].

3.2 Isolation and Identification of Observed Isolates

Pure bacterial isolates were identified based on their characteristics such as morphology, microscopy, staining potential and their biochemical reactions. The bacteria were stained using Gram's staining, spore staining and capsule staining methods [9].

3.3 Identification of Fungal Isolates

The samples were analyzed for fungal isolates on Sabouraud Dextrose Agar. On the establishment of growth after 2-4 days of incubation at room temperature, the plates were carefully examined and distinct growths were sub-cultured on fresh medium for purity. The fungi were then identified on the basis of their cultural characteristics and microscopy with reference to the methods described by Barnett et al. [10].

3.4 Antimicrobial Susceptibility Testing

This test was performed to assess the antibiotic susceptibility of the observed of the isolated bacteria using Kirby-Bauer Disc Diffusion method as described by Cheesbrough [8].

The broth culture of the test organisms of 4-6 hours was adjusted to 0.5 turbidity level of Macfarland standard using normal saline. About 0.5ml of the standardized cell suspension was spread evenly on the solidified agar medium and allowed to diffuse. Predetermined batteries of antimicrobial discs were placed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface using sterile forceps. The discs were distributed evenly so that they are not closer than 24 mm from centre to centre. The plates were inverted and incubated at 37°C for 24 h.

3.5 Reading of Plates and Interpreting Results

After 18-24 hours of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimetre, using sliding callipers, which were held on the back of the inverted Petri plate.

The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growths of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, were ignored. However, discrete colonies within a clear zone of inhibition were subcultured, re-identified and re-tested.

The sizes of the zones of inhibition were interpreted by referring to zone diameter interpretative standards and equivalent minimum inhibitory concentration breakpoints) of the Clinical and Laboratory Standards Institute (CLSI) [9].

3.6 Statistical Analysis

The results obtained in this study were subjected to standard statistical analysis by the use of correlation analysis, standard deviation and ANOVA. This was used to determine the significance of the results.

4. RESULTS

Table 1 reveals the total microbial counts of the ABSUTH samples. The total heterotrophic bacterial counts of the wastewater samples ranged from $5.4\pm0.5\times10^{7}$ cfu/ml to $8.3\pm0.5\times10^{10}$ cfu/ml for the laundry wastewater and collation point wastewater samples, respectively while the total coliform counts ranged from $2.3\pm0.1\times10^{1}$ cfu/ml to $4.1\pm0.1\times10^{8}$ cfu/ml for the laundry wastewater and collation point wastewater samples, respectively. The total faecal count had the least count of $2.4\pm1.2\times10^{3}$ cfu/ml (laundry wastewater sample) to $4.2\pm0.3\times10^{5}$ cfu/ml for collation point wastewater sample. Total fungal count ranged from $2.9\pm0.2\times10^{2}$ cfu/ml to $3.1\pm0.2\times10^{5}$ cfu/ml for laundry wastewater and laboratory wastewater samples.

Table 2 shows the total microbial counts of the soil samples. The total heterotrophic bacterial counts of the soil samples ranged from $3.2\pm0.1\times10^{6}$ to $8.4\pm1.6\times10^{12}$ cfu/g while the total coliform counts ranged from $1.0\pm0.0\times10^{2}$ cfu/g to $3.9\pm0.8\times10^{8}$ cfu/g. Total faecal count ranged from $1.0\pm0.0\times10^{2}$ cfu/g to $3.7\pm0.5\times10^{4}$ cfu/g. Total fungal count ranged from $2.4\pm0.5\times10^{7}$ cfu/g to $4.1\pm0.5\times10^{6}$ cfu/g.

Tables 3 and 4 show the rate of occurrence of the different isolates in the wastewater and soil samples. *Bacillus* species had the highest rate of occurrence as it was observed in all the wastewater samples while *Erwinia* species had the least occurrence rate as it was seen in only one of the wastewater samples. *Bacillus* species and *Rhizopus* species had the highest occurrence rates in the soil samples while *Salmonella* species had the least rate of occurrence.

Tables 5 and 6 show the mean and percentage antibiotics susceptibility pattern of bacterial isolates from ABSUTH wastewater. The results revealed that Ciprofloxacin produced the highest zones and percentage inhibition of 34.1±0.6 mm to 33.7±1.8 mm (100%) against *Bacillus* species and *E.* coli, respectively while Streptomycin and Chloramphenicol were highly resistant against all the isolates with the exception of *Streptococcus* species and *Enterococcus* species, respectively.

Figs. 1 and 2 indicate the percentage occurrence of the organisms within the wastewater and soil samples respectively. *Bacillus* species had the highest percentage occurrence rate (100%) while *Erwinia* species and *Serratia* species showed the least percentage rate of occurrence (20%) in the wastewater samples. *Rhizopus* species and *Bacillus* species produced the highest percentage rate of occurrence in the soil samples (100% respectively) while *Salmonella* species and *Trichophyton rubrum* showed the least percentage rate of occurrence (20% respectively).

5. DISCUSSION

This research was conducted to investigate the various effects of hospital wastewater generated from Abia State University Teaching Hospital, Aba on the microbiological parameters on the receiving environment (soil). There was a significant increase in most of the microbiological parameters studied with slight fluctuation. The hospital wastewater was observed to play a significant role in on the qualities of the parameters studied.

Table 1. Total microbial counts of ABSUTH waste water samples

Samples	Parameters (cfu/ml)			
	THBC	TCC	TFCC	TFC
A	$6.9\pm0.8\times10^{8}$	$3.0\pm0.0\times10^{3}$	$2.9\pm0.2\times10^{3}$	$3.0\pm0.2\times10^{3}$
B	$5.4\pm0.5\times10^{7}$	$2.3\pm0.1\times10^{3}$	$2.4\pm1.5\times10^{2}$	$2.9\pm1.2\times10^{2}$
C	$6.0\pm0.0\times10^{7}$	$3.0\pm0.0\times10^{4}$	$3.0\pm0.6\times10^{2}$	$3.1\pm0.2\times10^{5}$
D	$8.3\pm0.5\times10^{10}$	$4.1\pm0.1\times10^{8}$	$4.2\pm0.1\times10^{5}$	$2.9\pm0.2\times10^{2}$
E	$1.1\pm0.0\times10^{3^*}$	$0.6\pm0.2\times10^{1^*}$	ND	$1.0\pm0.0\times10^{2^*}$

Values are means of three replicates and are expressed as mean ± standard deviation.
At P<0.05, there is no significant difference except values with asterisk (*)

Keys:

Samples
A= Laboratory Waste Water Sample
B= Laundry Waste Water Sample
C= Mortuary Waste Water Sample
D= Collation Point
E= Control
ND= Not Detected

Parameters
THBC= Total Heterotrophic Bacterial Count
TCC= Total Coliform Count
TFCC= Total Faecal Count
TFC= Total Fungal Count

Table 2. Total microbial counts of the soil samples

Samples	Parameters (cfu/g)			
	THBC	TCC	TFCC	TFC
P_1	$8.4\pm1.6\times10^{12}$	$3.9\pm0.8\times10^{8}$	$3.7\pm0.5\times10^{4}$	$3.4\pm0.5\times10^{8}$
P_2	$7.1\pm0.1\times10^{10}$	$3.0\pm0.0\times10^{5}$	$2.8\pm0.5\times10^{4}$	$3.2\pm0.0\times10^{7}$
P_3	$5.0\pm0.0\times10^{8}$	$2.3\pm0.1\times10^{4}$	$2.3\pm0.1\times10^{2^*}$	$2.4\pm0.5\times10^{7}$
P_4	$3.2\pm0.1\times10^{6^*}$	$1.0\pm0.0\times10^{2^*}$	$1.0\pm0.0\times10^{2^*}$	$5.4\pm0.5\times10^{6}$
P_{VALUE}	P< 0.05	P<0.05	P< 0.05	P< 0.05

Values are means of three replicates and are expressed as mean ± standard deviation.
At P<0.05, there is no significant difference except values with asterisk (*)

Keys:

Samples
P_1= Point Of Discharge of the Waste Water
P_2= 100M away
P_3= 200M away
P_4= Control (UNPOLLUTED SOIL SAMPLE)

Parameters
THBC= Total Heterotrophic Bacterial Count
TCC= Total Coliform Count
TFCC= Total Faecal Count
TFC= Total Fungal Count

Table 3. Occurrence of the organisms within the waste water samples

Organisms	Samples				
	A	B	C	D	E
Erwinia species	-	+	-	-	-
Serratia species	-	+	-	-	-
Enterococcus species	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	-
Streptococcus species	+	-	-	+	-
Escherichia coli	+	+	+	+	-
Salmonella species	+	+	-	+	-
Pseudomonas aeruginosa	+	+	+	+	-
Proteus species	+	+	+	+	-
Staphylococcus epidermidis	+	+	+	+	-
Neisseria species	+	-	-	+	-
Actinomycetes species	-	-	+	+	-
Shigella species	+	+	-	+	-
Bacillus species	+	+	+	+	+
Enterobacter sp.	+	+	-	+	-
Aspergillus niger	-	-	+	+	-
Candida species	+	+	+	+	-
T. rubrum	+	+	-	+	-

Keys: A= Laboratory Waste Water Sample; B= Laundry Waste Water Sample; C= Mortuary Waste Water Sample; D= Point Where the Waste Water Meet; E= Control (Source of Water Supply to the Hospital); += Positive; -= Negative

Table 4. Occurrence of the organisms within the soil samples

Organisms	Samples			
	A	B	C	D
Erwinia species	+	+	+	-
Serratia species	+	+	-	-
Enterococcus species	+	+	-	-
Staphylococcus aureus	+	+	+	
Streptococcus species	+	+	+	-
Escherichia coli	+	+	+	-
Salmonella species	+	-	-	-
Pseudomonas aeruginosa	+	+	+	-
Proteus species	+	+	+	-
Staphylococcus epidermis	+	+	+	+
Neisseria species	+	+	-	-
Actinomycetes species	-	-	+	+
Shigella species	+	-	-	-
Bacillus species	+	+	+	+
Enterobacter sp.	+	+	-	-
Aspergillus niger	+	+	-	-
Candida species	+	+	-	+
T. rubrum	+	-	-	-
Penicillium species	+	+	+	+
A. fumigates	+	+	-	-
Rhizopus species	+	+	+	+

Keys: $P_{1=}$ Point Of Discharge of the Waste Water; $P_{2=}$ 100M away; $P_{3=}$ 200M away; P_4= Control (unpolluted soil sample); += Positive; -= Negative

Table 5. Mean antibiotic susceptibility pattern of bacterial isolates from ABSUTH wastewater (mm)

Organisms	No. of Isolates	Ciprofloxacin (10µg)	Norfloxacin (10µg)	Gentamicin (10µg)	Lincocin (20µg)	Streptomycin (10µg)	Rifampicin (20µg)	Chloramphenicol (30µg)	Ampiclox (20µg)	Floxapen (20µg)	Penicillin (10µg)	Drovid (10µg)	Augmentin (10µg)	Oflaxacin (10µg)
Staphylococcus aureus	6	28.0±1.9	19.1±1.1	17.3±0.8	9.1±1.2	1.0±2.3	10.3±0.1	0.0±0.0	3.0±1.7	18.4±1.0	2.0±1.7	17.8±0.5	30.1±0.9	11.3±1.9
Bacillus spp	9	34.1±0.6	18.0**±**1.2	5.0±2.6	12.0±1.0	0.0±0.0	19.6±1.5	0.0±0.0	12.0±1.3	13.0±1.0	11.0±1.6	20.0±0.5	33.0±0.5	26.8±2.0
Escherichia coli	7	33.7±1.8	27.9±1.0	18.0±1.3	2.9±2.0	5.1±1.8	26.4±1.9	6.0±0.9	4.5±0.4	12.0±0.7	2.0±0.2	11.9±1.0	30.0±0.7	26.0±0.8
Proteus species	7	26.6±1.1	30.0**±0.3**	18.2±1.8	19.0±1.7	6.0±1.0	18.8±0.5	0.0±0.0	2.9±0.8	10.1±1.9	2.3±1.0	19.2±0.5	31.7±1.1	32.0±1.5
Staphylococcus epidermidis	8	30.9±1.9	29.8**±0.6**	10.9±1.9	4.0±1.2	1.2±0.8	13.0±1.2	0.0±0.0	3.1±0.5	20.9±0.9	3.1±1.7	13.2±0.9	30.9±1.0	21.2±1.1
Streptococcus species	5	27.0±0.5	18.6±1.8	6.0±0.5	10.6±1.9	13.6±1.1	20.0±1.0	0.0±0.0	2.0±0.6	9.0±1.0	0.0±0.0	15.0±07	31.8±1.2	20.9±1.3
Neisseria species	4	15.6±1.1	10.6±0.9	13.6±0.9	3.0±1.0	0.0±0.0	16.0±1.2	0.0±0.0	18.0±1.0	10.0±0.5	15.0±1.0	9.0±0.9	32.8±1.1	16.9±1.0
Actinomycetes isreali	4	9.4±1.5	3.6±1.9	2.0±1.8	2.1±1.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	20.7±1.0	2.9±1.3
Serratia species	3	33.3±1.8	19.6±1.0	9.6±1.0	18.5±1.7	0.0±0.0	10.0±0.5	11.0±1.0	19.0±0.8	18.9±1.2	19.0±1.0	20.0±1.0	31.3±1.3	21.9±1.9
Erwinia spp	4	30.6±0.9	24.6±1.9	18.6±2.1	14.0±1.2	0.0±0.0	8.0±1.0	0.0±0.0	0.0±0.0	11.0±1.3	0.0±0.0	16.0±0.5	29.9±1.9	15.9±1.0
Pseudomonas aeruginosa	7	19.5±1.7	21.5±1.0	20.6±1.1	23.5±1.5	9.0±2.0	17.9±0.5	0.0±0.0	22.3±1.0	14.5±1.0	9.0±1.2	22.1±1.0	20.7±1.0	16.9±1.3
Enterococcus faecalis	5	31.4±1.2	26.1±1.3	19.4±0.5	20.6±1.1	0.0±0.0	20.0±1.0	17.3±0.5	13.0±1.0	30.5±1.0	15.0±1.7	30.0±0.9	30.7±1.9	30.5±1.8
Salmonella spp	4	32.6±1.8	25.0±0.9	20.6±1.0	3.0±1.8	0.0±0.0	22.7±1.9	20.0±0.8	10.0±0.5	26.5±2.0	0.0±0.0	30.6±1.2	31.0±1.2	29.9±1.0
Shigella spp	4	30.9±2.0	24.6±1.6	11.0±0.5	2.6±1.9	0.0±0.0	21.9±1.0	9.0±1.9	11.0±0.7	26.5±2.0	13.0±1.0	26.9±1.3	30.9±1.3	28.7±1.1

Interpretative standard: Clinical and Laboratory Standards Institute (CLSI) (2006). Key: Mean±SEM

Table 6. Percentage antibiotic susceptibility pattern of bacterial isolates from ABSUTH wastewater

Organisms	No of isolate	Ciprofloxacin (10µg)	Norfloxacin (10µg)	Gentamicin (10µg)	Lincocin (20µg)	Streptomycin (10µg)	Rifampicin (20µg)	Erythromycin (30µg)	Chloramphenicol (30µg)	Ampiclox (20µg)	Floxapen (20µg)	Penicillin (10µg)	Drovid (10µg)	Augmentin (10µg)	Oflaxacin (10µg)
Proteus species	7	85.7	100	57.1	57.1	14.2	57.1	57.1	0.0	0.0	28.6	0.0	57.1	100	100
Staphylococcus aureus	6	100	66.7	50.0	16.7	0.0	33.3	75.0	0.0	0.0	50.0	0.0	50.0	83.3	33.3
Staphylococcus epidermidis	8	100	100	37.5	0.0	0.0	37.3	62.5	0.0	0.0	62.5	0.0	37.5	100	62.5
Escherichia coli	7	100	100	14.2	28.5	14.2	85.7	57.1	14.2	14.2	57.1	0.0	57.1	100	85.7
Bacillus spp	9	100	W3	0.0	22.2	0.0	55.6	22.2	0.0	55.6	55.6	22.2	66.7	100	88.9
Streptococcus spp	5	80.0	60.0	20.0	20.0	60.0	60.0	80.0	0.0	0.0	20.0	0.0	40.0	100	60.0
Neiseria spp	4	50.0	25.0	50.0	0.0	0.0	50.0	25.0	0.0	50.0	25.0	50.0	25.0	100	50.0
Actinomycetes isreali	4	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0
Serratia spp	3	100	66.7	33.3	66.7	0.0	33.3	66.7	33.3	66.7	66.7	66.7	66.7	100	66.7
Erwinia spp	4	100	75.0	50.0	25.0	0.0	25.0	25.0	0.0	0.0	25.0	0.0	50.0	100	50.0
Pseudomonas aeruginosa	7	57.1	71.4	71.4	85.7	14.3	57.1	0.0	14.3	71.4	57.1	14.3	71.4	57.1	57.1
Enterococcus faecalis	5	100	100	40.0	40.0	0.0	60.0	100	40.0	33.3	100	40.0	100	100	100
Salmonella spp	4	100	100	75.0	0.0	0.0	75.0	100	75.0	25.0	75.0	0.0	100	100	100
Shigella spp	4	100	100	25.0	0.0	0.0	75.0	100	25.0	25.0	75.0	25.0	75.0	100	100

Interpretative standard: Clinical and Laboratory Standards Institute (CLSI) (2006)

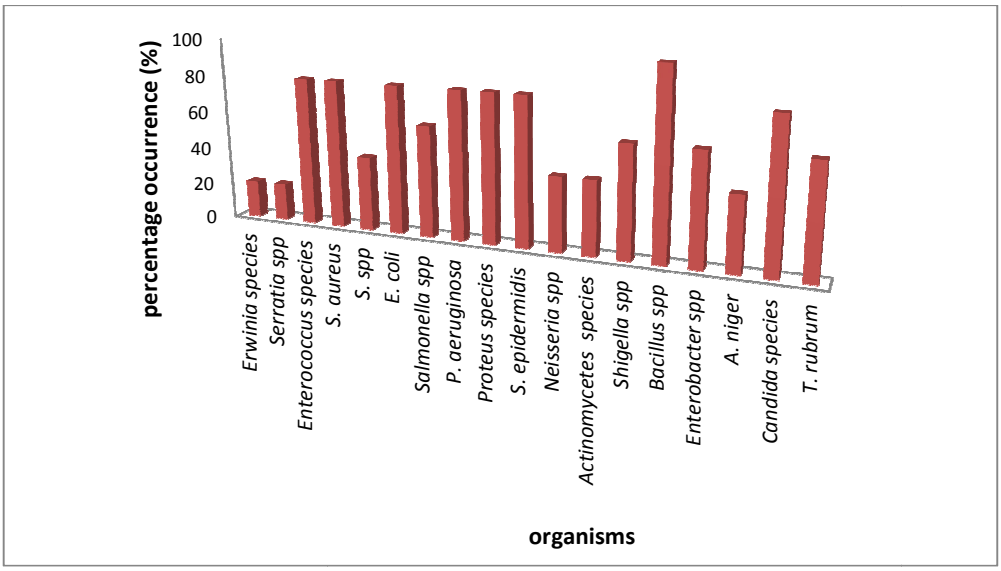

Fig. 1. Total percentage occurrence of the organisms within ABSUTH wastewater samples

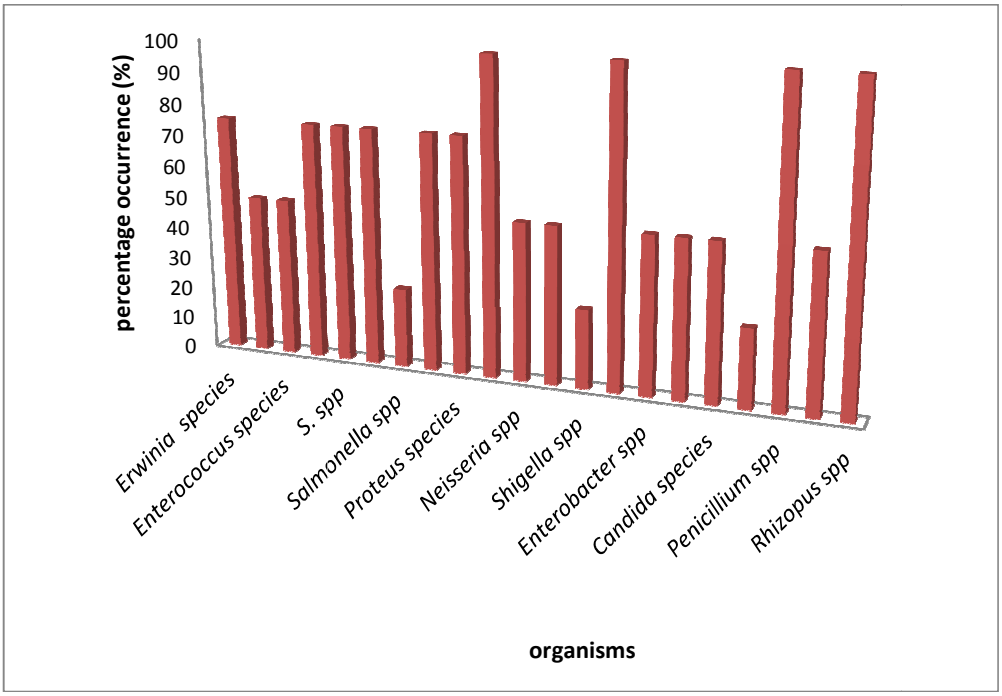

Fig. 2. Total percentage occurrence of the organisms within the soil samples

The highest value of total coliform count is $4.1\pm0.1\times10^{8}$ cfu/ml obtained from the collation point wastewater samples. The least value is $2.3\pm0.1\times10^{3}$ cfu/ml, occurred in laundry wastewater samples. The control has a value of $0.6\pm0.2\times10^{1}$ cfu/ml which is far below the toxicity level set by the WHO standard [11]. Total faecal count recorded the highest value $4.2\pm0.1\times10^{5}$ cfu/ml from the collation point wastewater samples while the least value which is $2.4\pm1.5\times10^{2}$ cfu/ml was obtained from the laundry wastewater samples. Total faecal count was not detected in the control sample. The total fungal count had the highest value of $3.1\pm0.2\times10^{5}$ cfu/ml from the mortuary wastewater samples and the least value from the collation point wastewater sample. The total bacterial count of the soil samples ranged from

$8.4\pm1.6\times10^{12}$ cfu/g– $3.2\pm0.1\times10^{6}$ cfu/g (point of discharge of the waste water and control soil sample respectively) while total coliform count recorded the highest value ($3.9\pm0.1\times10^{8}$ cfu/g) at the point of discharge of the wastewater and least value ($1.0\pm0.0\times10^{2}$ cfu/g) from the control sample (unpolluted soil sample). The total faecal count had the highest value at sample P1 ($3.7\pm0.5\times10^{4}$ cfu/g) while the least value is recorded from the control soil sample ($1.0\pm0.0\times10^{2}$ cfu/g). The control sample had the highest value of $5.4\pm0.5\times10^{6}$ cfu/g for the total fungal count while the least value $2.4\pm0.5\times10^{7}$ cfu/g was recorded from soil sample collected 100M away from the point of discharge of the wastewater. The introduction of wastewater in the environment brings about the increased amount of organic matter and essential nutrients, which influence the changes in the microflora. Aluyi et al. [12] noted that high counts of bacterial load reflected the level of pollution in the environment that is an indication of the amount of organic matter present. These results correlate with the findings of the influence of hospital wastewater in the University of Benin Teaching Hospital (UBTH), Benin City environment [1]. When evaluating the effects of hospital wastes on microbial communities, it is important to note that, target organisms vary between hospital wastes. Indigenous communities of bacterial and fungal populations are very complex and they have the important task of cycling nutrients.

The presence of high coliforms densities in the wastewater samples during sampling periods is an indication of faecal pollution of the environment due to human activities. Aluyi et al. [12] reported high faecal load with a high concentration of *E. coli* in Udu River, Warri, Delta State, Nigeria, which was attributed to human activities. In a similar study conducted by Chukwu et al. [13] to assess the influence of hospital wastewater on soil physicochemical properties in Aba, Abia State revealed some degree of variation among the sampling points.

The percentage occurrence of the isolated microbial spectrum within the wastewater and soil samples revealed that *Bacillus* species had the highest percentage occurrence rate (100%) while *Erwinia* species and *Serratia* species showed the least percentage rate of occurrence (20%) in the wastewater samples. *Rhizopus* species, *Staphylococcus epidermidis* and *Bacillus* species produced the highest percentage rate of occurrence in the soil samples (100% respectively) while *Salmonella* species and *Trichophyton rubrum* showed the least percentage rate of occurrence (20% respectively).

The high occurrence of the *Bacillus* species could be attributed to the fact that the organisms are ubiquitous in nature and include both free-living (non-parasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores which the bacteria can reduce themselves to and remain in a dormant state for very long periods. *S. epidermidis* is first among the causative agent of nosocomial infections and accounts for more than 50% of the late-onset sepsis episodes in neonates. *S. epidermidis* often causes infections in immune-compromised patients. The frequency of *S. epidermidis* infections is increasing, mainly due to concurrent advances in medical practice with more people undergoing and surviving intensive care treatment, acquiring prosthesis, and the increased survival of patients with a compromised immune system, such as preterm neonates and HIV patients. These infections are generally hospital-acquired [14]. The high percentage occurrence of this organism in the investigated samples, therefore, is not surprising.

The mean and percentage antibiotics susceptibility pattern of bacterial isolates from ABSUTH wastewater revealed that Ciprofloxacin produced the highest zones and percentage inhibition of 34.1±0.6 mm to 33.7±1.8 mm (100% to100%) against *Bacillus* species and *E.* coli respectively while Streptomycin and Chloramphenicol were highly resistant against all the isolates with the exception of *Streptococcus* species and *Enterococcus* species, respectively. Most of the bacterial isolates showed different forms of sensitivity to the antibiotics, the majority of which are susceptible to the antibiotics tested upon while a few were resistant to the antibiotics. This outcome corroborates with findings of Nain et al. [15] and Diwan et al. [16] in India.

6. CONCLUSION

Conclusively, it was observed that hospital wastes have a negative influence on the microbiological parameters of the environment. The microbial load parameters suggest that the activities of hospital wastes in the environment are a major health and environmental threat, which therefore call for a proper regulatory system on disposal of hospital waste in the

world, especially in the developing countries like Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ekhaise FO, Omavwoya BP. Bacteriological and Physicochemical studies of wastewater discharged from university of Benin Teaching Hospital (UBTH), Benin City into the Environment. Nigerian Journal of Microbiology. 2008; 22(1):1732-1736.
2. Prayitno, Kusuma Z, Yanuwiadi B, Laksmono R. Study of hospital wastewater characteristic in Malang city. International Journal of Engineering Science. 2013;2: 13-16.
3. Radha KV, Kalaivani K, Lavanya R. A case study of Biological waste management in hospitals. Global Journal of Health Science. 2009;1(1):82-88.
4. Mohsen B, Mohsen S. Investigation of metals accumulation in some vegetables irrigated with waste water in Shahre Rey-Iran and Toxicological Implications. American-Eurasian Journal of Agriculture and Environmental Science. 2008;4(1):86-92.
5. Wyasu G, Okereke NZJ. The influence of hospital wastewater and food samples growth within Ahamdu Bello University Teaching Hospital, Zaria, Nigeria on its receiving environment. Advances in Applied Science Research. 2012;3(3): 1686-1690.
6. Faruk O, Nazim S, Mefinkara S. Monitoring of cadmium and micronutrients in spices commonly consumed in Turkey. Research Journal of Agriculture, Biological Science, 2006;2:223–226.
7. Egbu AU, Kalu EE, Eze MU. Nature of home based enterprises (HBEs) in Aba, Abia State, Nigeria. Journal of Geography and Regional Planning. 2016;9(5):70-76.
8. Cheesbrough M. Laboratory manual for tropical countries vol. 11. Microbiology Tropical Health Technology. ELBS, London; 2006.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Seventeenth Information Supplement. CLSI Document M 100-S17, Clinical and Laboratory Standards Institute Wayne Pennsylvaria; 2006.
10. Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and identification. Cambridge, UK: Cambridge University Press; 2000.
11. Ewa L, Radoslaw S, Malgorzata K. Study on waste water toxicity using Tox Trak method. Environmental Science and Pollution. 2016;23:9105-9113.
12. Aluyi ASA, Ekhaise OF and Adelusi MD. Effect of human activities and oil pollution on the microbiological and physiological quality of Udu River, Warri, Nigeria. Journal of Applied Sciences. 2008;6(5): 1214-1219.
13. Chukwu VA, Nwaugo VO, Ajunwa KV. Effects of ABSUTH Wastewater on Soil Physicochemical Properties in Aba, Abia State, Nigeria. Asian Journal of Applied Sciences. 2015;3(6):838-833.
14. Levinson, W. Review of Medical Microbiology and Immunology (11th ed.). 2010;94–99.
15. Nain VK, Khurana GS, Singh S, Vashitha A, Sangeeta AS, Aggarwal N, Arora A, Khan I, Thareja G, Gupta K, Jain R, Diwan P. Antibiotic resistance pattern in bacterial isolates obtained from different water samples of Delhi Region. DU Journal of Undergraduate Research and Innovation. 2015;1(3):219-225.
16. Diwan V, Tamhankar AJ, Khandal RK, Sen S, Aggarwal M, Marothi Y, Iyer RV, Sundblad-Tonderski K, Stalsby-Lundborg C. Antibiotics and antibiotic resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Biology. 2010;10(414):1471-2458.

Peer-review history:
The peer review history for this paper can be accessed here:
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