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Research Article

Antibiotic Sensitivity of Bacteria and Physicochemical Constituents of Medical Wastewater Discharged From Some Hospitals in Port Harcourt

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ABSTRACT

Medical wastewaters are potentially infectious wastes generated at health care facilities. Soil associated or under the disposed wastewater is one of the reservoirs of microbial life. The aim of this study is to determine the antibiotic sensitivity of bacteria and physicochemical constituents of medical wastewater discharged from some hospitals in Port Harcourt. Wastewater samples were collected for the period of one (1) year from the Rivers State University Teaching Hospital, New Mile I Hospital, and Model Primary Health Centre, Potts Johnson. The bacteriological and physicochemical characteristics including heavy metal content of the wastewater samples were determined using standard analytical procedures. Data obtained were analyzed using statistical package for Social Sciences (SPSS) version 21. Mean values of counts of; total heterotrophic bacteria of samples ranged from 1.31±0.26 x10^6CFU/ml to 1.84±0.38 x 10^6CFU/ml across the stations, total coliform ranged from 1.18±0.21 x 10^4CFU/ml to 1.44±0.19 x 10^4CFU/ml, and faecal coliform ranged from 6.95±0.03 x10^4CFU/ml to 8.45±1.34 x 10^4CFU/ml. The following bacteria were isolated; *Klebsiella oxytoca*, *Bacillus subtilis*, *Pseudomonas aeroginosa, Enterobacter siamensis, Staphyloccoccus aureus, Proteus mirabilis, Micrococcus luteus, Escherichia coli, Yersinia enterocolitica, Corynebacterium diphtheriae, Providencia vermicola, Salmonella typhi, Shigella dysenteriae and Vibrio cholerae*. Physicochemical constituents of medical wastewater showed normal values except magnesium for all sample locations and bromine for Model Primary Health Centre. Antimicrobial susceptibility profile of the Gram negative isolates showed that *E. coli* (66.6%) and species of *Proteus* (50.0%), *Vibrio* (50.0%), and *Pseudomonas* (25.0%) were sensitive to Ceftazidime, Cefuroxime, Gentamycin, and Ceftriazone. However, *Salmonella*, *Klebsiella*, *Enterobacter*, and *Yersinia* species were resistant to Ofloxacin, Augmentin and Nitrofurantoin. Four Gram positive isolates belonging to the genera of *Staphylococcus, Corynebacterium, Micrococcus*, and *Bacillus*, were sensitive to Ofloxacin and Ceftriaxone. It is therefore recommended that medical wastewater should be treated before disposal since it has a tendency to pollute the subsoil and groundwater due to leaching of the pollutants and the health risks it poses to the general public.

Keywords: Medical wastewater, heavy metals, potential pathogens, *Vibrio, E. coli*, antibiotic resistance, bromine.

Introduction

Medical waste is any kind of waste either solid or liquid containing harmful materials generated at health care facilities e.g. hospitals, primary health centres, and nursing homes. This waste consists of human tissues, contaminated blood, body fluids, discarded medicines, drugs, contaminated cotton and dressings. Medical wastes are potentially infectious waste generated at health care facilities (Yadavannavar *et al*., 2010). These wastes constitute a problem because of the epidemiological implications and without proper handling can leads to nosocomial infections among patients and hospital personnel (Coker and Sangodoyin, 2000). In addition to the risk for patients and personnel who handle these wastes, it poses a threat to public health and environment.

Improper disposal of wastewater may be hazardous if it leads to contamination of water supplies/local sources used by nearby communities.
The soil associated or under the disposed water is one of the reservoirs of microbial life, and contaminated water contains pathogenic microorganisms which are causative agents of different types of diseases. Potential infectious risks include the spread of infectious diseases and microbial resistance from health care establishments into the environment which can occur spontaneously through mutation or mobile genetic material called plasmid (Read and Woods, 2014).

Multidrug resistant bacteria have been revealed in different water sources including rivers, lakes, groundwater and drinking water (Hamelin *et al*., 2007; Coleman *et al*., 2012).

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Human and animal faecal matters from wastewater may contain numerous species of enteric pathogens capable of causing viral, bacterial and parasitic infections which goes directly into the environmental water, including discharged wastewater effluents (Gerba and Smith, 2005). The array and number of pathogens that may be present in municipal wastewater differ with the levels of prevalent diseases in the community, commercial and industrial discharges, and seasonal factors (Godfree and Farrell, 2005).

Concerns about health risks associated with the discharge of inadequately treated wastewater effluents that may harbour antimicrobial resistant pathogens and other biological and chemical contaminants into the environment have renewed awareness in the effects of treatments on microbial pathogens (Godfree and Farrell, 2005).

The improper disposal of liquid waste may cause negative impact on the water quality as different pollutants may leach out from the waste dumping sites into the groundwater. Al Raisi *et al*. (2014) assessed and found that heavy metals in leachate were exceeding the drinking water standards. The concentrations of Al, V, Cr, Mn, Co, Ni, Ba, Pb, and Fe were found to be 2.050, 0.9775, 2.800, 0.503, 0.128, 0.773, 0.8575, 0.130, and 39.25 mg/L, respectively.

Heera and Rajor (2014) reported that incineration of medical waste contains a high concentration of heavy metals and polycyclic aromatic hydrocarbons (PAHs), and results in unfavourable amounts of hazardous materials and may pollute surface and groundwater.

Segregation, storage and safe disposal of the waste is the key to the effective management of biomedical waste in a workplace (Shrestha *et al.*, 2017). Biomedical waste treatment refers to the processes of eradicating the deleterious effects of the waste. Incineration, Autoclaving, irradiation and chemical treatments are the most common methods used for treatment and decontamination of biomedical waste (Shrestha *et al.*, 2017). Hence, it is important to decontaminate liquid waste before disposal into the environment.

The aim of this study is to determine the impact of medical wastewater disposal on soil by the determination of the antibiotic sensitivity of bacteria and physicochemical constituents of medical wastewater discharged from medical facilities in Port Harcourt.

Materials and Methods

Ethical Approval for the Study

Ethical clearance was obtained from the various hospitals prior to the commencement of the study.

Sampling Locations

Medical wastewater samples for this study were collected from three (3) different Hospitals located in Port Harcourt, namely; Rivers State University Teaching Hospital, New Mile 1 Hospital and Model Primary Health Centre, Potts Johnson.

Collection of Medical Wastewater Sample

Medical wastewater samples were aseptically collected from the bottle traps of wash basins for four (4) times from four (4) different sections (Laboratory, Post Operative Ward, Maternity Ward and Female Medical Wards) in each of the hospitals, using the method of Odokuma and Okpokwasili (1993). The samples were aseptically poured into sterile 1 litre bottles and their caps replaced. The samples were placed in a cooler containing ice blocks and immediately transported to the laboratory for analysis. Control water samples were collected from the tap at the entrance of the hospitals.

Physicochemical parameters

Physiochemical parameters such as Temperature, pH, Electric conductivity, Turbidity, Salinity, Chloride, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), and Biological Oxygen Demand (BOD), of the water samples were determined using standard methods as described by APHA 2012.

Preparation of Samples and Serial dilution

The samples were processed using the method of (Prescott *et al*., 2005). An aliquot of 10 ml of each medical wastewater sample was added to separate 90ml of normal saline. The various samples were then serially diluted using the ten-fold serial dilution method as described by (Prescott *et al.*, 2005).

Ten fold serial dilutions were carried out on the various processed samples by transferring 1ml from stock solution containing 10ml of the liquid samples to the next test tube containing 9ml of diluents. The samples were diluted up to 10^{-6} serially to reduce a dense culture of cells to a more usable concentration or countable colonies usually 30 to 300 colonies (Ogbonna and Inana, 2019).

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Each dilution will reduce the concentration of bacteria by a specific amount. Thus, prior to inoculation of the samples to the respective culture media used in this study, serial dilutions were carried out using standard method as adopted by Ogbonna *et al*., (2008).

Media and Diluents Preparation

The physiological saline used as diluent was prepared according to method of Cheesbrough, (2006) obtained by dissolving 8.5 grams of sodium chloride in to 1000ml of distilled water and sterilized in an autoclave at 121ºC at 15 psi for 15 minutes. Media used in this research include Nutrient Agar (NA) for the determination of Total Heterotrophic Bacteria, Mannitol salt agar (MSA) for determination of *Staphylococcus* species, MacConkey agar for determination of total coliforms, Eosin methyl Blue (EMB) for determination of feacal coliforms, *Salmonella* and *Shigella* agar (SSA) for determination of *Salmonella* and *Shigella* species, and Thiosulphate Citrate bile salt (TCBS) was used for determination of *Vibrio* species (Cheesbrough, 2006). All media were prepared and sterilized according to the manufacturer's instructions.

Purification of Isolates

After incubation, pure isolates were obtained by picking (with sterile inoculation loop) distinct culturally and morphologically different colonies from the various media plates. These were subjected to streaking on sterile nutrient agar in plates until pure distinct colonies were formed.

Identification of Bacterial Isolates

The discrete bacteria isolated from water samples were characterized based on their cultural morphology which includes colour, texture, shape, size, elevation, etc while, biochemical tests include; Gram's reaction, motility, catalyse, oxidase, spore formation, indole production, methyl red, citrate utilization, Voges-Proskauer test and sugar fermentation of the discrete bacterial isolates. Results obtained were compared with the recommendation by Cowan and Steel (1994) for the identification of the bacterial isolates. Isolates were also subjected to Automated Biometric Identification System (ABIS) tool to identify them to species level.

Antibiotics Susceptibility Testing

The antibiotic susceptibility pattern of the isolates to common antibiotics for Gram negative bacteria was evaluated using Cefuroxime (5µg), Ofloxacin (5µg), Ceftazidime (30µg), Gentamycin (10µg), Cloxacillin (30µg), Augmentin (30µg), Nitrofurantion (30µg), and Ceftriazone (30µg). The susceptibility of the Gram positive isolates to Ofloxacin (5µg), Augmentin (30µg), Ceftazidime (30µg), Gentamycin (10µg), Erythromycin (5µg), Cloxacillin (30µg), Cefuroxime (5µg), and Ceftriazone (30µg) was lso assayed, using the Kirby-Bauer disc diffusion technique as adopted by Adebayo-Tayo *et al*, (2012).

The inoculum for the sensitivity was prepared by adjusting the turbidity of the bacterial suspension to 0.5 MacFarland's standard (10^8cells/ml) (CLSI, 2017). The bacterial inoculum was spread over the surface of solid Mueller-Hinton agar (Difco Laboratories, Michigan, USA). The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps, and incubated for 24 hours at 37ºC (Iroha *et al*., 2009). Interpretation of results was based on the inhibition zones in accordance with the recommended breakpoints (Cheesbrough, 2006; CLSI, 2017).

Results

The result of the bacteria populations (CFU/ml) of medical wastewater samples from the different hospitals in Port Harcourt studied is presented in Table 1. The Total Heterotrophic Bacterial count ranged from 1.31±0.26 x10^6CFU/ml to 1.84±0.14 x10^6CFU/ml in waste water samples from New Mile I Hospital, Model Primary Health Centre, Potts Johnson, and RSU Teaching Hospital respectively, Total Coliform was 1.18±0.21 x10^4CFU/ml to 1.44±0.19 x10^4 CFU/ ml, Faecal coliform was 6.95±1.03 x10^4CFU/ml to 8.45±1.34 while counts of *Staphylococcus* was 1.02±0.03 x10^4CFU/ml to 6.45±4.12 x10^4CFU/ml. The counts of *Salmonella* was 3.10±0.97 x10^4 CFU/ ml to 5.64±0.81 x 10^4CFU/ml. *Shigella* counts were 4.10 ± 0.14 to5.90 ± 0.75, The counts of *Vibrio* was 2.37± 0.10 x10^4 CFU/ml to3.60±0.52 x10^4 CFU/ ml in wastewater samples from New Mile I Hospital, Model Primary Health Centre, Potts Johnson and Rivers State University Teaching Hospital respectively. There was a significant difference in the Total Heterotrophic Bacteria, Total Coliform, Faecal Coliform, *Salmonella*, *Shigella* and *Vibrio* counts across the different hospitals at $p \leq 0.05$.

The result of physicochemical constituents of medical wastewater samples from different Hospitals in Port Harcourt is shown in Table 2. The results revealed that there is statistical significance across the three Health Facilities.

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Table 1: Bacterial Population of Wastewater Samples from Different Hospitals in Port Harcourt

Hospital	Bacterial Counts						
	Total Heterotrophic Bacteria (×10⁶CFU/ml)	Total Coliform	Faecal Coliform	Staphylococci	*Salmonella*	*Shigella*	*Vibrio*
				(×10⁴CFU/ml)			
Control	0.85±0.07[ab]	0.04±0.01[a]	0.25±0.21[a]	0.40±0.00[a]	0.50±0.29[a]	0.80±0.14[a]	0.95±0.21[a]
MPHCPJ	1.84±0.38[c]	1.44±0.19[ab]	8.45±1.34[ab]	1.21±0.17[a]	5.65±0.81[ab]	5.90±0.75[ab]	3.60±0.52[c]
NM1	1.31±0.26[c]	1.18±0.21[a]	6.95±1.03[b]	6.45±4.12[a]	3.10±0.97[b]	4.12±0.87[a]	2.37±1.00[ab]
RSUTH	1.43±0.07[a]	1.34±0.03[c]	7.70±0.14[c]	1.02±0.03[a]	3.60±0.14[c]	4.10±0.14[c]	3.50±0.28[b]
P-value	0.013	0.000	0.000	0.069	0.000	0.000	0.011

*Means with same alphabet across the columns shows no significant difference at (p≥0.05).
Key: MPHCPJ= Model Primary Health Centre Potts Johnson, NM1= New Mile 1 Hospital; RSUTH= Rivers State University Teaching Hospital.

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Table 2: Physicochemical constituents of wastewater samples from different Hospitals in Port Harcourt

Parameter	Model Primary Health Center	New Mile I Hospital	University Teaching Hospital	WHO Standard	P-value
Temperature (°C)	26.52±0.02[a]	28.14±0.13[b]	28.59±0.12[c]	20-33	0.001
Ph	5.04±0.01[a]	5.77±0.71[a]	5.71±0.08[a]	6.5-8.5	0.292
DO (mg/l)	3.49±0.08[a]	4.18±0.06[c]	3.21±0.08[b]	14	0.002
BOD (mg/l)	1.87±0.02[a]	1.46±0.15[b]	0.77±0.03[c]	<5	0.003
COD (mg/l)	3.76±0.08[b]	3.13±0.01[a]	1.52±0.02[a]	10	0.000
Bromine (mg/ml)	2.23±0.04[a]	0.69±0.03[a]	1.25±0.00[b]	1.5	0.000
Chlorine (mg/l)	0.83±0.04[a]	1.09±0.01[a]	0.53±0.04[b]	5	0.001
Conductivity (S/m)	923.13±5.48[a]	489.00±29.69[b]	624.00±0.00[c]	500	0.005
TDS (mg/l)	585.00±35.36[b]	303.50±20.51[a]	424.00±79.19[c]	900	0.027
Turbidity (NTU)	14.66±0.08[b]	17.01±2.35[a]	9.98±0.67[c]	NS	0.034
Nitrogen (mg/l)	0.004±0.001[a]	0.002±0.000[a]	0.003±0.001[a]	5	0.125
Phosphorus (mg/l)	0.37±0.01[a]	0.28±0.02[a]	0.31±0.08[a]	NS	0.268
Potassium, K (mg/l)	12.16±0.14[a]	13.45±4.24[a]	7.72±0.36[a]	NS	0.193
Magnesium, Mg (mg/l)	13.57±0.48[a]	13.61±3.55[a]	11.66±0.33[a]	10	0.614

Key: DO=Dissolved Oxygen, BOD=Biological Oxygen Demand, COD=Chemical Oxygen Demand, TDS= Total Dissolved Oxygen and NTU=Nephelometric turbidity units

Mean value of the Temperature (°C) ranged from 26.52±0.02, to 28.59±0.12, Values of pH ranged from 5.04±0.10 to 5.77±0.71. The Electric conductivity ranged from 489.00±29.69 to 923.13±5.48(S/m). Total dissolved solid (TDS) ranged from 303.50±20.51to 585.00±35.36 (mg/l) Dissolved oxygen (DO) ranged from 3.21±0.08 to 4.18±0.06 (mg/l). Biological oxygen demand (BOD) ranged from 0.77±0.03 to 1.87±0.02(mg/l), Chemical oxygen demand (COD) ranged from 1.50±0.02 to 3.76±0.08(mg/l), Turbidity (NTU) 9.98±0.67 to17.01±2.35(NTU), Bromine 0.69±0.03 to 2.23±0.04(mg/l), Potassium, K (mg/l) 7.72±0.36 to 13.45±4.24(mg/l), Chlorine 0.53±0.04 to 1.09±0.01(mg/l), Nitrogen (mg/l) 0.002±0.000 to 0.004±0.001(mg/l), Phosphorus (mg/l) 0.28±0.02, 0.37±0.01(mg/l), Magnesium, Mg (mg/l) 11.66±0.33 to 13.61±3.55(mg/l) for New Mile 1 Hospital, Model Primary Health Center, Potts Johnson and Rivers State University Teaching Hospital respectively.

The mean value of Lead, Chromium, Cadmium and Nickel in medical wastewater in all the hospitals were <0.001mg/l Copper(mg/l) <0.015, <0.051, <0.001, Zinc (mg/l), <0.190, <0.384, <0.352, Iron (mg/l), <0.026, <0.299, <0.001, (mg/l, <0.001, <0.001, <0.001, Manganese (mg/l) <0.906, <0.154, <0.001 for New Mile 1 Hospital, Model Primary Health Center, Potts Johnson, and Rivers State University Teaching Hospital respectively are presented in Table 3.

The result of the distribution and occurrence of bacterial isolates from wastewater samples from the three different medical facilities in Port Harcourt is shown in Table 4.

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Table 3: Heavy Metal Content of medical wastewater samples from different Hospitals in Port Harcourt

Parameter (mg/l)	Model Primary Health Center	New Mile I Hospital	University Teaching Hospital	WHO Standard	P-value
Lead	0.001±0.000	0.001±0.000	0.001±0.000	0.01	0.000
Chromium	0.001±0.000	0.001±0.000	0.001±0.000	0.05	0.000
Cadmium	0.001±0.000	0.001±0.000	0.001±0.000	0.003	0.000
Copper	0.052±0.001	0.016±0.001	0.001±0.000	0.5	0.000
Zinc	0.385±0.001	0.192±0.003	0.353±0.001	1.0	0.000
Iron	0.299±0.001	0.027±0.001	0.001±0.000	1.0	0.000
Nickel	0.001±0.000	0.001±0.000	0.001±0.000	0.02	0.000
Manganese	0.156±0.003	0.906±0.001	0.001±0.000	0.1	0.000

Table 4: Distribution (%) of bacterial isolates from wastewater samples from the different Hospitals

Bacterial Isolate	Distribution (%) of bacterial isolates from wastewater samples		
	New Mile I Hospital	Model Primary Health Center	University Teaching Hospital
Klebsiella oxytoca	1(16.7)	0(0.00)	0(0.00)
Bacillus subtilis.	0(0.00)	1(16.7)	0(0.00)
Pseudomonas aeruginosa	0(0.00)	1(16.7)	0(0.00)
Enterobacter siamensis	1(16.7)	0(0.00)	0(0.00)
Staphylococcus aureus	0(0.00)	1(16.7)	0(0.00)
Proteus mirabilis	0(0.00)	0(0.00)	1(33.3)
Serratia marcescens	0(0.00)	0(0.00)	0(0.00)
Micrococcus luteus	1(16.7)	0(0.00)	0(0.00)
Escherichia coli	2(33.3)	0(0.00)	0(0.00)
Yersinia enterocolitica	0(0.00)	1(16.7)	0(0.00)
Corynebacterium diphtheriae	0(0.00)	1(16.7)	0(0.00)
Providencia vermicola	0(0.00)	0(0.00)	1(33.3)
Salmonella typhi	0(0.00)	1(16.7)	0(0.00)
Shigella dysenteriae	1(16.7)	0(0.00)	0(0.00)
Vibrio cholera	0(0.00)	0(0.00)	1(33.3)

The bacteria isolated and identified during the study were four Gram positive bacteria belonging to the genera: *Micrococcus*, *Bacillus*, *Corynebacterium* and *Staphylococcus*. Nine Gram negative bacteria isolated include *Escherichia coli,* and species of *Pseudomonas*, *Proteus*, *Enterobacter*, *Yersinia*, *Providencia*, *Salmonella*, *Shigella*, and *Vibrio*.

The results of the antibiotics sensitivity profile of the Gram negative isolates from the medical wastewater samples from the different Hospitals in Port Harcourt is presented in Table 5. The antibiogram of Gram negative bacterial isolates from the medical wastewater samples from the different hospitals showed that all the gram negative bacterial isolates were susceptible to only three antibiotics namely; Nitrofurantoin, Ofloxacin and Ceftriaxone out of the eight (8) different types of antibiotics that the isolates were subjected to.

On the other hand, the results of the antibiotics sensitivity profile of the Gram positive isolates from the medical wastewater samples from the different Hospitals in Port Harcourt are presented in Table 6.

The result revealed that the Gram positive bacterial isolates were susceptible to only two antibiotics namely; Ceftriaxone and Ofloxacin out of the eight (8) different types of antibiotics that the isolates were subjected to during the study.

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Table 5 Susceptibility pattern of Gram negative isolates from medical wastewater Model Primary Health Center, Potts Johnson

Antibiotic	*Pseudomonas aeruginosa* n-2			*Enterobacter siamensis* n-1			*Proteus mirabilis* n-1			*Yersinia enterocolitica* n-2			*Salmonella typhi* n-3		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
CAZ	0(0.00)	2 (100)	0(0.00)	1(100)	(0.00)	(0.00)	0(0.00)	0(0.00)	1 (100)	2(100)	(0.00)	(0.00)	0(0.00)	3(100)	0(0.00)
CRX	0(0.00)	2 (100)	0(0.00)	1(100)	(0.00)	(0.00)	0(0.00)	0(0.00)	1 (100)	2(100)	(0.00)	(0.00)	0(0.00)	3(100)	0(0.00)
GEN	0(0.00)	2(100)	0 (0.00)	1(100)	(0.00)	(0.00)	1(100)	0(0.00)	(0.00)	1(50.0)	1(50.0)	(0.00)	3(100)	0(0.00)	0(0.00)
CXM	0(0.00)	0 (00.0)	1 (50.0)	1(100)	(0.00)	(0.00)	0(0.00)	0 (0.00)	1 (100)	0(0.00)	(0.00)	2 (100)	0(0.00)	1(33.33)	2(66.67)
OFL	0(0.00)	2 (100)	0(0.00)	1(100)	(0.00)	(0.00)	1(100)	0(0.00)	(0.00)	0(0.00)	0(0.00)	(100)	3(1000)	0(0.00)	0(0.00)
AUG	2(0.00)	0 (0.00)	0(0.00)	0(0.00)	(100)	(0.00)	1(100)	(0.00)	(0.00)	0(0.00)	(0.00)	(100)	3(1000)	0(0.00)	0(0.00)
CTR	1(50.0)	1(50.0)	0(0.00)	1(100)	(0.00)	(0.00)	1(100)	(0.00)	(0.00)	1(50.0)	(0.00)	1 (50.0)	3(1000)	0(0.00)	0(0.00)
NIT	0(0.00)	1(50.0)	0(0.00)	1(100)	(0.00)	(0.00)	0(0.00)	(0.00)	1 (100)	1(50.0)	(0.00)	1 (50.0)	2(66.7)	1(33.33)	0(0.00)

Key: S- Sensitive, I- Intermediate, R- Resistant. CAZ - Ceftazidime, CRX- Cefuroxime, GEN- Gentamycin, CXM -Cloxacillin, OFL Ofloxacin, AUG- Augmentin, CTR-Ceftriazone, NIT -Nitrofurantoin

Table 6: Susceptibility pattern of Gram negative bacterial isolates from medical wastewater from New Mile I Hospital

Anti-biotic	*Klebsiella oxytoca* n-1			*Enterobacter siamensis* n-1			*E. coli* n-2			*Shigella dysenteriae* n-2			*P. aeruginosa* n-1			*Yorsinia enterocolitica* n-1			*Providencia vermicola* n-1		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
CAZ	1(100)	0 (0.00)	0(0.00)	1(100)	0(0.00)	0 (0.00)	0(0.00)	0(0.00)	1 (100)	2(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
CTX	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
GEN	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(500)	1(500)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)
CXM	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	1(50.0)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
OFL	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
AUG	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	1(50.0)	1(50.0)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)
CTR	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(50.0)	0(0.00)	1(50.0)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
NIT	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)

Key: S = Sensitive, I = Intermediate, R = Resistant. CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamycin, CXM = Cloxacillin, OFL = Ofloxacin, AUG = Augmentin, CTR = Ceftriazone, NIT = Nitrofurantoin

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Table 7: Susceptibility pattern of Gram negative bacterial isolates from medical wastewater from Rivers State University Teaching Hospital

Anti-biotic	*P. aeruginsa* n-1			*Serattia marcescens* n- 1			*E. coli* n-2			*Proteus mirabilis* n-1			*Providencia vermicola* n-1			*Vibrio cholerae* n-2			*Salmonella typhi* n-1			*Shigella dysentriae* n-1		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
CAZ	0(0.00)	1 (100)	0(0.00)	1(100)	0(0.00)	0 (0.00)	0(0.00)	0(0.00)	2 (100)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
CTX	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
GEN	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(50.0)	0(0.00)	1(50.0)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)
CXM	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	1(50.0)	0(0.00)	1(50.0)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)
OFL	1(100)	0(00.0)	0(0.00)	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(50.0)	0(0.00)	1(50.0)
AUG	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
CTR	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1 (100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
NIT	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)

Key: S- Sensitive, I- Intermediate, R- Resistant. CAZ - Ceftazidime, CRX- Cefuroxime, GEN- Gentamycin, CXM -Cloxacillin, OFL Ofloxacin, AUG- Augmentin, CTR- Ceftriazone, NIT –Nitrofurantoin

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Table 8: Susceptibility pattern of Gram positive bacterial Isolates from medical wastewater from New Mile I Hospital

Antibiotics	*Micrococcus luteus* n – 1			*Bacillus subtilis* n - 1			*Staphylococcus aureus* n - 1		
	S	I	R	S	I	R	S	I	R
OFL	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
AUG	1 (100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
CAZ	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)
GENT	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)
ERY	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
CXC	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)
CTR	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)

Key: S- Sensitive, I- Intermediate, R- Resistant. CAZ - Ceftazidime, CRX- Cefuroxime, GEN- Gentamycin, CXM -Cloxacillin, OFL Ofloxacin, AUG- Augmentin, ERY- Erythromycin, CTR-Ceftriazone

Table 9: Susceptibility Pattern of Gram Positive Isolates from Model Primary Health Center, Potts Johnson

Antibiotics	*Bacillus subtilis* n – 1			*Staphylococcus aureus* n - 2			*Corynebacterium diphtheriae* n - 3		
	S	I	R	S	I	R	S	I	R
OFL	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
AUG	0(0.00)	0(0.00)	1(100)	2(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)
CAZ	0(0.00)	1(100)	0(0.00)	1(50.0)	1(50.0)	0(0.00)	2(66.67)	0(0.00)	1(33.33)
Gent	1(100)	0(0.00)	0(0.00)	0(0.00)	1(50.0)	1(50.0)	1(33.33)	0(0.00)	2(66.67)
ERY	1(100)	0(0.00)	0(0.00)	1(50.0)	1(50.0)	0(0.00)	1(33.33)	1(33.33)	1(33.33)
CXC	0(0.00)	1(100)	0(0.00)	0(0.00)	1(50.0)	1(50.0)	0(0.00)	2(66.67)	1(33.33)
CRX	1(100)	0(0.00)	0(0.00)	1(50.0)	1(50.0)	0(0.00)	2(66.67)	1(33.33)	0(0.00)
CTR	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)

Key: S = Sensitive, I =Intermediate, R= Resistant. CAZ = Ceftazidime, CRX =Cefuroxime, GEN = Gentamycin, CXM = Cloxacillin, OFL = Ofloxacin, AUG = Augmentin, ERY = Erythromycin, CTR = Ceftriazone

Discussion

Wastes associated with health care facilities are potential sources of introducing microorganisms into the environment. Wastewaters generated from hospitals are discharged into the environment without any treatment (Sharma *et al*., 2010). This may have direct link to the increased prevalence of antibiotic resistant microbial strains in the environment which has over the years generated public health concerns as organisms has the potential to exchange resistance through horizontal gene transfer (Sharma *et al*., 2010). This present study has revealed the antibiotic resistance and sensitivity of microorganisms and the physicochemical constituents of medical wastewater discharged from some hospitals in Port Harcourt, Nigeria. The temperature of these wastes offer very appealing conditions for microbial proliferation as most of them are lower than 30˚C and the pH of the wastewaters are almost neutral. Physicochemical constituents of wastewater are an important factor that affects the rate of many biological and chemical processes and also the amount of oxygen that can dissolve in the water (Placide *et al*., 2016). The total heterotrophic bacteria (THB) counts of wastewater from the hospitals studied showed varying viable bacteria loads. New Mile I Hospital and Model Primary Health Centre, Potts Johnson has the highest number of organisms followed by Rivers State University Teaching Hospital.

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This could be attributed to the fact that the wash areas are not much in these two hospitals so more patients tend to utilize the few wash areas as compared to Rivers State University Teaching Hospital which is a tertiary hospital where there are lots of places to use despite the influx of patients.

Isolates found in wastewater from the three (3) health facilities showed fourteen (14) isolate, ten (10) Gram negative and four (4) Gram positive and their percentage occurrence showed *Micrococcus luteus* (16.7%), *Escherichia coli* (33.3%)from New Mile I Hospital and species of *Proteus*, *Providencia* and *Vibrio* from the Rivers State University Teaching Hospital were 33.3% each. While percentage occurrence of species of *Klebsiella*, *Bacillus*, *Shigella*, *Salmonella*, *Pseudomonas*, *Enterobacter*, *Staphylococcus*, *Yersinia*, and *Corynebacterium*, were 16.7% each. Similar reports by Ekhaise and Omavwoya (2008) in Benin Hospital showed that the bacterial genera, *Klebsiella*, *Pseudomonas* and *Serratia* were the most frequently distributed isolates in the hospital wastewater.

In another study, *Pseudomonas* spp. was found to be the most prevalent by 20.7% (Ashfaq *et al*., 2013). In the study conducted by Oyeleke and Istifanus (2009), the most predominant pathogens isolated from hospital wastes were *Bacillus* and *S. aureus* (80 to 90%); however, findings by Oyiasogie *et al.* (2010) showed that *P. aeruginosa* was among the highest Gram negative organism isolated from hospital waste accounting for about 25.00% overall of all the isolates. In a study carried out in Erbil city, Rhizgari by Aziz *et al*. (2014) revealed that *E. coli* was mostly isolated (100%) from a hospital wastewater. It is widely accepted that these pathogens are the major cause of hospital acquired infections which is in agreement with this study that *E. coli* strains were obtained (13%) from both hospitals being second to *Providencia* species. The percentage occurrence of bacterial isolates from medical wastewater contain more of Gram negative bacteria than Gram positive bacteria which corroborates with the findings of previous researchers (Maina *et al*., 2018, Omoni *et al*., 2015). Most of these bacteria are known to cause nosocomial infections (Maina *et al*., 2018).

The physicochemical constituents of wastewater from the three (3) health facilities in Port Harcourt showed varying results. The pH values were slightly acid though within permissible limit.

Temperature of wastewater was also within permissible limit. Values of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) recorded were within WHO standard. Bromine was highest at the Model Primary Health Centre followed by Rivers State University Teaching Hospital and New Mile I Hospital recorded the least. WHO standard for Chlorine was not specified though values from NMI had the highest followed by MPHC and RSUTH. Conductivity values were above permissible limit in all the hospital wastewater samples. Total Dissolved Solid (TDS) result showed that all the stations values were within WHO standard. Turbidity is the degree of clearness of water, there was no standard for turbidity but from the values gotten, New Mile I Hospital has the highest value followed by Model Primary Health Center and the University Teaching Hospital. Nitrogen was below WHO standard in all Health Facilities. Phosphorus and Potassium were not specified. Magnesium (13.61±3.55mg/l) from Health Facilities was above WHO standard.

The high values recorded for bromine (2.23±0.04 mg/l), conductivity (923.13±5.48mg/l) and magnesium (13.61±3.55mg/l) in this research work corroborates with the analysis done by Akan *et al*., 2008 who states that high values were also recorded.

Metallic trace elements like heavy metal tend to adsorb from the water column onto the surfaces of fine particles and generally move the sediments and affect the environment and to some extent, the food chain if toxic levels are released thus posing a health risk (Ekoe-Bessa *et al*., 2020, Mamat *et al*., 2002, Niu *et al*., 2020). Heavy metal content of wastewater from the three (3) Health Facilities showed all the eight (8) metals Lead (mg/l), Chromium (mg/l), Cadmium (mg/l), Copper (mg/l), Zinc (mg/l), Iron (mg/l), Nickel (mg/l) and Manganese(mg/l) studied.

The heavy metal with the highest value was Manganese (0.906±0.001mg/l) which was recorded at New Mile I Hospital. This is in agreement with the research done by Amfo-Out *et al*. (2015) who revealed average metal concentration in ashes of biomedical waste except manganese which was high. High values of these parameters indicates higher amount of impurities and may cause negative effect on water quality and in turn be dangerous to humans and the ecosystem.

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The antibiotic susceptibility and resistant has threatened the success of medical interventions at all levels of health care and create a set of specific challenges for clinical, therapeutic and public health interventions with local, national and global dimension (WHO, 2001). Bacteria that belong to the normal flora in humans become exposed to antibiotic compounds every time antibiotics are used and the most significance resistance has been emerging among these microorganisms (WHO, 2001). Results showing varying response to antibiotics were observed with the bacterial isolates from this study. *Vibrio cholerae* showed 50% resistant to nitrofurantoin, ceftazidime, cefuroxime and cloxacillin and 50% sensitive to gentamycin and ofloxacin. *Staphylococus aureus* was 33.3% resistant to erythromycin, cloxaccilin and cefuroxime, *Escherichia coli* was 66.6% resistant to ceftazidim and nitrofurantoin. *Klebsiella oxytoca* showed resistant to cloxacillin, ofloxacin and augmentin and sensitive to ceftriaxone and cefuroxime. This is in agreement with study carried out by Rastegar *et al*, 2021 who reported that *Klebsiella pneumoniae* and *Vibrio cholerae* were resistant to cloxacillin, nitrofurantoin and ceftazidin.

Generally, antibiotics susceptibility pattern from wastewater from the three (3) health facilities showed multi-drug resistant as some of these isolates (*Vibrio cholerae, Staphylococus aureus,* and *Klebsiella oxytoca*) were resistant to more than two (2) antibiotics.

In conclusion, the polluted world of today, correct methods of handling the waste generated have become essential. Proper methods of water disposal have to be undertaken to ensure that it does not affect the immediate environment or cause health hazards to the people living there. At the hospital-level treatment of wastewater has to be done before its discharge into the environment.

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