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EVALUATION OF WASTEWATER FROM A PUBLIC HEALTHCARE FACILITY IN BENIN CITY, NIGERIA: A CASE STUDY OF ITS PHYSICOCHEMICAL, BACTERIOLOGICAL QUALITIES AND OCCURRENCE OF EXTENDED SPECTRUM BETA LACTAMASE BACTERIAL ISOLATES

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ABSTRACT

This study evaluated the physicochemical and bacteriological qualities of wastewater generated from a public health facility between June and November, 2018. Standard analytical and bacteriological techniques were used to investigate the qualities of the effluent from two separate points. Antibiotic susceptibility study was carried out using Kirby Bauer disc diffusion method. Results obtained showed temperature ranged from 28.69 - 28.75 ^{0}C, pH 6.99 - 7.04, Biochemical Oxygen Demand 161.31 -164.25 mg/ml, Chemical Oxygen Demand 324.38 -327.63 mg/l, phosphate 145.15 - 146.13 mg/l, Electrical Conductivity 231.69 - 232.53 µS/cm, Settleable Solid 206.44 - 207.88 mg/l and Total Suspended Solid 79.81 -81.05 mg/l. The highest concentration of heavy metal was recorded with iron (12.79-13.11 mg/l). The total heterotrophic bacterial counts ranged from 0.39 - 138.6 x 10^7 cfu/ml, Coliform counts ranged from 0.3 - 204 x 10^6 cfu/ml and the Staphylococcal counts ranged from 0.67 - 22 x 10^5 cfu/ml. The results of the antibiotic susceptibility tests showed that the bacterial isolates possessed 29.21 % resistant to septrin, 5.62 % to chloramphenicol, 24.72 % to sparfloxacin, 6.74 % to ciprofloxacin, 22.47 % to augmentin, 6.74 % to gentamicin, 14.61 % to perfloxacin, 14.61 % to tarivid, 10.11 % streptomycin and 8.99 % to ampicillin. Multiple antibiotic resistance were observed in *Klebsiella* sp., *Escherichia coli* and *Staphylococcus epidermidis* and were found to be extended-spectrum beta-lactamase positive. The presence of a diverse group of multi drug-resistant bacteria in the wastewater could play a major role in the dissemination and spread of disease-causing pathogens in the environment.

Keywords: Bacteriological, Evaluation, Hospital effluents, Physicochemical, Qualities

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INTRODUCTION

The importance of the environment in the global development of clinically relevant antibiotic resistance is being increasingly recognized. (Singer *et al*., 2016). The contributions of different sources of antibiotic-resistant bacteria in the environment (Larsson *et al.*, 2018) is one of the proposed critical knowledge gaps and research needs related to the environmental dimensions of antimicrobial resistance, and one of these sources are hospital effluents or wastewater. Any water whose quality has been impaired by anthropogenic influences (human, chemical, and microbiological agents) and is discharged into the environment is referred to as wastewater (Ekhaise and Omavwoya, 2008).

Hospital wastewater is discharged from all hospital activities, both medical and non-medical, including activities in surgery rooms, examination rooms, laboratories, nursery rooms, radiology rooms, kitchens, and laundry rooms. The consumption of water in hospitals in industrialized countries varies from 400 to 1,200 L per bed per day (Emmanuel *et al*., 2005) , whereas in developing countries this consumption seems to be between 200 and 400 L per bed per day (Ul Haq *et al*., 2012).

Hospital wastewater is ranked as a special category of waste water because of their highly hazardous and toxic character, which contain cocktail of antibiotics, disinfectants, metabolized drugs, and sensitive and resistant bacteria from hospitalized patients (Le *et al*., 2016). Large quantities of chemicals are used in hospitals for patient care and disinfection are partially metabolized and residual quantities reach effluents exposing bacteria to a wide range of antibiotics, heavy metals and biocides that could act as a selective pressure for the development of resistance. This situation may be worsened when effluents from healthcare facilities are directly discharged with no prior treatment in the wastewater network.

Hospital effluents are a huge contributor to environmental pollution and underground water contamination if not properly treated (Bauer *et al*., 2000). The nature of such bacteria found in hospital effluents may be altered by the direct or indirect effect of hospital effluents components (Amouei *et al*., 2015). These components can be found in a wide range of concentrations and can be as a result of the size of a hospital, the bed density, number of inpatients and outpatients, the number and the type of wards, the number and types of services, the country and the season and can lead to bacteria with high antibiotic resistance like Extended-Spectrum Beta-Lactamase Organism (ESBL) (Emmanuel *et al*., 2005).

Extended-Spectrum Beta-Lactamase producing clinical isolates are members of the Enterobacteriaceae family, especially *Klebsiella pneumoniae* and *Escherichia coli*, this represent one of the most important world problems of β-lactam antimicrobial resistance, commonly used in the treatment of many bacterial nosocomial and community infections (Mahyi *et al*., 2009). At the level of a wider geographic scale, the incidence of ESBL-producing organisms is difficult to resolve due to various reasons to include difficulty in detecting ESBL production and inconsistencies in reporting (Steward *et al*., 2000). In 2017 the World Health Organization published a list of antibiotics-resistant "priority pathogens". The most critical group of all includes multidrug resistant (MDR) bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters. These include Acinetobacter spp., *Pseudomonas* spp. and various Enterobacteriaceae (*Klebsiella* spp., *E. coli*, *Serratia* spp. and *Proteus* spp.) producing extended spectrum beta-lactamases (ESBL) or carbapenemases (WHO, 2015). Extended Spectrum Beta Lactamase (ESBL) producing bacteria cause resistance to

β-lactam antibiotics containing an oxyimino group (e.g., ceftazidime, cefotaxime, aztreonam) together with resistance to other classes of non-penicillin antibiotics, including fluoroquinolones (FQ), aminoglycosides, trimethoprim/sulfa-methoxazole and ß-lactam/ß-lactamase inhibitor combinations. These bacteria are responsible for host prolonged hospital stay, increased treatment costs, morbidity, and mortality (Pilmis *et al*., 2018). Recent studies have shown the high frequency of human intestinal carriage of Extended Spectrum Beta Lactamase-producing *E. coli* in both hospital and community settings (Hocquet *et al*., 2016)

Studies have shown that hospital effluents in Nigeria and other developing countries are poorly treated and discharged into the immediate receiving environment (Ngwuluka *et al*., 2011) and these have been recognized as a growing public health threat. This study aimed at assessing the physicochemical and bacteriological qualities of effluents discharged from a public health facility in Benin City.

MATERIALS AND METHODS

SAMPLING SITE AND COLLECTION

In this study, the selected hospital was a premiere public health facility located at Ugbowo, Benin City, Edo State, Nigeria with GPS coordinates of 6°23′26″N 5°36′44″E and considered the busiest health facility with a bed size of 910.

A total of sixteen samples were collected at point 1 (inlet) which conveys wastewater from the hospitals treatment facilities into the central sewage underground tank; and point 2 (outlet) which is the outlet point of the sewage underground tank between June, 2018 and November, 2018. The samples were collected in 250 ml-sized sterile bottles containing 0.2 ml of 3% w/v sodium thiosulphate and immediately transported in ice jackets to the laboratory for physicochemical and bacteriological analysis (Nunez and Moretton, 2007). Hospital wastewater is a huge contributor to environmental pollution and underground water contamination when not treated before discharge. Due to various reasons which include quantities of components (antibiotics, heavy metals, and biocides), HWW could act as a selective pressure for the development of antibiotics resistance. This situation may be worsened when wastewater from healthcare facilities is directly discharged with no prior treatment in the wastewater network (Devarajan *et al*., 2016).

DETERMINATION OF THE PHYSICOCHEMICAL PARAMETERS

Parameters such as pH, Colour, electrical Conductivity, Temperature, Dissolved Oxygen, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) were all measured according to protocols described previously (AOAC, 2005; Begum and Harikrishnarai, 2008).

DETERMINATION OF THE HEAVY METALS

Five millilitres (5 ml) of the wastewater sample was taken from the preserved wastewater and put in a beaker. Ten (10) ml of nitric perchloric acid, ratio 2:1 was added to the sample and digested at 105oC in a fume cupboard. The cooled digest was transferred into a 100 ml standard volumetric flask and was made up to 100 ml mark with distilled water. Heavy metals such as iron (Fe), chromium (Cr), cadmium (Cd), and zinc (Zn) were thereafter analysed using Atomic Absorption Spectrophotometer (AAS) PG 550 (APHA, 2012).

ENUMERATION OF TOTAL VIABLE BACTERIAL COUNTS

The heterotrophic bacterial counts were carried out adopting the pour plate technique (Cheesbrough (2006), Zheng *et al*., 2018). A10-fold serial dilution of samples was carried out using physiological saline, and pours plated in Nutrient Agar, Mannitol Salt Agar, MacConkey agar and Eosin Methylene Blue Agar. Plates were incubated for 48 hours at 30ºC in duplicate. The number of colonies on duplicate plates having 30–300 colonies were counted and reported as cfu/ml. After incubation, the various isolates were further identified and characterized.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The sensitivity pattern of the isolates was determined using the Kirby-Bauer disk diffusion method (Murray, 2003). The bacterial isolates were transferred into Nutrient Broth under aseptic conditions and incubated overnight. The turbidity of the broth cultures was adjusted to match an opacity standard (108cfu/ml) and the resultant broth culture was then plated onto Mueller Hinton agar. Commercially available antibiotics discs containing different concentrations were placed onto Mueller Hinton agar and incubated for 24hrs. The sensitivity pattern of the isolates was determined by measuring the zones of inhibition with a calibrated ruler and was interpreted according to Clinical Laboratory Standards (CLSI, 2014) criteria. The tested organisms were then organized into 'sensitive' (S), 'intermediate' (I), or 'resistant' (R).

PLASMID CURING

Plasmid curing of isolates was carried out using acridine orange and elevated temperature methods. The elevated temperature was followed by adjusting the temperature for the growth of isolates at 44°C. In addition, to perform plasmid curing by acridine orange, a serial double dilution of 0.05 μg mL–1 of acridine orange was made, then 200 μL of bacteria culture (turbidity equal to 0.5 Mcfarland tube) was added into the different dilutions and the suspension tubes were incubated at 37°C. After 24 h the Minimal Inhibitory Concentration (MIC) and Subminimal Inhibitory Concentration (SIC) of acridine orange were determined based on inhibition growth dilution and a dilution under minimal inhibitory concentration, respectively (Baserisale *et al*., 2015).

EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) TESTING

Enterobacteriaceae isolates were subjected to Double-Disc Synergy Test (DDST) on Nutrient Broth agar with a 30-μg disk of cefotaxime (and/or ceftriaxone and/or ceftazidime and/or aztreonam) and a disk of amoxicillin-clavulanate (containing 10 μg of clavulanate) positioned at a distance of 30 mm (center to center) (Coque *et al*., 2008).

RESULTS AND DISCUSSION

The findings revealed pH (6.99±0.26 to 7.04±0.20) to indicate an alkaline waste water, with high Biological Oxygen Demand (161.31±27.40 to 164.25±27.40 mg/l) and Chemical Oxygen Demand (324.38±15.84 to 327.63±15.56 mg/l) values which were recorded to be higher than their permissible limits (30-50 and 60-90 mg/l) respectively (Table 1). The high levels of Biochemical Oxygen Demand and Chemical Oxygen Demand indicated

the presence of a high organic content resulting from the excessive waste that is generated from the hospital activities. Electrical conductivity measures the water's ability to conduct electricity, which provides a measure of dissolved ionic substances in water. The conductivity value of samples analysed were higher than standards set by Nigeria Regulatory Agency (DPR, 2002). However, in other to tell when the conductivity value of tested waste water is high or low, a baseline studies must have been conduct to establish a standard value for the said water over time (Yazdankhah *et al*., 2014). A very higher conductivity value is an indication that there are lots chemicals dissolved in the hospital wastewater. The physical parameters analysed in this study presented values higher than established permissible limits of effluent into the environment (DPR, 2002).

The heavy metals values (Table 1) were iron (12.79±10.32 to 13.11±10.60 mg/l), chromium (0.006±0.02 to 0.06±0.02 mg/l), cadmium (0.02±0.29 to 0.02±0.27 mg/l), zinc (5.80±1.08 to 5.72±1.19 mg/l). Heavy metals reported in this study were relatively higher than those set by environmental regulation agencies. Heavy metals could act as a selective pressure for the development of resistance and are a major concern in the treatment of hospital effluents and wastewater in general due to their toxic and detrimental effects (Yazdankhah *et al*., 2014).

The results of bacteriological analysis as shown in Tables 2-8, revealed the total heterotrophic bacterial counts ranged from 1.7 ± 0.13 x 10^7 cfu/ml to 11.3 ± 1.2 x 10^7 cfu/ml in point 1 (inlet) and 0.39 ± 0.11 x 10^7 cfu/ml to 13.8 ± 4.3 x 10^7 cfu/ml in point 2 (outlet). The coliform counts ranged from 1.8 ± 0.35 x 10^6 cfu/ml to 11.3 ± 1.1 x 10^6 cfu/ml in point 1 and 0.3 ± 0.04 x 10^6 cfu/ml to 14.0 ± 2.1 x 10^6 cfu/ml in point 2. The total staphylococcal counts ranged from 0.80 ± 0.23 x 10^5 cfu/ml to 22.7 ± 3.53 x 10^5 cfu/ml in point 1 and 0.67 ± 0.23 x 10^5 cfu/ml to 21.3 ± 3.52 x 10^5 cfu/ml in point 2. The isolates and their percentage frequency of occurrence from the two collection points were *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter* sp. and *Bacillus subtilis*. Gram-negative bacterial isolates were observed to be more prevalent with a total percentage frequency of 79.77% as against Gram positive bacterial isolates with 20.22%. A serious concern regarding hospital effluents is the high content of enteric pathogens which could be easily transmitted through water (Chitnis *et al*., 2004). Effluents from health facility where patients with enteric diseases are hospitalized often times presents an interesting challenge to public health due to the possibility of an infectious outbreak within the community as a result of the abundance of pathogenic organisms of the effluents (Chitnis *et al*., 2004).

The percentage resistance (Table 6) of the bacterial isolates against tested antibiotic were 29.21 % against septrin, 5.62 % against chloramphenicol, 24.72 % against sparfloxacin, 6.74 % against ciprofloxacin, 22.47 % against augmentin, 6.74 % against gentamicin, 10.11 % against streptomycin, 8.99 % against ampicillin and 14.61 % against pefloxacin. The result from the multiple antibiotics resistant index (MARI) of isolates from this study was significant at above 0.20 for *Klebsiella* sp. (0.9) *Staphylococcus epidermis* (0.9) and *Escherichia coli* (0.5) except for *Proteus* sp. (0.1). The resistance to more than one antibiotic represented a public health concern. The resistance demonstrated by isolates from this study to common antibiotics especially by Gram-negative bacterial isolates indicate a serious public health risk as detailed by global health regulatory bodies like the Centre for Disease Control and World Health Organization (Reygaert, 2018). Gram-negative bacteria present a challenge in the treatment of clinical infections globally due to the propensity of these organisms to rapidly develop resistance against antibiotics in use.

From this study, the Enterobactericeae demonstrated multiple drug resistance before and after curing and were subjected to ESBL test. An organism is interpreted as producing an extended-spectrum beta-lactamase (ESBL) if there is an increase in zone size of ≥5mm between the combination disc compared to that of the cephalosporin alone. Findings from this study revealed that the isolated and tested *Escherichia coli* and *Klebsiella* sp. were ESBL positive (Table 8). Extended-spectrum β-lactamases (ESBLs) producing organisms are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Extended-spectrum β-lactamases (ESBLs) represent an impressive example of the ability of Gram-negative bacteria to develop new antibiotic-resistance mechanisms in the face of the introduction of new antimicrobial agents and causing several diseases ranging from uncomplicated urinary tract infections to life-threatening sepsis (Rawati *et al*., 2010). Consequently, the isolation of ESBL organisms in hospital effluents is therefore of public health risk, which calls for urgent environmental review and concern.

Table 1: Physico-chemical parameters of hospital effluent

Parameters	INLET (Point 1)	OUTLET (Point 2)	T	P	*Regulatory Standards
Temperature (^{0}C)	28.69±1.15	28.75±0.92	0.140	0.889	40
pH	7.04±0.20	6.99±0.26	-0.621	0.539	6-9
Biochemical Oxygen Demand (mg/l)	161.31±27.40	164.25±27.40	0.303	0.764	30-50
Chemical Oxygen Demand (mg/l)	324.38±15.84	327.63±15.56	0.405	0.688	60-90
Phosphate (mg/l)	145.15±193.30	146.13±194.44	0.014	0.989	Not Stated
Turbidity (NTU)	307.31±72.05	301.00±72.27	-0.091	0.928	Not Stated
Electric Conductivity (uS/cm)	231.53±31.24	233.69±34.62	-0.072	0.943	Not Stated
Settleable solid (mg/l)	206.44±60.48	213.88±58.25	0.068	0.946	30
Total Suspended Solid (mg/l)	79.81±51.32	84.05±51.79	0.068	0.946	25
Iron (mg/l)	12.79 ± 10.32	13.11±10.60	-0.087	0.931	0.3
Chromium (mg/l)	0.006 ± 0.02	0.06 ± 0.02	0.364	0.719	0.05
Cadmium (mg/l)	0.20 ± 0.29	0.20 ± 0.27	0.045	0.965	0.003
Zinc (mg/l)	5.80 ± 1.08	5.72 ± 1.19	0.213	0.833	5

*DPR (2002)

Table 2: Total Heterotrophic Counts of Hospital Effluent

Sampling Period (Weeks)		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
1-4	THC ($\times 10^7$)	2.70± 0.13	0.39± 0.11	9.33±15.3	40.00±6.93	1.70±0.13	2.50±0.35	1.70±0.35	23.30±1.76
5-8	THC ($\times 10^7$)	2.50±0.13	2.53±0.71	5.73±18.80	2.00±0.46	4.00±12.70	0.40±0.23	4.40±0.46	3.73±0.35
9-12	THC ($\times 10^7$)	3.70±0.35	4.0±10.60	10.0±0.04	1.60±0.60	9.40±9.80	10.93±0.50	8.73±11.60	9.60±13.80
13-14	THC ($\times 10^7$)	11.30±11.2	11.0±29.00	1.9±0.46	13.80±14.30	5.70±3.53	4.30±0.35	5.20±0.69	4.80±10.10

Table 3: Total Coliform Counts of Hospital Effluent

Sampling Period (Weeks)		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
1-4	TCC ($\times 10^6$)	11.3.±10.10	14.0±23.10	6.30±0.93	3.60±1.40	1.60±4.62	2.30±3.53	6.00±0.69	3.60±10.90
5-8	TCC ($\times 10^6$)	9.30± 3.53	2.67± 1.30	34.7±7.42	0.30±0.04	10.67±4.10	2.40±1.30	5.70±1.40	1.90±0.48
9-12	TCC ($\times 10^6$)	3.80±7.90	1.90±0.48	6.00±6.11	4.27±0.48	3.37±5.81	3.33±1.41	8.30±9.30	12.40±32.30
13-14	TCC ($\times 10^6$)	1.80±0.35	2.30±70.50	3.6±0.71	38.70±5.81	2.70±3.52	11.0±30.0	4.70±8.11	4.40±6.11

Table 4: Total Staphylococal Count of hospital effluent

Sampling Period (Weeks)		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
1-4	TSC $(\times 10^5)$	0.90± 0.27	0.80±0.23	1.70±0.13	13.80±1.2	21.30±9.33	2.00±0.46	1.10±0.29	0.67±0.23
5-8	TSC$(\times 10^5)$	2.90±0.35	1.20±0.23	6.30±0.67	0.80±0.40	0.80±0.23	2.00±0.23	4.50±0.74	2.50±0.81
9-12	TSC$(\times 10^5)$	2.50±0.80	3.47±1.20	1.33±0.58	2.27±0.35	2.33±4.81	2.80±0.05	1.20±0.23	0.93±0.13
13-14	TSC $(\times 10^5)$	14.70±1.33	21.30±3.52	1.07±0.13	5.33±1.33	22.7±3.53	20.00±4.62	0.40±0.00	9.3±1.33

Table 5: Percentage frequency of occurrence of the bacterial isolates

Bacterial isolates	Inlet	Outlet
Proteus sp.	1(1.12%)	2 (2.25%)
Escherichia coli	9 (10.11%)	21 (23.59%)
Klebsiella sp.	14(15.73%)	13 (14.61%)
Enterobacter sp.	5 (5.62%)	6 (6.74%)
Staphylococcus aureus	3 (3.37%)	3 (3.37%)
Staphylococcus epidermidis	2 (2.25%)	4 (4.49%)
Bacillus subtilis	5 (5.62%)	1 (1.12%)

Table 6: Antibiotic sensitivity patterns of the bacterial isolates

Gram Negative	No. of isolate	MARI	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Proteus sp.	3	0.1	0(0)	0(0)	1(33.33)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Escherichia coli	30	0.6	10(33.3)	0(0)	4(13.3)	2(6.7)	4(13.3)	8(26.7)	0(0)	0(0)	0(0)	2(6.7)
Klebsiella sp.	27	0.3	6(22.2)	10(37.0)	5(18.5)	1(3.7)	1(3.7)	10(R)	4(14.8)	10(R)	10(R)	3(11.1)
Enterobacter sp.	11	0.4	5(45.5)	0(0)	4(36.4)	2(18.2)	0(0)	0(0)	0(0)	0(0)	0(0)	2(18.2)

Gram Positive	No. of isolate	MARI	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Staphylococcus aureus	6	0.9	2(33.3)	2(33.3)	3(50)	0(0)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	1(16.7)
Staphylococcus epidermidis	7	0.1	1(14.3)	2(28.6)	3(42.7)	1(14.3)	1(14.3)	1(14.3)	1(14.3)	1(14.3)	1(14.3)	1(14.3)
Bacillus subtilis	5	0.5	2(40)	0(0)	2(40)	0(0)	1(20)	0(0)	0(0)	1(20)	1(20)	0(0)

Legend: S = sensitive, R = resistant, I = intermediate, SXT = septrin 30 µg, CH = chloramphenicol 30 µg, SP = sparfloxacin 10 µg, CPX = ciprofloxacin 10 µg, AU = augmentin 10 µg, CN= gentamicin 10 µg, PEF = perfloxacin 10 µg, OFX = tarivid 10 µg, S = streptomycin 30 µg. S = ≥ 18mm, I = 15 – 17mm, R = ≤ 14mm

MAR index ≥ 0.2 (public health significance)

Gram Negative	No. of isolate	MARI	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Proteus sp.	1	0.1	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Escherichia coli	5	0.5	1(20)	0(0)	3(60)	2(40)	1(20)	2(40)	0(0)	0(0)	0(0)	2(40)
Klebsiella sp.	3	0.9	1(33.3)	1(33.3)	2(66.7)	1(33.3)	1(33.3)	0(0)	3(100)	1(33.3)	1(33.3)	3(100)
Enterobacter sp.	2	0.4	1(50.0)	0(0)	2(100)	1(50.0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)
Gram Positive			SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Staphylococcus aureus	1	0.3	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0))	0(100)	0(0)
Staphylococcus epidermidis	1	0.9	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)
Bacillus subtilis	1	0.3	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)

Legend: SXT = septrin 30 µg, CH = chloramphenicol 30 µg, SP = sparfloxacin 10 µg, CPX = ciprofloxacin 10 µg, AU = augmentin 10 µg, CN= gentamicin 10 µg, PEF = perfloxacin 10 µg, OFX = tarivid 10 µg, S = streptomycin 30 µg. S = ≥ 18mm, I = 15 – 17mm, R = ≤ 14mm, MAR index ≥ 0.2 (public health significance)

Table 8: Oxoid ESBL combination disc test results

Gram Negative	Cefpodoxime/ clavulanic acid (CD01) 10/1µg	Cefpodoxime (CPD10) 10µg	Difference	Interpretation
Escherichia coli	17mm	10mm	17-10=7mm	Positive
Klebsiella sp.	25mm	0mm	25-0=25mm	Positive

CONCLUSION

In conclusion, this study has revealed the chemical and bacteriological composition of hospital wastewater as a public health threat to the receiving community. The wastewater discharged from this hospital contains disease-causing bacteria and chemical components which include heavy metals with toxicological implications. The occurrence of extended spectrum beta lactamase producing bacteria in this study further explains the seriousness of its public health risk due to limitations in therapeutic option and huge cost in treatments associated with infections. Consequent to the above, strict monitoring and enforcement components should be implemented by appropriate regulatory agency to ensure adequate treatments of the generated wastewater before it's discharged into the receiving environment.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest.

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