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ASSESSMENT OF WASTEWATER AND MICROBIOLOGICAL CHARACTERISTICS FROM SELECTED HOSPITALS IN UMUAHIA METROPOLIS

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Abstract
There is an increasing universal awareness of environmental problems arising as a result of COVID- 19 pandemic and pollution especially in Nigeria. Among the source of this problem is effluent discharge from industries, particularly hospitals in arable farmlands and environs. Two outstanding hospital were purposively selected; Madonna Catholic Hospital and Abia Specialist Hospital in Umuahia, Abia State. Their wastewater samples were collected from three different wards; maternity, general private, and general out-patients department (GOPD) wards. Results obtained show significant variation in physiochemical properties in some wards and heavy metals across all wards. Seven bacteria species; *Staphylococcus aureus*, *Escherichia coli*, *Klesbsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacteriod sp* and *Streptococcus pyogenes* and one fungi specie- *Candida albican* were recorded from the samples. The bacterial load in Madonna ranged from 209.04 to 232.95cfu/ml in January, February, and March each and was statistically the same in the three wards (p>0.05). Fungi load ranged from 1.58 to 2.35cfu/ml in January, February, and March each and also significantly different at (p>0.05). The frequency of microbial characteristics isolated in the two hospital wastewater ranged from 33 to 100% with 100% of Staphylococcus aureus and Escherichia coli in all the wards, while other species varied significantly between 67 and 33% each. The results of the isolated bacteria from hospital wastewater showed resistivity to the tested antibiotics, and as therapeutic agents. Therefore, results call for need for urgent attention to be given to the discharge of wastewater from hospitals to ensure that food production around the environment is not contaminated.

Keywords: Hospital wastewater, resistant microorganisms, environmental monitoring, emerging contaminants

Introduction

Wastewater is any water contaminated as a result of being used for domestic, industrial, commercial, agricultural activities of humans. Wastewater can be characterized based on their source (Teklehaimanot *et al*., 2015). The development of medical and hospital related services and products and the resultant increased discharge of hospital wastewater directly into the environment has received increasing attention in the recent times (Emmanuel *et al*., 2005; Tchobanoglous *et al*., 2003; Amouei *et al*., 2010; Ekhaise and Omavwoya, 2008; Mahvi *et al*., 2009; Verlicchi *et al*., 2010). According to Gurel (2007), over 3000 chemical substances are used in human and veterinary medicine; including aquaculture and farming practices. The absence of specific pretreatment technologies for HWW also increased the frequency of gastro enteric viruses in aquatic bodies (Ibrahim *et al*., 2018). The direct discharge of hospital wastewater (HWW) into municipal wastewater containing disease-causing parasites has also increased the risk of skin infections and other harmful diseases in humans (Okjokwu and Inabo, 2014). Antibiotics are one of the most important medicines in hospitals. The estimates of global antibiotic consumption vary from 100,000 to 200,000 tonnes (Wise, 2002). The primary sources of antibiotic contamination of the environment are, waste from pharmaceutical plants (Larsson *et al*., 2007) and disposed unused antibiotics and excretion by humans and animals. Much of the antibiotics used in humans and animals are not metabolized and significant. The occurrence of antibiotics in the aquatic environment might promote the selection of antibiotic resistance genes and antibiotic resistant bacteria. This exacerbates the situation of antibiotic resistance, which is an increasingly a serious threat to global public health. This study is aimed to identify the environmental problems caused by the act of discharge of hospital wastewater (HWW) into the environment without proper management and treatment, and also, its adverse health implications.

Materials and Methods

Study Area

The study was carried out in Umuahia, capital of Abia State. Umuahia is bounded by Port-Harcourt to its South and Enugu city to its north. It has a population of 359,230 according to the 2006 Nigerian Population Census. Umuahia is located in the lowland rainforest zone of Nigeria, which lies between Latitude 05029′ to 05042′ North and Longitude 07029′ to 07033′ East. The area has an average rainfall of 2,238mm per year that is distributed over seven months rainy season period. It has bimodal peaks, the first occurring in the month of June or July and the second in the month of September. Its minimum and maximum temperatures are 23°C and 32°C respectively and a relative humidity of 60-80% (Ozabor and Nwagbara, 2018). Madonna Catholic Hospital (MCH), Abia Specialist hospital/ General hospital, Amachara are two among the biggest and most visited private and government owned hospitals in Umuahia respectively.

Collection of Wastewater Sample

In each of the selected hospitals, wastewater samples was collected from three different wards namely; maternity, general private, and GOPD wards for Madonna Catholic Hospital and general ward, laboratory section and dialysis unit for Abia Specialist Hospital. The wastewater samples were collected directly using clean sample bottles at the point the water is exiting the hospital ward building. The sites of the wastewater discharge were randomly selected, and samples were collected three times every month for three months. Sampling takes place in the morning. Each time the wastewater is collected, it will be handled to minimize degradation or alteration and taken to the laboratory without delay to determine their physico-chemical, heavy metals residues and microbiological properties.

Physicochemical Analysis of the Hospital Wastewater

The physico-chemical analysis of the wastewater samples were carried out following standard analytical methods for the examination of water and wastewater (APHA, 2001). Temperature, total dissolved solids, dissolved oxygen, conductivity, and pH were determined *in situ* using portable meters, while Chemical Oxygen Demand (COD), Biological Oxygen demand (BOD), nitrates and sulphates were analysed in the laboratory using spectrophotometer.

Microbiological analysis of the Hospital Wastewater

Inoculation and identification of microorganism: Pour plate technique was employed in the enumeration of the bacteria isolates. Exactly 1.0ml of wastewater sample was dispensed into sterile petri dish containing molten nutrient agar, Maconkey agar, Manitol salt agar and Sabouraud dextrose agar. Each plate was swirled gently for easy mixing of the wastewater (inoculum) and the media, and incubated aerobically at 37°C for 24 to 48 hours. All the bacterial counts were counted and recorded as colony forming units per ml (cfu/ml). The bacteria was characterized using cultural identification, Gram staining test and other biochemical tests including; Catalase, Starch hydrolysis, Coagulase, Indole, Citrate, Motility, Oxidase, Urease and Sugar fermentation according to Bergey's Manual of Determinative Bacteriology (9th edition).

***Multiple tubes fermentation techniques for coliform*:** The most probable number technique (MPN) was used to determine the coliform in the wastewater samples using the method of APHA (2001). The most probable number (MPN) of coliforms in the wastewater sample was estimated by the number of positive tubes corresponding with standard MPN statistical table and recorded as MPN/100ml.

Fungal count:For the fungal count, the sample dilutions were incubated in sterile *Sabouraud* Dextrose Agar plates supplemented with 0.05mg/ml chloramphenicol to suppress the growth of bacteria and spread with a sterile bent glass rod. The incubation period lasted for 4 days at room temperature. The fungal isolates were identified using their growth rate, colony morphology and microscopic morphological features.

***Antibiotic sensitivity test*:** Antibiotic susceptibility of the bacteria isolates was assayed according to the Kirby – Bauer disc diffusion method (Bauer *et al*., 1996). All the plates were incubated for 20 minutes before inoculation and placement of antibiotic disc to allow excess moisture to dry. After the drying, a single loop of each isolate was striked aseptically on the surface of agar plates and antibiotic sensitivity disc that contains Septrin (30µg), Chloramphenicol (30µ), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxicillin (30µg), Augmentin (25µg), Gentamycin (10µg), Pefloxacin (10µg), Tarivid (30µg) and Streptomycin (30µg) were aseptically laid on the surface of plates. The plates were incubated at 35°C for 24 hours. After the incubation, zone of growth of inhibition around each disc was measured and used to classify the organisms as sensitive or resistant to an antibiotic according to the interpretive standard of the clinical and Laboratory standards institute (CLSI).

Data Analysis

Mean and standard deviation were used to summarise the physico-chemical parameters, level of heavy metals and in the wastewater samples during the sampling period, including that of the microorganisms isolated. ANOVA will be used to compare the mean value of the physico-chemical parameters and the level of heavy metals of the wastewater's samples ($P < 0.05$). The Tukey-HSD test was considered for multiple comparisons within the treatment groups. All the data analysis was carried out using Statistical Package for Social Sciences (SPSS) version 22.

Results and Discussion

The result of the mean value of the physico-chemical characteristics of wastewater discharge in January, February and March 2021 from Madonna Catholic Hospital Umuahia Abia State are presented in Table 1.

Table 1: Mean ± Standard Deviation Value of Physico-chemical Characteristics from Madonna Hospital Wastewater within January to March

Parameters	Maternity ward	Private ward	GOPD	FEPA	WHO
pH	6.66 ± 0.1^{ax}	6.72 ± 0.09^{ax}	6.68 ± 0.09^{ax}	6-9	6.5-9.2
Temperature (°C)	20.48 ± 0.28^{ax}	20.49 ± 0.33^{ax}	19.94 ± 0.32^{ax}	<30	-
DO (mg/L)	14.41 ± 0.14^{by}	13.49 ± 0.42^{abz}	12.48 ± 0.25^{ay}	-	-
BOD (mg/L)	118.98 ± 1.55^{cz}	89.49 ± 1.25^{bz}	63.64 ± 1.51^{ay}	1	-
COD	694.65 ± 6.27^{ax}	748.59 ± 7.51^{bz}	685.33 ± 7.64^{ay}		-
Potassium (µg/L)	6.46 ± 0.13^{aby}	6.69 ± 0.15^{by}	6.05 ± 0.13^{az}	-	-
Nitrate (µg/L)	1.35 ± 0.02^{bx}	1.41 ± 0.03^{by}	1.24 ± 0.02^{ax}	20	200
Sulphate (µg/L)	15.4 ± 0.31^{ay}	16.02 ± 0.38^{ay}	15.86 ± 0.43^{ay}	500	20
EC (µS/cm)	240 ± 25.93^{ax}	222.33 ± 6.06^{ax}	217.67 ± 16.91^{ax}	-	300
TDS (mg/L)	600.33 ± 28.26^{ax}	413.67 ± 36.33^{ax}	488.33 ± 124.45^{ax}	2000	500

Results indicate mean ± standard error; For each parameter, a, b, c were used to compare each ward in the hospital, while x,y, z were used to compare each month. Parameters with different letters are significantly different (p<0.05). World Health Organization (WHO), Federal Environmental and Protection Agencies (FEPA)

The result from analyses of wastewater from Madonna, shows variation in pH and temperature, nitrate, sulphate, among the wards were not significantly different (P>0.05) (Table 1). They were equally less than FEPA and WHO recommended standard. The highest level of DO and BOD noted in Maternity ward were not significant (P>0.05). There is no significant different in potassium at (P >0.05), while electrical conductivity was below WHO standard and also not significant (P>0.05). TDS was observed to be higher (600.33mg/L) in maternity ward, compared to 500 permissible limits of WHO, but within FEPA recommended limit, while that in Private ward and GOPD ward were below FEPA AND WHO permissible limits.

Table 2: Mean ± Standard Deviation Value of Physicochemical Characteristics from Abia Specialist Hospital Wastewater within January to March

Parameters	Maternity	Private ward	GOPD	FEPA	WHO
pH	6.83 ± 0.1^{ax}	6.87 ± 0.1^{ax}	6.91 ± 0.1^{ax}	6-9	6.5-9.2
Temperature (°C)	21.69 ± 0.36^{ay}	23.62 ± 0.33^{by}	21.93 ± 0.33^{ay}	<30	-
DO (mg/L)	14.04 ± 0.12^{ay}	15.06 ± 0.22^{bz}	14.61 ± 0.13^{abz}	-	-
BOD (mg/L)	209.54 ± 3.74^{bx}	190.44 ± 2.98^{ax}	191.94 ± 3.21^{ax}	1	-
COD	594.83 ± 9.26^{ax}	615.02 ± 9.87^{ax}	608.63 ± 10.36^{ax}		-
Potassium (µg/L)	7.97 ± 0.19^{aby}	7.3 ± 0.11^{ay}	8.3 ± 0.17^{by}	-	-
Nitrate (µg/L)	1.37 ± 0.02^{ax}	1.33 ± 0.02^{ax}	1.32 ± 0.02^{ax}	20	200
Sulphate (µg/L)	17.12 ± 0.31^{ay}	17.3 ± 0.29^{ay}	17.09 ± 0.29^{ay}	500	20
EC (µS/cm)	203 ± 22.48^{ax}	187.33 ± 25.39^{ax}	238 ± 14^{ax}	-	300
TDS (mg/L)	628.33 ± 118.49^{ax}	643.33 ± 76.47^{ax}	526.67 ± 88.26^{ax}	2000	500

Results indicate mean ± standard error; For each parameter, a, b, c were used to compare each ward in the hospital, while x,y, z were used to compare each month. Parameters with different letters are significantly different (p<0.05). World Health Organization (WHO), Federal Environmental and Protection Agencies (FEPA)

The result from analyses of wastewater from Abia Specialist Hospital, had little or no variation in all the parameters between and within the wards, with no significant difference (P>0.05) (Table 2). However, the pH, nitrate, sulphate, EC were within the FEPA and WHO limits, while DO, COD were significantly different among the wards. There was no significant difference in TDS in all the wards and result shows that the values were below the limit recommended by FEPA and above the limits recommended by WHO.

Table 3: Result of Microbial Load in Madonna Catholic Hospital Wastewater

Month	Ward	Bacteria load × 10^7	Fungi load × 10^4
January	Maternity	225.45 ± 9.78^{ax}	2.7 ± 0.12^{by}
February		209.04 ± 9.07^{ax}	1.65 ± 0.07^{ax}
March		222.95 ± 9.67^{ax}	1.69 ± 0.07^{ax}
January	Private	220.46 ± 9.56^{ax}	1.74 ± 0.08^{ax}
February		232.58 ± 10.09^{ax}	1.68 ± 0.07^{ax}
March		226.16 ± 9.81^{ax}	1.58 ± 0.07^{ax}
January	GOPD	227.23 ± 9.86^{ax}	2.05 ± 0.09^{ay}
February		232.95 ± 10.1^{ax}	2.35 ± 0.1^{by}
March		217.24 ± 9.42^{ax}	1.65 ± 0.07^{ax}

Results indicate mean ± standard error; For each parameter, a, b, c were used to compare each ward in the hospital, while x,y, z were used to compare each month. Parameters with different letters are significantly different (p<0.05)

The bacterial load from Madonna ranged from 220.46×10^7 to 227.23×10^7cfu/ml (January) 209.04×10^7 to 232.95×10^7(February) and 217.24×10^7 to 226.16×10^7cfu/ml in March and had no significant difference in the three wards at each month ($p>0.05$) (Table 3). The fungi load ranged from 1.74 to 2.7×10^4 cfu/ml (January), 1.65 to 2.35×10^4cfu/ml (February), and 1.58 to 1.69×10^4cfu/ml in March and was significantly different between Maternity ward, and GOPD at ($p>0.05$).

Table 4: Result of Microbial Load in Abia Specialist Hospital Wastewater

Month	Ward	Bacteria load × 10^7	Fungi load × 10^4
January	General	240.43 ± 10.43^{ax}	2.19 ± 0.09^{cy}
February		218.67 ± 9.48^{ax}	0.92 ± 0.04^{ax}
March		216.89 ± 9.41^{ax}	1.99 ± 0.09^{by}
January	Laboratory	216.89 ± 9.41^{ax}	0.72 ± 0.03^{ax}
February		225.45 ± 9.78^{ax}	1.67 ± 0.07^{cz}
March		238.65 ± 10.35^{ax}	1.11 ± 0.05^{ay}
January	Dialysis	213.32 ± 9.25^{ax}	1.26 ± 0.06^{bx}
February		241.86 ± 10.49^{ax}	1.33 ± 0.06^{bx}
March		230.09 ± 9.98^{ax}	1.24 ± 0.05^{ax}

In the samples from Abia Specialist Hospital, bacterial load ranged from 213.32 to 240.43×10^7cfu/ml, 218.67 to 241.86×10^7cfu/ml, 216.89 to 238.65×10^7 cfu/ml in January, February, and March, respectively, and was statistically the same in the three wards at each month ($p>0.05$) (Table 4). The fungi load ranged from 0.72 to 2.19×10^4cfu/ml, 0.92 to 1.67×10^4 cfu/ml and 1.11 to 1.99×10^4cfu/ml in January, February, and March, respectively, and was significantly different among the three wards in January and February. It also differed between General ward and others in March.

Tables 5 and 6 show the result of the biochemical characterization and physical identification of pure microbial isolates in the wastewater samples from Madonna Hospital and Abia Specialist Hospital; the presence of nine bacteria species and two fungi species were recorded. In addition to the biochemical characteristics indicated in *Staphylococcus aureus* appeared deep yellow; *Escherichia coli* as mucoid white (yellowish) colonies; *Klebsiella spp* bright pink, *Pseudomonas aeruginosa* greenish-blue; *Proteus vulgaris* formed large milk coloured colonies; while *Bacteriodes* appeared as large brown flat colonies. *Streptococcal* colonies appeared whitish and glisten. On Sabourand Dextrose Agar (SDA), fungal yeast cell appeared as smooth large elevated whitish-brown colonies. This is an indication of the presence of only *Candida albican.*

Table 5: Frequency of Characterization and identification of pure microbial isolates from Madonna Hospital wastewater

Bacteria	Gram stain	Motility	Indole	Starch hydrolysis	Catalase	Coagulase	Urea	Oxidase	Lactose	Citrate
Staphylococcus aureus	+	-	-	-	+	+	-	-	+	-
Escherichia coli	-	+	+	-	-	-	-	-	+	-
Klesbsiella pneumonia	-	-	-	-	-	-	+	-	+	+
Pseudomonas aeruginosa	-	+	-	-	-	-	-	+	-	-
Proteus vulgaris	-	+	+	-	-	-	+	-	-	+
Bacteriodesspp	-	+	-	-	-	-	+	-	-	+
Streptococcus pyogenes	+	-	-	-	-	-	-	-	+	-

+ = *Positive reaction, - = Negative reaction*

Table 6: Frequency of Characterization and identification of pure microbial isolates from Abia Specialist Hospital Wastewater

Bacteria	Gram stain	Motility	Indole	Starch hydrolysis	Catalase	Coagulase	Urea	Oxidase	Lactose	Citrate
Staphylococcus aureus	+	-	-	-	+	+	-	-	+	-
Escherichia coli	-	+	+	-	-	-	-	-	+	-
Klesbsiella pneumonia	-	-	-	-	-	-	+	-	+	+
Pseudomonas aeruginosa	-	+	-	-	-	-	-	+	-	-
Proteus vulgaris	-	+	+	-	-	-	+	-	-	+
Becteriod spp	-	+	-	-	-	-	+	-	-	+
Streptococcus pyogenes	+	-	-	-	-	-	-	-	+	-

+ = *Positive reaction, - = Negative reaction*

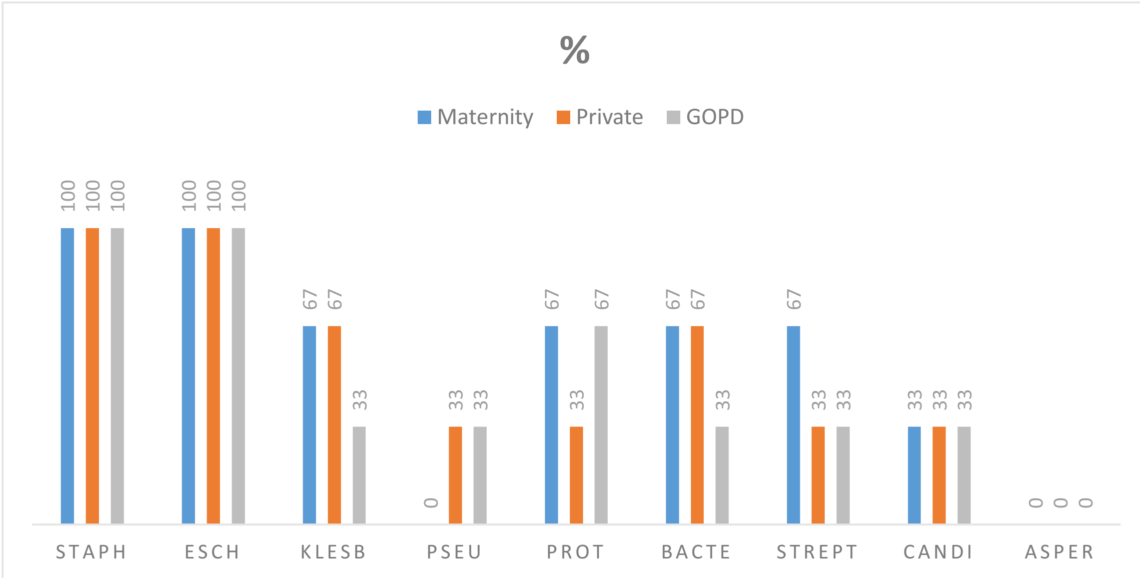

Figure 1: Frequency of Microbial Isolates in Madonna Catholic Hospital Wastewater

In Figure 1, *Escherichia coli* and *Staphylococcus aureus* had 100% occurrence in all the wastewater samples from all the wards. This is followed by equal % of 67 of *Klesbsiella Spp,* in Maternity and private wards, 67% *Proteus spp, in* maternity and GOPD, 67% of *bacteriodes spp* was observed in maternity and private wards. Others were 67% of *streptococcus spp* only in maternity ward; 33% of *Klesbsiella,* in GOPD ward; 33% of *Klesbsiella spp and Pseudomonas* each were absent in maternity ward, and present in private and GOPD wards; 33% of *Proteus spp* in maternity and Private wards, 33% *bacteriodes spp*, only in GOPD ward; 33% of *streptococcus* in Private and GOPD wards; while 33% of *candidiasis* were noted in all the wards each; while *Aspergillus spp* was not found in wastewater sample in all the wards.

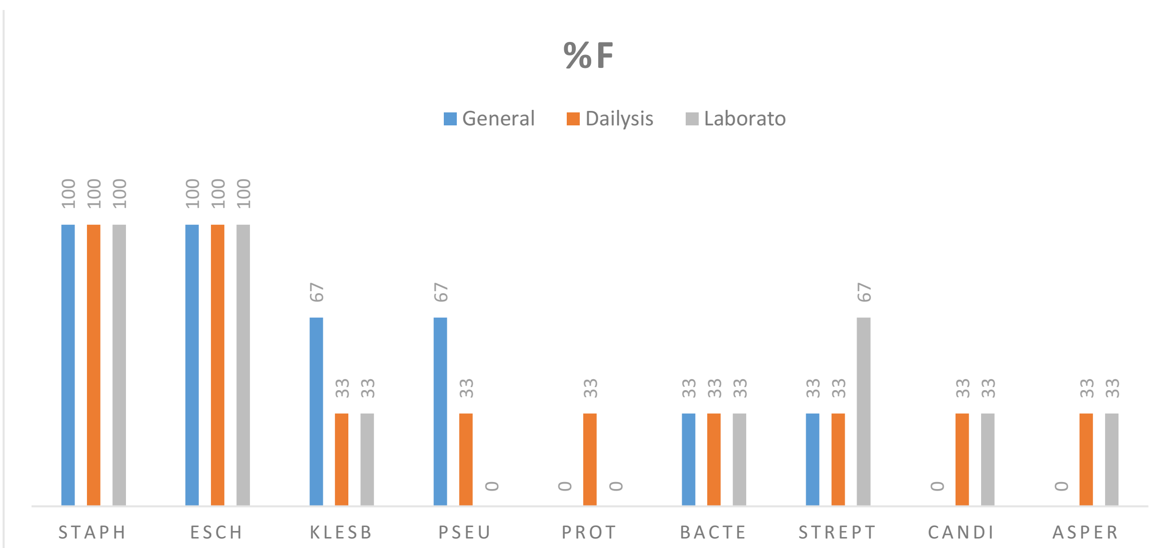

Figure 2: Frequency of Occurrence of Microbial Isolates in Abia Specialist Hospital Wastewater

In Figure 2, *Staphylococcus aureus* and *Escherichia coli* had 100% occurrence in all the wastewater samples from all the wards; followed by 67% of *Klesbsiella Spp,* and *Pseudomonas sp* each, observed in General ward, with 67% *streptococcus spp* in lab.; 33% of *Klesbsiella,* was observed in Dialysis and Laboratory wards each, also 33% *Pseudomona spp* in Dialysis and absent in Lab; 33% of *Proteus spp* was present in Dialysis but absent in General ward and laboratory; 33% of B*acteriodes spp* were noted in all the wards with 33% *streptococcus spp* in both General and Dialysis wards; same 33% of *Candidiasis spp* and *Aspergillus spp* were noted in both Dialysis and Laboratory but absent in the general ward.

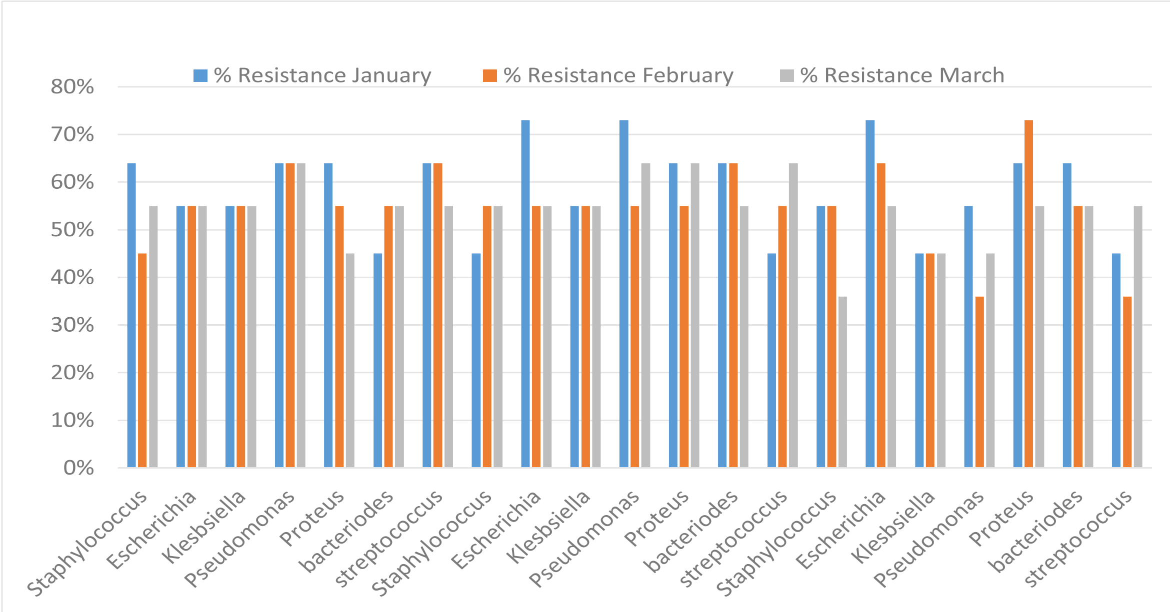

Figure 3: Antibiotic Sensitivity of bacteria isolated from Madonna Hospital Wastewater

The percentage of the isolates from Madonna hospital wastewater that are resistant to the tested antibiotics indicates they have become resistant to some of the tested antibiotics, and are thus multi-resistant to these therapeutic agents for *Staphylococcus*; Erythromycin, Amoxicillin, Augmentin, Septrin, Ceprofloxacin and Chloramphenicol. *Escherichia was* resistant to Ampicillin, Augmentin, Amoxicillin, Septrin and Ceprofloxacin. *Klesbsiella* was resistant to Ofloxacin, Erythromycin, Amoxicillin, Ceprofloxacin, Peflacin and Chloramphenicol. *Pseudomonas* was resistant to Streptomycin, Ampicillin, Gentamycin, Erythromycin, Amoxicillin, Septrin and Chloramphenicol. *Proteus* was resistant to Ampicillin, Erythromycin, Amoxicillin, Septrin, Ceprofloxacin, Peflacin and Chloramphenicol. *Bacteriodes* was resistant to Ampicillin, Ofloxacin, Gentamycin, Erythromycin and chloramphenicol. *Streptococcus* was resistant to Ofloxacin, Gentamycin, Amoxicillin, Augmentin, Septrin, Ceprofloxacin and Peflacin.

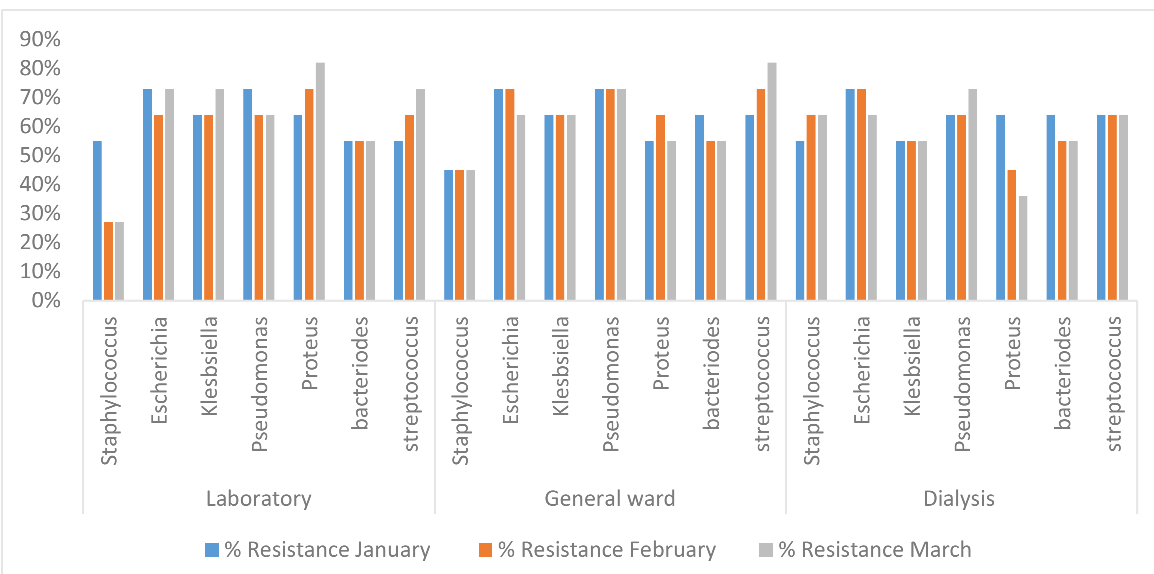

Figure 4: Antibiotic Sensitivity of bacteria isolated from Abia Specialist Hospital

Figure 4 shows the results of antibiotic sensitivity of bacteria isolated from Abia Specialist hospital wastewater. The percentage resistance indicates the extent the bacteria have become resistant to the tested antibiotics and are thus multi-resistant to these therapeutic agents. These drugs would be ineffective as treatments for infections caused by these pathogens. *Staphylococcus* was resistant to Ampicillin, Nalidixic Acid, Augmentin, Rifampicin, Ciprotab and Chloramphenicol. *Escherichia was* resistant to Ampicillin, Ofloxacin, Levofloxacin, Augmentin, Rifampicin, Ciprotab, Peflacin, and Chloramphenicol. *Klesbsiella* was resistant to Ofloxacin, Gentamycin, Nalidixic Acid, Levofloxacin. Rifampicin, Ciprotab, Peflacin and Chloramphenicol. *Pseudomonas* was resistant to Streptomycin, Ampicillin, Ofloxacin, Gentamycin, Levofloxacin, Augmentin, Rifampicin, and Chloramphenicol. *Proteus* was resistant *to* Streptomycin, Ampicillin, Nalidixic Acid, Levofloxacin, Augmentin, Rifampicin, Ciprotab, Peflacin, and Chloramphenicol. *Bacteriodes* was resistant to Ampicillin, Ofloxacin, Gentamycin, Levofloxacin, Rifampicin, and Chloramphenicol. *Streptococcus* was resistant to Ampicillin, Ofloxacin, Nalidixic Acid, Rifampicin, Chloramphenicol, Ciprotab, Peflacin, and Augmentin.

Table 7: Mean values of Heavy Metals in Madonna Hospital Wastewater

Heavy metals (mg/L)	Maternity ward	Private ward	GOPD	FEPA	WHO
Arsenic	0.058 ± 0.005^{by}	0.053 ± 0.004^{bxy}	0.018 ± 0.002^{ax}	-	-
Cadmium	0.074 ± 0.006^{by}	0.027 ± 0.002^{ay}	0.032 ± 0.003^{ay}	<1	0.01
Lead	0.02 ± 0.002^{ax}	0.03 ± 0.003^{ay}	0.071 ± 0.006^{bx}	<0.05	0.05
Mercury	0.007 ± 0.001^{by}	0.004 ± 0^{ay}	0.006 ± 0.001^{aby}	0.05	-
Chromium	0.002 ± 0^{ax}	0.007 ± 0.001^{by}	0.003 ± 0^{ax}	<1	-
Nickel	0.028 ± 0.002^{ax}	0.048 ± 0.004^{by}	0.045 ± 0.004^{bxy}	<1	-
Iron	0.06 ± 0.005^{bx}	0.02 ± 0.002^{ax}	0.071 ± 0.006^{bz}		-

Results in Table 7 shows that Arsenic, Lead, Cadmium, Mercury, Chromium, Nickel were significantly different (P<0.05) among the wards and are within the limit of FEPA and WHO. Iron concentration in Private and GOPD ward were also significantly different (P<0.05), however, iron in Maternity ward was not significant (P>0.05).

Table 8: Mean values of Heavy Metals in Abia Specialist Hospital Wastewater

Heavy metals (mg/L)	General ward	Dialysis ward	Laboratory ward	FEPA	WHO
Arsenic	0.071 ± 0.006^{by}	0.01 ± 0.001^{ax}	0.01 ± 0.001^{ax}	-	-
Cadmium	0.01 ± 0.001^{az}	0.01 ± 0.001^{ay}	0.01 ± 0.001^{ay}	<1	0.01
Lead	0.081 ± 0.007^{by}	0.081 ± 0.007^{by}	0.03 ± 0.003^{ay}	<0.05	0.05
Mercury	0.006 ± 0^{bx}	0.008 ± 0.001^{bx}	0.003 ± 0^{ax}	0.05	-
Chromium	0.01 ± 0.001^{ax}	0.01 ± 0.001^{ax}	0.02 ± 0.002^{bx}	<1	-
Nickel	0.052 ± 0.004^{bx}	0.049 ± 0.004^{by}	0.014 ± 0.001^{ax}	<1	-
Iron	0.03 ± 0.003^{abx}	0.04 ± 0.003^{bx}	0.025 ± 0.002^{ax}		-

The level of arsenic and lead in wastewater from general ward and laboratory respectively as well as chromium and nickel in wastewater from Dialysis ward differed significantly from other wards at (p<0.05) (Table 8) . The level of iron in wastewater differed only between samples from dialysis and laboratory (p<0.05).

The pH of wastewater was slightly acidic and within the range reported by Ekhaise and Omavwoya (2008) and Mesdaghinia *et al*. (2009). pH of water is a critical factor that influences the solubility of heavy metals in water and the growth of microorganisms (Hu *et al*. (2004), while Temperature influenced the level of dissolved oxygen (DO) in water which in turn could promote the growth of aerobic or anaerobic microbes. The pH of the water is known to affect the availability of micronutrients and trace and heavy metals. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are estimates of the degree of organic and inorganic pollution respectively. BOD measures the amount oxygen required by microorganisms to breakdown organic matter, while COD measures the amount of oxygen required to degrade inorganic matters (Chukwura and Okpokwasili, 1997). The concentrations of BOD and COD in all the sampling points were higher than the values of Mojeed *et al.* (2018). High BOD and COD concentrations observed in the wastewater reflect the presence of diverse inorganic and organic substances including; pharmaceuticals, radionuclides, solvents and disinfectants used for medical purposes. The BOD values reported in this study is lower than the mean values of 291mg/L reported by Sarafraz *et al*. (2007) in wastewaters from Hormozgan hospitals, and 444.3mg/L reported by Mesdaghinia *et al*. (2009) in Teheran hospital. On the other hand, the COD values reported in this study is similar to the mean COD of 628mg/L reported by Sarafraz *et al*. (2007), but lower than the mean COD (792 mg/L) reported by Mesdaghinia *et al.* (2009). Nitrate and sulphate play important roles in the recycling of nitrogen and sulphur respectively. In this study, the concentration of potassium, nitrate, and sulphate, was lower than FEPA (1991) acceptable limits with some degree of variation among the sampling points. Therefore, the level of potassium, nitrate and sulphate produced in the hospitals if discharged into water bodies may not adversely affect the environment and human health as supported by Orias *et al*. (2013). Other parameters used in defining wastewater are electrical conductivity and total dissolved solids, while electrical conductivity was above the FEPA permissible limits of 125mg/l, total dissolved solids was below the permissible limits of 2000mg/L set by FEPA (1991). In general, the hospital wastewater if disposed untreated may adversely impact the environment given that some physicochemical properties are above the permissible regulatory standards. This agrees with the findings of Emmanuel *et al*. (2009) and Verlicchi *et al*. (2010). Microbial pollutants are indicators of water quality. Indicator microorganisms can also serve as indicators of pollution sources (Paillard *et al.,* 2005). For example *coliforms, Escherichia coli*, and *streptococci* are used as indicators of faecal contamination of water sources (Momba and Mfenyana, 2005). In this study, the presence of pathogenic microorganisms is a potential risk to the recipient environmental matrices according to

Oyeleke and Istifanu (2009). Pathogenic organisms pose great harm to the public and environmental health. Aluyi *et al*. (2006) reported that high bacteria counts are an indication of the presence of organic matter and pollution of the environment. The microbes found in the hospital wastewater are consistent with the findings of previous studies (Giroletti and Lodola 1993; Oyeleke, 2009). A study reported on HWW of Brazil also confirmed the presence of other bacterial species including *Citrobacter freundii, Klebsiella ornithinolytica, Proteus mirabilis, Pantoea agglomerans,* and *Serratia rubidace* (Changes *et al*., 2011). Hospital wastewater may not be favorable for the growth of fungi; the occurrence of fungi was low in this study and is consistent with the report of Eze and Onwurah (2015). In all, the presence of micro-organisms in the hospital wastewater may be linked to the diverse forms of disease brought in by patients. However, bacterial and fungal populations may play important role in cycling nutrients such as phosphate, nitrates and sulphates in the environment, thus; making them available for use by other life forms (Hocquest *et al*., 2016). The extensive use of antibiotics in medicine and veterinary practice has led to the incidence and spread of antibiotic resistance bacteria. The use, misuse and under-use of antibiotics are responsible for resistance development to bacterial antimicrobials worldwide. In this study, varying degree of multidrug resistant bacteria was observed amongst the isolates from the hospital wastewater. Lateef (2004) reported that in developing countries, drugs are available to the public and thus people may practice self – administration of antibiotics and further increase the prevalence of drug resistant strains. Similar occurrence of antibiotic-resistant bacteria was reported in hospital wastewater (Rabbani *et al.,* 2017; Sharmin *et al.,* 2018; Wang *et al.,* 2018). In this study, *E. coli* had the highest resistance to the all drugs tested; this could be attributed to the abundant nature of *E. coli* in environment and its easy adaptability. Similar trend of high resistance pattern of *E. coli* from hospital sewage have been reported (Rabbani *et al.,* 2017; Sharmin *et al.,* 2018; Wang *et al.,* 2018). Multiple drug resistance is an extremely serious public health problem and it has been found to be associated with the outbreak of major epidemic throughout the world. Thus, the multiple – drug resistance shown by these pathogens are worrisome and of public health concern (Lateef, 2004). The occurrence of multiple antibiotic resistant pathogenic bacteria encountered in this study represents a well-known phenomenon that carries a negative impact for public health, an observation that it is in consonance with the reports of Torogolu *et al*. (2005). The presence of heavy metals in hospital wastewater is a major concern due to their toxic effects. This in addition to the fact that heavy metals are non-biodegradable implies that the discharge of the wastewater into the environment possess danger because they can bio-accumulate in exposed organisms and become biomagnified in the food chain to toxic levels (Hussein *et al.,* 2005). In this study, heavy metals were found in the wastewater at levels which fall within the World Health Organization (WHO, 2013) and Federal Environmental and Protection Agencies (FEPA, 1991) the permissible limits. In aquatic ecosystems, heavy metals greatly depress the number of living organisms. Heavy metals have negative effect on the growth of aquatic organisms and can cause serious upsets in biological wastewater treatment plants.

Conclusion

In recent times, the emphasis towards management of HWW and various studies focused on the microbial communities present in wastewater have increased immensely. The pathogenic microbes present in HWW have affected human health since decades and antibiotic resistance microbes are also increasing significantly. The development of resistance toward antibiotics has been observed worldwide and has challenged both public and animal health. The use and release of various antibiotic agents in different settings have not only led to the prevalence of Antibiotic-resistance genes in the environment but also spread and emergence of resistant bacteria. This has caused increased resistance in human pathogens and thus, making infections caused by them difficult to deal with, leading to higher mortality rates. Also Long term exposure to heavy metals can bio-accumulate in the environment leading to diverse human health problems such as high blood pressure, cancer, heart disease, affect reproduction and nerve effects etc. On the other hand it can lead to growth inhibition, affect photosynthesis and reduction of various species of animals and plants within the locality. More so the presence of microorganisms in the hospital wastewater may cause diarrhea, fever, cramps, vomiting among many other health challenges. There is also the challenge of indiscriminate release of antibiotics through the HWW, leading to antibiotics resistance bacterial. The basic responsibility for pollution control lies with the legislature, there is need to comply with all the waste water regulations and guidelines to ensure that the effluents standards set by international and national regulatory bodies are not exceeded. The state environmental agencies should also conduct a periodical monitoring of these hospitals to enforce compliance to these regulations, and sensitization of both health workers and patients in the appropriate way to discharge off their wastewater, for sustainable environment and total wellbeing of the general public. Hospital wastewater should be treated before discharging into the environment. This can be done by proper regulatory procedures which the management of the hospitals should monitor effectively; through the engagement of qualified professionals responsible for management of the environment.

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