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# Antibiotic Susceptibility Pattern of Hospital Wastewaters' Bacteria from Oyun Local Government Area of Kwara State, Nigeria.

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## ABSTRACT

The increasing prevalence of antibiotic resistance is a major health problem worldwide. This study determined the prevalence and antibiotic susceptibility patterns of bacteria isolated from hospital wastewaters collected from selected hospitals in Oyun Local Government Area of Kwara State, Nigeria. A cross sectional study design was conducted from April, 2018 to March, 2019. A total of 18 composite samples were aseptically collected, transported and analyzed for enumeration of microorganisms, bacteriological identification and susceptibility testing following standard procedures. The Global Positioning System (GPS) coordinates of each site location was equally taken and data obtained were entered and analyzed using SPSS version 20. The means bacterial count population of wet season samples ranged between 9±1.00 and 270±24.46 (x $10^5$cfu/ml), while that of dry season samples ranged between 68±2.00 and 280±17.32 (x $10^5$cfu/ml) respectively. Among the total samples, 25 bacterial isolates were detected and 13(52%) were from wet season samples and 12(48%) were from dry season samples. The most frequently isolated bacteria from wet season samples were *Alcaligenes faecalis* 8(61.5%) followed by *A. aquatilis* 2(15.4%) *Providentia rettgeri* 2(15.4%) and *Streptomyces* sp. 1(7.7%). Similarly in dry season samples *A. faecalis* 9(75.0%) and *P. rettgeri* 3(25.0%) were frequently detected. The findings from antibiotic susceptibility pattern on both the wet and the dry seasons isolates indicated that ofloxacin (OFL) demonstrated highest antimicrobial potency against the test isolates, with Zone inhibition diameters (mm) (resistant ≤12, intermediate 13-15 and susceptible ≥ 16**),** while cefixime (CXM) has the least potency with Zone inhibition diameters (mm) (resistant ≤15, intermediate 16-18 and susceptible ≥ 19), among the selected antibiotics. Thus, hospital wastewater should be prevented from getting into municipal water supply to avoid infections associated with *A. faecalis, A. aquatilis, P. rettgeri* and *Streptomyces* sp.

**Key words**

Hospital wastewaters, wet season, dry season, bacteria, antibiotics

## 1.1 INTRODUCTION

Hospital wastewater is an ideal media for microorganisms and carries the resistant gene into the sewage system (Abdel-Rouf *et al.,* 2012; Fekadu *et al.,* 2015). Hospital wastewater inhabits a numerous persistent chemical compounds and complex mixtures of organic matter such as pharmaceuticals, radionuclides, detergents, antibiotics, antiseptics, surfactants, solvents, medical drugs heavy metals, radioactive substances (Aurelien *et al.,* 2013; Ferrando-Climent *et al.,* 2014) and microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* (Anitha and Jayraaj, 2012; Ferrando-Climen*et al.,* 2014**)**.

Large quantities of antibiotics are daily used for patient care and control of infection in hospitals and appreciable concentration of these antibiotics is excreted through faces and urine of patient and reaches liquid wastes. Hence, wastewater from hospitals contains resistant gene and antibiotic residues that inhibit the growth of susceptible microorganism through selection pressure (Beyene and Redaie, 2011; Stalder *et al.*, 2014). Also, some of these compounds and microorganisms defecated by patients are found in Hospital wastewater and then, enter the municipal sewer network without preliminary treatment. For this reason, this composition leads to broad levels of toxicity, genotoxicity and organic load and consequently, results to wholly adverse impact on the natural ecosystem and inherent health hazard to human (Wilde *et al.*, 2013).

Hospitals release large amounts of microbial and chemical agents in their wastewaters, inherent chemicals present in hospital wastewater belong to different groups, such as antibiotics, X-ray contrast agents, disinfectants and pharmaceuticals. Many of these chemical compounds resist normal wastewater treatment. They end up in surface waters where they can negatively affect the aquatic ecosystem and hinder the food chain. Humans are particularly at receiving end by the drinking water produced from surface water (Pauwels and Verstraete, 2006). Resistant bacteria in the environment carry transmissible gene, by acting as a vector or reservoir of resistant gene (Pandey *et al.*, 2011; Keen and Patrick, 2013).

Hospitals also release the most dangerous micro-organisms threating human health, and wastewaters are one of the most serious pollutants discharging to the environment (Diwan *et al.*, 2010; Pandey *et al.*, 2011). Microbial agents of special concern are multi-resistant microbial strains. These microbial agents are allegedly accused to contribute to the spread of antibiotic resistance (Pauwels and Verstraete,2006). The multidrug resistance pattern seen in microbial isolates from hospital wastewater include most of the antibiotics used presently. Therefore the problem is the transfer of this resistance to pathogenic microorganism to lead currently available antibiotics to be vain (Pauwels and Verstraete, 2006). The aim of this study is to investigate the microorganisms available in hospital wastewaters analyzed and determine the susceptibility pattern of bacteria isolated to some commonly used antibiotics.

### 1.2 Scope of the Study

The investigation on the a microbiological study of hospital wastewater on the study site in this work is based on qualitative analyses just to further establish the claims of previous researchers

## 2.0 MATERIALS AND METHODS

### 2.1 Study site

This study was conducted on wastewater samples collected from Oyun Local Government Area of Kwara State, Nigeria

### 2.2 Population size of the study

Eighteen (18) wastewater samples collected during wet and dry seasons from Oyun Local Governments Area of Kwara State between June – August, 2018 and October –December, 2018 formed the population size of this study.

### 2.3 Inclusion criteria

Those included in this project were all the hospitals located within Oyun Local Government Area of Kwara State, Nigeria whose owners allowed the collection of wastewater samples for this research work.

### 2.4 Exclusion criteria

Those excluded were those hospitals within Oyun Local Governments Area of Kwara state whose owners did not give consent.

### 2.5 Collections of hospital wastewater Samples

Two sets of Nine (9) hospital wastewater samples were collected from Oyun Local Government Area of Kwara State, Nigeria. The first set of nine samples (**wet season samples**) were collected between the months of April – October, 2018 while the second set of nine samples (**dry season samples**) were collected between the months of November, 2018 – March, 2019. The samples were collected from hospital laboratory units into wide mounted sterile plastic containers with screw cap tops (universal bottles) corked tightly. The containers were labeled with date, time and sites of collection, and transported inside ice berg to Microbiology laboratory for culturing of bacteria

### 2.6 Isolation and Enumeration of Bacterial Colonies from Hospital Wastewater Samples

Fivefold serial dilution was carried out on collected wastewater samples, Aliquot of 1 ml of the diluents were pipetted into Petri-dishes and pour plated with about 20 ml of molten nutrient agar at about 45 $^0$C and allowed to gel, the isolation of bacteria from wastewater was done according to the methods of Cheesbrough (2010). Colonies were counted using a colony counter.

### 2.7 Preparation of Pure Isolates of Bacterial Colonies from Hospital Wastewater Samples

After 24 hours of incubation, pure isolates were prepared from the mixed culture with the aid of sterile wire loop, a district colony was taken and streaked on a freshly prepared solidified nutrient agar and incubated for 24 hours at 37 $^0$C to get pure and distinct colonies. This was repeated several times until satisfactory pure isolates were obtained.

### 2.8 Conventional identification of Bacterial Isolates in Hospital Wastewater Samples

The identity of all isolates was determined using standard conventional methods as reported by Cheesbrough (2010). The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 hours and subsequently sub-cultured on to differential selective media such as Eosin Methylene blue agar and MacConkey agar. Biochemical tests were also carried out on distinct colonies to further ascertain the identity of the isolates. The bacterial isolates were tentatively identified by means of morphological characteristics, cellular and biochemical tests. Morphological characteristics were observed for each bacterial colony after 24 hours of growth. The appearance of the colony of each isolate on the media was studied and the characteristics observed include; cell shape, elevation, edge, optical characteristics, consistency colony surface and pigmentation. Biochemical tests carried out include; catalase, production of hydrogen sulphide ($H_2S$), indole, urease, methyl red, oxidase, coagulase, motility, citrate utilization, methyl red, voges-proskauer, starch hydrolysis and sugar fermentation. The results were compared with Bergey's Manual of Determinative Bacteriology (Fawole and Oso, 2007).

### 2.9 Molecular Identification of Bacterial Isolates

#### 2.9.1 Extraction of DNA using CTAB method

DNA was extracted from hospital wastewater isolates by a standard CTAB genomic DNA isolation method (Doyle and Doyle, 1990) as follows: 1ml of 24 hour broth culture was transferred into 1.5ml eppendorf tube and spun at 14,000rpm for 30 minutes (to harvest the cell). 400µl of a pre-warmed CTAB buffer (at 60 $^0$C) containing Proteinase k and β-mercapto

ethanol was added. Then 75 µl of 10% SDS (sodium deodycylsulphate) was added and heated in water bath at 65 $^0$C for 30 minutes. 500 µl chloroform was added and mixed for 15minutes (to purify the DNA) spun at 10,000 rpm for 10mins. The supernatant was collected in eppendorf tube to which 500 µl isopropanol and 1µl (100mg/ml) RNase were added and incubated for 30 min at 37 $^0$C. The resultant mixture was kept at -20 for 24 hours, spun at 10,000rpm for 10 minutes. The supernatant was gently decanted and the pellet was washed with 200 µl of 70% ethanol, gently mixed and spun at 10,000rpm for 5mins. The extracted DNA was air dried for 30 minutes to 1 hour (to eliminate all traces of alcohol) and finally re-suspended in 200 µl of sterile distilled water

### 2.9.2 Quantification of Extracted DNA

Quantification of DNA concentration and purity of the samples were measured using Nano-Drop® 2000 spectrophotometer. The ratio of 260/280 absorbance was used to assess the purity of DNA with ratios ~1.8 being accepted as pure

### 2.9.3 PCR analysis of 16S

PCR analysis was run with a universal primer called 16S. The PCR mix comprises of 1µl of 10 x buffer, 0.4 µl of 50mM $MgCl_2$, 0.5 µl of 2.5mMdNTPs, 0.05 µl of 5 units/µlTaq with 2 µl of template DNA and 6.05 µl of distilled water to make-up 10 µl reaction mix. The PCR profile used is initial denaturation temperature of 94 $^0$C for 3minutes, followed by 30 cycles of 94 $^0$C for 60 seconds, 56 $^0$C for 60 seconds, 72 $^0$C for 120seconds and the final extension temperature of 72 $^0$C for 5 minutes and the 10 $^0$C on hold for few hours.

### 2.9.4 Purification of PCR products

The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10 µl of the PCR product, 1 µl of 2M NaAct pH 5.2 was added, followed by 20 µl Absolute Ethanol, kept at -20 $^0$C for 1hr, spun at 10,000 rpm for 10 mins, washed with 70% ethanol and then air dried. Re-suspended in 5µl sterile distilled water and kept at 4 $^0$C for sequencing.

## 2.10 Standardization of Inoculums for Antibiotic Sensitivity Test (0.5 McFarland standard)

About 0.1 ml of 1 % Barium chloride was added to 9.9 ml of 1 % surphuric acid which was later reconstituted into 10 ml of sterile distilled water to make 0.5 ml McFarland standard solution. The broth culture of 24 hours test organism was then compared in terms of turbidity to 0.5 % McFarland. A loopful of the standardized culture was used for antibiotic sensitivity assay.

## 2.11 Antibiotics Sensitivity Test

The antibiotic sensitivity of the bacterial species isolated was performed on Mueller-Hinton agar (MHA) (Merck) plates by disk diffusion method as described by the National Committee for Clinical Laboratory Standard Institute (CLSI, 2017). A 0.1 ml of each bacterial isolate was seeded into each of the Petri dishes containing Mueller-Hinton agar and allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: Ceftazidine (CAZ, 30µg), Cefuroxime (CRX, 30µg), Gentamicin (GEN, 10µg), Ceftriaxone (CTR, 30µg), Erythromycin (ERY, 5µg) Cloxacillin (CXC, 5µg), Ofloxacin (OFL, 5µg), Augmentin (AUG, 30µg), Cefixime (CXM, 5µg), Nitrofrantion (NIT, 300µg), Ciprofloxacin (CPR, 5µg) (Liverpool L9 7AR, UK) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and incubated at 37 $^0$C overnight. Zones of inhibition after incubation were observed and the Interpretation was made using susceptibility breakpoints of CLSI,

2017 and diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a metal caliper and the isolates were classified as sensitive, intermediate and resistant.

### 2.12 Data Quality Assurance

Sample collection, handling, transportation and microbiological analysis and interpretation of results were carried out using standard operating procedures (SOPs). Prior to the actual work Reagents, media and antimicrobial disks were checked for expiry date, damage and storage problems. Laboratory equipments were properly cleaned and sterilized before use. Media preparation was made based on the respective manufacturer's directions. 5 % of media per batch/prepared was incubated overnight for sterility check.

### 2.13 Data analysis

Data obtained were analyzed using analysis of variance (ANOVA) and mean separated using Duncan's Mean Multiple Rang Test (IBM-SPSS) 20 version). Differences were considered significant at $p < 0.05$.

## 3.0 RESULTS

### 3.1 Mean Count of Bacteria Isolated from Hospital Wastewaters

There were significant differences ($p < 0.05$) among the means counts of bacteria in the entire samples. Samples from site D recorded highest means bacterial count (270±26.46 x $10^5$cfu/ml) followed by that collected from site C (220±72.11 x $10^5$cfu/ml) during the wet season periods. Also site C recorded highest means bacterial count (280±17.32) and site H recorded the least means bacterial count (68±2.00 x $10^5$cfu/ml) during the dry session periods (Table 1).

### 3.2 Bacteria Isolated from Hospital Wastewaters

Using conventional and molecular methods of bacterial identification, *A. aquatilis* strain YFMCD4.2 were found present in the sample from sites A and G., *A. faecalis* strain HX2016003 were found in the sample from sites A, C and F during the wet season period. On the other hand *A. faecalis* strain JBW4 were found in the samples from sites A, C and E, *A. faecalis* strain HX2016003 were found present in the samples from sites B, D and F, *P. rettgeri* strain RB151 were found in the samples from sites B, D and G (Table 2).

### 3.3 Prevalence/Percentage of Bacteria Present in Hospital Wastewater

Of the total bacteria isolated 13(100%), during wet season periods, *A. faecalis* HX2016003 and *A. faecalis* JBW4 have the highest prevalence of 3(23.08%) and 3(23.08%) respectively, followed by *A. faecalis* C10, *A. aquatilis* strain YFMCD4, *P. rettgeri* strain RB151 and *S. nodosus* strain N56010 with percentage occurrences of 2(15.39%), 2(15.39%), 2(15.39%) and 1(7.69%) respectively. Whereas of total bacteria isolated 12(100%) during the dry season period, *A. faecalis* JBW4, *A. faecalis* HX2016003, *A. faecalis* C10 and *P. rettgeri* RB151 were all of the same percentage occurrences of 3(25%), 3(25%), 3(25%) and 3(25%) respectively (Table 3).

### 3.4 Antibiotics susceptibility pattern of bacterial Isolates from hospital wastewater

The findings from *in-vitro* antibiotic susceptibility pattern on the bacteria isolates in hospital wastewater samples collected during wet season periods revealed that OFL (ofloxacin) demonstrated highest antimicrobial activities, followed by GEN (gentamicin) with percentages susceptibilities of 100 and 84.6 respectively, while NIT (nitrofurantoin), CPR (ciprofloxacin), CTR (ceftriaxone), AUG (augmentin), CXM (cefixime), CAZ (ceftazidine),

CRX (cefuroxime), CXC (cloxacillin) and ERY (erythromycin) displayed least susceptibility of 0.0 percentage (Table 4).

The findings form *in-vitro* antibiotic susceptibility pattern on the bacteria isolated from samples obtained during dry season periods indicated that OFL (ofloxacin) and GEN (gentamicin) demonstrated highest antimicrobial activities with percentage susceptibility of 100 followed by NIT (nitrofurantoin) with percentage susceptibility of 50 (Table 5).

### 3.5 Antibiotics Sensitivity Profile of Bacterial Isolates from Hospital Wastewater

All bacterial isolates from samples collected during wet season periods were found to be 100% resistant to Ceftazidine, Cefuroxime, Erythromycin, Cloxacillin, Augmentin and Cefixime, and they were equally found to be 100% sensitive to Ofloxacin. *A. faecalis* C10 and *A. faecalis* JBW4 were found to be 100% sensitive to Gentamicin and Nitrofurantoin while only *A. faecalis* C10 was 100% sensitive to Ciprofloxacin. (Table 6)

All bacterial isolates from samples collected during the dry season periods were found to be 100% resistant to Ceftazidine, Cefuroxime, Erythromycin, Cloxacillin, Augmentin and Cefixime, and they were equally found to be 100% sensitive to Gentamicin and Ofloxacin., *A. faecalis* JBW4 and *A. faecalis* HX2016003 were found to be 100% sensitive to Nitrofurantoin. Also among the isolates only *A. faecalis* JBW4 was 100% sensitive to Ciprofloxacin (Table 7)

The total resistance of bacterial isolates from wet season samples was higher for Ceftazidine, Cefuroxime, Erythromycin Cloxacillin, Augmentin and Cefixime 13/13 (100%) followed by Ceftriaxone 8/13 (61.5%) and Nitrofurantoin 7/13 (53.8%). However, relatively lower resistances were observed among bacterial isolates to Gentamycin 0/13 (0.0%), Ofloxacin 0/13 (0.0%) and Ciprofloxacin 0/13 (0.0%) (Figure 1).

The total resistance of bacterial isolates from the dry season samples was higher for Ceftazidine, Cefuroxime, Erythromycin, Cloxacillin, Augmentin and Cefixime 12/12 (100%) followed by Ceftriaxone 9/12 (75.0%) and Nitrofurantoin 6/12 (50.0%). However, relatively lower resistance was observed among bacterial isolates to Gentamicin 0/12 (0.0%), Ofloxacin 0/12 (0.0%) and Ciprofloxacin 0/12 (0.0%) (Figure 2).

**Table 1:** Mean Count of Bacteria Isolated from Hospital Wastewaters from Oyun Local Government Area of Kwara State, Nigeria.

| S/N | Samples' Sites | Samples Sites' Coordinates | Wet season | Dry season |
|---|---|---|---|---|
| | | | Mean Population x $10^5$cfu per ml | Mean Population x $10^5$cfu per ml |
| 1 | A | Lat. 8.13363 Long. 4.71133 | 170±26.46$^{b}$ | 160±20.00$^{b}$ |
| 2 | B | Lat. 8.08573 Long. 4.59696 | 35±5.00$^{a}$ | 101±18.19$^{a}$ |
| 3 | C | Lat. 8.107606 Long. 4.65911 | 220±72.11$^{bc}$ | 250±65.57$^{d}$ |
| 4 | D | Lat. 8.1779983 Long. 4.739203 | **270±26.46$^{c}$** | **280±17.32$^{d}$** |
| 5 | E | Lat. 8.1638913 | 170±30.00$^{b}$ | 193±15.13$^{bc}$ |

| | | | | |
|---|---|---|---|---|
| | | Long. 4.7314038 | | |
| 6 | F | Lat. 8.17066<br>Long. 4.73158 | 174±64.09[b] | 234±28.36[cd] |
| 7 | G | Lat. 8.17422<br>Long. 4.73334 | **9±1.00[a]** | 70±10.00[a] |
| 8 | H | Lat. 8.25128<br>Long. 4.66282 | 19±1.75[a] | **68±2.00[a]** |
| 9 | I | Lat. 8.25239<br>Long. 4.65358 | 190±10.00[b] | 230±26.46[cd] |

Values with the same alphabet in the same column are not significantly different while values with different alphabet are significantly different ($\alpha < 0.05$)

**Table 2: Bacterial Isolated from hospital wastewater sample collected from Oyun Local Government Area Kwara State Nigeria.**

| S/N | Samples' Sites | Wet Season Microorganisms | Dry Season Microorganisms |
|---|---|---|---|
| 1 | A | *A.aquatilis* (Strain YFMCD4.2)$OYW_1$<br>*A. faecalis* (Strain HX2016003)OYW1 | *A. faecalis* (Strain JBW4)$OYD_1$ |
| 2 | B | *A. faecalis* (Strain JBW4)$OYW_1$ | *A. faecalis* (Strain HX2016003)$OYD_1$ |
| 3 | C | *A. faecalis* (Strain HX2016003)$OYW_2$<br>*P. rettgeri* (Strain RB151)OYW1 | *A. faecalis* (Strain JBW4)$OYD_2$<br>*P. rettgeri* (Strain RB151)OYD1 |
| 4 | D | *A. faecalis* (Strain C10) $OYW_1$<br>*St. sp*. (strain N562010)OYW1 | *A. faecalis* (Strain HX2016003)$OYD_2$<br>*P.rettgeri* (Strain RB151)OYD2 |
| 5 | E | *A. faecalis* (Strain JBW4)$OYW_2$ | *A. faecalis* (Strain JBW4)$OYD_3$ |
| 6 | F | *A. faecalis* (Strain HX2016003)$OYW_3$<br>*P. rettgeri* (Strain RB151)OYW2 | *A. faecalis* (Strain HX2016003)$OYD_3$ |
| 7 | G | *A.aquatilis* (Strain YFMCD4.2)$OYW_2$ | *A. faecalis* (Strain C10) $OYD_1$<br>*P. rettgeri* (Strain RB151)OYD3 |
| 8 | H | *A. faecalis* (Strain C10) $OYW_2$ | *A. faecalis* (Strain C10) $OYD_2$ |
| 9 | I | *A. faecalis* (Strain JBW4)$OYW_3$ | *A. faecalis* (Strain C10) $OYD_3$ |

**KEY:**
*A. aquatilis = Alcaligenesaquatilis, A. faecalis = Alcaligenes faecalis,*
*P. rettgeri = Previdenciarettgeri, St. nodosus= Streptomyces nodosus*
OYW = Oyun wet isolate, OYD =Oyun dry isolate,

**Table 4: Percentage Susceptibility, intermediary and resistance of Antibiotics on Normal Isolates of hospital wastewater samples collected from Oyun Local Government Area between months of wet season periods**

| *S/N* | *Antibiotics* | *No of tested isolates* | *No of susceptible isolates* | *No of intermediate isolates* | *No of resistant isolates* | *% Susceptibility* | *% intermediary* | *% Resistance* |
|---|---|---|---|---|---|---|---|---|
| 1 | Ceftazidine | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 2 | Cefuroxime | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 3 | Gentamicin | 13 | 11 | 2 | 0 | 84.6 | 15.4 | 0 |
| 4 | Ceftriaxone | 13 | 0 | 5 | 8 | 0 | 38.5 | 61.5 |
| 5 | Erythromycin | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 6 | Cloxacillin | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 7 | Ofloxacin | 13 | 13 | 0 | 0 | 100 | 0 | 0 |
| 8 | Augmentin | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 9 | Cefixime | 13 | 0 | 0 | 13 | 0 | 0 | 100 |
| 10 | Nitrofurantoin | 13 | 6 | 0 | 7 | 46.2 | 0 | 53.8 |
| 11 | Ciprofloxacin | 13 | 3 | 10 | 0 | 23 | 77 | 0 |

**Table 5: Percentage Susceptibility, intermediary and resistance of Antibiotics on Normal Isolates of hospital wastewater samples collected from Oyun Local Government Area between months of November 2018 – March, 2019**

| *S/N* | *Antibiotics* | *No of tested isolates* | *No of susceptible isolates* | *No of intermediate isolates* | *No of resistant isolates* | *% Susceptibility* | *% intermediary* | *% Resistance* |
|---|---|---|---|---|---|---|---|---|
| 1 | Ceftazidine | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 2 | Cefuroxime | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 3 | Gentamicin | 12 | 12 | 0 | 0 | 100 | 0 | 0 |
| 4 | Ceftriaxone | 12 | 0 | 3 | 9 | 0 | 25 | 75 |
| 5 | Erythromycin | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 6 | Cloxacillin | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 7 | Ofloxacin | 12 | 12 | 0 | 0 | 100 | 0 | 0 |
| 8 | Augmentin | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 9 | Cefixime | 12 | 0 | 0 | 12 | 0 | 0 | 100 |
| 10 | Nitrofurantoin | 12 | 6 | 0 | 6 | 50 | 0 | 50 |
| 11 | Ciprofloxacin | 12 | 3 | 9 | 0 | 25 | 75 | 0 |

**Table 6: Antibiotic Sensitivity Profile of Isolates of Hospital Wastewater Sample collected from Oyun Local Government Area during wet season periods**

| Bacterial Isolates | | Antibiotics Used N (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CAZ | CRX | GEN | CTR | ERY | CXC | OFL | AUG | CXM | NIT | CPR |
| | **S** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) |
| **AaYFMCD4OYW(2)** | **I** | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 2(100) |
| | **R** | 2(100) | 2(100) | 0(0) | 2(100) | 2(100) | 2(100) | 0(0) | 2(100) | 2(100) | 2(100) | 0(0) |
| | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 0(0) |
| **AfHX2016003OYW(3)** | **I** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(100) |
| | **R** | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) |
| | **S** | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 2(100) | 2(100) |
| **AfC10OYW(2)** | **I** | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| | **R** | 2(100) | 2(100) | 0(0) | 0(0) | 2(100) | 2(100) | 0(0) | 2(100) | 2(100) | 0(0) | 0(0) |
| | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 3(100) | 0(0) |
| **AfJBW4OYW(3)** | **I** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(100) |
| | **R** | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 0(0) | 0(0) |
| | **S** | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) |
| **PrRB151OYW(2)** | **I** | 0(0) | 0(0) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 2(100) |
| | **R** | 2(100) | 2(100) | 0(0) | 0(0) | 2(100) | 2(100) | 0(0) | 2(100) | 2(100) | 2(100) | 0(0) |
| | **S** | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 1(100) | 1(100) |
| **StsN562010OYW(1)** | **I** | 0(0) | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| | **R** | 1(100) | 1(100) | 0(0) | 0(0) | 1(100) | 1(100) | 0(0) | 1(100) | 1(100) | 0(0) | 0(0) |

**Key**: AaYFMCD4OYW = *Alcaligenesaquatilis* (Strain YFMCD4.2) Oyun Wet Isolate, AfHX2016003OYW = *Alcaligenesfaecalis* (Strain HX2016003) Oyun Wet Isolate AfJBW4OYW = *Alcaligenesfaecalis* (Strain JBW4) Oyun Wet Isolate , AfC10OYW = *Alcaligenesfaecalis* (Strain C10) Oyun Wet Isolate, PrRB151OYW = *Providentiarettgeri* (Strain RB151) Oyun Wet Isolate, StsN562010OYW =*Streptomyces* sp. (strain N562010) Oyun Wet Isolate, R = Resistant, S = Susceptible I= intermediate , Ceftazidine (CAZ, 30µg), Cefuroxime (CRX, 30µg), Gentamicin (GEN, 10µg), Ceftriaxone (CTR, 30µg), Erythromycin (ERY, 5µg) , Cloxacillin (CXC, 5µg), Ofloxacin (OFL, 5µg), Augmentin (AUG, 30µg), Cefixime (CXM, 5µg), Nitrofurantoin (NIT, 300µg), Ciprofloxacin (CPR, 5µg)

**Table 7: Antibiotic Sensitivity Profile of Isolates of hospital wastewater sample collected from Oyun Local Government Area during the dry season periods**

| Bacterial Isolates | | Antibiotics Used N (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CAZ | CRX | GEN | CTR | ERY | CXC | OFL | AUG | CXM | NIT | CPR |
| **AfJBW4OYD(3)** | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 3(100) | 3(100) |
| | **I** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| | **R** | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 0(0) | 0(0) |
| **AfHX2016003OYD(3)** | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 0(0) |
| | **I** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(100) |
| | **R** | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) |
| **AfC10OYD(3)** | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 3(100) | 0(0) |
| | **I** | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(100) |
| | **R** | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 0(0) | 0(0) |
| **PrRB151OYD(3)** | **S** | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 0(0) |
| | **I** | 0(0) | 0(0) | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(100) |
| | **R** | 3(100) | 3(100) | 0(0) | 0(0) | 3(100) | 3(100) | 0(0) | 3(100) | 3(100) | 3(100) | 0(0) |

**Key:**
AfJBW4OYD =*Alcaligenesfaecalis* (Strain JBW4) Oyun Dry Isolate, AfHX2016003OYD =*Alcaligenes faecalis* (Strain HX2016003) Oyun Dry Isolate, PrRB151OYD =*Previdenciarettgeri* (Strain RB151) Oyun Dry Isolate , AfC10OYD =*Alcaligenesfaecalis* (Strain C10) Oyun Dry Isolate, R = Resistant, S = Susceptible I= intermediate, Ceftazidine (CAZ, 30µg), Cefuroxime (CRX, 30µg), Gentamicin (GEN, 10µg), Ceftriaxone (CTR, 30µg), Erythromycin (ERY, 5µg) Cloxacillin (CXC, 5µg), Ofloxacin (OFL, 5µg), Augmentin (AUG, 30µg), Cefixime (CXM, 5µg), Nitrofurantoin (NIT, 300µg), Ciprofloxacin (CPR, 5µg)

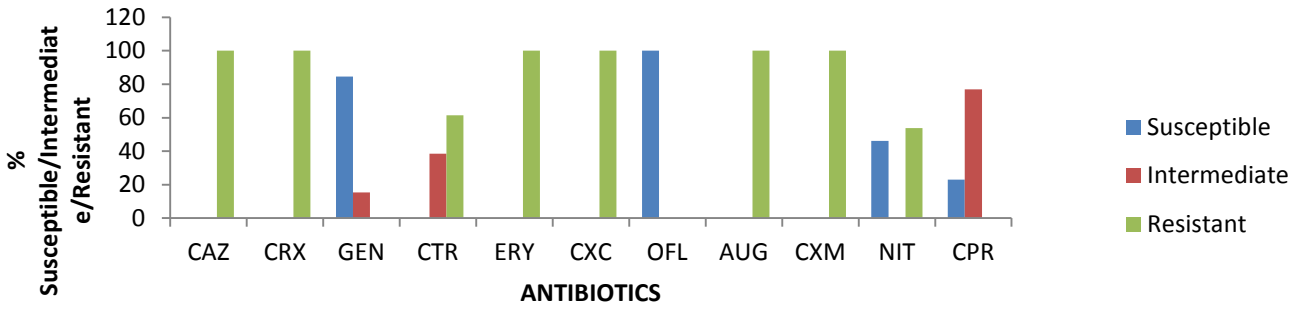


**Fig 1: Total Antibiotic Sensitivity Profile of Isolates of hospital wastewater sample collected from Oyun Local Government Area during wet season periods**

Ceftazidine (CAZ, 30µg), Cefuroxime (CRX, 30µg), Gentamicin (GEN, 10µg), Ceftriaxone (CTR, 30µg), Erythromycin (ERY, 5µg), Cloxacillin (CXC, 5µg), Ofloxacin (OFL, 5µg), Augmentin (AUG, 30µg), Cefixime (CXM, 5µg), Nitrofurantoin (NIT, 300µg), Ciprofloxacin (CPR, 5µg)

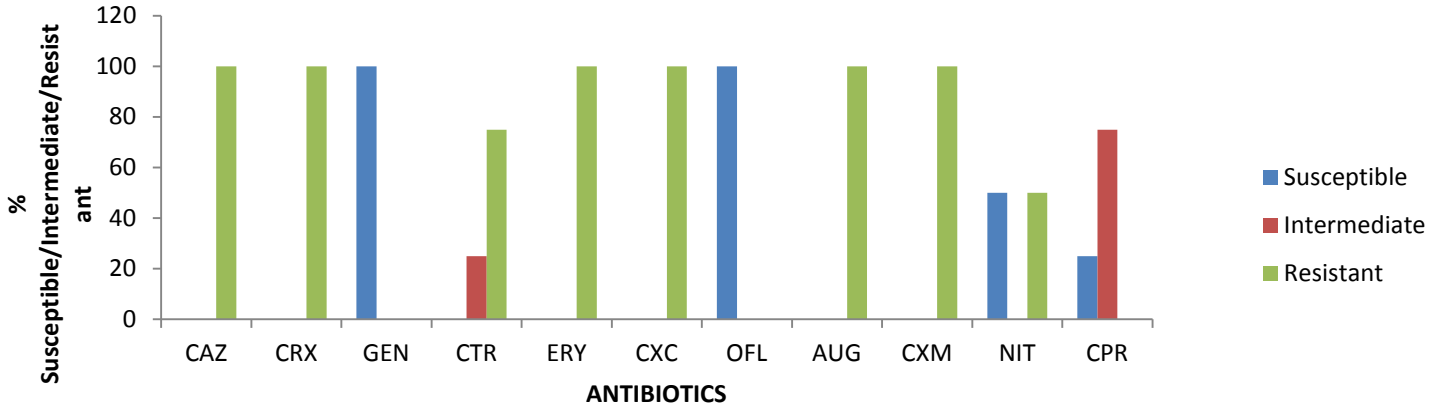


**Fig 2: Total Antibiotic Sensitivity Profile of Isolates of hospital wastewater sample collected from Oyun Local Government Area during dry season periods**

Ceftazidine (CAZ, 30µg), Cefuroxime (CRX, 30µg), Gentamicin (GEN, 10µg), Ceftriaxone (CTR, 30µg), Erythromycin (ERY, 5µg) Cloxacillin (CXC, 5µg), Ofloxacin (OFL, 5µg), Augmentin (AUG, 30µg), Cefixime (CXM, 5µg), Nitrofurantoin (NIT, 300µg), Ciprofloxacin (CPR, 5µg)

**Table 3: Prevalence/Percentage of Bacteria present in Hospital Wastewaters collected from Oyun Local Government Area of Kwara State**

| Bacterial isolates ( Wet season) | N (%) | Bacterial isolates ( Dry season) | N (%) | Total (%) |
|---|---|---|---|---|
| *A. faecalis* 3(HX2016003) | 3(23.08) | *A. faecalis* 3(HX2016003) | 3(25.00) | 6(24.00) |
| *A. faecalis* 3(JBW4) | 3(23.08) | *A. faecalis* 3(JBW4) | 3(25.00) | 6(24.00) |
| *A. faecalis*2(C10) | 2(15.39) | *A faecalis* 3(C10) | 3(25.00) | 5(20.00) |
| *A. aquatilis* 2(YFMCD4) | 2(15.39) | - | 0(0.00) | 2 (8.00) |
| *P. rettgeri* 2(RB151) | 2(15.39) | *P. rettgeri* 3(RB151) | 3(25.00%) | 5(20.00) |
| *Streptomyces* sp. 1(N562010) | 1(7.69) | - | 0(0.00%) | 1(4.00) |
| **Total** | 13(100) | | 12(100. %) | 25(100) |

N = number of isolate, % = percentage present, - = absent

### 4.1 Discussion

The were significant difference ($p < 0.05$) among the means bacterial populations of the entire samples. The present study showed that mean bacterial plate counts for the wet season ranged between 9±1.00 and 270±26.46 (x $10^5$ cfu/ml) and that of dry season samples ranged between 68±2.00 and 280±17.32 (x $10^5$ cfu/ml) respectively. And these values exceeded the permissible limit of Environment Protection Agency, EPA [2002] and Health Protection Agency, HPA [2005](<1000 cfu/ml) and also failed to fulfill the requirements of the revised guidelines on the quality of treated wastewater used in agriculture, in public parks (<5 × $10^3$cfu/100ml) (Carr *et al*. (2004). High density of bacteria of 270±26.46 x $10^5$ cfu/ml and 280±17.32 x $10^5$ cfu/ml both during wet and dry season periods respectively was an indication of environmental pollution due to human activities.

The findings from this study agreed with 1.6 x $10^6$ cfu/ml recorded by Tsegahun *et al.* (2017) on wastewater at Ayder Referral Hospital, Mekelle North Ethiopia. The variations observed in the values of the mean bacterial populations among hospitals in Oyun Local Government Area may be due to variation in the rate of people's patronage at different hospitals which is a function of location, accessibility, health care facility and personnel, this accounts for high microbial means population recorded in site D which happened to be one of the major in the Local Government Area. Also it was observed that higher density of microbial population was obtained during dry season than wet season, because preference of specific microorganism to specific temperature ranges for growth and activity can have impacts on the composition of the microbial community (Fierer and Schimel 2003; Singh *et al.*, 2010).

The bacterial isolates from this study vary from that obtained by Tsegahun *et al*. (2017) on wastewater from Ayder Referral Hospital, Mekelle North Ethiopia, where *Klebsiella* spp 10 (20%), *P. aeruginosa* 8(16%), *S. aureus* 8(16%), *E. coli* 7(14%) and *Salmonella* spp 6(12%) were detected, the results also disagrees with the result of Fekadu *et al.* (2015) who reported presence of *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *S. aureus* from effluent collected from Ethiopia, Hawassa University Referral Hospital, Likewise different from the study in India by Chitnis *et al.* (2000) that showed large numbers of enteric-bacteria *S. aureus* and *P. aeruginosa*. The result is also somewhat dissimilar to work of Danchaivijitr *et al.* (2005) and Salem *et al*. (2011) who claimed availability of pathogenic bacteria like *Vibrio* spp. and *Salmonella* spp. in Thailand and Tunisia hospital effluents respectively.. The

absence of some pathogenic bacteria in the hospital wastewater analyzed may be due to variation in geographical and climatically condition as shifting of microbial community occurs in favor of the species which are better adapted to higher temperatures and have accelerated rates of growth (Castro *et al.*, 2010; Fierer and Schimel 2003; Singh *et al*. 2010) More so, inter specific competition among microorganisms may cause shift in microbial community, such that microorganisms that compete favorably among the mixed community due to several factors such as population density, inhibitory metabolites, etc. will be prevailing. The highest prevalence of *A. faecalis* may be due to the fact that it is highly associated with urinary tract infection (UTI) which is not uncommon in hospital environment, and production of toxic metabolites might also favor availability of *Streptomyces* sp.

The resistance of isolates obtained during the wet season periods to ceftazidine, cefuroxime, erythromycin, cloxacillin, augmentin and cefixime is similar to that obtained in India where isolates showed simultaneous resistance for ampicillin, ampicillin with clavulanic acid, cotrimoxazole, tetracycline, first, second and third generation cephalosporins in the final effluent of wastewater treatment plant (Katouli *et al.*,2012). Study in Alexandria, Egypt also showed the presence of antibiotic resistant extended spectrum B-lactamase (ESBL) producing bacteria at the end of wastewater purification process (Amine, 2013), posing a risk of its spread to the environment and subsequent human and animal exposure. Overall resistance of bacteria isolated during wet season periods for ceftriaxone, nitrofurantoin and ciprofloxacin were found to be 61.5%, 53.8% and 0.0% respectively. Similarly, all bacterial isolates from dry season samples were found to be 100% resistant to ceftazidine, cefuroxime, erythromycin, cloxacillin, augmentin and cefixime, and they were equally found to be 100% sensitive to gentamicin and ofloxacin. Similar results was reported by (Iweriebor *et al.* (2015) from Alice, Eastern Cape province of South Africa and European countries [Servais and. Passerat, (2009)], where higher rate of resistance in bacterial isolates from final effluent of wastewater treatment plant was found. Higher percentage resistance of isolates from hospital wastewater suggests that, hospital wastewater could have contributed massively to the resistances observed among the isolates from the final effluent. These can be due to the fact that, hospital wastewater contains a diverse group of pathogenic, commensal and environmental bacteria. This characteristic composition makes sewage particularly suitable ecological niche for the growth and spread of antibiotic resistance due to selection pressure and horizontal gene transfer (Davies and Davies, 2010; Periasamy and Sundaram, 2013; Cantona *et al.*, 2013)

## 4.2 Conclusion and Recommendations

In the present study, dry season hospital wastewaters have more bacterial load than wet season hospital wastewater samples.The organisms isolated from the hospital wastewaters analyzed include, *Alcaligenes faecalis, A. aquatilis, Providencia rettgeri* and *Streptomyces* sp. And *A. faecalis* were the most predominant among the isolates from hospital wastewater samples analysed followed by *P. rettgeri*, which exceeded the WHO, HPA, EPA and FAO standard permissible levels. Ofloxacin had the highest potency followed by gentamycin while augmentin, cefixime, ceftazidine and cloxacillin had the least potency among the selected antibiotics. The high prevalence of drug resistant isolates from hospital wastewater samples suggesting their persistence in the hospital environment, and their ability to spread antibiotic resistance due to selection pressure and horizontal gene transfer. Therefore Liquid waste treatment system should be developed to disinfect pathogens in hospital wastewater effluent before discharging into municipal water supply.

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### 4.4 Disclaimer
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