Appraisal on Microbiological Qualities of Hospital Wastewaters from Off a Local Government Area of Kwara State, Nigeria

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Authors’ contributions
This work was carried out in collaboration among all authors. Author UKM designed the study, carried out microbiological analyses of the samples and wrote the first draft of the manuscript, author ADJ. Managed the microbiological analyses of the samples author EFO managed the literature searches and the three of them read and approved the final manuscript.

Article Information
DOI: 10.9734/JAMB/2021/v21i930381
Editor(s): (1) Dr. Simone Aquino, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Brazil. (2) Sayed Mohammad Reza Khoshroo, Islamic Azad University, Iran. (3) Talib M. Albayati, University of Technology, Iraq.
Reviewers:
Complete Peer review History: https://www.sdiarticle4.com/review-history/72670

ABSTRACT

Aims: This study investigates the microorganisms associated with hospital wastewaters collected from the Offa Local Government Area of Kwara State, Nigeria, during the wet and dry seasons.

Study Design: This project was a cross sectional descriptive study in which subjects were hospital wastewater samples collected from the study site.

Place and Duration of Study: The samples were analyzed in the department of microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Methodology: Microbiological study of one hundred and twenty-six samples of hospital wastewater collected both during wet season and dry season periods in the years 2018 – 2019 from Offa Local Government Area of Kwara State, Nigeria, was carried out using conventional and molecular techniques respectively and the Global Positioning System (GPS) of each collection site was accurately recorded.

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Results: The microbial load of wet season samples collected from Offa Local Government Area ranged between 7±4,00 and 150±43.59 while that of dry season samples ranged between 10±2.00 x 10^5 and 225±67.27 (x 10^5 cfu/ml). The bacteria isolated from wet season samples included; Alcaligenes faecalis, A. aquatilis and Staphylococcus saprophyticus with percentage occurrences of 65.4, 19.2 and 15.4 respectively, while bacteria isolated from dry season sample were A. faecalis and S. saprophyticus with percentages occurrence of 79.17 and 20.89, respectively.

Conclusion: The findings from this study showed that hospital wastewater collected during dry season period had more bacterial load than that of wet season period. The findings also confirmed A. faecalis as the most predominant and prevailing bacteria inhabiting hospital wastewater. Thus, care must be taken by avoiding hospital wastewater from getting into the municipal water supply to prevent infections associated with A. faecalis, A. aquatilis and S. saprophyticus.

Keywords: Hospital wastewaters; wet season; dry season; global positioning system; bacteria.

1. INTRODUCTION

Hospital wastewater is an ideal medium for microorganisms [1,2] such as Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli [3,4]. Hospital wastewater inhabits 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 [4,5].

Large quantities of antibiotics are used daily for patient care and control infection in hospitals and an appreciable concentration of these antibiotics is excreted through feces and urine of patient and reaches liquid wastes. Hence, wastewater from hospitals contains resistant gene and antibiotic residues that inhibit the growth of susceptible microorganisms through selection pressure [6-7].

Many of these chemical compounds resist normal wastewater treatment. They end up in surface waters, which 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 [8].

Hospitals also release the most dangerous microorganisms threatening human health, and wastewaters are one of the most severe pollutants discharging to the environment [9-10]. The investigation of the microbiological study of hospital wastewater on the study site in this work is based on qualitative analyses to establish previous researchers’ claims further. Thus, the aim of this study is to investigate the microorganisms associated with hospital wastewaters collected from Offa Local Government Area of Kwara State Nigeria, during wet and dry season periods.

1.1 Scope of the Study

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

1.2 Justification of the Study

Hospitals release large amounts of microbial and chemical agents in their wastewaters. Many of these inherent microorganisms and chemicals present in hospital wastewater resist normal wastewater treatment. These agents 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.

2. MATERIALS AND METHODS

2.1 Study Area

Offa Local Government is one of the major Local Government Areas and one of the major Cities of Kwara State, situated in the North Central geographical zone of Nigeria. It is situated between latitude 8° 10’ 33” N, 238 Km North of Lagos at longitude 4° 43’ 02” E and 530 Km southwest of Abuja; The Federal Capital of Nigeria [11]. The yearly temperature ranges between 18.5-37.5°C, with a humidity of between 45-47%, and a peak annual rainfall of 200 cm [12]. The vegetation is essentially guinea savanna, a transition zone between the Sudan Savanna of the far North and the tropical rain forest vegetation of the South East. The population of Offa from the 2006 census was
estimated at 175,000, while the annual growth rate was estimated as 2.3%. Kwara State, Nigeria, where Offa is located has 2.37 million from the 2006 census [13].

2.2 Population Size of the Study

One hundred and twenty-six wastewater samples collected from forty-two location sites (three samples per site) during wet and dry seasons from Offa Local Governments Area of Kwara State, Nigeria, 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 Offa 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 Offa Local Governments Area of Kwara State, Nigeria whose owners did not give consent.

2.5 Collections of Hospital Wastewater Samples

Two sets of sixty-three (63) hospital wastewater samples from twenty-one (21) location sites were collected from Offa Local Government Area of Kwara State, Nigeria. The first set of sixty-three samples (wet season samples) were collected between April – October, 2018 while the second set of sixty-three samples (dry season samples) were collected between 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 packs to Microbiology laboratory, Obakekere, FUTA for culturing of bacteria.

2.5.1 Isolation and enumeration of bacterial colonies from hospital wastewater samples

Fivefold serial dilution was carried out on collected wastewater samples. An aliquot (1 ml) of the diluents was pipetted into Petri-dishes and pour plated with 20 ml of molten nutrient agar at 45°C and allowed to gel. The isolation of bacteria from wastewater was done according to methods of [14]. The emerged colonies were counted using colony counter, and values were recorded after 24 hours of incubation [15].

2.5.2 Preparation of pure isolates of bacterial colonies from hospital wastewater samples

A distinct colony was taken and streaked with a wire loop on a freshly prepared solidified nutrient agar and incubated for 24 hours at 37°C to get pure and distinct colonies. This was repeated several times until satisfactory pure isolates were obtained [15].

2.5.3 Conventional identification of bacterial isolates in hospital wastewater samples

Standard conventional methods, as reported by [14] were used for the identification of isolates. The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 hours and subsequently sub-cultured onto differential selective media, namely Eosin Methylene blue agar and MacConkey agar. The bacterial isolates were tentatively identified using 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 included cell shape, elevation, edge, optical characteristics, consistency colony surface, and pigmentation. Biochemical tests carried out include; catalase, production of hydrogen sulphide (H2S), 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 [16].

2.6 Molecular Identification of Bacterial Isolates

2.6.1 Extraction of DNA using CTAB method

Deoxyribonucleic acid (DNA) was extracted from hospital wastewater isolates by a standard CTAB genomic DNA isolation method [17] as follows: 1 ml of 24-hour broth culture was transferred into 1.5ml Eppendorf tube and spun at 14,000 rpm for 30 minutes (to harvest the cell). A 400 µl of a pre-warmed CTAB buffer (at 60°C) containing...
proteinase k and β-mercaptoethanol was added. Then 75 µl of 10% SDS (sodium deoxycholate) was added and heated in water bath at 65 °C for 30 minutes. A 500 µl chloroform was added and mixed for 15 minutes (to purify the DNA) spun at 10,000 rpm for 10 minutes. The supernatant was collected in an Eppendorf tube to which 500 µl isopropanol and 1µl (100 mg/ml) RNase were added and incubated for 30 min at 37 °C. The resultant mixture was kept at -20 for 24 hours, spun at 10,000 rpm 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 5 minutes. 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.

2.6.2 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.

2.6.3 Polymerase chain reaction analysis of 16S

Polymerase chain reaction (PCR) analysis was run with a universal primer called 16S. The PCR mix comprises of 1µl of 10 x buffer, 0.4 µl of 50 mM MgCl₂, 0.5 µl of 2.5 mM dNTPs, 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 was initial denaturation temperature of 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 60 seconds, 56 °C for 60 seconds, 72 °C for 120 seconds and the final extension temperature of 72 °C for 5 minutes and the 10 °C on hold for few hours.

2.6.4 Purification of Polymerase chain reaction products

The amplicon was further purified before the sequencing using 2 M Sodium Acetate wash techniques. To 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°C for 1 hr, spun at 10,000 rpm for 10 minutes, washed with 70% ethanol, and then air-dried. Re-suspended in 5 µl sterile distilled water and kept at 4°C for sequencing.

2.6.5 Preparation of sample for gene sequencer (ABI 3130xl machine)

The Cocktail mix was a combination of 9 µl of Hi Di Form-amide with 1 µl of Purified sequence, making 10 µl. The samples were loaded on the machine and the nucleotide sequences were obtained.

2.6.6 Polymerase chain reaction for sequencing

The PCR mix used includes 0.5 µl of Big Dye Terminator Mix, 1 µl of 5X sequencing buffer, 1 µl of the forward primers with 6.5 µl distilled water and 1 µl of the PCR product making a total of 10 µl. The PCR profile for sequencing is rapid profile. The initial rapid thermal ramp to 96 °C for 1 minute followed by 25 cycles of rapid thermal ramp to 96 °C for 10 seconds rapid thermal ramp to 50 °C for 5 seconds and rapid thermal ramp to 60 °C for 4 minutes, then followed by rapid thermal ramp to 4 °C and placed on hold for few hours.

2.6.7 Purification of polymerase chain reaction sequencing products

The PCR sequence product was also purified before the sequencing running using 2M Sodium Acetate wash techniques. To 10 µl of the PCR product, 1 µl of 2M Na Act pH 5.2 was added, followed by the addition of 20 µl absolute ethanol, the mixture was kept at -20 °C for 1hr, spun at 10,000 rpm for 10 minutes, washed with 70% ethanol and then air-dried. It was Re-suspended in 5 µl sterile distilled water and kept at 4 °C for sequencing running.

2.6.8 Data quality assurance

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

2.7 Data Analysis

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

### 3. RESULTS

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

Significantly, there are differences ($p<0.05$) in means count of bacteria in all wastewater samples. Sample from site A recorded the highest bacterial count ($150 \times 10^5$ cfu/ml), than that collected from site C with a bacterial count ($130 \times 10^5$ cfu/ml) during the wet season. On the other hand site C had the highest bacterial count ($225 \times 10^5$ cfu/ml) and site B had the lowest bacterial count ($10 \times 10^5$ cfu/ml) during the dry season (Table 1).

#### 3.2 Conventional Characterization of Bacterial Isolates from Hospital Wastewater

Using standard conventional methods, the bacteria suspected to be present in hospital wastewaters include: *Alcaligenes* spp, and *Staphylococcus* species (Table 2)

#### 3.3 Blast for Bacterial DNA Sequences

Using National Center for Biotechnology Information (NCBI) protocol, the results of bacterial DNA sequences blast is shown on Table 3.

#### 3.4 Bacteria Isolated from Hospital Wastewaters

In the entire 126 wastewater samples, *A. faecalis* was found present (both during the dry and wet season periods) except in sites J, O, Q and R (during the wet season) and sites O and P (during the dry season), *Staphylococcus saprophyticus* were found present alone in sites O and P (during the dry season), and also in sites O, Q and R (during the wet season). However, *A. aquatilis* was found alone in site J during the wet season (Table 4 and 5).

#### 3.5 Prevalence of Bacteria Present in Hospital Wastewaters

*Alcaligenes faecalis* made up 17(65.4%) of the total bacteria isolated during wet season and 19(79.2%) during the dry season. *A. faecalis* strain JF3 is more prevalent with percentage occurrence 26.9% during the wet season and strain G68 and KWW84 with 29.2 during dry season (Table 6).

### 4. DISCUSSION

Generally, in this study there was significant difference ($p<0.05$) in the means count of bacteria in all wastewater samples analyzed in this study. The values of bacterial plate counts recorded during dry and wet season period in this research exceeded the permissible limit of [18-19]. (<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) [20]. A high density of bacteria recorded during wet and dry season periods confirmed a high level of environmental pollution due to human activities. This finding agrees with a report [21] on wastewater at Ayder Referral Hospital, Mekelle North Ethiopia.

The differences in the values of the mean bacterial populations among hospitals in Offa 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 available. Also, a higher density of microbial population obtained during the dry season may be due to the preference of specific microorganisms to specific temperature ranges for growth and activity that can impact the microbial community's composition [22-23].

The bacterial isolates; *Alcaligenes faecalis*, *A. aquatilis* and *Staphylococcus saprophyticus* confirmed present in this study were different from that obtained by [21] on wastewater from Ayder Referral Hospital, Mekelle North Ethiopia, where *Klebsiella* spp, *P. aeruginosa*, *S. aureus*, *E. coli* and *Salmonella* spp. were detected. The findings in the study of [21] also disagree with the observation of [2] who reported the presence of *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *S. aureus* from effluent collected from Ethiopia, Hawassa University Referral Hospital. Also, findings from this study disagreed with the reports in India by [24] that confirmed large numbers of enteric-bacteria *S. aureus* and *P. aeruginosa*. Also dissimilar to the work of [25-26] who claimed availability of pathogenic bacteria like *Vibrio* spp. and *Salmonella* spp. in Thailand and Tunisia hospital effluents, respectively.
Table 1. The samples sites coordinates and mean count of bacteria isolated from hospital wastewaters from offa Local Government Area of Kwara State, Nigeria

| S/N | Samples’ Sites | Samples’ Sites Coordinates | Wet Season Mean Population x 10^5 cfu per ml | Dry Season Mean Population x 10^5 cfu per ml |
|-----|----------------|-----------------------------|---------------------------------------------|---------------------------------------------|
| 1   | A              | Lat. 8.15405, Long. 4.71693 | 150±43.59h                                  | 160±10.00g                                  |
| 2   | B              | Lat. 8.15445, Long. 4.72080 | 8±2.00a                                     | 10±2.00a                                    |
| 3   | C              | Lat. 8.15619, Long. 4.71465 | 130±43.59h                                  | 225±67.27i                                  |
| 4   | D              | Lat. 8.14967, Long. 4.72209 | 65±5.00cd                                   | 80±10.00bcd                                 |
| 5   | E              | Lat. 8.16362, Long. 4.72274 | 9±3.00a                                     | 13±3.00a                                    |
| 6   | F              | Lat. 8.15417, Long. 4.71595 | 14±2.00ab                                   | 17±1.00a                                    |
| 7   | G              | Lat. 8.13615, Long. 4.71401 | 90±19.08def                                  | 120±10.00f                                  |
| 8   | H              | Lat. 8.14714, Long. 4.7092  | 110±18.03fg                                  | 120±10.00f                                  |
| 9   | I              | Lat. 8.14577, Long. 4.70525 | 120±10.00g                                  | 202±9.17h                                   |
| 10  | J              | Lat. 8.15553, Long. 4.71627 | 80±10.00de                                   | 110±10.00ef                                 |
| 11  | K              | Lat. 8.13241, Long. 4.71317 | 7±4.00a                                     | 14±2.00a                                    |
| 12  | L              | Lat. 8.14735, Long. 4.71178 | 39±8.54bc                                    | 54±14.00bc                                  |
| 13  | M              | Lat. 8.14655, Long. 4.72694 | 65±5.00cd                                    | 83±3.00cde                                   |
| 14  | N              | Lat. 8.15125, Long. 4.70302 | 71±1.00d                                     | 74±3.46bcd                                  |
| 15  | O              | Lat. 8.14961, Long. 4.71268 | 103±13.00efg                                 | 115±5.00f                                   |
| 16  | P              | Lat. 8.14838, Long. 4.72694 | 110±10.00fg                                  | 120±10.00f                                  |
| 17  | Q              | Lat. 8.15884, Long. 4.72436 | 16±6.00ab                                    | 11±1.00a                                    |
| 18  | R              | Lat. 8.16861, Long. 4.71516 | 32±3.00ab                                    | 51±2.00b                                    |
| 19  | S              | Lat. 8.15529, Long. 4.71584 | 69±8.54d                                     | 89±16.52def                                 |
| 20  | T              | Lat. 8.16534, Long. 4.71088 | 30±5.00ab                                    | 20±4.58a                                    |
| 21  | U              | Lat. 8.15347, Long. 4.71563 | 16±3.46ab                                    | 190±10.00h                                  |

Values are mean ±SD of replicates (n=3).
Values with the same alphabet in the same column are not significantly different while, values with different alphabet are significantly different (α < 0.05).
Lat= latitude, Long=longitude, S/N = serial number.

However, the findings of this study agreed with the work of [21] where presence of *S. aureus* and CoNS (coagulase-negative *Staphylococcus*) in treated hospital wastewater collected from Ayder, Referral Hospital, Mekelle North, Ethiopia were confirmed. The absence of some pathogenic bacteria in the hospital wastewater analyzed may be due to variation in geographical
Table 2. Conventional characterization of bacterial isolates from hospital wastewater

| Serial number | Colonial morphology                        | Gram reaction | Structural arrangement | Catalase reaction | Indole test | Methy l red test | Simmon citrate test | Oxidase test | Spore | Nitrate reduction | Coagulase test | Hydrolysis | Motility | Glucose fermentation | Mannitol fermentation | Hydrogen sulfide | Microorganisms suspected |
|---------------|------------------------------------------|---------------|------------------------|-------------------|-------------|-----------------|---------------------|--------------|-------|-------------------|----------------|------------|----------|---------------------|------------------------|----------------|------------------------|
| 1             | Circular, opaque, convex yellowish, smooth colony | + Coci        | Clustered              | +                  | -           | -               | Na                  | Ab           | -     | -                 | Na             | Na         | Na       | G                   | Na                     | Na          | Staphylococcus spp. |
| 2             | Cream, circular, smooth, entire          | - Bacilli     | Singly                | +                  | -           | -               | -                   | Ab           | -     | Na                | urea           | Na         | Na       | Na                  | Na                     | Na          | Alcaligenes spp.     |

(*) = positive, (G) = gas, (-) = negative, (Nd) = not determined, (Na) = not applicable, (Ab) = absent, (P) = present (AG) = acid and gas
Table 3. Blast for bacteria DNA sequences

| S/N | Accession Number | Description                                                                 | Molecule type | Query ID     | Query length | E-value |
|-----|------------------|------------------------------------------------------------------------------|---------------|--------------|--------------|---------|
| 1   | FJ999731.1       | *Alcaligenes* sp. JF3 16S ribosomal RNA gene.                               | DNA           | FJ999731.1   | 1417         | 0.0     |
| 2   | MH106703.1       | *Alcaligenes aquatilis* strain YFMCD4.2 16S ribosomal RNA gene.             | DNA           | MH106703.1   | 1444         | 0.0     |
| 3   | MH362692         | *Staphylococcus saprophyticus* subsp. saprophyticus strain FELAO49 16S ribosomal RNA gene. | DNA           | MH362692     | 1430         | 0.0     |
| 4   | MH470268.1       | *Alcaligenes faecalis* subsp. phenolicus strain M4S3B1 16S ribosomal RNA gene. | DNA           | MH470268.1   | 1436         | 0.0     |
| 5   | MK968769         | *Alcaligenes faecalis* strain ISJ128 16S ribosomal RNA gene.                | DNA           | MK968769     | 1462         | 0.0     |
| 6   | KX44024          | *Alcaligenes faecalis* strain G68 16S ribosomal RNA gene.                  | DNA           | KX44024      | 1401         | 0.0     |
| 7   | KC212095         | *Alcaligenes faecalis* strain Z1116 16S ribosomal RNA gene.                | DNA           | KC212095     | 1407         | 0.0     |
| 8   | LK391651.1       | *Alcaligenes faecalis* partial 16S rRNA gene, isolate KWW 84              | DNA           | LK391651.1   | 1404         | 0.0     |
Table 4. Bacteria Isolated from hospital wastewater samples collected from Offa Local Government Area Kwara State Nigeria during wet season period

| S/N | Samples' Sites | Conventional identification | Molecular identification |
|-----|----------------|-----------------------------|--------------------------|
| 1   | A              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>1</sub> |
|     |                | A. aquatilis OFW<sub>1</sub> | A. faecalis(Strain JF3)OFW<sub>2</sub> |
| 2   | B              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>2</sub> |
| 3   | C              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>3</sub> |
|     |                | A. aquatilis OFW<sub>1</sub> | A. aquatilis(Strain YFMCD4.2)OFW<sub>2</sub> |
| 4   | D              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>4</sub> |
|     |                | S. saprophyticus OFW<sub>1</sub> | S. saprophyticus(Strain FELA049)OFW<sub>1</sub> |
| 5   | E              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain M453B1)OFW<sub>1</sub> |
| 6   | F              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain ISJ128)OFW<sub>1</sub> |
|     |                | A. aquatilis OFW<sub>1</sub> | A. aquatilis(Strain YFMCD4.2)OFW<sub>3</sub> |
| 7   | G              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>5</sub> |
| 8   | H              | A. faecalis OFW<sub>1</sub>  | A. faecalis(ISJ128)OFW<sub>2</sub> |
| 9   | I              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>6</sub> |
| 10  | J              | A. aquatilis OFW<sub>1</sub> | A. aquatilis(Strain YFMCD4.2)OFW<sub>4</sub> |
| 11  | K              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain JF3)OFW<sub>7</sub> |
|     |                | A. aquatilis OFW<sub>1</sub> | A. aquatilis(Strain YFMCD4.2)OFW<sub>5</sub> |
| 12  | L              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain M453B1)OFW<sub>2</sub> |
| 13  | M              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain M453B1)OFW<sub>3</sub> |
| 14  | N              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain M453B1)OFW<sub>4</sub> |
| 15  | O              | S. saprophyticus OFW<sub>1</sub> | S. saprophyticus(Strain FELA049)OFW<sub>2</sub> |
| 16  | P              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain ISJ128)OFW<sub>3</sub> |
| 17  | Q              | S. saprophyticus OFW<sub>1</sub> | S. saprophyticus(Strain FELA049)OFW<sub>3</sub> |
| 18  | R              | S. saprophyticus OFW<sub>1</sub> | S. saprophyticus(Strain FELA049)OFW<sub>4</sub> |
| 19  | S              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain M453B1)OFW<sub>5</sub> |
| 20  | T              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain ISJ128)OFW<sub>4</sub> |
| 21  | U              | A. faecalis OFW<sub>1</sub>  | A. faecalis(Strain ISJ128)OFW<sub>5</sub> |

A. aquatilis = Alcaligenes aquatilis, A. faecalis = Alcaligenes faecalis
S. saprophyticus = Staphylococcus saprophyticus, OFD = Offa Dry isolate, OFW = Offa Wet isolate, S/N = serial number, A – U = samples sites locations
Table 5. Bacteria Isolated from hospital wastewater samples collected from Offa Local Government Area Kwara State Nigeria during dry season period

| S/N | Samples’ Sites | Conventional identification | Molecular identification |
|-----|----------------|----------------------------|--------------------------|
| 1   | A              | A. faecalis OFD₁           | A. faecalis(Strain G68)OFD₁ |
| 2   | B              | A. faecalis OFD₂           | A. faecalis(Strain G68)OFD₂ |
| 3   | C              | A. faecalis OFD₃           | A. faecalis(Strain G68)OFD₃ |
| 4   | D              | A. faecalis OFD₄           | A. faecalis(Strain Z1116)OFD₁ |
| 5   | E              | A. faecalis OFD₅           | A. faecalis(Strain G68)OFD₄ |
| 6   | F              | A. faecalis OFD₆           | A. faecalis(Strain Z1116)OFD₂ |
| 7   | G              | A. faecalis OFD₇           | A. faecalis(Strain G68)OFD₅ |
| 8   | H              | A. faecalis OFD₈           | A. faecalis(Strain KWW84)OFD₁ |
| 9   | I              | A. faecalis OFD₉           | A. faecalis(Strain KWW84)OFD₂ |
| 10  | J              | A. faecalis OFD₁₀          | A. faecalis(Strain G68)OFD₆ |
| 11  | K              | A. faecalis OFD₁₁          | A. faecalis(Strain Z1116)OFD₃ |
| 12  | L              | A. faecalis OFD₁₂          | A. faecalis(Strain G68)OFD₇ |
|     |                | Staphylococcus spp. OFD₁₃ | S. saprophyticus (Strain FELA049)OFD₁ |
| 13  | M              | A. faecalis OFD₁₄          | A. faecalis(Strain KWW84)OFD₃ |
| 14  | N              | A. faecalis OFD₁₅          | A. faecalis(Strain KWW84)OFD₄ |
| 15  | O              | Staphylococcus spp. OFD₁₆ | S. saprophyticus (Strain FELA049)OFD₂ |
| 16  | P              | Staphylococcus spp. OFD₁₇ | S. saprophyticus (Strain FELA049)OFD₃ |
| 17  | Q              | A. faecalis OFD₁₈          | A. faecalis(Strain Z1116)OFD₄ |
| 18  | R              | A. faecalis OFD₁₉          | A. faecalis(Strain KWW84)OFD₅ |
| 19  | S              | A. faecalis OFD₂₀          | A. faecalis(Strain Z1116)OFD₅ |
|     |                | Staphylococcus spp OFD₂₁  | S. saprophyticus (Strain FELA049)OFD₄ |
| 20  | T              | A. faecalis OFD₂₂          | A. faecalis(Strain KWW84)OFD₆ |
| 21  | U              | A. faecalis OFD₂₃          | A. faecalis(Strain KWW84)OFD₇ |
|     |                | Staphylococcus spp. OFD₂₄ | S. saprophyticus (Strain FELA049)OFD₅ |

A. aquatilis = Alcaligenes aquatilis, A. faecalis = Alcaligenes faecalis
S. saprophyticus = Staphylococcus saprophyticus, OFD = Offa Dry isolate, OFW = Offa Wet isolate, S/N = serial number, A – U = samples sites locations
Table 6. Prevalence/Percentage of bacteria present in hospital wastewaters collected from Offa Local Government Area of Kwara State, Nigeria

| Bacterial isolates (Wet season) | N (%) | Bacterial isolates (Dry season) | N (%) | Total (%) |
|-------------------------------|-------|-------------------------------|-------|-----------|
| A. faecalis 7(JF3)            | 7(26.92) | -                             | 0(0.00) | 7(14.00) |
| -                             | 0(0.00) | A. faecalis 7(G68)           | 7(29.17) | 7(14.00) |
| A. faecalis 5(M4S3B1)         | 5(19.23) | -                             | 0(0.00) | 5(10.00) |
| -                             | 0(0.00) | A. faecalis 5(Z1116)         | 5(20.83) | 5(10.00) |
| A. faecalis 5(ISJ128)         | 5(19.23) | -                             | 0(0.00) | 5(10.00) |
| -                             | 0(0.00) | A. faecalis 7(KWW84)         | 7(29.17) | 7(14.00) |
| A. aquatilis 2(YFMCD4)        | 5(19.23) | -                             | 0(0.00) | 5(10.00) |
| S. saprophyticus4(FELA049)    | 4(15.39) | S. saprophyticus 5(FELA049)  | 5(20.83) | 9(18.00) |
| Total                         | 26(100.00) |                               | 24(100) | 50(100.00) |

N = number of isolate, % = percentage present, - = absent
and climatic condition as shifting of microbial community occurs in favour of the species which are better adapted to higher temperatures and have accelerated rates of growth [27,23,22]. More so, inter-specific competition among microorganisms may cause a shift in microbial community, such that microorganisms that compete favorably among the mixed community due to several factors such as population density, inhibitory metabolites, and so on will be prevailing. The highest prevalence of A. faecalis may be because it is highly associated with urinary tract infection (UTI), which is not uncommon in hospital environment, and production of toxic metabolites might also favour the survival of Staphylococcus spp.

5. CONCLUSION AND RECOMMENDATIONS

In this study, dry season hospital wastewaters had more bacterial load than wet season hospital wastewater samples. The bacteria isolated from the hospital wastewaters analyzed included: Alcaligenes faecalis, A. aquatilis and Staphylococcus saprophyticus. Alcaligenes faecalis were the most predominant, followed by S. saprophyticus among the isolates from wastewater samples. The microbial load of wastewater samples exceeded the WHO, HPA, EPA and FAO standard permissible levels. The availability of these microbes suggests their persistence in the hospital environment. Therefore, hospital health workers should improve personal hygiene and routine daily disinfection of the hospital environment to reduce the microbial load of hospital effluents. Also, wastewater should be treated appropriately in conformity with an acceptable standard to mitigate the risk of reinfections by the isolates.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ACKNOWLEDGEMENT

We sincerely acknowledge the assistance of the following people: Head of Department of Department of microbiology, Federal University of Technology, Akure, Ondo State, Nigeria in person of Prof. M. K. Oladunmoye and all technical staff of the same department for their moral support and assistance in making available the laboratory equipment and other facilities used towards the achievement and success of this work. May God Almighty provide for your needs (Amen).

DISCLAIMER

The products used for this research are commonly and predominantly used in our research area and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but the advancement of knowledge. Also, the research was not funded by the producing company; instead, it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:https://www.sdiarticle4.com/review-history/72670