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Appraisal on Microbiological Qualities of Wastewaters from Offa 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.

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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 10^5cfu/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^{0}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-tree (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 45^0 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 ^{0}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 ^{0}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 (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 [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^{0}C) containing

proteinase k and β-mercaptoethanol was added. Then 75 µl of 10% SDS (sodium deodycylsulphate) was added and heated in water bath at 65 ^{0}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 ^{0}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 $MgCl_2$, 0.5 µl of 2.5 mMdNTPs, 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 ^{0}C for 3 minutes, followed by 30 cycles of 94 ^{0}C for 60 seconds, 56 ^{0}C for 60 seconds, 72 ^{0}C for 120 seconds and the final extension temperature of 72 ^{0}C for 5 minutes and the 10 ^{0}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^{0}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^{0}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 ^{0}C for 1 minute followed by 25 cycles of rapid thermal ramp to 96 ^{0}C for 10 seconds rapid thermal ramp to 50 ^{0}C for 5 seconds and rapid thermal ramp to 60 ^{0}C for 4 minutes, then followed by rapid thermal ramp to 4 ^{0}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 ^{0}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 keep at 4 ^{0}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)

20 version). 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 x 10^5cfu/ml), than that collected from site C with a bacterial count(130 x 10^5cfu/ml) during the wet season. On the other hand site C had the highest bacterial count (225 x 10^5 cfu/ml) and site B had the lowest bacterial count (10 x 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 × 10^3cfu/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	Dry Season
			Mean Population x 10^5 cfu per ml	Mean Population x 10^5 cfu per ml
1	A	Lat.8.15405 Long. 4.71693	150±43.59^{h}	160±10.00^{g}
2	B	Lat. 8.15445 Long. 4,72080	8±2.00^{a}	10±2.00^{a}
3	C	Lat. 8.156139 Long. 4.71465	130±43.59^{gh}	225±67.27^{i}
4	D	Lat. 8.14967 Long. 4.72209	65±5.00^{cd}	80±10.00^{bcd}
5	E	Lat. 8.16362 Long. 4.72274	9±3.00^{a}	13±3.00^{a}
6	F	Lat. 8.15417 Long. 4.71595	14±2.00^{ab}	17±1.00^{a}
7	G	Lat. 8.13615 Long. 4.71401	90±19.08^{def}	120±10.00^{f}
8	H	Lat 8.14714 Long. 4.7092	110±18.03^{fg}	120±10.00^{f}
9	I	Lat. 8.14577 Long. 4.70525	120±10.00^{g}	202±9.17^{hi}
10	J	Lat. 8.15553 Long. 4.71627	80±10.00^{de}	110±10.00^{ef}
11	K	Lat.8.13241 Long. 4.71317	7±4.00^{a}	14±2.00^{a}
12	L	Lat. 8.14735 Long. 4.71178	39±8.54^{bc}	54±14.00^{bc}
13	M	Lat. 8.14655 Long. 4.72694	65±5.00^{cd}	83±3.00^{cde}
14	N	Lat. 8.15125 Long. 4.70302	71±1.00^{d}	74±3.46^{bcd}
15	O	Lat. 8.14961 Long. 4.71268	103±13.00^{efg}	115±5.00^{f}
16	P	Lat. 8.14838 Long. 4.72694	110±10.00^{fg}	120±10.00^{f}
17	Q	Lat. 8.15884 Long. 4.72436	16±6.00^{ab}	11±1.00^{a}
18	R	Lat. 8.16861 Long. 4.71516	32±3.00^{ab}	51±2.00^{b}
19	S	Lat. 8.15529 Long. 4.71584	69±8.54^{d}	89±16.52^{def}
20	T	Lat. 8.16534 Long. 4.71088	30±5.00^{ab}	20±4.58^{a}
21	U	Lat. 8.15347 Long. 4.71563	16±3.46^{ab}	190±10.00^{h}

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 ($\alpha < 0.05$)
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	Shape	Structural arrangement	Catalase reaction	Indole test	Methyl red test	Simmon citrate test	Oxidase test	Vegetative hyphae	spore	Nitrate reduction	Coagulase test	hydrolysis	Motility	Glucose fermentation	Mannitol fermentation	Hydrogen sulfide	Microorganisms suspected
1	Circular, opaque, convex yellowish, smooth colony	+	Cocci	Clustered	+	-	-	Na	-	Ab	-	-	-	Na	-	Na	G	Nd	*Staphylococcus* spp.
2	Cream, circular, smooth, entire	-	Bacilli	Singly	+	-	-	-	+	Ab	-	Na	-	urea	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_1	*A. faecalis*(Strain JF3)OFW_1
		A. aquatilis OFW_1	*A. aquatilis* (Strain YFMCD4.2)OFW_1
2	B	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_2
3	C	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_3
		A. aquatilis OFW_1	*A. aquatilis* (Strain YFMCD4.2)OFW_2
4	D	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_4
		S. saprophyticus OFW_1	*S. saprophyticus* (Strain FELA049)OFW_1
5	E	*A. faecalis* OFW_1	*A. faecalis* (Strain M453B1)OFW_1
6	F	*A. faecalis* OFW_1	*A. faecalis*(Strain ISJ128)OFW_1
		A. aquatilis OFW_1	*A. aquatilis* (Strain YFMCD4.2)OFW_3
7	G	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_5
8	H	*A. faecalis* OFW_1	*A. faecalis*(ISJ128)OFW_2
9	I	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_6
10	J	*A. aquatilis* OFW_1	*A. aquatilis*(Strain YFMCD4.2)OFW_4
11	K	*A. faecalis* OFW_1	*A. faecalis*(Strain JF3)OFW_7
		A. aquatilis OFW_1	*A. aquatilis* (Strain YFMCD4.2)OFW5
12	L	*A. faecalis* OFW_1	*A. faecalis* (Strain M453B1)OFW_2
13	M	*A. faecalis* OFW_1	*A. faecalis* (Strain M453B1)OFW_3
14	N	*A. faecalis* OFW_1	*A. faecalis* (Strain M453B1)OFW_4
15	O	*S. saprophyticus* OFW_1	*S. saprophyticus* (Strain FELA049)OFW_2
16	P	*A. faecalis* OFW_1	*A. faecalis*(Strain ISJ128)OFW_3
17	Q	*S. saprophyticus* OFW_1	*S. saprophyticus* (Strain FELA049)OFW_3
18	R	*S. saprophyticus* OFW_1	*S. saprophyticus* (Strain FELA049)OFW_4
19	S	*A. faecalis* OFW_1	*A. faecalis* (Strain M453B1)OFW_5
20	T	*A. faecalis* OFW_1	*A. faecalis*(Strain ISJ128)OFW_4
21	U	*A. faecalis* OFW_1	*A. faecalis*(Strain ISJ128)OFW_5

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* OFD_1	*A. faecalis*(Strain G68)OFD_1
2	B	*A. faecalis* OFD_2	*A. faecalis*(Strain G68)OFD_2
3	C	*A. faecalis* OFD_3	*A. faecalis*(Strain G68)OFD_3
4	D	*A. faecalis* OFD_4	*A. faecalis*(Strain Z1116)OFD_1
5	E	*A. faecalis* OFD_5	*A. faecalis*(Strain G68)OFD_4
6	F	*A. faecalis* OFD_6	*A. faecalis*(Strain Z1116)OFD_2
7	G	*A. faecalis* OFD_7	*A. faecalis*(Strain G68)OFD_5
8	H	*A. faecalis* OFD_8	*A. faecalis*(Strain KWW84)OFD_1
9	I	*A. faecalis* OFD_9	*A. faecalis*(Strain KWW84)OFD_2
10	J	*A. faecalis* OFD_{10}	*A. faecalis*(Strain G68)OFD_6
11	K	*A. faecalis* OFD_{11}	*A. faecalis*(Strain Z1116)OFD_3
12	L	*A. faecalis* OFD_{12}	*A. faecalis*(Strain G68)OFD_7
		Staphylococcus spp. OFD_{13}	*S. saprophyticus* (Strain FELA049)OFD1
13	M	*A. faecalis* OFD_{14}	*A. faecalis*(Strain KWW84)OFD_3
14	N	*A. faecalis* OFD_{15}	*A. faecalis*(Strain KWW84)OFD_4
15	O	*Staphylococcus* spp. OFD_{16}	*S. saprophyticus* (Strain FELA049)OFD_2
16	P	*Staphylococcus* spp. OFD_{17}	*S. saprophyticus* (Strain FELA049)OFD_3
17	Q	*A. faecalis* OFD_{18}	*A. faecalis*(Strain Z1116)OFD_4
18	R	*A. faecalis* OFD_{19}	*A. faecalis*(Strain KWW84)OFD_5
19	S	*A. faecalis* OFD_{20}	*A. faecalis*(Strain Z1116)OFD_5
		Staphylococcus spp OFD_{21}.	*S. saprophyticus* (Strain FELA049)OD_4
20	T	*A. faecalis* OFD_{22}	*A. faecalis*(Strain KWW84)OFD_6
21	U	*A. faecalis* OFD_{23}	*A. faecalis*(Strain KWW84)OFD_7
		Staphylococcus spp. OFD_{24}	*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 reinventions 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).

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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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