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Detection of *Enterococcus faecalis* and Antibiotic Resistance Genes in Wastewater Facilities from Two Nigerian University Campuses

Olubunmi M. Emurotu[1]*, Kalu J. Ndukwe[3], Olumorin I. Owemidu[2], Foluke R. Aminu[1]

[1]*Department of Microbiology, Kogi State University, Anyigba, Nigeria*
[2]*Department of Physiology, Kogi State University, Anyigba, Nigeria*
[3]*Department of Microbiology, University of Nigeria, Nsukka, Nigeria*

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ABSTRACT

Enterococcus faecalis is a familiar urinary tract infection (UTI) pathogen of bacteria origin. This uropathogen is becoming more resistant to currently prescribed antimicrobials. Use of water from hostel drains and wastewater treatment plants (WTP) in the irrigation of vegetable farms favours horizontal gene transfer (HGT), and transfer of antimicrobial resistance (AMR) pathogens from UTI to the gut microbiome. This work aimed at evaluating WTP and hostel drains from two campuses (University of Nigeria, Nsukka- UNN and Kogi State University, Anyigba- KSU) as reservoirs for this bacterial uropathogenic and antibiotic resistance genes (ARGs). *E. faecalis* was isolated, identified and confirmed using oxoid Chromogenic UTI agar and other biochemical tests. Twenty-one (21) multidrug-resistant isolates were screened for antibiotic-resistance genes. All the isolates were confirmed to be *Enterococcus* based on the presence of the 16SrRNAgene. More than 85 and 66 (%) of the isolates were confirmed to have Enterococcal surface protein D-ala-D-ala ligase (*Ddl*) and Cytolysin (*ClyA*) respectively. Indicating that the isolates were of the genus *Enterococcus* and *E. faecalis* species. The *Van B* and *Van X* genes were absent, 9.52%, 95.2% and 14.3% of the species having the *Van A*, *Ant(2)-la* and *Acc(3)-lla* genes respectively. All isolatesfrom UNN-WTP influent and effluent showed total resistance to ceftazidime (CAZ), penicillin G (P), erythromycin (E), meropenem (MEM) and cefotaxime (CTX) while isolates from both campuses' wastewater drains also resisted (100%) MEM, P and CAZ antibiotics, 100% and 91.68% resistance to E and 100% and 92.6% resistance to CTX respectively.

Keywords*:Enterococcus faecalis*, Urinary tract infection, Antibiotic resistance genes, wastewater, wastewater facilities

Introduction

One common bacterial disease that people habitually experience is urinary tract infections (UTI). Although several microbes are responsible for its development, *Staphylococcus saprophyticus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis* and *Proteus mirabilis*, are the most implicated [1, 2]. *Enterococcus faecalis* is the organism accountable for UTIs that occur most regularly.[3, 4] According to reports, urogenital tract infections result in over 8 million hospital admissions each year. [5] These infections can occasionally be incredibly challenging to treat, [6] as numerous *Enterococcus faecalis* isolates are resistant to conventional medications. Antimicrobial resistance (AMR) in Enterobacteriaceae is a serious public health problem, particularly in developing countries.[7, 8] The financial burden of these diseases is rising significantly as a result of the uropathogenic increased recurrence rate and resistance to antibiotic treatment.[1]

*Corresponding author. E mail: bunmi_emurotu@yahoo.com
Tel: 08100262786

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The most common gram-positive infectious pathogens that are clinically important and frequently cause UTIs are *Enterococci* and *Staphylococcus saprophyticus*).[4]*Enterococcus* strains linked to pathogens of the gastrointestinal tract (GIT) could be the source of UTIs.[8, 9] It is possible for uropathogens and gastrointestinal strains that are sensitive to water settings to acquire antibiotic-resistance genes from antibiotic-resistant bacteria.[10] The two *Enterococcus species* that cause enterococcal infections frequently are *Enterococcus faecalis* and *Enterococcus faecium*.[11] Enterococci are developing seriously increased antibiotic resistance faster than the rate of illness worldwide. Enterococci are seen to be defiant whenever resistance genes (RG) are found within chromosome or when determinants responsible for resistance found within plasmids (or transposons) are acquired.[12] Infections arising from enterococcal have inherent resistance determinants to many antibiotics.[13]

AMR has been outlined by WHO as global hazard to public health, endangering the perfect treatment and prevention of parasitic, bacterial, viral, and fungal infections.[14,15] Therefore, findings into its avoidance and abatement is highly crucial. AMR increases worldwide and obtainable information suggests that this problem is not country-specific or specific to a region, it affects countries that are industrialized and not industrialized. High resistance has been observed in bacteria that cause familiar infections acquired in hospital and community like pneumonia and UTI present in the whole six WHO regions (South-East Asia, Eastern Mediterranean, Americas, Africa, Europe, and Western Pacific).[15] Over the years, several epidemics are connected to impure food and drinking water, watering with water contaminated by animal faeces or surface runoff.[16, 17]

Wastewater treatment facilities are reported as store house for genes that are antibiotic-resistant and are peculiar to man and animal

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microbes.[18, 19] Excreted urine and faeces may contain either antibiotic-resistant bacteria or unabsorbed (antibiotic) residues which ultimately enter wastewater treatment plants (WTP) through domestic sewer lines.[20] Different classes of residues have been regularly observed in municipal effluents namely β-lactam, lincosamide, sulphonamides, tetracyclines, macrolides, and fluoroquinolones.[21] Because of the increase in antibiotic-resistant microorganisms, there is growing anxiety about the commonness of antibiotics in settings.[20] Nearly the entire crisis has link linked to availability of transferable plasmids that encodes multidrug-resistance and spreading within various species of enterobacterial apprehension.[22-24]WTP effluents and wastewater discharged directly into the hostel drain at the University of Nigeria Nsukka are used to irrigate vegetables and other food crops in the locality while at Kogi State University hostel drains are not controlled but are often washed away by rain into different farmlands. Wastewater treatment plant (WTP) effluents and wastewater discharged directly into hostel drains at the University of Nigeria Nsukka and Kogi State University, Anyigba are used to irrigate vegetables and other food crops in the localities. The use of such waters presents risks for GIT and UT infections after consumption of such vegetables, and may also, favour the spread of antimicrobial resistance through horizontal gene transfers.[25]Dearth of data occurs on availability and widespread of pathogens urinary tract conditions and genes that are antibiotic resistant in aquatic environments.

The work aimed at evaluating wastewater environments at the University of Nigeria, Nsukka (UNN) and Kogi State University, (KSU) Anyigba as reservoirs of bacterial uropathogens and antibiotic resistance genes. The specific objectives were to:(i) isolate presumptive uropathogenic *Enterococcusfaecalis* from wastewater samples collected from UNN-WTP and Hostel drains at UNN and KSU; (ii) identify and characterize the isolates, via Gram-stain and other biochemical tests that are; (iii) determine also set side by side the antibiotic susceptibility patterns of enterococcal isolates from different locations as well as confirm isolates as *E. faecalis* and (iv) characterize confirmed isolates for virulence and antibiotic resistance genes.

Materials and Methods

Study Area

Two University environments, the University of Nigeria, Nsukka (UNN) and Kogi State University, Anyigba (KSU) were selected. UNN is a first-generation University, located at (6°52'10.07"N; 7°24'2.46"E) in Southeast, Nigeria, and KSU is a state-owned university located at (7°29'3.60"N; 7°10'51.62"E) in Anyigba, Kogi State, North central, Nigeria (Figure 1). Wastewater irrigation around the UNN WTP and hostel drains has been in existence since 1976 at UNN and since 2000 in KSU where wastewater from the WTP and hostel drains obtained by using directly, containers and buckets are often used to irrigate vegetable farms.

Study Design and Samples Collection

Wastewater influent and effluent and hostel drain samples at UNN were collected at designated points while at KSU hostel drains were collected. Over the course of a 9months sampling period, 63 samples were randomly obtained in total. At KSU a total of 27 hostel drain samples were collected, while at UNN 18 hostel drains with 9 samples each of influent and effluent from WTP were collected. KSUis located in North Central Nigeria, and the WTP and hostel drain at UNN are in South-East Nigeria. At the WTP facility as well as hostel drains, 250 mlinfluent and final effluent wastewater were collected. At various points where they discharged into the main drain, the hostel drains were gathered. Samples were gathered in sanitized 250 mL bottles, correctly labelled, and delivered to the lab for microbiological examination while being maintained in ice boxes.

Presumptive Characterization of Enterococcus

Using Chromogenic UTI agar (Oxoid/Thermo Fisher Scientific, USA), *Enterococcus* was recovered from each sample. Then, separate Gram-staining and common biochemical tests were performed on each isolate.[26]The presumed isolates were then maintained for further testing and molecular confirmation inside tryptic-soy-broth (Oxoid, UK) with glycerol (15%) (stored inside Eppendorf bottles and refrigerated). As a control (positive), *Enterococcus faecalis* (ATCC 29212) was employed. Based on colour that developed on the Oxoid UTI chromogenic agar plates, organisms were identified. *Enterococcus faecalis* is indicated by a turquoise colour.

Antimicrobial Susceptibility Testing

Antibiotic sensitivity tests were done on all confirmed bacterial isolates, following Kirby-Bauer disc-diffusion technique[27] combined with the evaluation methods and rules of Clinical and Laboratory Standards Institute[28]. A total of 15 antimicrobial susceptibility test discs (OxoidThermoFisher Scientific, United Kingdom) were used. They were tested for their susceptibilities to Amocycillin\Clavulanic acid (AMC 30µg), Ciprofloxacin (CIP 5µg), Gentamycin (CN 30µg), Ampicillin (AMP10µg), Meropenem (MEM 10µg), Streptomycin (S 10µg), Erythromycin (E 15µg), Cefotaxime (C TX 30µg); Sulphamethoxazole/Trimethoprim (SXT 25µg), Vancomycin (VA 30µg), Levofloxacin (LEV 1µg), Penicillin G (P 10µg), Ceftazidime (CAZ 30µg), Nitrofurantoin (F 300 µg) and tetracycline (TE 30µg)

Virulence Factor and Antibiotic Resistance GenesCharacterization
Genomic DNA Extraction

As stated in,[29] genomic-DNA was obtained out of pure culture of each isolate that grew in nutrient-agar for 24 h at 37°C by conventional method of boiling, as narrated by[29] into a ml of type one purified water, a loopful of bacterial cells was dissolved. At 100^{o}C for 5 min, suspensions of bacteria were allowed to cool for 10 min before centrifuging (5 min), 12,000 x g for 5 min in order to separate debris therein. Then, extracted DNA was cleaned using GeneJET Gel Extraction Kit (ThermoFisher Scientific, USA) and stored at -20^{0}C for PCR analysis.

Polymerase Chain Reactions and Gel Electrophoresis

The PCR assays were done using *MyGene* gradient-t*hermal cycler*, (*Model-MG96G;*Long gene-Scientific Instruments Co., Ltd, USA). The reaction mixtures were made up of half a 0.5µl each of primers of oligonucleotide (Inqaba Biotech South Africa), twenty-five microliter of Master Mix PCR (Thermo Scientific, USA), 14 µl of water that is nuclease-free, as well as 10 µl template DNA to make up an aggregate of 50 µl reaction volume.

The cycling conditions in PCR (Initial-activation at 94^{o}C (3 min), then 30 cycles of denaturing to the end (94^{o}C) for 1 min, annealing (55^{o}C) at a minute, extension(74^{o}C)at a minute as well as final-elongation, 74^{o}C for another 9 minutes.[26]*E. faecalis* (ATCC 29212) wasutilized for controls that are positive and negative for *E. faecalis* generic identification. The primers of the oligonucleotide sequence used, the targeted genes and the products of amplification expected are shown on Table 1.

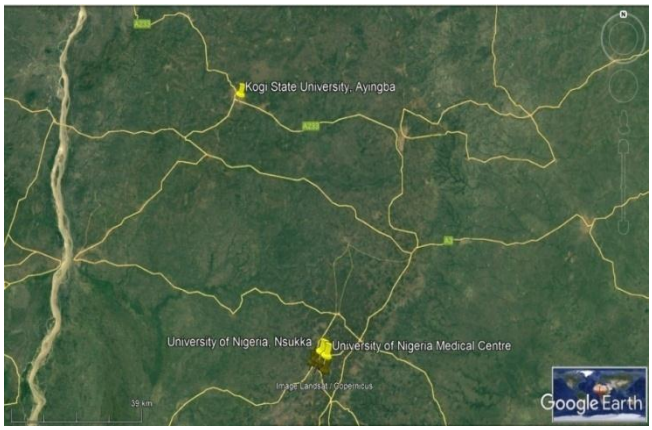

Figure 1: Map representation of Study area/Sampling sites (Source: Google Earth Access date: 8/12/2021)

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Gel electrophoresis consisting 10µl of template DNA, 6µl positive-control and 3 µl ladder of DNA (1 Kb + ladder of DNA; Invitrogen), were done on agarose gels 2.5% (w/v) at 100V for 30 min in 1×-TBE buffer (0.002MEDTA and 0.09MTrisborate,pH 8). Then the gels were viewed and snapped using DigiDoc-It® Imaging System (Thomas Scientific, USA).

Screening for antibiotic Resistance Genes (ARGs)

The isolates were screened for *ant*(2)-Ia, *van*A, *van*B and *van*X resistance genes by a hot start PCR procedure as described by.[29]The amplicons-sizes and primers are given in Table 2. PCR-reactions were done with 20µl volume harbouring 10 µl 1×Quick Taq® HS DyeMix (ThermoFisher, USA), 0.4 µl of each of the primers (Inqaba, West Africa), 7.7 µl of sterile water and 1.5 µl template. This profile of temperature was used: initial denaturation for five minutes at 94^{o}C then 30 cycles of 94° 30 s, thereafter, annealing for 30 s at 55^{o}C, then extension at 74°C in 50 s with 74°C for 7 min for final elongation, final hold and storage was at 4°C. PCR amplification was done in the My-gene-series Peltier thermal cycler (Model MG96G) Long Gene Scientific Instruments Co. Ltd, USA. The PCR byproducts were cataphoresed via gels of agarose of 1.5% (w/v) run on 1× TAE-buffer (40mM-Tris, 20mM-Acetate and 1mM-EDTA pH8.6) at 100V in 30 min. The gels obtained were stained with red-safe nucleic acid staining solution (Intron-Biotechnology, Koria) then products were visualized beneath UV light and documented using DigiDoc-It® Imaging System (Thomas Scientific, USA).

Statistical Analysis

Data were analysed to determine statistical significance using analysis of variance. Results as well as *P* values of <0.05 were regarded significant.

Table 1: Primers for Characterizing *E.faecalis*

DNA target	Virulence factor/gene product		Primer sequence (5'to 3')	Product size(bp)	Thermocycling conditions	References
Ddl	D-ala-D-ala	F	ATCAAGTACAGTTAGTCTTTATTAG	941	Initial activation 94^{o}C;in 3 min,	29, 30
	ligase	R	ACGATTCAAAGCTAACTGAATCAGT		then, 30 cycles comprising	
*Cyl*A	Cytolysin	F	ACTCGGGGATTGATAGGC	700	denaturing 94^{o}C in 1 min,	
		R	GCTGCTAAAGCTGCTT		annealing at 55^{o}C for 1 min,	
Esp	Enterococcal	F	AGATTTCATCTTTGATTCTTGG	510	extension74°C at 1 min and	29
	surface protein	R	AATTGATTCTTTAGCATCTGG		final elongation 72°C in 9 min	

Results and discussion

Total Enterococcus Count from Two Sampling Points in UNNWTP and Two Campuses Hostel Drains

The *Enterococcus* count between the influents and effluents in the UNN campus ranges from 3.2x10^{6} to 5.8 x10^{7} (cfu/ml). More *Enterococcus* were observed in the influent (3.8x10^{7}-5.8x10^{8}cfu/ml) with a mean count of 3.08x10^{7} cfu/ml,than in the effluent (3.2x10^{6} to 5.7 x10^{7}cfu/ml) with a mean count of 4.68x10^{7}cfu/ml Figure.2 (A). However, there was a significant difference between the two sampling points at P≤0.05 in the months of August, September, October, December, January and March (Table S1). The fluctuation and high number of *Enterococcus* in sewage influents and effluents above the acceptable threshold may be linked to the lopsided influx of industrial and domestic effluent into sewage; and inefficiency of treatment plant and protocol. In the comparison of the *enterococcus* count between the two campuses' hostel drains, there was higher number of the organism (9.55×10^{4}-1.87×10^{5}cfu/ml)from the UNN campus when compared with that from KSU (3.67×10^{3}-1.26×10^{5}cfu/ml) with a mean colony count of 1.47×10^{5} and 9.15×10^{4}cfu/ml respectively. Interestingly more of the organism was observed close to and during the dry season than in the rainy season, Figure 2 (B).However, there was no significance statistically at P≤0.05 in the enterococcal load between the campuses within the months of July, October, November, and February (Table S2).

Total Enterococcus Count between the Two Campuses Female and Male Hostel Drains

The number of E*nterococcus* in-between female hostel drains from both campuses was observed to be higher in the water samples from UNN female hostel drains with a mean colony count of 2.91×10^{5}cfu/mlwith the highest count (3.7×10^{5}cfu/ml) observed in September, and the lowest colony count of 1.9×10^{5}(cfu/ml) where observed in the three months of February, July and November. However, the mean colony count of 2.65×10^{5} (cfu/ml) and the highest (3.7×10^{5}) and lowest (3.6×10^{3}) colony counts of *Enterococcus* were observed in the water samples from the female hostel on KSU campus (Figure 3A). There was also no significant difference statistically at P≤0.05 in the enterococcal load between the female hostels from both campuses except in the month of March (Table S3).

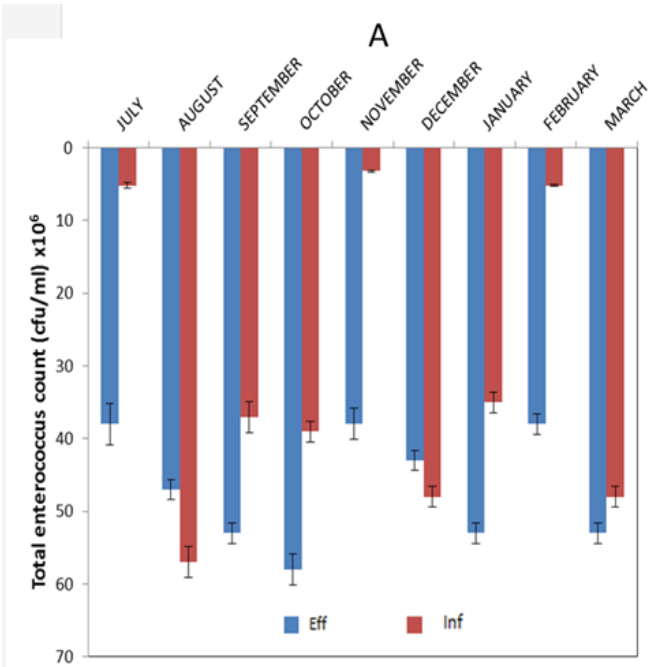

Figure 2a: Comparative study of total *Enterococcus* count from two sampling points in UNN (A) and the two different campuses (B)

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The highest enterococcal count of 5.2×10^3cfu/mlbetween the male hostel drains of the two campuses was observed in samples from Alvan-Ikoku hostel in UNN, this was followed by that from Ocheja in KSU (4.5×10^3cfu/ml) and Dangana(3.8×10^3cfu/ml); with mean colony count of 2.9×10^3, 3.81×10^3, and 3.39×10^3 (cfu/ml)respective (Fig.ure 3B). The level of significance statistically at $P\leq0.05$ was high all through the months except for the month of December (Table S4).

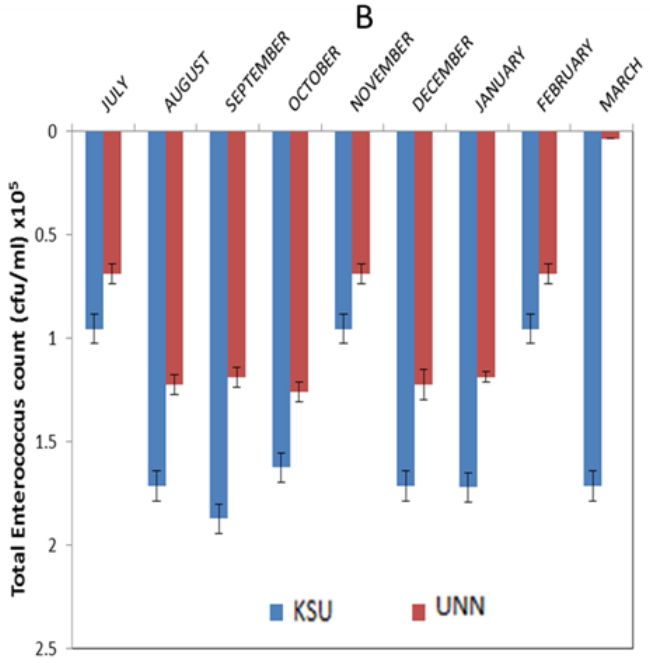

Figure 2b: Comparative study of total *Enterococcus* count from two sampling points in UNN (A) and the two different campuses (B)

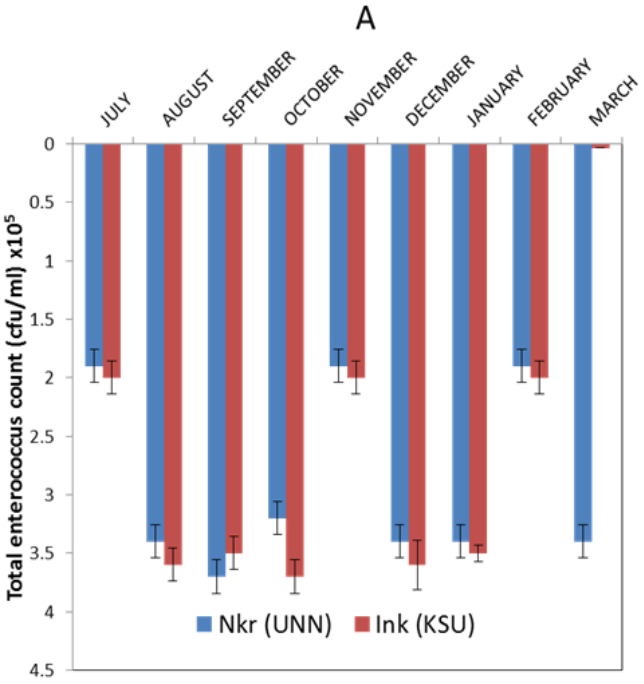

Figure 3a: Comparative study of total *Enterococcus* count between the two campuses female (A) and male (B) hostel drains

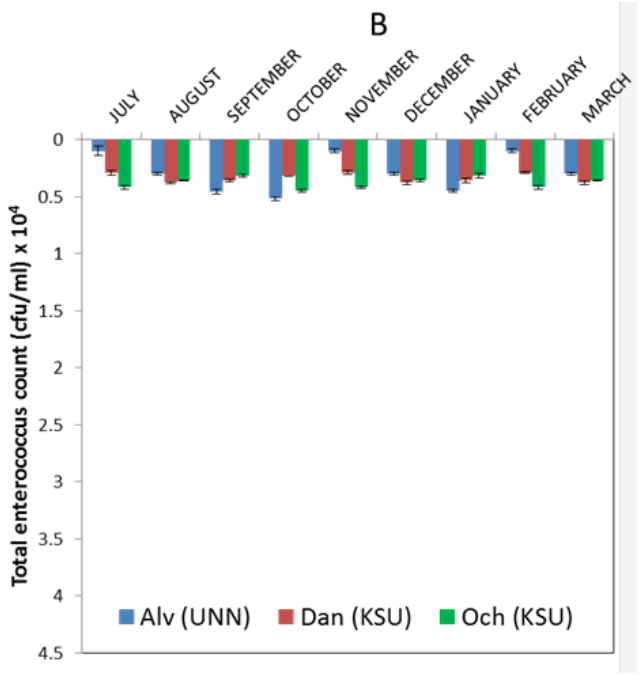

Figure 3b: Comparative study of total *Enterococcus* count between the two campuses female (A) and male (B) hostel drains

Mean Colony Counts of Viable E. faecalis from July 2017-March 2018
In this study, there were fluctuations in the mean colony counts of viable *E. faecalis* within the months. The microbial load of more than $10^7$was counted in the months of August (1.5×10^7 cfu/ml), October (1.4×10^7cfu/ml), September (1.3×10^7 cfu/ml), January (1.27×10^7 cfu/ml) and March (1.45×10^7). The lowest count was observed in the month of December with a 1.31×10^6 cfu/ml (Table 3). The irregularity in number of bacterial counts in the wastewater is probably due to the irregular influx of domestic, agricultural, and industrial effluents into sewage. The seemingly high bacterial count within the months is due to the high number of effluents and influents from UNN-WTP.

Isolation, Molecular Identification and Characterization of Enterococcus faecalis Isolates and the Antibiotic Resistance Genes
Total isolates
A total of 906 *E. faecalis* were isolated out of the two campuses that were used for this study. Out of which 192 and 167 isolates were from UNN-WTP effluent and influent, respectively; while 147 isolates were obtained from the hostel drains in the UNN campus (92 from Nkrumah and 55 from AlvanIkoku). The remaining 400 isolates were from hostel drains in the KSU campus with the highest load (150) coming from Inikpi, followed by Ocheja with 131 isolates and Dangana with 119 isolates.

Molecular characterization
Out of the 906 *E. faecalis* isolated, 63 were selected for antibiotics susceptibility tests and 21 were selected for molecular analyses.

Antibiotic Resistance Prevalence
Comparison of Percentage Antimicrobial-Resistance Profile of Enterococcus faecalis isolates from UNN-WTP Influent and Effluent
In comparing, percentage antimicrobial-resistance profile of *E. faecalis* isolates from UNNWTP influent and effluent, there is no significant difference between the isolates of *E. faecalis* from both the UNNWTP influent as well as effluent at $p>0.05$ (Figure 4).

Comparison of Percentage Antimicrobial-Resistance Profile of E. faecalis isolates from UNN and KSU wastewater Drains
In comparing the percentage antimicrobial resistance profile of *E. faecalis* isolates from UNN and KSU wastewater, there was no

significant difference between the *E. faecalis* isolates from both Universities at p>0.05 (Figure 5).

Susceptibility patternsof 63 isolates in response to 15 different antibiotics are shown in Table 3. Results for the antimicrobial sensitivity tests revealed that isolates *of E. faecalis* were 100% resistant to MEM, P, and CAZ. Others had 96.8 % and 95.2% for CTX and E respectively. *E. faecalis* isolates were most susceptible to LEV (100%), VA (90.5%), F (90.5%) and AMC and TE which both had 88.9% susceptibilities (Table 3).

In comparing, the antimicrobial resistance profile there is no significant difference between the isolates *of E. faecalis* from both the UNNWTP influent and effluent (Table 4).

In comparing the antimicrobial resistance profile of *E. faecalis* isolates from UNN and KSU wastewater, there was no significant difference between the *E. faecalis* isolates from both Universities (Table 5).

Multidrug Resistance (MDR) Patterns of E. faecalisisolates from UNN and KSU wastewater Drains

The multidrug resistance patterns of *E. faecalis* isolates from both campuses showed resistance to mostly MEM, CAZ, P, CTX, and E. The multidrug resistance index of isolates from this study were from 0.33 to 0.73, with the broad range found in isolates from both male (Ocheja) and female (Inikpi) hostels of KSU. Although there was a high *E. faecalis* load in the influents and effluents from WTP in UNN, majority of these isolates showed MAR index range between 0.33 and 0.53 (Table 6).

Antibiotics resistance gene

Twenty-one multidrug-resistant isolates of *Enterococcus faecalis* were screened for antibiotic resistance genes. The isolates had (100%) 16S rRNA, (*RrS)* and Enterococcal surface protein (*esp*); 85.7% with D-ala-D-ala ligase (*Ddl*) and 66.7% with Cytolysin (*ClyA*) indicating they were members of the genus *Enterococcus* and the specie, *E. faecalis (plates 1-2)*. The *Van B* and *Van X* targeted genes were absent in the *Enterococci faecalis* isolates. However, 2(9.52) had *Van A* gene, 20(95.2), and 8(38.1) possessed the *Ant(2)-la* and *Acc(3)-lla* respectively table 7; plate 3. The non-detection of antimicrobial resistance genes ARGs (*Van B* and *Van X*) among *Enterococcus spp*. isolates may be due to the isolates being phenotypically susceptible to vancomycin antimicrobials[38,39] as was observed during susceptibility testing. Other virulence genes screened and confirmed of the *Enterococcus* isolates were *Ant(2)-la* and *Acc(3)-lla*.

The presence of pathogens in environment is of great worry because of their impact on human wellbeing. The persistence of *Enterococcus faecalis* in the environment is often employed as an indicator to predict the presence of pathogens in the environment, especially in surface water. Wastewater treatment plants have been shown as potential point sources of pathogens in surface water.[40] Antimicrobial susceptibility tests carried out on the isolates revealed different degrees of the isolates'resistance. The result of this study on the antimicrobial susceptibility pattern of *Enterococcus faecalis* showed that the organism was 100% resistant to MEM, P, and CAZ. Levofloxacin (LEV) was observed to be the most active because the isolates had 0% resistance to it. The result showed that *Enterococcus faecalis* was resistant to multiple antibiotics. Similar work carried out had reported 100% resistance to tetracycline, ciprofloxacin, penicillin, erythromycin and gentamycin.[41]There have been reports of isolates exhibiting varying levels of responses to some classes of antibiotics. In one of the reports, tetracycline was listed as the least potent followed by ampicillin.[42] Also, other studies have observed similar results.[43,44]

Table 2: Primers for Detecting Antibiotic Resistance Genes

Primers name	Primers Sequence (5′—3′)	Amplicon size (bp)	Thermocycling conditions	References
ant(2)-laF	CATCATGAGGGAAGCGGTG	787	Initial activation step 94°C	31
ant(2)-laR	GAGTACCTTGGTGATCTCG		in 5 min.Then, 30 cycles	
vanA-F	TTGCTCAGAGGAGCATGACG	957	comprising of denaturing	32, 33
vanA-R	TCGGGAAGTGCAATACCTGC		94°C in a min, annealing	
vanB-F	CTTAACGCTGCGATAGAAGC	947	60°C in a min, extension	33
vanB-R	CTG ATGGATGCGGAAGATAC		74°C in 2 min and final	
aac(3)-l1aF	ATATCGCGATGCATACGCGG	877	elongation 74°C in 15 min	34, 35
aac(3)-l1Ar	GACGGCCTCTAACCGGAAGG		and final hold at 4^0C	
vanX-F	TGGGACGCTAAATATGCCAC	511	(indefinite)	36,37
vanX-R	ATACATAGTGCCACCATTCG			

Table 3: Mean Viable *E. faecalis* Colony Counts from July 2017-March 2018(cfu/ml)

Months/year	Inf	Eff	Nkr	Alv	Dan	Och	Ink	MEAN
July 2017	3.8×10^7	5.2×10^6	1.9×10^5	1.0×10^3	2.9×10^3	4.2×10^3	2.0×10^5	6.23×10^6
August 2017	4.7×10^7	5.7×10^7	3.4×10^5	3.0×10^3	3.8×10^3	3.6×10^3	3.6×10^5	1.5×10^7
September 2017	5.3 ×107	3.7×10^7	3.7 x105	4.6 x103	3.6 x103	3.2 x103	3.5 x105	1.3×10^7
October 2017	5.6×10^7	3.9×10^7	3.2×10^5	5.2×10^3	3.2×10^3	4.5×10^3	3.7×10^5	1.4×10^7
November 2017	3.8×10^7	5.8×10^6	1.9×10^5	1.0×10^3	2.9×10^3	4.2×10^3	2.0×10^5	5.94×10^6
December 2017	4.3×10^7	4.8×10^7	3.4×10^5	3.0×10^3	3.8×10^3	3.6×10^3	3.6×10^5	1.31×10^6
January 2018	5.3×10^7	3.5×10^7	3.4×10^5	4.5×10^3	3.6×10^3	3.2×10^3	3.5×10^5	1.27×10^7
February 2018	3.8×10^7	5.2×10^6	1.9×10^5	1.0×10^3	2.9×10^3	4.2×10^3	2.0×10^5	6.23×10^6
March 2018	5.3×10^7	4.8×10^7	3.4×10^5	3.0×10^3	3.8×10^3	3.6×10^3	3.6×10^5	1.45×10^7

KEYS: Inf -WTP influent; Eff-WTP Effluent; Nkr-Nkruma female hostel; Alv-AlvanIkoku male hostel; Dan-Dangana male hostel; Och-Ocheja male hostel and Ink-Inikpi female hostel

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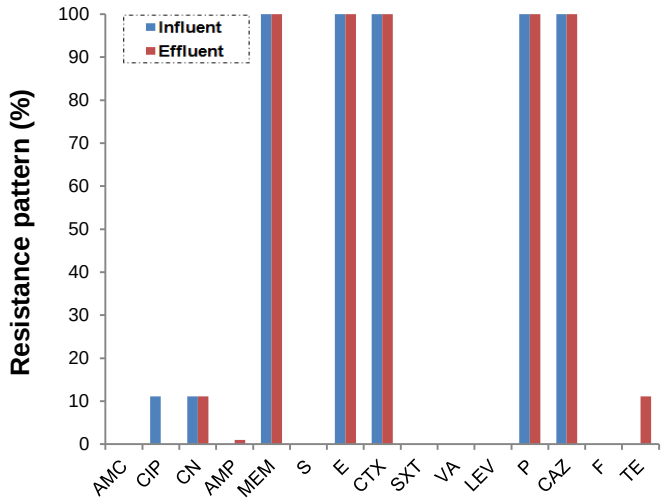

Figure 4: Comparison of percentage antimicrobial resistance profile of *Enterococcus* isolates from UNN-WTP influent and effluent.

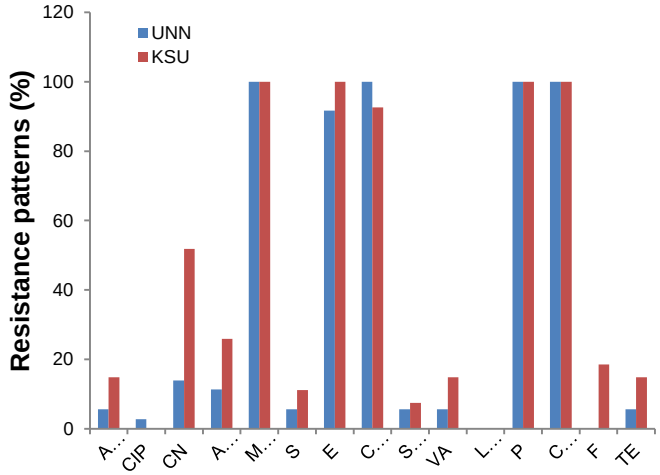

Figure 5: Comparison of percentage antimicrobial resistance profile of *Enterococcus* isolates from UNN and KSU

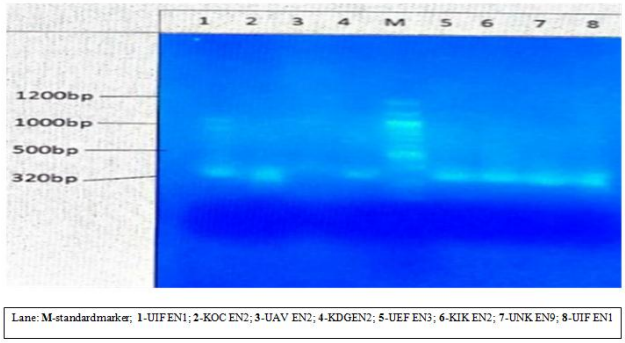

Image 1: Gel electrophoresis image of 16SrRNA gene

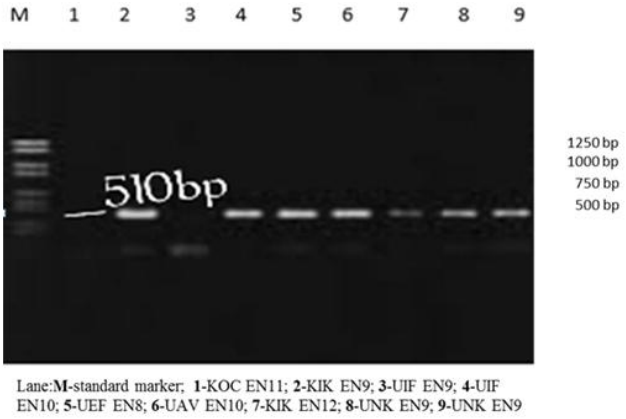

Image 2A: Gel electrophoresis image of Enterococcal surface protein (*esp* gene)

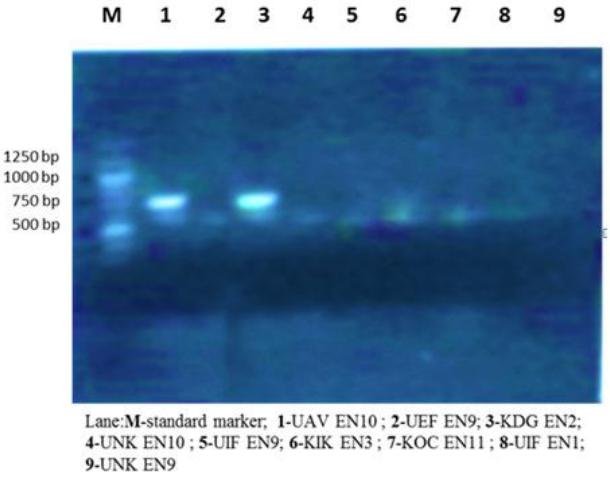

Image 2B: Gel electrophoresis images of Cytolysin gene (***CylA***)

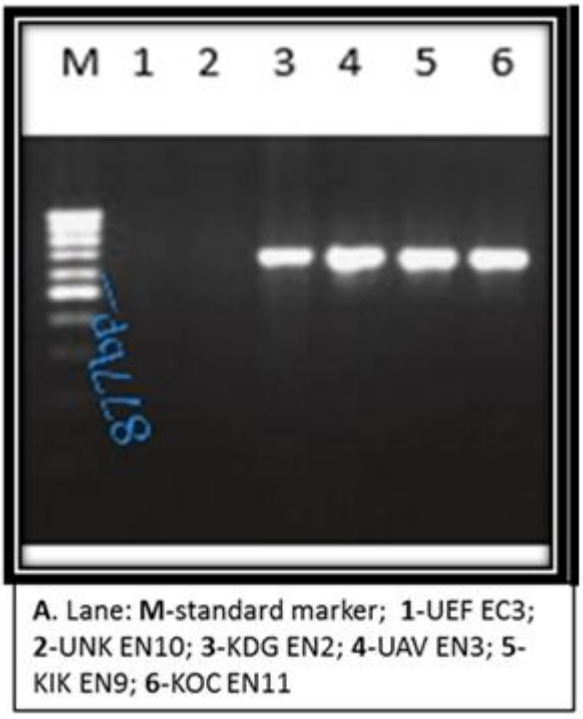

Image 3A: Gel electrophoresis image of antibiotics resistance genes *aac(3)-lla* gene

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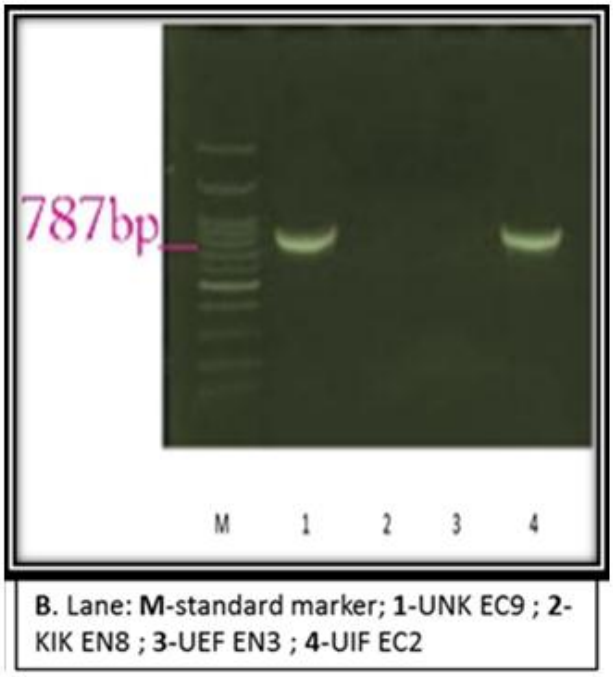

Image 3B: Gel electrophoresis image of antibiotics resistance genes *ant(2)-la* gene

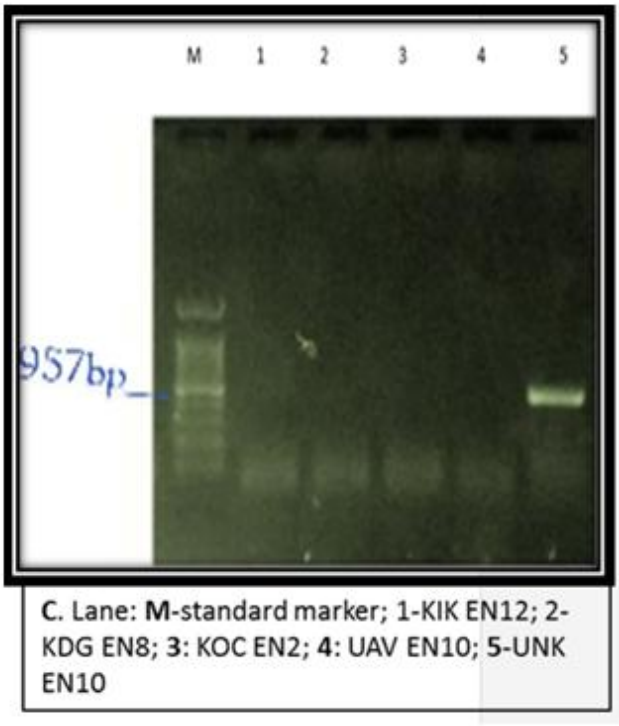

Image 3C: Gel electrophoresis image of antibiotics resistance genes *van A* gene

Keywords:
UIF-UNN WTP influent; UEF-UNN WTP effluent; UAV-UNN AlvanIkoku male hostel; UNK-UNN Nkruma female hostel; KDG-KSU Dangana male hostel; KOC-KSU Ocheja male hostel; KIK-KSU Inipki female hostel; $_{EN}$-Enterococcus; $_{EC}$-E.coli; 1, 2, 3, 7, 8, 9, 10, 11, 12- January 2018, February2018, March,2018 July2017,August2017,September2017,October2017,November2017,December2017

Chigor *et al* 2010[10] have also reported that antibiotic susceptibility tests they carried out on water isolates revealed high levels of resistance in aquatic isolates.

The observed resistance frequency of *Enterococcus faecalis* isolates (55.2%) from UNN hostel drains and that of KSU hostel drains (52.6%) is not significant ($p>0.05$). The presence of *Enterococcus faecalis* in the hostel drains could cause harmful effects on the receiving water body,[45] as results indicate a high percentage level of resistance to antibiotics drugs. The detection of the *Enterococcus faecalis* pathogen in the hostel drains may be attributed to antibiotics in urine, spilt and expired drugs that are carelessly discarded into washbasins.[46]

The detection of pathogens *Enterococcus faecalis* in the effluent (35.8%) of WTP has been linked to the inefficient removal of these pathogenic bacteria by the treatment process. In their research, they reported the detection of pathogenic bacteria in the wastewater treatment facility at Hawassa University Referral Hospital (HURH). Similarly, Iweriebor *et al*. 2015[46] have also reported the inefficient Fort Hare wastewater treatment plant following the detection of the presence of *Enterococcus* spp. in the final effluent discharged to receiving water bodies.[46] The result of this study is in agreement with this report as the final effluent released into the water body at UNN-WTP indicates the presence of *Enterococcus faecalis* with a 35.8% resistance to antibiotics. The result of this study has also revealed the inefficiency of the treatment plant process. Hence the final effluent discharged may cause harmful effects on the receiving water body.[45] A good number of studies have revealed that WWTPs can provide a favourable environmental condition that is needed for antibiotic-resistance gene transfer[47,48,49,46] and as reservoirs of antibiotic-resistant bacteria.[50] The isolation and confirmation of *Enterococcus faecalis* in the isolates from hostel drains and WTP (influent and final effluent) especially the hostel drains should be of major concern. This is so as *Enterococcus faecalis* has been linked with infections like urinary tract infections, neonatal infections, central nervous system (CNS) infections, endocarditis, bacteremia, and abdominal and pelvic infections.[51]Hence farmlands that receive this kind of drain when watched off by rain or serve as a source of irrigation could eventually be absorbed by food crops and can cause infections. The genes *RrS*, *Ddl*, *ClyA*, *hlyA* and *esp* were detected. The virulence factor *esp* is described as a human-specific marker used for microbial source tracking.[13,52,53,54] The presence of these genes in isolates is regarded as a good indicator of human faecal contamination in water bodies.[54,55] Virulence factor enterococcal surface proteins (*esp*) play a significant role in enterococcal adhesion to collagen and the extracellular matrix.[46] The presence of resistant *Enterococcus faecalis* in the hostel drains and the observation of the resistant patterns showed similarities in resistant patterns with WTP final effluent discharged.

In this study, microbial counts range from 1.0 $x10^3$(cfu/ml) (AlvanIkoku male hostel, UNN) to 5.8 $\times10^7$ (cfu/ml) (WTP effluent, UNN). The fluctuation in the number of bacterial counts in the wastewater may be due to the irregular influx of domestic, agricultural and industrial effluents into sewage. In the present study, bacterial counts tend to get high in the warm and dry seasons because bacteria can survive and reproduce more successfully under warm conditions due to free sediment-water interactions and less available dilution in summer. Generally, AlvanIkoku male hostel, UNN had the lowest bacterial count, while samples from WTP effluent, UNN had the highest counts. In comparing the hostels, Ink---Inikpi female hostel (KSU) had the highest and AlvanIkoku male hostel, UNN had the lowest) reason being that Inikpi hostel is closer to the electricity powerhouse of the university and the animal farmhouse is situated in the same region (exposed to more animal dung run-off, hydrocarbons from diesel, engine oil and smoke which microbes use as food substrate during biodegradation). Also, open defecation is very common among those female hostellers. All these and more cause a rise in the microbial count. Similarly in another study, observed *E. coli* 1.00 to 530×10^7 MPN/100 mL. Coliform 5.00 to 720×10^7 MPN/100 mL, Leptothrix sp. Nil to 42×10^5 MPN/100 mL and, fecal streptococci 9.00 to 700×10^3 MPN/100 mL, in sewage water of Aligarh. Coliform, Fecal streptococci and *E. coli* form 13 to 214.6×10^6 MPN/100 mL, and 8.7 to 47×10^6 MPN/100 mL respectively in sewage of Ahmedabad.[54]

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From the pond waters of Aligarh Kalidah, (that receives sewage) hashigher coliforms 210 to 480×10^5 MPN/100 mL, faecal streptococci 220 to 480×10^2 MPN/100 mL, *E. coli* 60 to 290×10^5 MPN/100 mL, Zoogloea sp. 102 to 290 $\times10^5$/100 mL and Leptothrix sp. 263 to 640 $\times10^5$ /100 mL.[55] The fluctuation in the number of bacteria in sewage may be due to the irregular surge of domestic and industrial effluents into sewage. Therefore, hostel drains could be seen to be a huge source of antibiotic-resistant bacteria in the environment. Therefore, more work should be carried out in this regard.

Conclusion

Pathogenic *Enterococcus feacalis* isolated from hostel drains and WTP were found to be resistant to meropenem, penicillin G, ceftazidime, cefotaxime erythromycin and other commonly used antibiotics. However, the number of antibiotics that were active increased on the isolates on the final effluent. The susceptibility patterns indicated a potential possibility of antibiotic resistance dissemination in aquatic environments. The contribution of individual point sources like hostel drains to the microbial load of surface water varies. Inefficient WTPs were also observed with the discharge of final effluents with unacceptable microbial counts into the receiving water bodies.

Table 4: Comparative Antibiotics Susceptibility Pattern of *E. faecalis* from Influent and Effluent of WTP in UNN

Treatment	**Influent**			**Effluent**			**Statistical significance**
ANTIBIOTICS	**S**	**R**	**I**	**S**	**R**	**I**	**Chi-square**
AMC	9	0	0	9	0	0	*NA
CIP	8	1	0	8	0	1	0.331
CN	8	1	0	7	1	1	0.929
AMP	8	0	1	6	2	1	0.131
MEM	0	9	0	0	9	0	*NA
S	9	0	0	9	0	0	*NA
E	0	9	0	0	9	0	*NA
CTX	0	9	0	0	9	0	*NA
SXT	9	0	0	9	0	0	*NA
VA	9	0	0	9	0	0	*NA
LEV	9	0	0	9	0	0	*NA
P	0	9	0	0	9	0	*NA
CAZ	0	9	0	0	9	0	*NA
F	9	0	0	9	0	0	*NA
TE	9	0	0	8	1	0	0.303

NA: Not Applicable

Table 5: Comparative antibiotics susceptibility pattern of *E. faecalis* from drains at both UNN and KSU campuses

Treatment	**KSU**			**UNN**			**Statistical significance**
ANTIBIOTICS	S	R	I	S	R	I	**Pearson Chi-square**
AMC	23	4	0	15	2	1	0.3407
CIP	22	0	5	15	0	3	*NA
CN	12	14	1	15	3	0	0.0003
AMP	17	7	3	10	4	4	0.6089
MEM	0	27	0	0	18	0	*NA
S	24	3	0	14	4	0	*NA
E	0	27	0	0	15	3	*NA
CTX	2	25	0	0	18	0	*NA
SXT	24	2	1	14	2	2	0.4960
VA	23	4	0	16	2	0	*NA
LEV	27	0	0	18	0	0	*NA
P	0	27	0	0	18	0	*NA
CAZ	0	27	0	0	18	0	*NA
F	21	1	5	18	0	0	0.0358
TE	21	4	2	17	1	0	0.0580

NA: Not Applicable

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Table 6: Multidrug Resistance (MDR) Patterns of *Enterococcus faecalis* Isolates from this Study

Sampling Area	Campus	Sampling point	Resistant isolates	Antibiotics resistant	Numberof antibiotics resisted (%)	MAR Index range
Female Hostels Drains	UNN	Nkrumah	UNK_{EN}^{3} UNK_{EN}^{10} UNK_{EN}^{12} UNK_{EN}^{9} UNK_{EN}^{11}	MEM, CAZ, P, CTX, E	5(33.3)	0.33-0.67
	KSU	Inikpi	KIK_{EN}^{9} KIK_{EN}^{12} KIK_{EN}^{8} KIK_{EN}^{3} KIK_{EN}^{1} KIK_{EN}^{10}	MEM, CAZ, P, CTX, E, CN	6(40.0)	0.33-0.73
Male Hostels Drains	UNN	Alvan-ikoku	$UAV_{EN}^{2}UAV_{EN}^{9}$ UAV_{EN}^{10} UAV_{EN}^{1} UAV_{EN}^{3}	MEM, CAZ, P, CTX, E	5(33.3)	0.33-0.67
	KSU	Ocheja	$KOC_{EN}^{3}KOC_{EN}^{1}$ $KOC_{EN}^{2}KOC_{EN}^{8}$ KOC_{EN}^{11}	MEM, CAZ, P, CTX, E	5(33.3)	0.33-0.73
	KSU	Dangana	$KDG_{EN}^{8}KDG_{EN}^{7}$ $KDG_{EN}^{10}KDG_{EN}^{2}$ KDG_{EN}^{12}	MEM, CAZ, P, CTX, E	5(33.3)	0.33-0.67
WTP	UNN	Influent	UIF_{EN}^{8} UIF_{EN}^{3} UIF_{EN}^{9} UIF_{EN}^{11} UIF_{EN}^{10} UIF_{EN}^{1}	MEM, CAZ, P, CTX, E,	5(33.3)	0.33-0.53
	UNN	Effluent	UEF_{EC}^{3} UEF_{EC}^{1} UEF_{EC}^{9} UEF_{EC}^{8} UEF_{EC}^{2} UEF_{EC}^{7} UEF_{EC}^{10}	MEM, CAZ, P, CTX, E,	5(33.3)	0.33-0.53

Table 7: Detected genes for antibiotic resistance in isolates of *E. faecalis*

Genes	Present No (%)	Absent No (%)	Pearson Chi-square/ fisher's exact test
ant(2)-la	20(95.2)	1(4.8)	0.000
aac(3)-lla	8(38.1)	13(61.9)	
Van A	2(9.5)	19(90.5)	

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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