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Molecular Assessment of Methicillin-Resistant Staphylococcus Aureus Strains in Domestic Effluents of a University Community Akure, Nigeria

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ABSTRACT

Domestic effluents were collected from the kitchen, bathroom, laundry of students' hostels and the effluents were bacteriologically analysed. The molecular identity and methicillin-resistant gene assay of selected multidrug resistant Staphylococcus aureus strains were conducted via 16S rRNA sequencing. Bathroom effluents obtained from Jadesola hostel had the highest staphylococcal count of 38.04±2.31 CFU/100 ml, while Adeniyi hostel had the least at 1.25±0.05 CFU/100 ml. All presumptive Staphylococcus species isolated from the domestic effluents produced a coagulase-positive outcome. The domestic effluents sourced 2 m away from the original source in FUTA hostels had the Staphylococcus aureus percentage occurrence of 15 % (45.76) whilst a low percentage occurrence was recorded in tap water obtained from the hostel locations at 1 % (8.48%). Staphylococcus aureus isolated in effluents from Abiola male hostel and FUTA staff quarters were resistant to oxacillin at 11.50±0.55 and 12.00±0.00 mm respectively. Staphylococcus aureus strain 1, 2, 6, 7, 8, 13 and 14 were positive with Mec A gene bands at approximately 300 base pairs. The Staphylococcus aureus strains isolated from this study showed phenotypic resistance to oxacillin, a drug proxy of methicillin in Staphylococcal therapy. The unsystematic expulsion of untreated domestic effluents into water channels should be prohibited.

Keywords: *Domestic Effluents; Staphylococcus Aureus; Resistant Genes; Resistant; Susceptible*.

1.0 Introduction

Staphylococcus aureus is considered as one of the bacterial commensals, however it may act as a virulent pathogen threatening both animals and humans [1] [2] [3]. It causes a variety of hospital and community acquired clinical infections in humans, including skin, soft tissue, and pleuropulmonary infections [4]. The ability of S. aureus to outwit the immune system, above and beyond its multidrug resistance (MDR) phenotype makes it as one of the most intractable pathogenic bacteria in the history of antibiotic chemotherapy [5].

The spread of methicillin-resistant S. aureus (MRSA) has become a significant concern for both animal and human health worldwide [6] [7]. Methicillin resistance in S. aureus is predominantly mediated by the expression of mecA gene, which is located on a mobile genetic element; the staphylococcal cassette chromosome mec (SCCmec), encoding an altered penicillin-binding protein (PBP2a) with an exceedingly low susceptibility to beta-lactam antibiotics. Thus, S. aureus will be practically resistant to most beta-lactam antibiotics [8]. Currently, as water shortages expand, treated municipal wastewater is increasingly used for applications including landscape and crop irrigation, groundwater recharge, and snowmaking [9] [10]. During these activities, individuals applying, using, or coming in contact with reclaimed wastewater could potentially be exposed to MRSA and other bacteria that may remain in treated wastewater [11]. The emergence of Methicillin-resistant Staphylococcus aureus (MRSA) poses a serious public health threat. Strains of S. aureus resistant to β-lactam antibiotics are known as methicillin-resistant Staphylococcus aureus (MRSA) [12]. First described as a cause of nosocomial infection in hospital settings, now MRSA has gained attention as community pathogen [13].

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On the other hand, resistance to vancomycin is accomplished by horizontal transfer of a plasmid-born transposon carrying vanA gene from vancomycin-resistant Enterococcus to S. aureus crosswise the genus barrier [5]. In the present study, evaluation occurrence of MRSA and methicillin-susceptible S. aureus (MSSA) in domestic wastewater at the Federal University of Technology Akure (FUTA) student hall of resident in Ondo State, Nigeria. To further assess the MRSA strains, isolates were characterized by staphylococcal cassette chromosome mec (SCCmec) typing. Beside the determination of its antimicrobial susceptibility pattern, the prevalence of both mecA genes among some selected methicillin-resistant isolates was investigated.

2.0 Materials and Methods

2.1 Sample location depiction

Federal University of Technology, Akure (FUTA) was used as sampling locations with a major focus on halls of residence where most of the domestic effluents were being generated. FUTA is a leading University of Technology located in the largest city and capital of Ondo State, located in South-west Nigeria (Figure 1). The residents on campus are majorly staff and students from different part of the country. Akure lies about 70°15 north of the equator and 50°15 east Meridian.

2.2 Wastewater effluents collection

During this research, seventy-two (72) samples of domestic effluents coupled with twelve (12) tap water samples were collected from six (6) different halls of residence and staff quarters in FUTA (Jibowu hostel, Akindeko hostel, Abiola hostel, Adeniyi hostel and two staff quarters). During the period of sample collection, it was observed that the domestic effluents are mostly generated early in the morning and later in the evening but early in the morning was chosen in order to enable the transportation of the samples to the laboratory for further research analysis.

The effluents were aseptically collected from the collection points and two (2) meters away from collection point into sterile sampling bottles labeled appropriately with the source, time and date of collection. They were transported to microbiology research laboratory of Federal University of Technology, Akure within one hour of collection for microbiological analyses.

2.3 Isolation of staphylococcus from domestic effluents

Bacteriological examinations were carried out using standard methods for aerobic bacteria and membrane filtration was used to recover S. aureus from wastewater (effluents) samples collected in sterile bottle was gently shake. Briefly, 100 mL of each sample were vacuum filtered through a 0.45-µm, 47-mm mixed cellulose ester filter (Millipore, Billerica, MA). Filters were then placed on mannitol salt agar (MSA) plates and incubated at 37°C for 24-48 hr. aliquot (1.0 ml) was transferred into the test tube containing 9.0 ml of sterile distilled water and diluted serially in one-tenth stepwise to 10-4 dilution factor and 1.0 ml each of dilution 10-3 and 10-4 was pure plated on Nutrient agar, selective and differential media (Manitol Salt agar), the plates were inverted and incubated aerobically at 37°C for 24 - 48 hours after which the plates were examined for growth.

Resulting yellow colonies growing on a yellowish medium were considered presumptive S. aureus, while, the result on Nutrient agar was used to determine the microbial count for each sample. Staphylococcus aureus was confirmed using Gram stain, coagulase, catalase tests and streaking on blood agar [14] [15].

2.4 Enumeration of bacterial colony

Calculation of colony forming unit (CFU) per milliter (ml) for Staphylococcus species was recorded in standard form [16]. The number of colonies on each plate was recorded utilizing a colony counter (J-2 PEC MEDICAL, New Jersey, USA) [17]. Calculation of colony forming unit (CFU) per gram for the bacteria was based on the formula:

CFU = Number of colonies × volume of the sample suspended Dilution factor

2.5 Standardization of inoculum (McFarland turbidity standard)

The Method modified by Bayode et al. (2021), was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of

bacterial cells. In this method, fifty milliliter (50ml) of a 1.175% (wt/vol) dehydrates Barium chloride (BaCl2.2H2O) solution was added to 99.4ml of 1% (vol/vol) sulfuric acid. McFarland standard tube was then sealed with Paraffin to prevent evaporation and stored in the dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1cm light path. The 0.5 McFarland standards were vigorously agitated before use [16].

2.5.1 Antimicrobial susceptibility test

Antibiotic resistance of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method [18]. The suspension of the test organism in nutrient broth was matched with 0.5 McFarland turbidity standards to give concentration of 1.5 × 108 CFU/ml, 0.5ml of the suspension was transferred to prepared Mueller-Hinton agar and spread with a sterilized glass spreader, excess suspension was drained. The surface of the agar was allowed to dry and antibiotic disc was aseptically picked and gently placed on top of agar plate by sterile forceps. The inoculated plates were incubated at 37oC for 18 hours, after incubation a clear zone of no growth in the immediate vicinity of an antibiotic disk was measured and recorded as zone of inhibition. The zones of inhibition were compared with Clinical Laboratory Standards interpretative chart, [19] and interpreted as resistance, intermediate and susceptible.

The following clinical antibiotics, with their concentrations given in parentheses were used in the antibiograms for gram negative bacteria; Tetracycline (30µg), Ofloxacin (30µg), Gentamicin (20µg), Chloramphenicol (30µg), Augmentin (30 µg), Ceftriazone (30 µg), Nitrofuration (300 µg), Cotrimozazole (25 µg), Ciprofloxacin (10 µg) and Amoxicillin (30µg) while gram positive was tested against Cotrimozazole (25 µg), Erythromycin (10µg), Gentamicin (20µg), Augmentin (30 µg), Streptomycin (10 µg), Cloxacilin (5 µg) Tetracycline (30µg) and Chloramphenicol (30µg). Multidrug resistance was defined in this study as resistance to three or more antibiotics tested. Also, methicillin resistance was defined in this study as resistance to oxacillin antibiotics.

2.5.2 Multiple antibiotic resistance (MAR) index

The MAR index was calculated to compare the resistance level of isolates across different areas

Figure 1: Locality Map of Federal University of Technology Akure Showing Diverse Sites of Domestic Effluents Collected

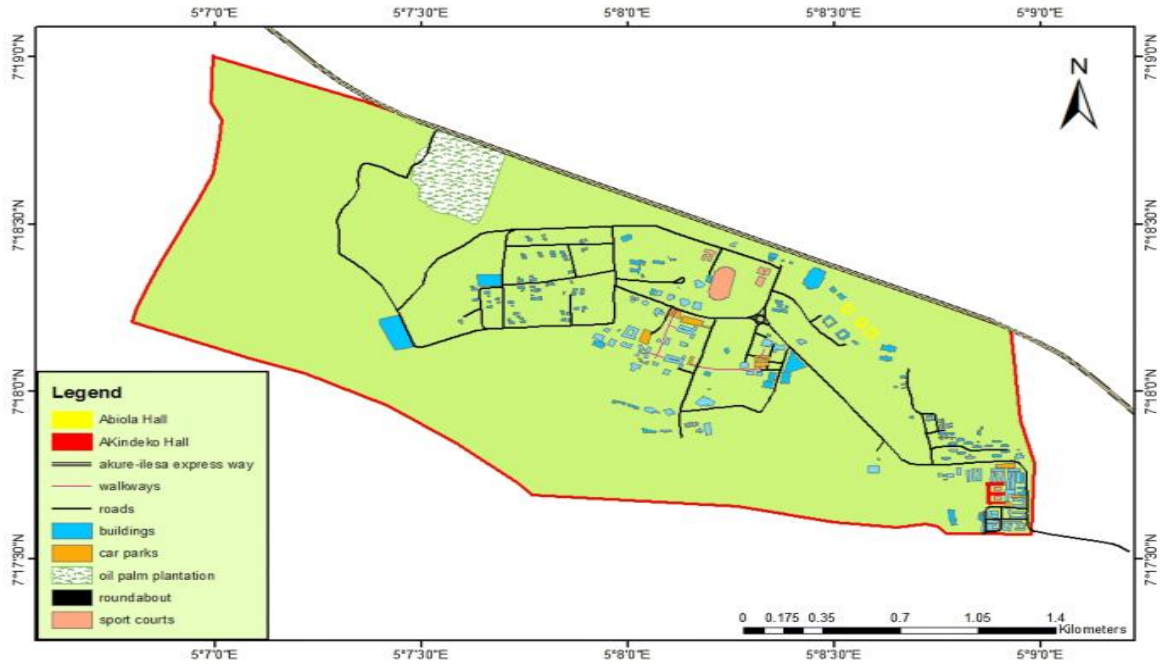


Table 1: Total Staphylococcal Counts of Domestic Effluents Collected from FUTA Campus, Akure

Sources of effluents		Effluents' source	2m away from the source	Tap water
Laundry (Cfu/100ml)	Female Hostel (Jadesola)	13.30 ± 0.26^{d}	7.00 ± 0.42^{b}	0.00 ± 0.00^{a}
	Female Hostel (Jibowu)	9.00 ± 1.03^{c}	28.03 ± 1.31^{c}	2.01 ± 0.01^{b}
	Male Hostel (Abiola)	6.01 ± 0.11^{b}	8.02 ± 0.08^{b}	0.00 ± 0.00^{a}
	Male Hostel (Adeniyi)	1.25 ± 0.05^{a}	2.00 ± 0.01^{a}	0.00 ± 0.00^{a}
	Staff Quarters	2.50 ± 0.21^{b}	1.00 ± 0.00^{a}	1.00 ± 0.00^{b}
Bathroom (Cfu/100ml)	Female Hostel (Jadesola)	38.04 ± 2.31^{f}	10.03 ± 0.03^{b}	0.00 ± 0.00^{a}
	Female Hostel (Jibowu)	5.00 ± 1.06^{b}	6.01 ± 0.01^{b}	0.00 ± 0.00^{a}
	Male Hostel (Abiola)	12.06 ± 2.44^{d}	25.05 ± 0.22^{c}	0.00 ± 0.00^{a}
	Male Hostel (Adeniyi)	29.50 ± 1.19^{e}	42.21 ± 0.37^{d}	0.00 ± 0.00^{a}
	Staff Quarters	6.02 ± 0.18^{b}	6.00 ± 0.00^{b}	0.00 ± 0.00^{a}
Kitchen (Cfu/100ml)	Female Hostel (Jadesola)	5.00 ± 0.00^{b}	4.03 ± 0.01^{a}	0.00 ± 0.00^{a}
	Female Hostel (Jibowu)	17.01 ± 0.06^{d}	189.06 ± 1.36^{e}	0.00 ± 0.00^{a}
	Male Hostel (Abiola)	3.00 ± 0.00^{b}	2.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Male Hostel (Adeniyi)	4.00 ± 0.00^{b}	4.01 ± 0.39^{a}	0.00 ± 0.00^{a}
	Staff Quarters	18.03 ± 0.01^{d}	6.05 ± 0.04^{b}	0.00 ± 0.00^{a}

and sample types using the equation MAR index = a/b. Where "a" represents number of antibiotics to which isolates were resistant, "b" represents the number of antibiotics to which isolates were exposed [20].

2.6 Molecular characterization of wound bacteria

2.6.1 DNA extraction of bacteria isolates

Genomic extraction and purification of Deoxyribonucleic Acid (DNA) is virtually the basis of all procedures in forensic molecular biology. DNA extraction method used and DNA concentration were evaluated through the steps described by Jena bioscience Kit™. The steps were classified into three (3) which are lysis, precipitation and hybridization as demonstrated by Bunyan et al. [21].

2.6.2 PCR amplification

Polymerase chain reaction was carried out using the method described by Abayasekara et al. [22]. The 16SrRNA gene of the bacteria was amplified using the primer pair 27F-5′-AGAGTTTGATCCTGGCT CAG-3', and 1492R 5'GGTTACCTTGTTACGAC TT-3'.

Thermal cycling was conducted in an eppendorf vapo protect thermal cycler (Nexus Series) for an initial denaturation of 95 °C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95 °C; 1 minute at 61 °C and 1 minute 30 Seconds at 72 °C. This was followed by a final extension step of 10 minutes at 72 °C.

The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

2.6.3 Sequencing and blasting

Polymerase chain reaction (PCR) products were purified with Exo sap and then subjected to DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2). The basic local alignment search tool was carried out using the NCBI (National Commission for Biotechnological Information) gene bank as conducted by as conducted by Abdulkasim et al. [23].

2.7 Data analysis

Data was statistically analyzed using SPSS version 20, percentage resistance was separated using Duncan's New Multiple Range test and significant difference will be value at $p \leq 0.05$.

3.0 Result

3.1 Staphylococcal load of domestic effluents from FUTA campus

Table 2: Cultural, Morphological and Biochemical Characteristics of Staphylococcus Aureus Isolated from Domestic Effluents Collected from FUTA Campus, Akure

Gram reaction	Cell shape	Haemolysis on blood agar	Oxidase	Catalase	Sugar fermentation						Coagulase	DNASE	Presumptive identification	Sources of isolates
					Lactose	Sucrose	Glucose	Manitol	Maltose	Galactose				
Positive	Cocci	+		+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Female Hostel (Bathroom)
Positive	Cocci	+	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Female Hostel (Kitchen)
Positive	Cocci	+	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Male Hostel (Laundry)
Positive	Cocci	+	-	-	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Male Hostel (Bathroom)
Positive	Cocci	+	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Staff Quarters (Kitchen)
Positive	Cocci	+	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Female Hostel (Laundry)
Positive	Cocci	+	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Male Hostel (Kitchen)
Positive	Cocci	-	-	+	+	+	+	+	+	+	+	+	Staphylococcus aureus	FUTA Staff Quarters (Bathroom)

Bathroom effluents (source) obtained from Jadesola hostel had the highest staphylococcal count of 38.04±2.31 CFU/100 ml while Adeniyi hostel had the least count at 1.25±0.05 CFU/100.

Adeniyi hostel also recorded a high staphylococcal count of 29.50±1.19 CFU/100 ml. Jibowu hostel had the highest staphylococcal count of 189.06±1.36 CFU/100 ml for effluents obtained 2 m away from the source in the kitchen area while Abiola hostel had the least at 2.00±0.05 CFU/100 ml.

The staphylococcal counts of bathroom effluents obtained from all hostel locations 2 m away from the source ranged 1.25±0.05 CFU/100 to 42.21±0.37 CFU/100 ml. Additionally, the staphylococcal counts of laundry effluents ranged from 1.00±0.00 to 28.03±1.31 CFU/100 ml. The tap water of all sample locations produced one or no staphylococcal count as shown in Table 1. Values are presented as mean±SE, values in the same column carrying same superscript are not differently significantly according to new Duncan's Multiple Range test at $p<0.05$

3.2 The biochemical characteristics of Staphylococcus aureus of effluents from FUTA hostels

All presumptive Staphylococcus species isolated from the effluents samples all produced a coagulase-positive test result being a major confirmatory test for Gram positive cocci Staphylococcus aureus as displayed in Table 2.

3.3 Percentage occurrence of Staphylococcus aureus in domestic effluents from FUTA hostels

The domestic effluents source and 2 m away from the source in FUTA hostels recorded Staphylococcus aureus percentage occurrence of 15 (45.67 %) whilst a low percentage occurrence of S. aureus 1 (8.48 %) was recorded in tap water obtained from the hostel locations in both campuses as shown in Table 3.

3.4 Antibiotic sensitivity of domestic effluents from FUTA hostels laundry and kitchen

Table 3: Percentage Occurrence of Staphylococcus Aureus in Domestic Effluents Collected from FUTA Campus, Akure

Sources of effluents		Effluents' source	2m away from the source	Tap water	Total
Laundry	FUTA Female Hostel (Jadesola)	+	+	-	2
	FUTA Female Hostel (Jibowu)	+	+	+	3
	FUTA Male Hostel (Abiola)	+	+	-	2
	FUTA Male Hostel (Adeniyi)	+	+	-	2
	FUTA Staff Quarters	+	+	-	2
Bathroom	FUTA Female Hostel (Jadesola)	+	+	-	2
	FUTA Female Hostel (Jibowu)	+	+	-	2
	FUTA Male Hostel (Abiola)	+	+	-	2
	FUTA Male Hostel (Adeniyi)	+	+	-	2
	FUTA Staff Quarters	+	+	-	2
Kitchen	FUTA Female Hostel (Jadesola)	+	+	-	2
	FUTA Female Hostel (Jibowu)	+	+	-	2
	FUTA Male Hostel (Abiola)	+	+	-	2
	FUTA Male Hostel (Adeniyi)	+	+	-	2
	FUTA Staff Quarters	+	+	-	2
Total (%)		15(45.76)	15(45.76)	1(8.48)	31

All Staphylococcus aureus strains from FUTA hostels were susceptible to rocephin except the strain from FUTA female hostel which was resistant at 13.00±0.68 mm. Staphylococcus aureus isolated in effluents from Jadesola female hostel, Adeniyi male hostel, Abiola male hostel and FUTA staff quarters were sensitive to septrin at 24.05 ± 1.32 mm, 22.40±0.38 mm, 23.00±0.78 mm and 22.50±0.55 mm respectively.

All Staphylococcus aureus strains from FUTA hostels were susceptible to erythromycin and perfloxacin except the strain from FUTA female hostel. All Staphylococcus aureus strains from the hostels in FUTA were susceptible to gentamycin except strains of waste water effluents from Jadesola female hostel as demonstrated in Table 4. All Staphylococcus aureus strains isolated from domestic effluents from FUTA Abiola male hostel was resistant to septrin at 15.00±0.00 mm whilst other S. aureus strains were sensitive to other strains. All S. aureus strains from the kitchen effluents were either resistant or displayed intermediate sensitivity to oxacillin as illustrated in Table 5.

3.5 Multiple antibiotic resistance (MAR) index of Staphylococcus aureus strains from FUTA hostels' domestic effluents

The MAR index of Staphylococcus aureus strains from FUTA hostels' laundry domestic effluents ranged from 0.09-0.64 (Table 6) whilst the MAR index of S. aureus strains from FUTA hostels' kitchen effluents ranged from 0.09-0.36 (Table 7).

Table 4: Antibiotic Susceptibility Patterns of Staphylococcus Aureus in Domestic Effluents (Laundry) Collected from FUTA Campus, Akure

	Mean zones of inhibition (mm)				
Antibiotics	**FUTA Female Hostel (Jadesola)**	**FUTA Female Hostel (Jibowu)**	**FUTA Male Hostel (Abiola)**	**FUTA Male Hostel (Adeniyi)**	**FUTA Staff Quarters**
Oxacillin	16.42±0.15b (I)	17.00±0.56b (I)	11.50±0.55a (R)	16.05±0.42b (I)	12.00±0.00a (R)
Septrin	24.05±1.32c (S)	15.50±0.33b (I)	22.40±0.38c (S)	23.00±0.78c (S)	22.50±0.55c (S)
Erythromycin	24.00±0.86b (S)	13.12±0.62a (I)	21.50±0.61b(S)	23.50±0.32b (S)	22.50±0.32b (S)
Perfloxacin	24.50±0.55b (S)	12.01±0.14a (R)	23.61±0.62b(S)	24.00±0.00b (S)	24.41±0.43b (S)
Gentamicin	23.33±0.36c (S)	12.00±0.00a (R)	19.50±0.42c (S)	22.50±0.03c (S)	20.00±0.00c .(S)
Ampiclox	13.51±1.11a (R)	10.05±0.03a (R)	13.00±0.00a (R)	13.05±0.05a (R)	13.50±0.21a (R)
Zinnacef	21.00±0.29c (I)	13.50±0.02a (R)	22.00±0.00c (I)	23.50±0.71c (S)	18.05±1.06b (I)
Amoxicillin	20.42±0.91b (S)	12.00±0.41a (R)	13.05±0.31a (R)	13.06±0.44a (R)	20.00±0.15b (S)
Rocephin	23.00±0.00c (S)	13.00±0.68a (R)	15.15±0.42a (R)	18.50±0.37b (I)	22.50±0.44c (S)
Ciprofloxacin	24.00±0.00b (S)	19.04±0.62a (I)	23.33±0.21b (S)	24.00±0.62a (S)	24.00±0.00b (S)
Streptomycin	23.06±0.53b (S)	14.57±1.32a (R)	21.50±1.05b (S)	23.50±0.42b (S)	24.50±0.27b (S)

Values are presented as mean±SE, values in the same row carrying same superscript are not different significantly according to new Duncan's Multiple Range test at p<0.05

3.6 The molecular identity of Staphylococcus aureus strains from domestic effluents

The gel electrophoresis image of DNA fragments of the Staphylococcus species with clear bands observed in isolate codes including; 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18 as displayed in Plate 1.

3.7 Detection of mec a gene in Staphylococcus aureus strains associated with domestic effluents from FUTA and elizade university students' campus

Staphylococcus aureus strain 1, 2, 6, 7, 8, 13 and 14 were positive with electrophoresis DNA resistant Mec A gene bands at approximately 300 base pairs. Staphylococcus aureus strain 3, 4, 5, 9, 10, 11, 12, 15, 16, 17, 18, 19, and 20 were negative with no band observed for Mec A gene as displayed in Plate 2.

4.0 Discussion

Effluent generation is an important part of anthropogenic activities which could facilitate the development and spread of antibiotic resistance bacteria (ARB). Staphylococcus aureus and S. sciuri isolates were recovered in this study as it was also reported in effluents in Nigeria and Spain [24] [25] [26]. In FUTA students' hostel domestic effluents, the highest load of S. aureus was found in Kitchen effluent (2 m away from the source), which could be as a result of poor personal hygiene of the student utilizing the kitchen and the type of detergent used in cleaning the kitchen utensils.

Table 5: Antibiotic Susceptibility Patterns of Staphylococus Aureus in Domestic Effluents (Kitchen) Collected from FUTA Campus, Akure

Antibiotics	Mean zones of inhibition (mm)				
	FUTA Female Hostel (Jadesola)	FUTA Female Hostel (Jibowu)	FUTA Male Hostel (Abiola)	FUTA Male Hostel (Adeniyi)	FUTA Staff Quarters
Oxacillin	0.00±0.00a (R)	16.22±0.16c (I)	13.44±0.32c (R)	15.06±0.31c (R)	10.00±0.00b (R)
Septrin	23.34±0.02c (S)	24.00±0.00c (S)	15.00±0.00a (I)	19.71±0.42b(S)	24.41±0.32c (S)
Erythromycin	22.06±0.14a (S)	24.03±1.27a (S)	20.34±0.74a (S)	23.18±0.33a (S)	24.06±1.11a (S)
Perfloxacin	24.31±0.68a (S)	24.33±0.42a (S)	23.38±0.62a (S)	24.24±0.16a (S)	24.00±0.00a (S)
Gentamicin	21.00±0.13a (S)	23.00±0.00a(S)	20.00±0.00a (S)	23.00±0.00a (S)	22.31±0.58a (S)
Ampiclox	18.41±0.11b (S)	13.07±0.29a(R)	13.11±0.31a (R)	23.01±1.04c (S)	10.06±0.41a (R)
Zinnacef	20.00±0.00b (S)	21.00±0.00b (S)	14.03±0.55a (R)	23.31±0.28b (S)	21.31±0.21b (I)
Amoxicillin	13.00±0.00a (R)	20.05±0.54b(S)	13.00±0.00a (R)	24.00±0.00b (S)	13.00±0.00a (R)
Rocephin	23.24±0.31b(S)	23.03±0.62b (S)	18.50±0.31a (S)	24.22±0.06b (S)	22.50±0.31b (S)
Ciprofloxacin	22.01±1.24b (S)	24.01±0.44b(S)	21.73±0.41b (S)	24.55±0.42b (S)	24.42±1.38b (S)
Streptomycin	23.00±0.00a (S)	23.02±0.62a(S)	23.06±0.58a (S)	24.00±0.00a	24.06±0.02a (S)

Values are presented as mean±SE, values in the same row carrying same superscript are not different significantly according to new Duncan's Multiple Range test at p<0.05

Based on available information, this is the first report that shows the detection of higher S. aureus in a specific distance away from the source, this has provided an insight and need for active surveillance at distance away from the effluent source to understand the burden of antimicrobial resistance in the community. The presence of S. aureus in 2 m away suggests that waste water could be a potential reservoir for S. aureus in the community. However, this report is similar to recent report from wastewater in Ile Ife [24] in South-west Nigeria.

The discharge of untreated wastewater into the environment has many serious implications on the receiving environment because it leads to high nutrient accumulation, reduced dissolved oxygen concentration as well as higher percentage of potentially pathogenic and other microbial population as reported by Adekanmbi et al. [27]. [27] [28] reported that the risks associated with pathogenic bacteria could increase due to the presence of nutrients in enormous concentration in wastewater. The presence of potential pathogens in water could signal a potential public health challenge especially since there is scarcity of potable water in the rural areas in so many developing countries of the world. This risk is even greater for opportunistic pathogens including Staphylococcus species.

In this study, a total of twenty Staphylococcus spp. was obtained, with 3 (15.0 %) being Staphylococcus aureus. Several authors have reported the occurrence of Staphylococcus spp. from water, sewage and wastewater. Among them are Bassey et al. [29] who reported that 33.3% of the total isolates in their study on wastewater in Awka metropolis, Nigeria were Staphylococcus spp. In

Table 6: Multiple Antibiotics Resistance (MAR) Index of Multidrug Staphylococcus Aureus Strains from FUTA Hostels' Laundry

Isolate codes	Resistant (a)	Tested (b)	MAR index (a/b)
FFHJ1	1	11	0.09
FFHJ2	7	11	0.64
FMHA1	4	11	0.36
FMHA2	2	11	0.18
FSQ	2	11	0.18

FFHJ1- FUTA female hostel (Jadesola); FFHJ2- FUTA female hostel (Jibowu); FMHA1- FUTA male hostel (Abiola); FMHA2- FUTA male hostel (Adeniyi); FSQ-FUTA staff quarters

Keys: a = classes of antibiotics to which the isolates were resistant; b = the total number of antibiotic classes to which the isolates were exposed

Table 7: Multiple Antibiotics Resistance (MAR) Index of Multidrug Resistant Staphylococcus Aureus Strains from FUTA Hostels' Kitchen

Isolate codes	Resistant (a)	Tested (b)	MAR index (a/b)
FFHJ1	1	11	0.09
FFHJ2	1	11	0.09
FMHA1	4	11	0.36
FMHA2	1	11	0.09
FSQ	3	11	0.27

FFHJ1- FUTA female hostel (Jadesola); FFHJ2- FUTA female hostel (Jibowu); FMHA1- FUTA male hostel (Abiola); FMHA2- FUTA male hostel (Adeniyi); FSQ-FUTA staff quarters

Keys: a = classes of antibiotics to which the isolates were resistant; b = the total number of antibiotic classes to which the isolates were exposed

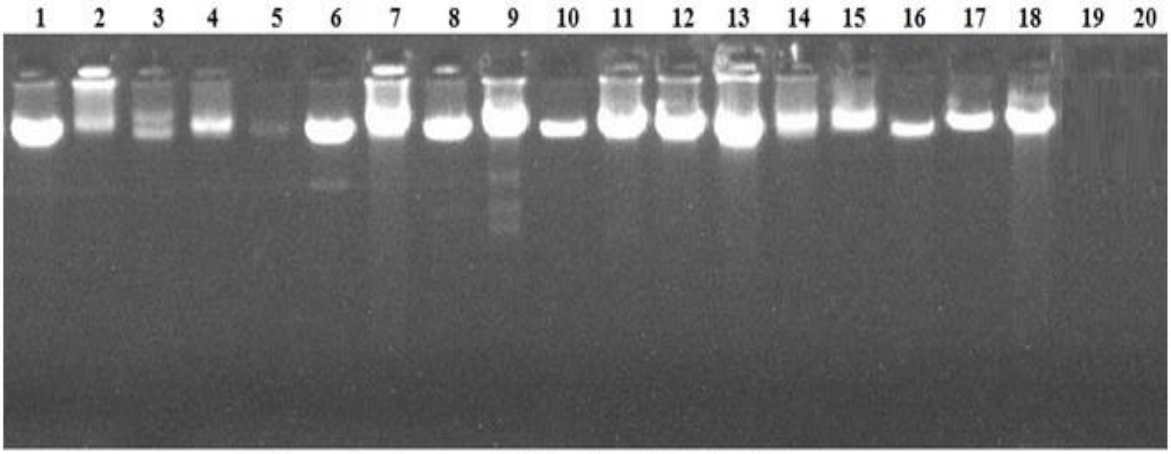
addition, Ayandiran et al. [30] reported the isolation of Staphylococcus aureus (3.57%) among other bacterial species from polluted Oluwa River, Nigeria. In a study carried out by Eze et al. in Ikwano, Abia state, Nigeria, 16.7% of isolated bacteria were Staphylococcus aureus [31] while Adekanmbi et al. [27] reported 36.2% Staphylococcus spp. in sewage and river water in 2012 and 2014 during the Schussen Aktivplus project.

Furthermore, in a study carried out in Durban, South Africa on treated wastewater and receiving surface water, [32] Ramessa and Olaniran (2019), reported the isolation of eighty isolates of Staphylococcus aureus that were methicillin resistant, while Naquin et al. [33] reported the presence of antibiotic resistant Staphylococcus aureus in raw and treated sewage of selected sewage treatment plants in the rural community of Thibodaux city in Louisiana, USA.

The kitchen is one of the most crucial areas that harbors and transmits infection. Germs are prevalent everywhere in the kitchen, in sink sponges, countertops, cutting boards, kitchen utensils, refrigerators, sinks, towels, and even stove tops [34].

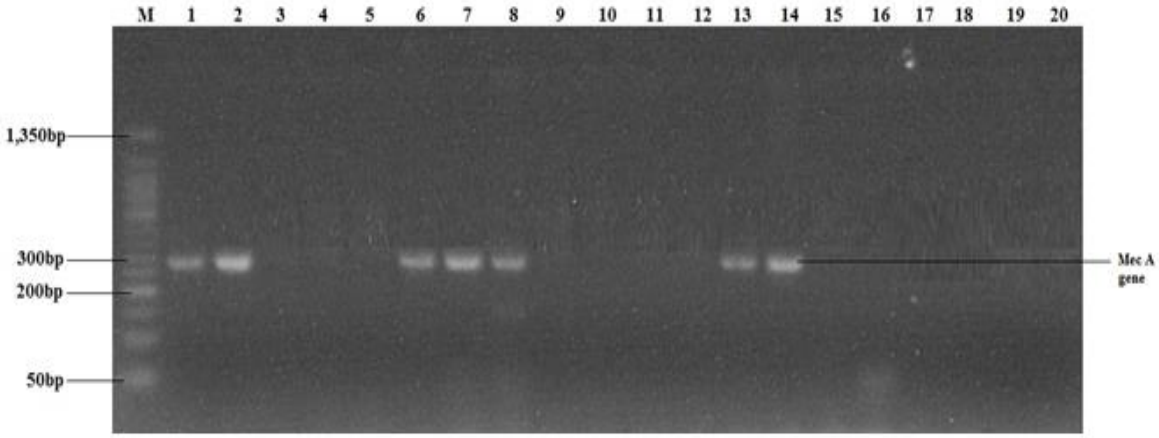
The study investigated that kitchens were contaminated with pathogenic microorganisms such as E. coli, Klebsiella spp., S. aureus, S. epidermidis, Salmonella spp., Shigella spp., and Micrococcus spp. The presence of S. aureus in some water source was also observed which could be as a result of absence

Plate 1: Gel Image Showing the Genomic DNA Extracted from Multidrug Resistant Staphylococcus Isolates



Gel image showing genomic DNA extracted from isolates.

Plate 2: Agarose Gel Electrophoresis of Amplified MEC a Gene (300 Bp) in Antibiotic Resistant Staphylococus Aureus in Domestic Effluents Collected From Elizade University (EU) and FUTA Campus, Akure



M = 50 bp ladder, Wells 1, 2, 6, 7, 8, 13 and 14 showed amplification of Mec A gene

of residual chlorine or concentrations below the recommended level.

Determination of antibiotic susceptibility pattern revealed that most Staphylococcus aureus strains tested were resistant to oxacillin. The improper use of antibiotics in human and livestock with substandard prescriptions by unqualified medical personnel along with poor diagnosis or lack of it all have been reported to be among the main factors contributing to the development of resistant staphylococcal infections [35].

In the laundry effluents, some isolates were resistant to Oxacillin, Septrin, Gentamicin, Ampiclox. In the bathroom effluents, some isolates were resistant to Oxacillin, Ampiclox, Amoxicillin and Rocephin. In the Kitchen effluents, some isolates were resistant to oxacillin, ampiclox and

Amoxicillin. This observation was similar to the report of Shittu et al. [24] in Nigeria and Said et al. [36] in Tunisia. In this study, there was little resistance to erythromycin and gentamycin in all the

Staphylococcus spp. and this does not corroborate the work of Goldstein et al. [37]; Adekanmbi et al. [27] who both reported that Staphylococcus species showed resistance toward these set of antibiotics in their study on samples collected from selected wastewater treatment plants. It is also not in accordance with Fagade et al. [38]; Adekanmbi et al. [27] who both reported 96% resistance to erythromycin in their study on environmental samples including polluted water. Estimated MAR index values (above 0.25) as observed amidst Staphylococus aureus strains from Students' hostel domestic effluents in this study as is an indicator of potentially risky to human health in the academic environment as supported by Azzam et al. [39]. They divulged the antibiotics resistance phenomenon and virulence ability in bacteria from water environment in their study.

This study also investigated the presence of mecA gene in the isolated S. aureus from domestic effluents which indicate the resistance of the isolates to methicillin and this is also in line with the report of Shittu et al. [24] and Rosenberg et al. [40]. This species has been described with carriage of the SCCmec-like mecA with a putative antibiotic resistance gene pool, with considerable ability to survive in various environments [41].

The presence of MRSA in domestic effluents is a public health concern as it indicates that the non-clinical environment could play a role in its transmission to humans [42].

5.0 Conclusion

The Staphylococcus aureus strains isolated from this study showed phenotypic resistance to oxacillin, a class of antibiotic as methicillin in Staphylococcal therapy.

This could present a potential public health challenge as the discharged untreated domestic effluents is connected to diverse water sources which could elicit contaminate useful water resources and air pollution in other parts of the University community. The unsystematic expulsion of untreated domestic effluents into water channels should be prohibited.

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