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Antibiogram and non-detection of *mecA* gene in *Staphylococcus* spp. isolated from a sewage-imparted stream within a University community

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ABSTRACT: Wastewater from households containing varying amount of chemicals such as antiseptics, antibiotics, bacteria and other toxic chemicals are directly discharged into the environment. The compounds present in the wastewater could play a role in the selection of antibiotic resistance in environmental bacteria and pose a public health risk to the environment. Water samples were obtained from five (5) selected points along the stream channel fortnightly for a period of four months. Isolation of *Staphylococcus* spp. was carried out on mannitol salt agar using the pour plate technique. Antibiotics susceptibility testing was done using the Kirby-Bauer disc diffusion method. Detection of *mecA* was carried out on methicillin resistant isolates by PCR using specific primers. A total of 53 *Staphylococcus* spp. were obtained from the wastewater sample; *Staphylococcus aureus* (79.2%), *S. epidermidis* (17%) and *S. saprophyticus* (3.8%). The antibiotics susceptibility test showed that 42% of the total isolates obtained were resistant to oxacillin, tetracycline (4%), chloramphenicol (4%), sulfamethoxazole/trimethoprim (2%) and linezolid (2%). There was no resistance to vancomycin, erythromycin, clindamycin, ciprofloxacin and gentamycin. None of the twenty-two methicillin resistant isolates in this study possessed m*ecA* gene. There is a need for adequate treatment of wastewater discharge before release into various receiving channels to prevent the increasing rate of antibiotics resistance develop in environmental bacteria.

Keywords: Water samples; *Staphylococcus* spp.; *mecA* gene; Sewage-imparted stream; University community; Nigeria.

1. INTRODUCTION

The rapid spread and geometric rise in the emergence of antibiotic resistance in human pathogens is a worrisome issue to global health. Several milieus such as the hospital environment, agricultural sector and other human-impacted ecosystem where antibiotics usage is paramount provide a platform for the selection of antibiotic resistant bacteria, while also favouring the promotion of genetic exchange among bacteria from several other environments. Aminov and Mackie [1], and Baquero et al. [2] have carried out studies focusing

on the roles being played by the wider environment and other closely connected habitats, such as the water bodies in the transfer of resistant traits in bacteria and also more importantly, the resistance genes. Drug overuse coupled with indiscriminate use of broad spectrum antimicrobials in the treatment of preventable infections have also been seriously implicated in the emergence of resistance to antibiotics [3]. The aforementioned factors together with inefficient waste and wastewater treatment protocols have been largely responsible for the sustenance of antibiotic resistance in bacteria and also the emergence of new ones.

The water environment provides a compartment for the co-existence of bacteria from human, environmental and animal origin, as the water bodies are continually fed with water from agricultural settings, households, industrial sources and the hospital environment [2]. This complex interaction could potentially result in the acquisition of resistance traits and genes by environmental bacteria [4], and secondly the selection of antibiotic resistant bacteria in polluted aquifers, as a result of the introduction of antimicrobial compounds into the system from agricultural and hospital settings where antibiotic usage is at its peak [5]. This eventually gives rise to the environmental spread of antibiotic resistant bacteria using a lot of mechanisms including mobile genetic elements (MGE).

Staphylococcus spp. are opportunistic pathogens and have been implicated as the cause of most clinically important infections such as septicaemia, soft tissues infection, skin infections and as one of the causative agents of pneumonia [6]. They can be introduced into surface water via the discharge of untreated wastewater and sewage from household, agricultural farms, industrial operations and many other anthropogenic activities. In Nigeria, wastewater from several sources including household, industrial, agricultural and clinical are directly discharged into water bodies [7], without any form of treatment or in cases where treatment facilities area available, are grossly inefficient. This singular action could pollute water sources (surface and underground), that are the mainstay of water supply for most communities and population.

This study therefore aimed at determining the antibiotic susceptibility profile of *Staphylococcus* spp. isolated from selected points on a stream imparted by the inflow of sewage and other wastes within a University community in South-west Nigeria and detecting the presence of methicillin resistance genes in the methicillin-resistant isolates.

2. MATERIALS AND METHODS

2.1. Description of the study site and sample collection

The study was carried out at selected points on a sewage-imparted stream within the University of Ibadan community in Oyo state. The state is located in the South-western part of Nigeria. Five points on the stream were chosen for the study. The stream receives heavy input of wastes and wastewater from bathing, washing and other domestic wastes from student halls of residence and residential buildings; including sewage from a broken sewerage along the course of the channel and other anthropogenic activities. The sampling points were chosen based on the inflow of input from different halls of residence, broken sewerage and the zoological garden. The description of the sampling points and activities carried out are shown in Table 1. Water samples were collected from the waste-imparted flowing stream at the five (5) different sampling points into pre-cleaned sterile polythene sample bottles, stored in ice pack and transported to the laboratory for analysis. Samples were analysed within four hours of collection. The frequency of sampling was fortnightly, covering a period of four months.

Table 1. Description of activities carried out around the selected sampling points.

Sampling point	Designation code	Description
Independence point	IND	It receives wastewater from independence hall and discharge outlet from black market into the flowing stream. It is located adjacent Zik hall.
Tafawa Balewa point	BAL	It receives wastewater from Independence hall, Tafawa Balewa hall and Nnamdi Azikwe hall which flows into the stream. The effluents being discharged in the flowing stream consist of wastewater released from various bathrooms and toilets, laundry and cafeteria.
Nnamdi Azikwe point	ZIK	There is entry of wastewater from a residential hall and other inlets including a broken sewerage and wastewater from the student cafeteria.
Zoological garden point	ZOO	It is located immediately after the Zoological garden, University of Ibadan. It receives input majorly from the Zoological garden.
Awba dam point	AWB	This site is a dam located within University of Ibadan. It collects all wastewater from different sources within the University community. All drainage channels and wastewater from the University community are emptied directly here.

IND: Independence point; BAL: Tafawa Balewa point; ZIK: Nnamdi Azikwe point; ZOO: Zoological garden point; AWB: Awba dam point.

2.2. Isolation and characterization of *Staphylococcus* spp. from water samples

The wastewater samples were serially diluted using saline water (0.9% NaCl) and aliquot (1 mL) of selected dilutions were plated out on Mannitol Salt Agar (Oxoid, Basingstoke, UK) using the standard pour plate technique [8]. The set-up was allowed to solidify, incubated at 37ºC for 24 hours and observed. Colonies presumptive of *Staphylococcus* spp. were selected and further sub-cultured to obtain pure colonies, which were stored on nutrient agar (Oxoid Basingstoke, UK) slant for further studies. The identities of the isolated bacteria were confirmed using morphological, biochemical and sugar fermentation tests including Gram staining, fermentation of different sugars, oxidase test, catalase test, coagulase test and haemolysis test etc. [9].

2.3. Susceptibility to antibiotics

The disc diffusion method [10] was used to determine the susceptibility of the isolates to selected antibiotics. Ten (10) different classes of antibiotics were tested against the organisms. The antibiotics used were tetracycline (30 µg), gentamicin (30 µg), erythromycin (15 µg), vancomycin (30 µg), oxacillin (1 µg), clindamycin (2 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), linezolid (30 µg), sulfamethoxazole/trimethoprim (25 µg), they were purchased from Oxoid, Basingstoke, UK). These antibiotics selected were based on the guidelines for antimicrobial susceptibility testing for *Staphylococcus* spp. by CLSI [11]. The antibiotics discs were placed with the aid of a forceps on Muller-Hinton agar plates already layered with an 18-24 hour old culture of the SRB using a sterile swab stick. The set-up was incubated aerobically for 18-24 hours at 35±2ºC. The inhibition zones were measured and interpreted using CLSI [11] guidelines. The results obtained were used in the construction of the pattern of resistance and determination of the percentage resistance of the *Staphylococcus* spp. to each antibiotic.

2.4. Detection of *mecA* gene in isolates showing phenotypic resistance to methicillin

The DNA of isolates showing resistance to methicillin was extracted using Quick-gDNA™ (Zymo Research Corporation, USA). The ultra pure DNA was stored at -80 °C for the detection of *mecA* gene. The oligonucleotide primers used were purchased from Inqaba Biotechnology, South Africa and are listed in Table 2. The PCR reaction mixture was 25 µL and it contained the following: PCR Master Mix (12.5 µL),

Nuclease-free water (7.5 µL), forward and reverse primers (1.0 µL each) and DNA template (3.0 µL). *Staphylococcus epidermidis* A8, which harboured the *mecA* gene as reported by Adekanmbi et al. [12], was used as the positive control. The PCR amplification conditions included an initial denaturation of 5 minutes at 95°C, followed by 30 cycles of denaturation at 94°C for 120 seconds, annealing at 57°C for 120 seconds and extension at 72°C for 50 seconds. The final extension step was for 7 minutes at 72°C.

Table 2. Oligonucleotide primers used in this study.

Target gene	Primers (Forward and Reverse)	Amplicon size	Reference
mecA	5' GATCTGTACTGGGTTAATCA 3' 5' CATATGACGTCTATCCATTT 3'	500 bp	[13]

3. RESULTS

3.1. Distribution and frequency of *Staphylococcus* spp. isolated in this study

A total of fifty-three (53) *Staphylococcus* spp. were recovered from the water samples collected from the flowing stream in this study. The biochemical tests confirmed all the isolates to be Gram-positive cocci, catalase positive and oxidase negative, while 79% (42) of the total isolates were coagulase positive and the remaining 21% (11) were coagulase negative. As shown in Figure 1, forty-two (42) of the isolates were identified as *Staphylococcus aureus;* with nine (9) being *Staphylococcus epidermidis* and the remaining two (2) identified to be *Staphylococcus saprophyticus*.

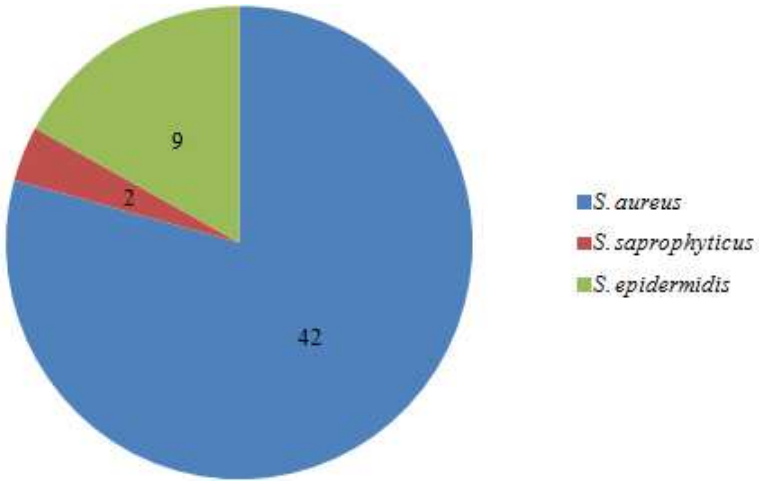

Figure 1. Distribution of the *Staphylococcus* spp. according to species.

The frequency of *Staphylococcus* species recovered from the water samples according to sampling points is shown in Figure 1. IND point had the highest number of *Staphylococcus aureus* with thirteen isolates, followed by ZOO with 12 isolates. BAL had 11 *Staphylococcus aureus*, ZIK (6) and none was recovered from AWB. Of all the sampling points, only ZOO had two isolates of *Staphylococcus saprophyticus*, while the other points had none. With the exception of BAL and AWB with no *Staphylococcus epidermidis*, the other three points IND, ZIK and ZOO had two, three and four isolates of *Staphylococcus epidermidis* respectively. It should be stressed that only from AWB was no isolate recovered at all.

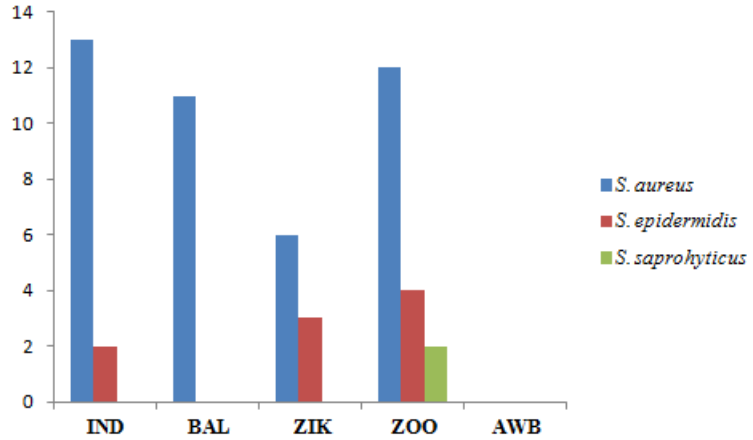

Figure 2. Frequency of *Staphylococcus* species recovered based on sampling points.
IND: Independence point; BAL: Tafawa Balewa point; ZIK: Nnamdi Azikwe point;
ZOO: Zoological garden point; AWB: Awba dam point.

3.2. Antibiotic susceptibility testing

The antibiotic susceptibility test result is shown in Table 3. With the exception of vancomycin, erythromycin, gentamycin, clindamycin and ciprofloxacin to which there were no resistance observed, there was resistance to the other antimicrobial. Twenty-two isolates (42%) were resistant to methicillin, while 4% of the isolates showed resistance to tetracycline and chloramphenicol respectively. There were 2% resistance each to linezolid and sulfamethoxazole-trimethoprim.

Table 3. Antibiotics susceptibility test results.

	Sensitive	**Intermediate**	**Resistant**
Antibiotics	**No (%)**	**No (%)**	**No (%)**
Vancomycin	53 (100)	0 (0)	0 (0)
Tetracycline	51 (96)	0 (0)	2 (4)
Erythromycin	52 (98)	1 (2)	0 (0)
Gentamicin	52 (98)	1 (2)	0 (0)
Clindamycin	44 (83)	9 (17)	0 (0)
Sulfamethoxazole/Trimethoprim	52 (98)	0 (0)	1 (2)
Linezolid	52 (98)	0 (0)	1 (2)
Chloramphenicol	50 (94)	1 (2)	2 (4)
Methicillin	15 (28)	16 (30)	22 (42)
Ciprofloxacin	51 (96)	2 (4)	0 (0)

Table 4 is showing the resistance of the three species of *Staphylococcus* to the tested antibiotics. There was no resistance to vancomycin, tetracycline, erythromycin, gentamicin, clindamycin, sulfamethoxazole/trimethoprim, linezolid, chloramphenicol and ciprofloxacin in the two isolates of *Staphylococcus saprophyticus* obtained, however one of the isolates was resistant to methicillin. In *S. epidermidis*, 44% of the isolates showed resistance to methicillin, while no resistance was observed to the remaining tested antibiotics. In the 42 isolates of *S. aureus* obtained, 40% showed resistance to methicillin, 5% to tetracycline and 2% respectively to linezolid and sulfamethoxazole/trimethoprim. There was no resistance observed to vancomycin, erythromycin, gentamicin, clindamycin, chloramphenicol and ciprofloxacin.

Table 4. Resistance to antibiotics in the three species of *Staphylococcus* spp. from the waste-imparted stream.

	S. aureus			*S. epidermidis*			*S. saprophyticus*		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Antibiotics	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**	**No (%)**
VAN	42 (100)	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
TET	40 (95)	0 (0)	2 (5)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
ERY	41 (98)	1 (2)	0 (0)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
GEN	41 (98)	1 (2)	0 (0)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
CLI	36 (86)	6 (14)	0 (0)	6 (67)	3 (33)	0 (0)	2 (100)	0 (0)	0 (0)
SXT	41 (98)	0 (0)	1 (2)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
LZD	41 (98)	0 (0)	1 (2)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
CHL	42 (100)	0 (0)	0 (0)	9 (100)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)
MET	10 (24)	15 (36)	17 (40)	4 (44)	1 (12)	4 (44)	1 (50)	0 (0)	1 (50)
CIP	40 (95)	2 (5)	0 (0)	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)

KEY: S: Sensitive; I: Intermediate; R: Resistant; VAN: Vancomycin; TET: Tetracycline; ERY: Erythromycin; GEN: Gentamicin; CLI: Clindamycin; SXT: Sulfamethoxazole/Trimethoprim; LZD: Linezolid; CHL: Chloramphenicol; MET: Methicillin; CIP: Ciprofloxacin.

3.3. Detection of methicillin resistance genes

None of the twenty-two isolates showing phenotypic resistance to methicillin harboured the methicillin resistance gene, *mecA* in this study. There was no amplification of the 500 bp fragment after the process of gel electrophoresis despite all the quality control measures and optimization procedures.

4. DISCUSSION

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 [14]. Rose et al. [15] 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 fifty-three *Staphylococcus* spp. were obtained, with 42 (79.2%) being *Staphylococcus aureus*. Several authors have reported the occurrence of *Staphylococcus* spp. from water, sewage and wastewater. Among them are Bassey et al. [16] who reported that 33.3% of the total isolates in their study on wastewater in Awka metropolis, Nigeria were *Staphylococcus* spp. In addition, Ayandiran et al. [17] 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. [18] in Ikwano, Abia state, Nigeria, 16.7% of isolated bacteria were *Staphylococcus aureus* while Heb and Gallert [19] 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, Ramessa and Olaniran [6], reported the isolation of eighty isolates of *Staphylococcus aureus* that were methicillin resistant, while Naquin et al. [20] 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.

In this study, there was no resistance to vancomycin, erythromycin, clindamycin, ciprofloxacin and gentamycin in all the *Staphylococcus* spp. and this does not corroborate the work of Goldstein et al. [21] who 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. [22] who reported 96% resistance to gentamycin and 86% resistance to erythromycin in *Staphylococcus* spp. in their study on environmental samples including polluted water. Moges et al. [23] reported that no isolates of *Staphylococcus* spp. was observed to be vancomycin resistant in isolates from non-hospital environments in Northwest Ethiopia which is in agreement with this study.

The resistance to tetracycline in the isolates from this study was 4%, while 2% were resistant to sulfamethoxazole/trimethoprim. As reported by Akanbi et al. [24], the percentage of antibiotic resistance for tetracycline and sulfamethoxazole/trimethoprim was 56.7% respectively in a study carried out on recreational waters and beach sand in South Africa. Dong et al. [25] reported a resistance of 1.5% to tetracycline in *Staphylococcus* spp. isolated from wastewater treatment plant and receiving river In addition, no resistance was obtained to sulfamethoxazole/ trimethoprim as reported by Faria et al. [26] in their study on the isolation of bacteria from wastewater and drinking water, which is lower compared to the percentage resistance to the antibiotic in this present study. Furthermore, 4% of the total isolates in this study were resistant to chloramphenicol and this does not correspond with the work of Shanthi et al. [27] who reported that the percentage resistance to chloramphenicol in *Staphylococcus* spp. isolated from tannery effluent in South Africa was 35%. Fagade et al. [22] also reported 76% resistance to chloramphenicol in *Staphylococcus* spp. isolated from environmental samples.

Of all the isolates, 42% (22) were resistant to methicillin. The resistance to methicillin was the highest reported in this study. Some authors have also reported a high level of resistance to methicillin in different studies. Notable among them is Akanbi et al. [24] who reported 73.3% resistance to methicillin in *Staphylococcus* spp. from recreational waters and beach sand in South Africa. Goldstein et al. [21] reported 50% resistance to oxacillin in their study on methicillin-resistant *Staphylococcus aureus* (MRSA) detected in the United States wastewater treatment plant. Hafsat et al. [28] also reported some level of resistance to methicillin (an analogue of oxacillin) in *Staphylococcus aureus* from sewage in Bolivia, while in Nigeria, Adekanmbi and Falodun [29] reported a 63.6% resistance to oxacillin (an analogue of methicillin) in *Staphylococcus* spp. isolated from wastewater from abattoir operations the same city and geographical region as this present study.

None of the twenty-two methicillin resistant isolates in this present study was detected to possess the methicillin resistance genes. This is not a strange phenomenon, as some studies have reported the absence of the genes despite the bacteria displaying phenotypic resistance to the antibiotic. The absence of *mecA* gene in methicillin resistant *Staphylococcus aureus* in some samples of clinical origin in the city of Shendi in Sudan was reported by Elhassan et al. [30]. Similarly, the absence and low occurrence of methicillin resistance genes in phenotypically methicillin resistant Staphylococci has been well reported [31, 32]. This might be attributed to the fact that other mechanisms apart from the possession of the methicillin resistance genes could be responsible for the observed resistance traits, and also presence of other variants of the same gene.

5. CONCLUSION

The bacteria isolated from this study showed phenotypic resistance to some commonly used antibiotics in *Staphylococcal* therapy, which might present a potential public health challenge as the discharged untreated

wastewater connects several adjoining water sources which are being used for other purposes in other parts of the University community. The indiscriminate discharge of untreated wastewater and other domestic waste into water channels should be discouraged.

Authors Contributions: AOA conceived and designed the study. AOA, IAO, OOA and AVO were involved in carrying out the laboratory experiment and acquisition of data. Author AOA analyzed and interpreted the data. AOA drafted the article. All authors critically revised the article and approved the final article for publication.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

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