Original Research Article

PREVALENCE AND ANTIBIOGRAM OF BIOFILM FORMING BACTERIA ASSOCIATED WITHTOILET SEATSIN DORMITORIES WITHIN A UNIVERSITY CAMPUS IN PORT HARCOURT, RIVERS STATE, NIGERIA

ABSTRACT

Public toilet facilities are major sources of microbial transmission infection. This study was conducted to determine the prevalence and antibiogram of biofilm forming bacterial species associated with toiletseats in University dormitories. A total of 24 samples were obtained from six (6) hostels aseptically. Isolates were enumerated using the standard plate count method, and identified phenotypically based standard conventional bacteriological procedures. Antibiotics susceptibility and biofilm production was evaluated using the Kirby-Bauer disk diffusionand the Congo red agar method, respectively. The data obtained from the study identified*Staphylococcus*spp.as the predominant isolate, having a prevalence of 58.3%, followed by *Bacillus* spp.(54.2%), while *Serratiamercensis*(4.2%) was the least encountered isolate. The data from the enumeration of the bacterial groups showed the bacterial counts ranged from 1±0.02x 10^5 cfu/ml to 2.5±0.03 x 10^5 cfu/ml, for the total heterotrophic bacterial count.The antibiogram obtained showed that the isolates were highly resistant to most of the antibiotics tested, as all the Gram positive isolates (100 %) were sensitive to five (i.e 62.5 %) of the antibiotics(Cefuroxime, Ceftaziidime, Augmentin, Cloxacillin and Ceftriaxone. The Gram negative isolates showed similar pattern where 100 % of the isolates were resistant to four (i.e 50%) of the antibiotics, (Augmentin, Ceftaziidime, Ciprofloxacin and Cefuroxime).The results showed that 57.1 % of the isolates produced biofilm. The study indicated a high rate of recovery of bacteria from the toilet seats in dormitories within the University. Proper sanitary measures should therefore be adopted to facilitate good hygiene and reduce the transmission of bacterial infections.

Comment [H1]: Reframe to give an ideal introduction with statement of problem and justification for the research.

Comment [H2]: Rephrase, state the standard methods.

Comment [H3]: Rephrase.

Comment [H4]: This is not justifying the resistance status of the isolates.

Comment [H5]: Any relationship between antibiotics resistance and biofilm formation?

Keywords:Antibiogram, biofilm formation,dormitories, prevalence, public toilets, toilet

seats, university campus.

1. INTRODUCTION

Public toilets in dormitories remain inan indispensable facility in university settings of most developing countries. This is due to the fact that most developing economies could hardly provide personal toilet facilities in the various rooms found in students' hostels. These public toilets have therefore become major sources of disease transmission amongst students. Several factors however responsible for the increasing incidence of toilet associated infections. Personal hygiene, lack of maintenance and cleaning of public toilets are frequently associated with the proliferation of toilet –related disease and medical conditions (1).

Microorganisms are important mediators of toilet related infections. Methicillin-Resistant *staphylococcus aureus* (MRSA) in hospital toilets had grabbed the headlines as it contributed to the death of 5,000 patients each year. But, there is a wide range of other bacteria and Viruses that are associated with dirty toilets wherever they are located including public toilets. The contributions of public toilets as means for continuous source of epidemics has made research in this area very important. The sources of pathogens in public toilets are usually from fecal and urine residues from humans accessing these facilities. This provide an ideal condition for the horizontal spread of pathogens, which may in turn bring about disease outbreak [3], that are usually occasioned by improper cleaning and disinfection of restroom facilities [5].

The use of public toilet can predispose and individual to a plethora of disease such as diarrhea, dysentery, skin infection, urinary tract infection, vaginal infections, etc. These infections are due to bugs/germs {for example bacteria, parasites and viruses} that are passed between people using public toilets. In a dirty toilet, these germs survive outside the human body on toilet seats, door handles and flush knobs of the water cisterns (wc).

Biofilm is a complex structure of microbiome having different bacterial colonies or single type of cells in a group that adhere to the surfaces. Bacterial biofilms are serious global health concern due to their abilities to tolerate antibiotics, host defense systems and other external stresses; therefore, it contributes to persistent chronic infections [6; 7].

Comment [H6]: can

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An antibiogram is a table that displays the antibiotic susceptibility pattern of specific microorganisms to different types of antimicrobial drugs [8]. According to theWHO Regional office for south East Asia (2011)[9], antimicrobial resistance (AMR) has surfaced as a global problem facing public health and as a result of this, there is prolonged illness and a higher risk of death since diseases arising from these resistant microorganisms do not respond to the antimicrobial drugs. Furthermore, the diseases caused by multidrug-resistant microorganisms which are virtually non-treatable are the most alarming. The development of antibiotic resistance in many bacteria constitutes serious problems in the control of infectious diseases [9].

In other to contain the spread of diseases arising from the use of public toilets, several users-based measures such as the use of disinfectants, wiping of toilet seat surfaces, flush before and after use, etc are adopted by the individuals or managers of toilet facilities. In spite of these measures, there is still documented evidence of increasing incidence of toilet related infections in most developing countries. This has therefore necessitated the need for continuous research in this area, in other to identify the bacterial drivers associated with spread of toilet associated infections among university students. This study was therefore, carried out to determine the prevalence and antibiogram of biofilm forming bacterial species associated with toilet seats in University dormitories.

2.0 MATERIALS AND METHOD

2.1 Description of study area and location

The study was carried out at a University campus in Port Harcourt, Rivers State, Nigeria. The study location involved six (6) different hostel toilets used by both the male and female students. The hostels included HostelsC, D, H, for girls, and Hostels E, F and G for boys.

2.2Method of Sample collection

A total of 24 samples were collected from six different hostel toilets withinuniversity. Water samples were taken from the interior the water cisterns (wc), and swab samples were as well taken from the toilet seat surfaces. Samples from solid surfaces (toilet seats) were collected with a sterile swab stick and water sample from the water cisterns (wc) were collected with new syringes for each toilets. The samples were collected within 7-8 AM. The samples were labeled properly and transported aseptically to the laboratory for microbiological procedure.

2.3 Characterization and identification of the isolates

The bacterial isolates were examined for colonial morphology as well as for cell morphology and biochemical characteristics.

This was carried out to group bacteria into gram positive and gram negative. A smear was made from a 24hours culture on properly labeled grease free glass slides. This was achieved by dropping one to two drops of water on the slide and emulsifying with a loop full of bacteria on the grease free glass slide. The smear was air dried and heat fixed by passing the slide under Bunsen burner flame three times each smeared slide was flooded with the primary stain (crystal violet) for 60seconds ,rinsed in slow running water .Smears were then flooded with lugol's iodine for 30 seconds and then rinsed in slow running tap water .The smear were then decolorized with 70% ethanol for 10 seconds and rinsed with slow running tap water and then flooded with a counter stain (safranin) for 30 seconds and again rinsed with slow running tap water. The slides was allowed to air dry on a slide rack. The stained smear was examined microscopically using oil immersion lens(x100) for better magnification. Purple or Violet colour showed gram positive while pink or red colour showed gram negative.

2.4Preservation of pure culture

The pure culture of the isolates were stored in 10 % (v/v) glycerol suspension at -4^{o}C in bijou bottles to prevent damage of the pure cultures for further analysis.

2.5Preparation of standard bacterial suspension

A 24-hour old pure culture of the test organism was emulsified in sterile nutrient broth tubes and adjusted to an equivalence of 0.5 McFarland's turbidity standard prepared by adding 99.4ml of 1% v/v solution of sulphuric acid and 0.6ml of 1% w/v Barium Chloride solution [10].

2.6Antibiotics sensitivity testing by the agar disk diffusion (Kirby Bauer disk diffusion) method

A sterile swab stick was dipped into the tube containing the bacterial suspension and its turbidity was equivalent to 0.5m McFarland turbidity. The swab stick was pressed against the tube above the fluid level to remove excess broth. The swab was used to streak over the entire plate surface evenly which contained already prepared Mueller- Hinton agar in three dimensions rotating the plate about 60^{o}C each time. The agar plate was allowed to dry for 5 minutes then the antimicrobial disk was impregnated to the agar using a sterile forcep on the surface of the inoculated plate 15mm away from the edge of the plate. Using the head of the sterile forcep the disk was slightly pressed down to ensure good contact with the agar. After applying the disk, the plates were incubated in an inverted position at 35^{o}C for 16 to 18 hours. After incubation, the test plates were examined to ensure confluence growth or near confluence. The diameter of each zone of inhibition was measured in ml using a ruler on the underside of the plate and recorded for reference purpose [11].

Comment [H9]: ml or mm?

2.7Test for biofilm formation

The Congo red agar (CRA) plates were inoculated with isolates and incubated aerobically for 24 to 48 hours at 37 degrees Celsius. Positive result was indicated by black coloration, and non-biofilm producers remained pink.

Comment [H10]: ^{0}C

The CRA plate test allows for the direct analysis of the colonies and identification of slime-forming strains (which appear as black colonies on the red agar) and non-slime-forming strains (red-colored colonies).

3.0 RESULTS

3.1Enumeration of Bacterial isolates across the hostels

Table 1 showed the Total heterotrophic and coliform bacterial counts of the samples obtained from the various hostels. The highest Total Heterotrophic (THB) bacterial count of 2.5±0.03 x 10^5cfu/ml was recorded at hostel E (boy's hostel). While hostel F (boys hostel) had the least count with 1.0±0.0210^{5}cfu/ml. Data obtained showed that hostel D (girls hostel) recorded the highest Total coliform count (29.0±0.01cfu/ml), while hostel H (boys hostel) had the least, with no presence of coliform detected during the sampling period.

Table 1Population of bacterial species in the various toilets

Sample ID	THBC (x 10^5cfu/ml)	TCC (x10^3cfu/ml)
C	2.0±0.01	7.0±0.02
D	2.3±0.04	29.0±0.01
H	1.5±0.01	0
E	2.5±0.03	9.0±0.06
F	1±0.02	7.0±0.05
G	2.4±0.07	28±0.01

4.3 PATTERN OF BACTERIAL DISTRIBUTION IN THE HOSTEL TOILETS.

Comment [H11]:

The results of the bacterial diversity (Table 2**)** showed that hostel H had the highest number of bacterial species, with a total of six (6) diverse species which included *Staphylococcus* spp., *Bacillus* spp, *Salmonella, Klebsiella*spp, *Escherichia coli* and *Shigella* spp. This was followed by hostel D having five (5) species, including *Staphylococcus* spp., *Bacillus* spp., *Salmonella* spp., *Shigella,* and *Serratiamercescens* and hostel E also having (5) species, including *Staphylococcus* spp., *Bacillus* spp., *Salmonella* spp. *Klebsiella*spp, *Escherichia coli*. The rest hostel had two (2) and four (4) species each. Table 3 shows the distribution of bacterial isolates in the different hostel water cisterns sampled.

Comment [H12]: Be consistent with font type.

4.4 PREVALENCE OF THE BACTERIAL ISOLATES IN THE HOSTEL TOILETS

The prevalence of the bacterial species is as shown in Table 3**.** The result showed that *Staphylococcus* spp was the highest, showing a frequency of 58.3%, as it occurred in all the six (6) hostels. *Bacillus spp.* was the second highest with frequency of 54.2%, the least occurring was *Serratiamercescens* with frequency of (1%). It also followed that the other bacterial species had variations in their frequency of occurrence. *Salmonella* spp. had a prevalence of 33.3 %, occurring in five (5) hostels ; *Shigella* had a prevalence of 16.7 %, occurring in three (3) hostels; *Klebsiella spp*. had a prevalence of 12.5 %, occurring in three (3) hostels and *Escherichia coli* with prevalence of 12.5, occurring in two (2) hostels.

Table 2 Distribution of bacterial species in the various toilet samples from the hostels

Isolate	**G**		**F**		**E**		**H**		**C**		**D**	
	water	swab	Water	swab	Water	swab	Water	Swab	water	swab	water	swab
Bacillus spp	++	-	++	+	++		++		++		++	
Staphylococcus spp	++	++	+	+	++	+	+	+		++		+
Escherichia coli						+	+					
Salmonella spp							+	+	+	+	+	
*Shigella*spp								+	+	+	+	
*Seratia*spp												+
*Klebsiella*spp				+	+			+				
TOTAL	2		4		5		6		4		5	

Comment [H13]: Key to the hostels represented with alphabets.

Table 3 Prevalence of the bacterial species in the hostel toilets

S/N	Isolate	Frequency (n=24)	% occurrence (x/n x 100)
1.	*Bacillus* spp.	13	54.2
2.	*Staphylococcus aureus*	14	58.3
3.	*Escherichia coli*	3	12.5
4	*Salmonella* spp.	8	33.3
5	*Shigella*spp.	4	16.7
6	*Saratiamercescens*	1	4.2
7	*Klebsiella*spp.	3	12.5

3.3 SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES TOANTIBIOTIC

The antibiotics susceptibility test was carried out to determine the level of susceptibility of the bacterial isolates from the water and swab samples of the water cisterns (wc). The antibiogram obtained showed that the isolates were highly resistant to most of the antibiotics tested. It further revealed that all the Gram positive isolates (100 %) were sensitive to five (i.e 62.5 %) of the antibiotics (Cefuroxime, Ceftaziidime, Augmentin, Cloxacillin and Ceftriaxone) as recorded in Table 4. The Gram negative isolates showed similar pattern as 100 % of the isolates were resistant to four (i.e 50%) of the antibiotics, (Augmentin, Ceftaziidime, Ciprofloxacin and Cefuroxime) as shown in Table 5.

Comment [H14]: This is not clear, were they sensitive or resistant to the antibiotics? Give a clearer explanation.

Table 4 SENSITIVITY PATTERN FOR GRAM POSITIVE BACTERIA

S/N	Probable isolate	CRX	CAZ	AUG	OFL	ERY	CXC	CTR	GEN
1	*Bacillus*spp	R	R	R	S	S	R	R	S
2	*Staphylococcus aureus*	R	R	R	S	R	R	R	S
3	*S. aureus*	R	R	R	S	I	R	R	S
4	*S. aureus*	R	R	R	R	I	R	R	R
5	*S. aureus*	R	R	R	S	I	R	R	S
6	*S.aureus*	R	R	R	S	R	R	R	I
7	*S.aureus*	R	R	R	S	R	R	R	S
	%R	100	100	100	14.3	42.9	100	100	14.3
	%S	0	0	0	85.7	14.3	0	0	71.4
	%I	0	0	0	0	42.9	0	0	14.3

KEY:

GEN: Gentamycin AUG: Augmentin CRX: Cefuroxime CAZ: Ceftaziidime OFL: Ofloxacin ERY: Erythromycin CXC:Cloxacillin CTR: Ceftriaxon

Table 5SENSITIVITY PATTERN GRAM NEGATIVE BACTERIA

Serial no	Probable isolate	OFL	AUG	NIT	CPR	CAZ	CRX	GEN	CXM
1	*E. coli*	S	R	S	S	R	R	S	R
6	*E.coli*	S	R	S	S	R	R	S	R
7	*E.coli*	S	R	S	S	R	R	S	R
2	*Salmonella*spp	S	R	S	S	R	R	R	R
5	*Salmonella*spp	R	R	S	R	R	R	R	R
4	*Serratiamarcescens*	S	R	R	S	R	R	S	R
3	*Shigella*spp	S	R	R	S	R	R	I	R
	%R	14.3	100	28.3	14.3	100	100	28.6	100
	%S	85.7	0	71.4	85.7	0	0	57.1	0
	%I	0	0	0	0	0	0	14.3	0

KEY:

AUG: Augmentin NIT: Nitrofurantoin CPR: Ciprofloxacin GEN: Gentamycin CXM: Cefuroxime CAZ: Ceftaziidime CRX: Cefuroxime OFL: Ofloxacin

Table 6Biofilm formation by Bacterial isolates

A biofilm test was carried out using the Congo red agar, eight out of fourteen (14) bacterial isolates produced biofilm following a 24hrs incubation period at 37 degrees Celsius. This represented 57.1% of the isolates.

Table 7 Biofilm forming Bacteria

Isolate number	Probable identity	BIOFILM PRODUCTION
1	*E.coli*	-
2	*Salmonella*spp	+
3	*Staphylococcus*spp	-
4	*Serratia*spp	+
5	*Bacillus*spp	-
6	*E.coli*	-
7	*Staphylococcus*spp	+
8	*Shigella*spp	+
9	*Staphylococcus*spp	-
10	*Staphylococcus*spp	+
11	*Salmonella*spp	+
12	*Staphylococcus*spp	-
13	*Staphylococcus*spp	+
14	*E.coli*	+
Percentage (%)		**57.1**

4.0 DISCUSSION

Public toilets have for decades remained an import source of disease transmission to humans due to the various daily activities that make sharing of toilets almost impossible in developing economies. This study reported the presence of bacterial species of different genera in university students' dormitories. The hostel toilets were observed to

harbor many pathogenic organisms and most of the isolates were from girls' hostel. This may be due to the high number of users and lack of proper sanitary measures. The students' population and traffic in the hostel toilets was also very high which could have resulted in high bacterial contamination of the toilets. A research conducted by a previous researcher [12]had observed that the toilets at Daeyang Luke Hospital which had more users were more contaminated than other bathroom with less users.

The Millennium Development Goal (MDG) targets for sanitation was to reduce by halve the problems by 2015. The country could not meet the MDG's target up till 2016 because most people still lacked access to good water supply which is complimentary to good toilet sanitation in several parts of the country [13; 14].

In this study, bacterial isolates of the following genera, *Staphylococcus, Bacillus, E.coli, Serratia, Klebsiella, Salmonella, Shigella*species were identified from the water and swab samples of the hostel toilets. The bacteria in this study could be pathogenic and responsible for a number of infections such as urinary tract infections, foodborne infections, and other related diseases. This agreed with earlier reports by [15] who reported some possible diseases caused by similar bacteria such as *Staphylococcus and E.coli* which causes food borne diseases and Urinary tract infections. Furthermore, *Bacillus* species are known to withstand high temperatures and adaptsto unfavorable conditions with the help of endospores. Thus, they would withstand heat and cause food borne illness. This study therefore collaborates with the findings by earlier researchers that noted the role of toilets in the university environments serving as a reservoir for bacterial [3; 8; 12] and fungal pathogens [16].

Antibiotics resistance is currently a public health concern as environmental isolates have been severally reported to be associated with antibiotics resistance [17; 18; 19]. These could however be due to contaminations of human origin. This present study reported high levels of antibiotics resistance in the study area which is similar to earlier report by from another University in Delta State, Nigeria [3].

Comment [H15]: Not cler.

Comment [H16]: This is not genera, it should just be Escherichia.

Comment [H17]: This is not necessary in this contest.

Biofilm production was reported in this study. Biofilm formation poses serious challenge in the management of public toilets as they aid the bacteria adhere to the toilet surfaces and resist removal by flushing. The biofilm also helps bacteria to survive in adverse environmental conditions, including the effect of disinfection, as well as aids antibiotics resistance. One of the antibiotics resistance mechanisms of biofilms communities is the uptake of resistance genes by horizontal gene transfer [20].This therefore makes the biofilm bacteria persist in the toilet facility and pose a public health threat to those accessing the facility. Women are among those with the highest risk as these bacteria may cause an imbalance in the vaginal micro flora of the students [21] and other women population [22]. This bacterial genera in public toilet facilities could also serve as means of transmission of urinary tract and other toilet associated infections. Biofilm bacteria may always contaminate the wounds of those accessing these toilets and cause further complicate wound management [23].

Comment [H18]: Reference this statement.

5.0 CONCLUSION

Public toilets have become a basic necessity in the life of students,as they spend a considerable number of time using the school facilities in course of their academic pursuit.This study has however, shown that the toilets in University dormitories harbor considerable number of bacterial organisms which are potential pathogens.

The hostel toilets of the University have been noted to pose a great challenge to the health of students. The study observed that the bacterial load varied depending on the gender of students accessing the facility.

Potential pathogens such as *Staphylococcus* spp., *Bacillus* spp, *Salmonella*, *Klebsiella*spp, *Escherichia coli*, *Serratiamercescens* and *Shigella*spp. isolated from these hostels validates the riskassociated with their usage. The presence of these organisms even in low numbers constitutes a major public health concern.

From this study, the water in the wc constituted a larger population of bacteria than the swabs. Therefore, it implies that the quality of water in the water cistern is critical in the spread of toilet acquired infections, and has the potential of being the leading cause of toilet associated infections.

The safety of public toilets cannot be over emphasized. Therefore sanitary measures as well as regular decontamination practices are recommended.

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