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Bacteriological Assessment of Toilet Seats in a Nigerian University

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Authors' contributions

This work was carried out in collaboration among all authors. Author TS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors APE and SSB managed the analyses of the study. Author APE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Exposure to enteric pathogens through direct contact with contaminated toilets surfaces and associated water is one of the major sources of disease transmission in public settings. The bacterial profile of toilet seats in students' dormitories was investigated to determine the pattern of bacterial contamination of public toilet seats in a university setting. Samples were collected from the male and female hostels in the University, and Total Heterotrophic Bacterial Count (THBC) as well as Fecal Coliform Counts (FCC) were carried out using standard microbiological procedures. The male hostels had a mean THBC of $11.4 \pm 4.9 \times 10^5$ cfu/ml and $2.7 \pm 0.7 \times 10^5$ cfu/ml for the water and swab samples collected from the toilet bowl (WC), respectively. The female hostels on the other hand had a mean THBC of $7.7 \pm 0.6 \times 10^5$ cfu/ml and $2.0 \pm 2.7 \times 10^5$ cfu/ml for the water and swab samples from the WC, respectively. The result also revealed that the water in the WC accounted for 80.7% of the bacterial isolates while the toilet seat surfaces accounted for 19.3%. However, there was a statistical difference in the bacterial counts between the male and female hostels as well as the water and swab samples from the WC (p < 0.05). A total of thirty seven

isolates (37) belonging to five (5) genera were identified as *Staphylococcus* spp. (32.4%), *Bacillus* spp (32.4%), *Klebsiella spp* (13.5%), *Escherichia coli* (13.5%), as well as *Coccobacilli* (8.2%). This research has shown the pattern of bacterial contamination of toilet seats and the potential pathogenic bacteria that may pose health challenges. Reduction in the number of students per toilet as well as proper sanitary practice is recommended, to prevent toilet associated infections amongst students.

Keywords: Bacterial profile; toilet seats; sanitary practice; fecal coliform; university setting.

1. INTRODUCTION

A toilet is simply a receptacle into which both solid and liquid waste of human origin, in the form of urine and excreta are discharged. A public toilet may therefore be defined as a facility shared or used by a group of persons in a public setting or environment. It is referred to as a public toilet when it is open to the public, shared by or accessible to a group of individuals. They may be situated in the markets, and transport centers, schools, eateries, hostels, offices, factories, schools, hospitals, factories, cinemas, restaurants, museums. places entertainment, railway stations, filling stations, etc [1]. These could be compartmented in a room or small building containing one or more toilets [2]. They could also be found as portable toilets at large outdoor events and demarcated into male, female and unisex sections [2].

The role of public toilets serving as vehicle for continuous source of epidemics has made research in this area very valuable. Shared toilets can also provide an ideal condition for the spread of pathogens from person to person [3].

Unhygienic use of the toilet facilities may cause urine and fecal residues after use to serve as a major reservoir or source of human pathogen, which may in turn bring about disease outbreak [4].

Biological hazards associated with public toilet usage may include bacterial, fungal and viral-mediated infections, which may be influenced by the number of users as well as the hygiene or sanitary behaviors of the individual users [5]. The toilets in university hostels are more or less public toilets since they are shared by a group of students in a particular block or wing.

Antibiotic resistance is a major health concern all over the world. Humans are in continuous contact with microbes and disease causing bacteria, and this can leading to increase in the resistance os bacterial to antibiotics. The

effectiveness of individual antibiotics may differ depending on the site or location of infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to inactive the antibiotic. This effectiveness can however be evaluated using different methods including the disk diffusion method [6].

Hazards associated with toilet seats and other fomites have been reported by researchers [7,8] but less attention has been given to toilet seats as inanimate objects which could harbor and transmit infectious agents [9]. The colonization of toilet seat surfaces is influenced by various factors or properties of the colonizing agent. These factors may include the physicochemical properties of substrata, such as net surface charge, surface hydrophobicity, surface free critical surface tension. surface energy, wettability, and surface molecular topography are related to bacterial attachment on surfaces. It is possible to alter surface colonization by manipulating surface physicochemical properties [10,11].

This work on the bacteriological survey of toilet seats was therefore carried out to determine the bacterial genera associated with public toilet seats used in a university setting.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Water samples were taken from the water closet (WC) and sterile swab sticks were used to collect samples from the toilet seat surface, covering a circumference of 131 cm.

Samples from solid surfaces (toilet seats) were collected with a sterile swab stick and water sample from the water closet were collected with sterile syringes for each of the toilets. The samples were collected within 7-8 AM each day.

The samples were collected from both the wings (A and B) in the male and female Hostel. The

female hostels had up and down wings while the male hostels had only down wings, A and B. equal number of samples were however collected from both male and female hostels.

2.2 Sterilization

All glasswares and media were sterilized in the autoclave at a temperature of 121°C for 15 minutes at 15psi (Pounds per Square Inch). Wire loops were sterilized by heating until red hot.

2.3 Bacteriological Analysis of Samples

2.3.1 Serial dilutions

- Samples from solid surfaces: Samples from solid surfaces were collected with sterile swab sticks and were properly labeled. A volume of 2 ml normal saline was poured into each swab stick and was allowed to stand for 5 minutes, from which a serial ten-fold dilution was carried out to 10⁻³.
- ➤ Water samples from the WC: Water samples collected from the WC were diluted by transferring 1 ml of the sample into test tubes containing 9 ml of sterile normal saline to make a serial 10 fold dilution to a dilution of 10⁻³.

2.3.2 Inoculation and Incubation

Inoculation was done by spread plate method using a bent glass rod. A 0.1 ml volume of the serially diluted samples was introduced to the plates containing the nutrient agar and MacConcey agar and was spread properly using bent glass rod. The plates were then incubated at 37°C for 24 hrs. MacConcey agar plates that showed no growth or insufficient growth were allowed to stay for 48 hrs.

2.4 Isolation of Pure Culture

To get a pure culture, an inoculum of the colonies was taken and subcultured on fresh agar plates using the streak plate method and incubated at 37°C for 24-48 hours.

2.5 Identification of Bacterial Isolates

The bacterial isolates were identified phenotypically by probing their cultural identities according to standard microbiological procedures, as described by Sampson et al. [12] and Akani et al. [13]. This was done using the spread plate method, by transferring an aliquot of

the serially diluted sample and spread over the surface of solid agar plates and incubated at 37°C for 24-48 hours, as explained in section 2.3.2, above. Microscopy (following Gram staining) as well as the cultural characteristics of the isolates were used alongside standard biochemical test [14], to identify the bacteria present in the samples.

2.6 Antibiotics Sensitivity Test

Antibiotics Sensitivity Test was performed according NCCLS [15]. A set of antimicrobial discs (multi-disc) was dispensed onto the surface of the agar plate inoculated with the isolates, using the Kirby Bauer disc diffusion method. Each disc was pressed down to ensure complete contact with the agar surface. Zones of inhibition were measured around the antibiotic disc using a meter rule and the result was recorded in millimeter (mm). The measurement included the diameter of the disc and susceptibility or resistance of the isolates was reported by referring to Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints of the NCCLS [15], and the organisms were reported as either susceptible, intermediate, or resistant to the agents that were tested.

3. RESULTS AND DISCUSSION

3.1 Enumeration of Bacteria Isolated from Toilet Seats

Water and swab samples were collected from water closets (WC) and seat surfaces, respectively from both male and female hostels in equal proportion, for bacteriological analysis. The laboratory analysis shows that the samples collected from Male Hostel 2, Wing B had the highest Total Heterotrophic Bacterial Count (THBC) of bacteria isolated from both the toilet seat surface and the of the water closet (WC). It had $28.1\pm0.2 \times 10^5$ cfu/ml and $7.2\pm0.3 \times 10^5$ cfu/ml for the samples collected from the toilet bowl, WC and the toilet seat-surface swab samples, respectively (Fig. 1). The least Total Heterotrophic Bacterial Count (THBC) was however observed in samples collected from the Female Hostel Down wing A, which had 1.1±0.3 x 10⁵cfu/ml, for bacteria isolated from the toilet seat surface. Male Hostel 1, Wing B had had similar low count of 1.2±0.1 x 10⁵ cfu/ml albeit from the water in the WC (Fig. 1).

Fecal Coliform Count (FCC) was also carried out. The results shows that the samples collected from Male Hostel 2 Wing B had the highest Fecal Coliform Count (FCC) of bacteria isolated from both the toilet seat surfaces and from the water in the toilet bowl. It had 26.4±0.3 x10⁵ cfu/ml for the samples isolated from the water in the toilet bowl and 2.3±0.4 x10⁵ cfu/ml for the samples isolated from the toilet seat surface (Table 1). The result also shows that Male Hostel 2 Wing A had 1.3±0.2 x10⁵ cfu/ml which represented the least FCC and was enumerated from the water in the WC. Female Hostel down Wing B had similar low level Fecal Coliform Count (FCC) of 1.0 x10⁵ cfu/ml but was however enumerated from the toilet seat surfaces (Table 1).

A comparative analysis of samples from the male and female hostels was carried out and it was discovered (as shown in Fig. 2) that the male hostels had more Total Heterotrophic Bacterial Count (THBC) than the female hostels. The result shows that male hostels had a Mean THBC of 11.4 \pm 4.9 x 10 5 cfu/ml and 2.7 \pm 0.7 x10 5 cfu/ml for the water and swab samples collected from the toilet bowl (WC), respectively. The female hostels on the other hand had a mean THBC of 7.7 \pm 0.6 x 10 5 cfu/ml and 2.0 \pm 2.7 x 10 5 cfu/ml for the water and swab samples from the WC, respectively.

The Fecal Coliform Count (FCC) for the samples shows that the male hostels had more Fecal Coliform than the female hostel, as shown in Table 1.

There was however, a significant difference in the bacterial counts between the male and female hostels as well as the water and swab samples from the WC (p < 0.05). This observation may be as a result of the number of students that use a particular toilet in the University studied. This is similar to the work of [16]. The researcher observed that the toilets at Daeyang Luke Hospital which had more users were more contaminated than other bathrooms with less users. This observation may also be as a result of lack of proper sanitary practices by the students. This is supported by the work of Mendes and Lynch [17], who reported that in order to maintain low bacterial populations and reduce cross-infection, daily cleaning of contact surfaces should be effected and a regular more extensive maintenance and disinfection programme (hygiene service) should employed in order to reduce contamination in all areas. Cortney et al. [18], reported that disinfecting of toilet is very important in order to reduce bacterial contamination and also the

disinfectant used for cleaning the toilets should be used according to the instructions of the producers in order produce its best effect.

The overall bacteriological analysis of the samples from the toilet seat surface and the water of the WC is as shown in Fig. 3, and it was observed that samples collected from the interior water of the WC had a higher bacterial contamination than the samples collected from the toilet seat surfaces. The water in the WC accounted for 80.7% of the bacteria enumerated from the toilet samples while the toilet seat surfaces accounted for 19.3% of the bacterial counts. This result indicates that the interior water of the WC was highly contaminated with bacteria and contact with this water is likely to cause infection on students. The higher bacterial load of the interior water in the WC than that of the toilet seat surfaces may be due to the fact that bacteria grow better on water than solid surface because they require moisture in order to grow.

3.2 Bacterial Diversity in the Various Toilets Studied

The bacterial genera isolated from the toilets in the student hostels were characterized and identified based on their microscopy, cultural and biochemical identities.

From Table 2, it is shown that Female Hostel up-Wing A, Female Hostel Down-wing B and Male Hostel 2 Wing B, all had a total of four (4) bacterial isolates and represented the highest number of bacterial diversity. This was the highest number of isolates for the samples collected from the interior water of the WC. It was also observed that the toilets in Female Up -Wing B, Female Hostel Down wing A, Male Hostel 1 Wing A and Male Hostel 1 Wing B, all had a total number of two (2) bacteria isolates which was the lowest number of isolates for the samples collected from the interior water of the WC.

In the overall, a total of thirty seven isolates (37) belonging to five (5) genera were identified as *Staphylococcus* spp. (32.5%), *Bacillus* spp (32.5%), *Klebsiella* spp (13.5%), *Escherichia coli* (13.5%), as well as *Coccobacilli* spp (8.2%). This shows that *Bacillus spp.* and *Staphylococcus spp.* had the highest percentage frequency while *Coccobacillus* spp had the lowest percentage distribution (Table 3). This result is similar to the work of Ejim et al. [19], on characterization of micro-organisms isolated from bathroom walls in a Nigerian university.

The presence of *Staphylococcus spp* indicates the possibility of human vectors involved, *Staphylococcus* spp. are usually found on the

skin or in the nose and infection by this bacteria may lead to skin infections, sepsis and other forms of infections.

Table 1. Number of students assigned to each of the toilets sampled

Toilets	MH2WB	MH2WA	MH1WB	MH1WA	FDWB	FDWA	FUWB	FUWA
Number	7	3	4	5	4	4	4	4
NB: FUWA=FEMALE HOSTEL UPWING A; FUWB= FEMALE HOSTEL UPWING B								
FDWA = FEMALE HOSTEL DOWNWING A; FDWB = FEMALE HOSTEL DOWNWING B								
MH1WA= MALE HOSTEL 1, WING A;								
MH1WB=MALE HOSTEL 1, WING B;								
MH2WA=MALE HOSTEL 2, WING A;								

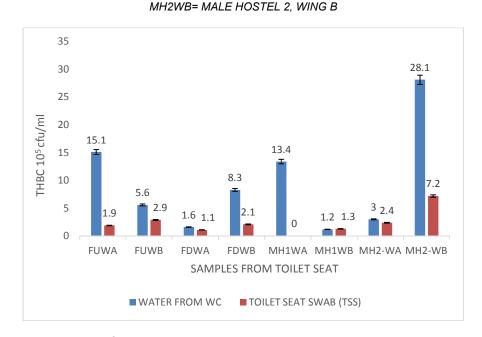


Fig. 1. Population of bacterial contaminants associated with the various toilet seats

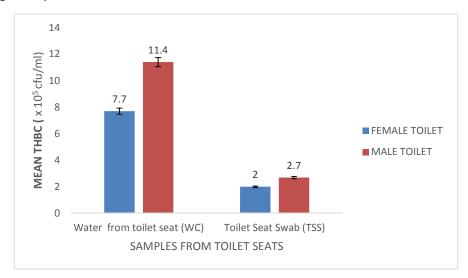


Fig. 2. Comparative index of the bacterial load in male and female toilets sampled

Table 2. Total heterotrophic bacterial and fecal coliform counts (x 10⁵cfu/ml) in the various toilets studied

S/N	Sample ID	FCC	ТНВС	Mean THBC (Male Vs Female)	Mean [%] THBC (WC Vs TSS)
	Water sample from	om toilet bo	wl		
1.	WC-FUWA	1.5	15.1		
2.	WC -FUWB	NG	5.6		
3.	WC -FDWA	NG	1.6	7.7	
4.	WC -FDWB	5.8	8.3		
5.	WC-MH1WA	NG	13.4	11.4	9.6 [80.7]
6.	WC-MH1WB	NG	1.2		
7.	WC-MH2-WA	1.3	3.0		
8.	WC-MH2-WB	26.4	28.1		
	Toilet Se	at Swab (TS	SS)		
9.	TSS-FUWA	NG	1.9		
10.	TSS-FUWB	NG	2.9		
11.	TSS-FDWA	NG	1.1	2.0	
12.	TSS-FDWB	1.0	2.1		
13.	TSS-MH1WA	NG	NG		2.3 [19.3]
14.	TSS-MH1WB	NG	1.3		- •
15.	TSS-MH2WA	NG	2.4	2.7	
16.	TSS-MH2WB	2.3	7.2		

NB: THBC = Total Heterotrophic Bacterial Count FCC = Fecal Coliform Count

NG = No Growth; FUWA = Female Hostel Upwing A; FUWB= Female Hostel Upwing B; FDWA= Female Hostel Downwing A; FDWB= Female Hostel Downwing B; MH1WA = Male Hostel 1, WING A; MH1WB= Male Hostel 1, Wing B; MH2WA= Male Hostel 2, Wing A, MH2WB = Male Hostel 2, Wing B

The presence of *Escherichia coli* indicates fecal contamination which also indicates the possibility of human vectors involved. Infection by *Escherichia coli* may cause diseases like Urinary tract infection (UTI), Pneumonia etc. *Klebsiella* spp. which also indicates a fecal contamination may cause diseases like pneumonia, urinary tract infection (UTI) etc.

Coliform bacteria, defined as rod-shaped, nonspore forming, motile or non-motile bacteria which ferment lactose with the production of acid and gas when incubated at 35-37°, can be found in restrooms mostly as fecal coliforms. Their occurrence could be related to improper disposal of sanitary waste. Escherichia and Enterococci species have been report to be dominant in rest rooms [20]. E. coli has been also reported as the main bacterium within the thermo tolerant coliform group, present in large numbers in feces at concentrations of about 109 bacteria per gram of fecal matter [21]. It does not multiply appreciably in the environment [22]. Most people are concerned about the health risk that coliform may pose. People exposed to coliform contaminated water may exhibit fever, diarrhea and abdominal cramps, chest pain, or hepatitis.

Coccobacilli are pleomorphic bacteria and members of this group include *Chlamydia trachomatis*, *Haemophilus influenza*, *Gardnerella vaginialis*, *Bordetella pertussis*, *Yersinia pestis and Brucella* spp. They are known to cause a variety of infections including pneumonia, whooping cough, bacterial vaginosis, plaque, plaque, and periodontitis, depending on the species involved [23].

The presence of *Coccobaccilli* spp in the male and female hostels is therefore of a public health importance and calls for more sanitary measures at these sites. Further research targeting the molecular identification of these bacteria is necessary, in order to know the proper identity of the bacteria as well as the specific possible associated infection. Coccobaccillus has also been isolated by previous researchers [19] from bathroom walls of public and private school buildings and this research therefore includes public toilet seats in the microbial ecology of Coccobaccilli.

As of 2017, an estimated 2.3 billion people lacked access to improved sanitation facilities, worldwide [24]. Inadequate access to sanitation

and hygiene facilities is known to be a leading cause of morbidity and mortality, particularly in low-income countries [25]. In fact, approximately 10% of the global burden of disease is thought to be attributed to inadequate water, sanitation, and hygiene (WASH), which is largely driven by increased exposure to human pathogens

transmitted via the fecal-oral route [26]. A lack of safely managed wash infrastructure has been identified by Flores et al. [27], as a possible source of exposure to enteric pathogens through improper hand hygiene, or through direct contact with contaminated toilets surfaces.

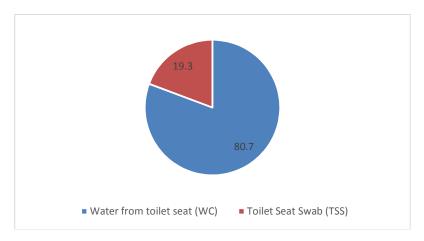


Fig. 3. Level of bacterial colonization of toilet seat surface and interior water

Table 3. Distribution of isolates in the various toilets

S/N	Sample ID	Bacterial isolates	Total number of isolates
1.	WC-FUWA	Bacillus spp., Staphylococcus spp., Escherichia coli, Klebsiella spp.	4
2.	WC-FUWB	Bacillus spp., Coccobacillus sp.	2
3.	WC-FDWA	Bacillus spp., Staphylococcus spp.,	2
4.	WC-FDWB	Staphylococcus spp., Escherichia coli, Klebsiella spp.	3
5.	WC-MH1WA	Coccobacillus spp., Staphylococcus spp.	2
6.	WC-MH1WB	Coccobacillus spp., Staphylococcusspp	2
7.	WC-MH2-WA	Bacillus spp., Staphylococcus spp., Klebsiella spp.	3
8.	WC-MH2-WB	Bacillus spp., Staphylococcus spp.,	2
9.	TSS-FUWA	Bacillus spp.,	1
10.	TSS-FUWB	Bacillus spp.,	1
11.	TSS-FDWA	Bacillus spp.,	1
12.	TSS-FDWB	Bacillus spp., Staphylococcus spp.,Escherichia coli, Klebsiella spp.	4
13.	TSS-MH1WA	Bacillus spp., Staphylococcus spp.	2
14.	TSS-MH1WB	Bacillus spp., Staphylococcus spp., Escherichia coli	3
15.	TSS-MH2WA	Staphylococcus spp.	1
16.	TSS-MH2WB	Bacillus spp., Staphylococcus spp., Escherichia coli, Klebsiella spp.	4

NB: FUWA = Female Hostel Upwing A, FUWB= Female Hostel Upwing B, FDWA= Female Hostel Downwing A, FDWB= Female Hostel Downwing B, MH1WA = Male Hostel 1, WING A, MH1WB= Male Hostel 1, WING B, MH2WA= Male Hostel 2, WING A, MH2WB = Male Hostel 2, WING B

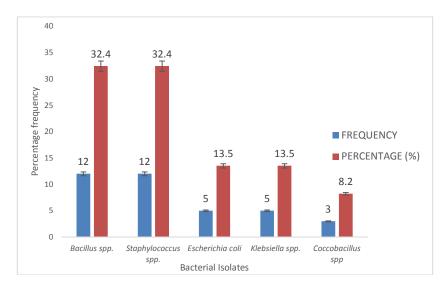


Fig. 4. Frequency of bacterial isolates from the various points sampled

Table 4. Percentage occurrence of bacterial isolates

Isolates	Frequency	Percentage (%)	
Bacillus spp.	12	32.4	
Staphylococcus spp.	12	32.4	
Escherichia coli	5	13.5	
Klebsiella spp.	5	13.5	
Coccobacillus spp	3	8.2	
Total	37	100	

Table 5. Susceptibility pattern of the isolates to some common antibiotics

S/N	Antibiotics	Isolates				
		Bacillus	Staphylococcus	Coccobacillus	Klebsiella	Escherichia
		spp.	spp	spp	spp	coli
1.	LEV 20 mcg	S	S	S	ND	ND
2.	APX 20 mcg	S	R	S	ND	ND
3.	RD 20 mcg	S	S	S	ND	ND
4.	AML 20 mcg	R	R	S	ND	ND
5.	E 30 mcg	S	S	S	ND	ND
6.	NB 10 mcg	S	S	S	ND	ND
7.	CH 30 mcg	S	S	S	ND	ND
8.	CN 10 mcg	S	S	S	S	S
9.	S 30 mcg	S	S	S	S	S
10.	CPX 10 mcg	S	S	S	S	S
11.	AU 30 mcg	ND	ND	ND	S	R
12.	SXT 30 mcg	ND	ND	ND	R	S
13.	PN 30 mcg	ND	ND	ND	S	R
14.	CEP10 mcg	ND	ND	ND	S	S
15.	OFX 10 mcg	ND	ND	ND	S	S
16.	NA 30 mcg	ND	ND	ND	S	S
17.	PEF 10 mcg	ND	ND	ND	S	S
No. of	resistance, R [%]	1 [10]	2 [20]	0	1[10]	2 [20]
No. susceptibility, S [%]		9 [90]	8 [80]	10 [100]	9 [90]	80 [80]

Key: CPX: Ciproflox, CN: Gentamycin, AML Amoxil, S: Streptomycin, RD: Rifampicin, E: Erythromycin, CH: Chloramphenicol, APX: Ampiclox, LEV: Levofloxacin, AU: Augmentin, SXT: Septrin, PN: Ampicillin, CEP: Ceporex, OFX: Tarivid, PEF: Reflacine, NB: Norfloxacin, NA: Nalidixic acid; R= Resistant, S = Susceptible, ND = Not determined

3.3 Antibiotics Susceptibility Pattern of the Bacterial Isolates from the Toilet Seat Samples

The antibiotics susceptibility test was carried out to determine the level of susceptibility of the bacterial isolates from the toilet seat samples. The antibiogram obtained (Table 4) showed that the isolates were highly susceptible to most of the antibiotics tested. Among the Gram positive isolates, Staphylococcus spp was the least susceptible (80%) as it was resistant to Ampiclox and Amoxil. This is similar to the work of [28]. The researcher also used 20 mg of Ampiclox and Amoxil and reported similar results. Bacillus spp. was however resistant to only Amoxil while Coccobacillus was susceptible (100%) to all the antibiotics. The Gram negative isolates showed similar susceptibility pattern as Escherichia coli was more resistant, 20% (to Augmentin and Ampicillin) than Klebsiella which was resistant, 10%, to only Septrin.

The difference in the susceptibility pattern of the isolates to the various antibiotics is probably dependent on the source of the bacterial contaminant as organisms without prior exposure may show low level susceptibility compared to pathogens of human origin with prior exposure [29].

4. CONCLUSION

This study on the bacteriological assessment of toilet seats in the student hostels has shown the bacterial profile of shared toilets in a university setting. The study shows that bacterial contamination was higher in the male hostel toilets compared to the female hotel toilet seats. The bacterial species isolated in this study are known to be associated with various forms of infection in human, and is therefore of a public health concern as student may get infected through the use of shared toilets in the university. Therefore, proper sanitary measure should be taken in order to prevent the outbreak and spread of infection among students dwelling in the dormitories.

From this study, the water in the WC (toilet seats) was found to contain higher bacterial population than the toilet seat surface swabs. This therefore implies that the water quality in the WC is critical in the epidemiology of toilet acquired infections, and therefore has the potential of being the leading cause of toilet associated infections.

From the results, it can also be deduced that the female hostel occupants may be adopting more sanitary measures than the male occupants in this area. Also, it was observed that the number of students assigned to a particular toilet influenced the bacterial profile of the toilet with a strong positive correlation. Therefore, the cleanliness of a toilet is partly dependent on the number of persons using the toilet as a result of differences in personal hygiene. The safety of toilets, including public toilets therefore, resides in the hands of the users of the toilet.

Proper sanitary practices should be adopted by students, in order to keep their toilets clean. Also, toilets should be cleaned daily in order to reduce the bacterial load of the toilets to avoid infection. Disinfectants should be used in cleaning the toilets rather than regular soaps in order to kill most of the bacteria.

University authorities should try to reduce the number of students using a particular toilet to prevent toilet associated infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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