

BACTERIOLOGICAL ASSESSMENT OF TOILET SEATS IN A NIGERIAN UNIVERSITY

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.

1.0 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, bars, museums, restaurants, places of 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 lead to an increase in the resistance of bacteria 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 inactivate 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 energy, critical surface tension, surface 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.

2.0 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.

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
 MH2WB = MALE HOSTEL 2, WING B

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 1ml 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 for 24hrs. MacConcey agar plates that showed no growth or insufficient growth were allowed to stay for 48hrs.

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 for 24-48 hours.

2.5 Antibiotics Sensitivity Test

The sensitivity of an isolated organisms was tested by placing antibiotic disc on culture plates then followed with the test organisms and by judging the degree of sensitivity by the size of inhibition zones resulting after 24 hours of incubation.

The isolated was placed into peptone water then incubated at 37°C for 3 – 4 hours. Sterile nutrient agar plate was dried in hot air over and then the plate was flooded with growth in peptone water. Antibiotic disc (multi-disc) was placed on the surface of the culture medium using the Kirby Bauer disc diffusion method and was incubated overnight at 37°C. Zones of inhibition were measured around the antibiotic disc using a meter rule and the result was recorded in millimeter (mm).

3.0 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 $28.1 \pm 0.2 \times 10^5$ cfu/ml and $7.2 \pm 0.3 \times 10^5$ cfu/ml for the samples collected from the toilet bowl, WC and the toilet seat-surface swab samples, respectively (Figure 1). The least Total Heterotrophic Bacterial Count (THBC) was however observed in samples collected from the Female Hostel Down wing A, which had $1.1 \pm 0.3 \times 10^5$ cfu/ml, for bacteria isolated from the toilet seat surface. Male Hostel 1, Wing B had had similar low count of $1.2 \pm 0.1 \times 10^5$ cfu/ml albeit from the water in the WC (Figure 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 $26.4 \pm 0.3 \times 10^5$ cfu/ml for the samples isolated from the water in the toilet bowl and $2.3 \pm 0.4 \times 10^5$ cfu/ml for the samples isolated from the toilet seat surface (Table 1). The result also shows that Male Hostel 2 Wing A had $1.3 \pm 0.2 \times 10^5$ 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 1.0×10^5 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 Figure 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 $11.4 \pm 4.9 \times 10^5$ 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 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 Ngonda 2017[12], he 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 *et al.* 1976 [13], 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 be employed in order to reduce contamination in all areas. Cortney *et al.* 2013 [14], 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 Figure 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.

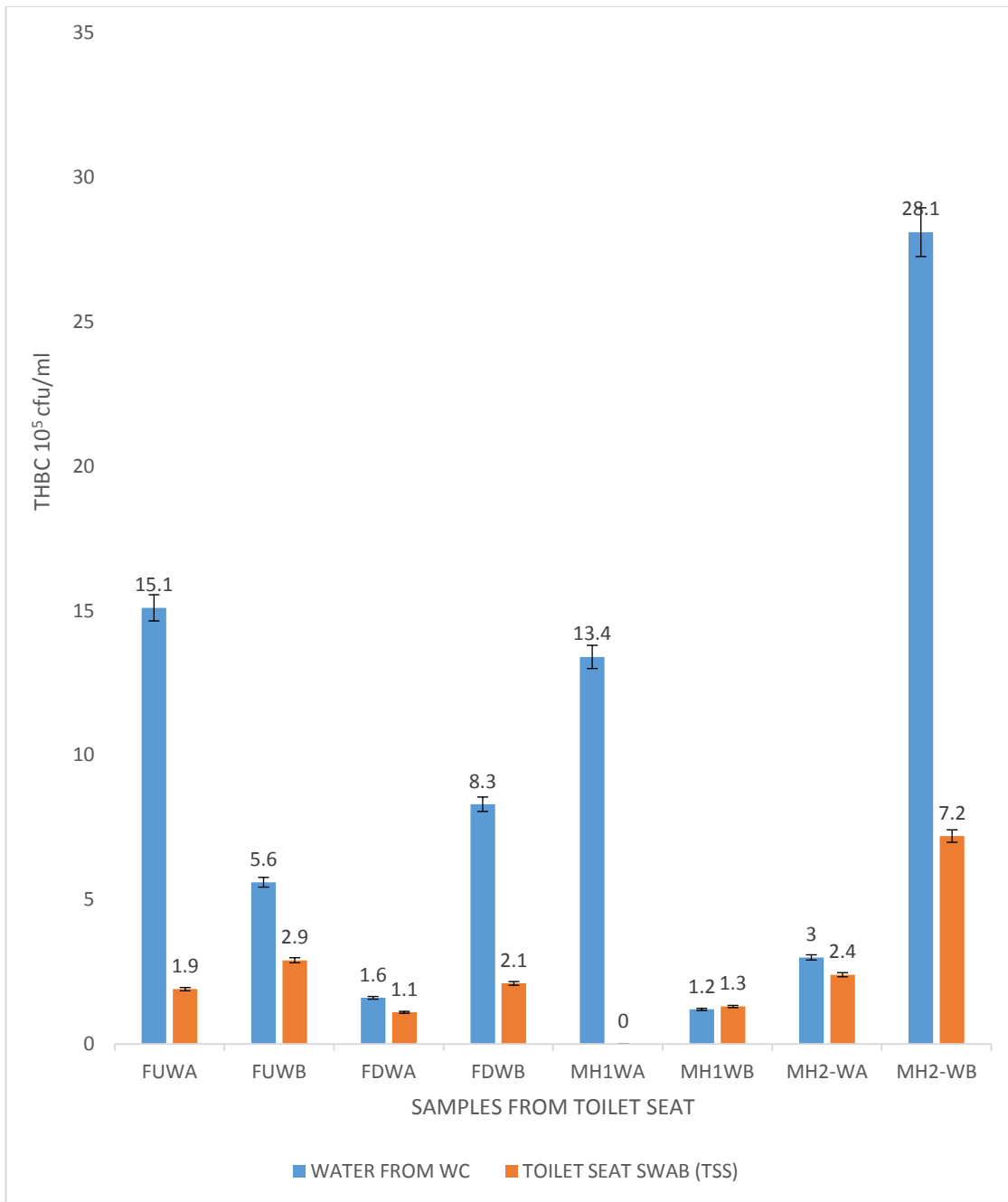


Figure 1: Population of bacterial contaminants associated with the various toilet seats

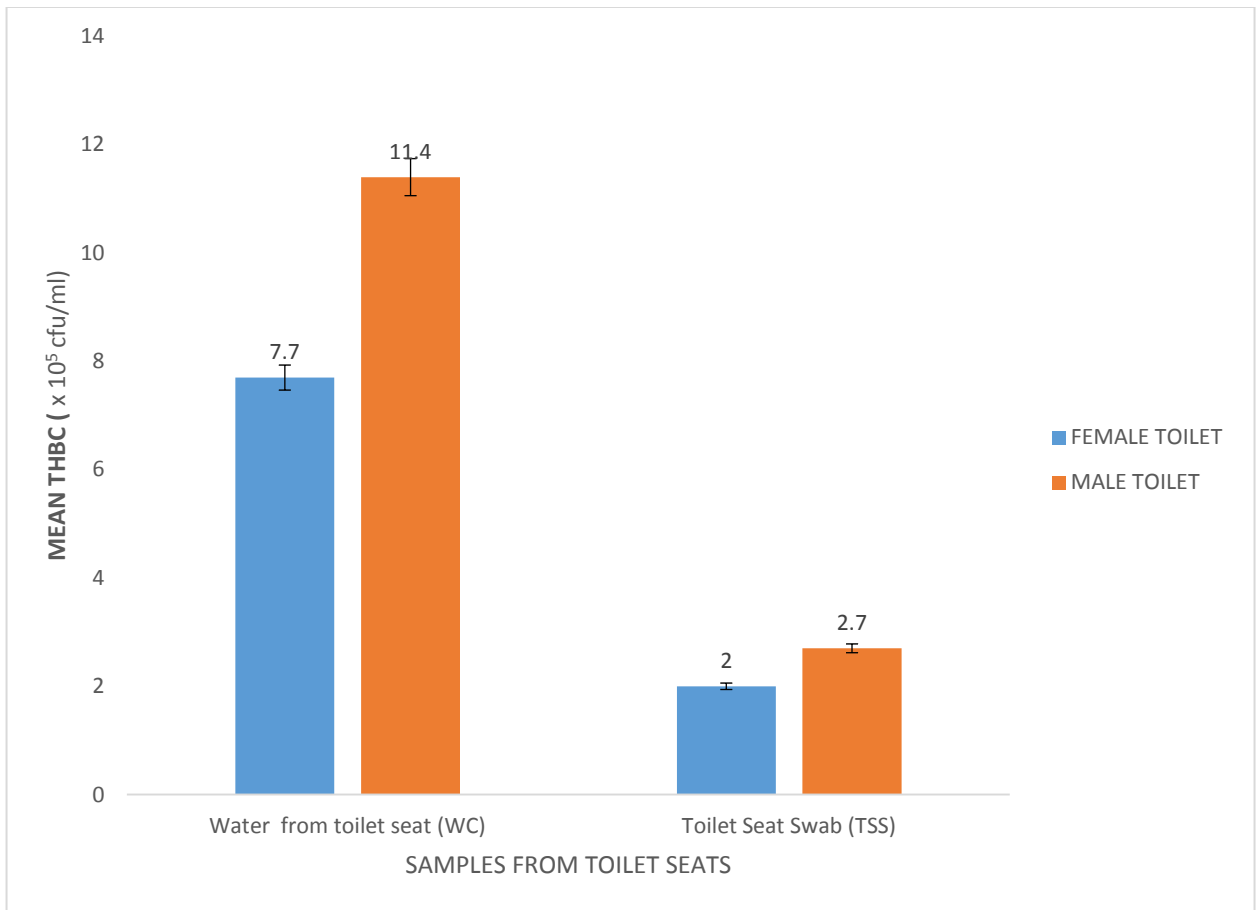


Figure 2: Comparative index of the bacterial load in male and female toilets sampled

UNDER PPL

Table 1: Total heterotrophic bacterial and fecal coliform counts ($\times 10^5$ cfu/ml) in the various toilets studied

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

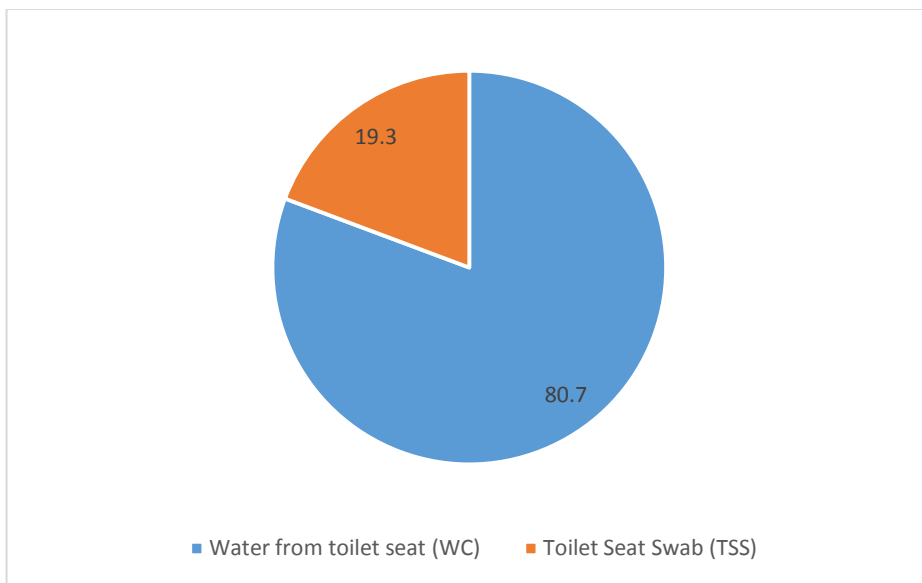


Figure 3: Level of bacterial colonization of toilet seat surface and interior water.

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 (8.2 %). This shows that *Bacillus* spp. and *Staphylococcus* spp. had the highest percentage frequency while Coccobacillus had the lowest percentage distribution (Table 3). This result is similar to the work of Ejim *et al.*, 2016, 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.

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, non-spore 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 [15]. *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 faecal matter [16]. It does not multiply appreciably in the environment [17]. 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 vaginalis*, *Bordetella pertussis*, *Yersinia pestis* and *Brucella* spp. They are known to cause a variety of infections including pneumonia, whooping cough, bacterial vaginosis, plaque, plaque, periodontitis, depending on the species involved [18].

The presence of Coccobaccilli 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 [20]. Inadequate access to sanitation and hygiene facilities is known to be a leading cause of morbidity and mortality, particularly in low-income countries [21]. 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 [22]. A lack of safely managed wash infrastructure has been identified by Flores *et al.*, 2011 [23], as a possible source of exposure to enteric pathogens through improper hand hygiene, or through direct contact with contaminated toilets surfaces.

Table 2: Distribution of isolates in the various toilets.

S/N	SAMPLE ID	BACTERIAL ISOLATES	TOTAL NUMBER OF ISOLATES
1.	WC-FUWA	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp.	4
2.	WC-FUWB	<i>Bacillus</i> spp., <i>Coccobacillus</i> sp.	2
3.	WC-FDWA	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp.,	2
4.	WC-FDWB	<i>Staphylococcus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp.	3
5.	WC-MH1WA	<i>Coccobacillus</i> spp., <i>Staphylococcus</i> spp.	2
6.	WC-MH1WB	<i>Coccobacillus</i> spp., <i>Staphylococcus</i> spp	2
7.	WC-MH2-WA	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Klebsiella</i> spp.	3
8.	WC-MH2-WB	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp.,	2
9.	TSS-FUWA	<i>Bacillus</i> spp.,	1
10.	TSS-FUWB	<i>Bacillus</i> spp.,	1
11.	TSS-FDWA	<i>Bacillus</i> spp.,	1
12.	TSS-FDWB	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Escherichia</i> <i>coli</i> , <i>Klebsiella</i> spp.	4
13.	TSS-MH1WA	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp.	2
14.	TSS-MH1WB	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Escherichia coli</i>	3
15.	TSS-MH2WA	<i>Staphylococcus</i> spp.	1
16.	TSS-MH2WB	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> 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

Table 3: Percentage occurrence of bacterial isolates.

ISOLATES	FREQUENCY	PERCENTAGE (%)
<i>Bacillus</i> spp.	12	32.4
<i>Staphylococcus</i> spp.	12	32.4
<i>Escherichia coli</i>	5	13.5
<i>Klebsiella</i> spp.	5	13.5
<i>Coccobacillus</i> spp	3	8.2
TOTAL	37	100

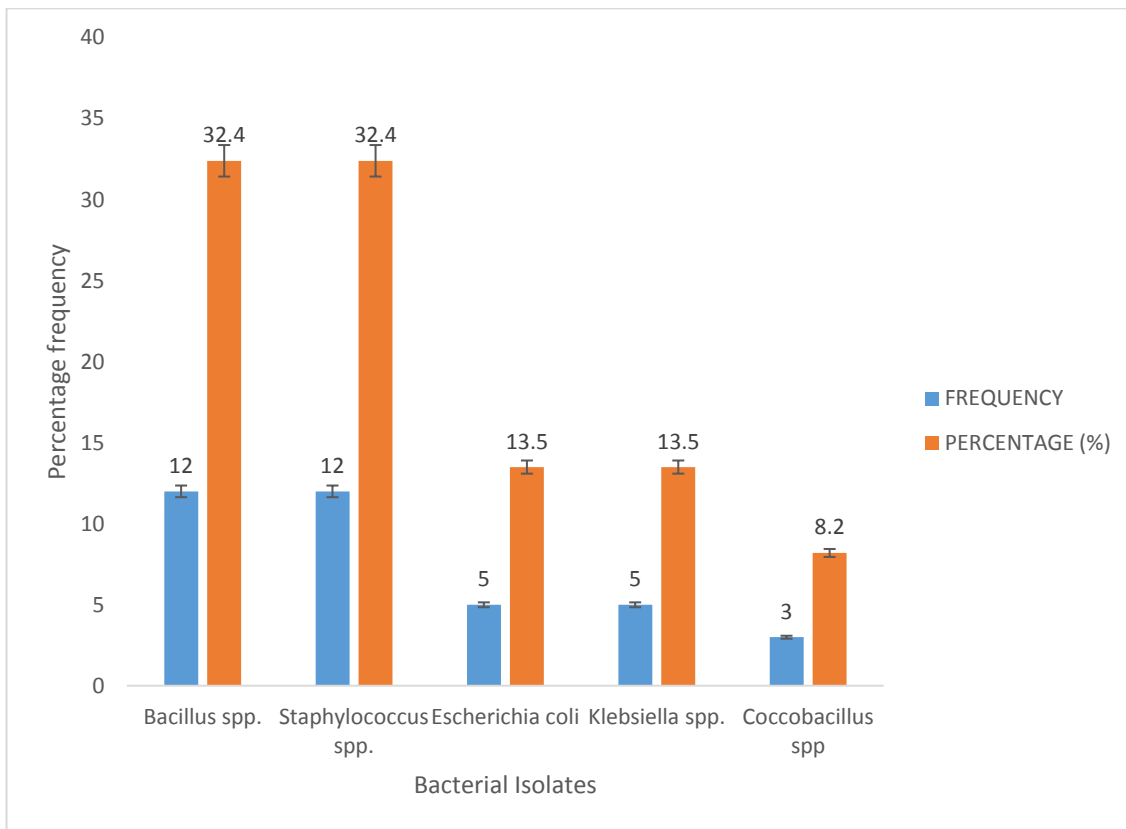


Figure 4: Frequency of bacterial isolates from the various points sampled

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 Owuna et al 2015 [24]. 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 properly 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 [25].

Table 4 Susceptibility pattern of the isolates to some common antibiotics.

S/N	ANTIBIOTICS	ISOLATES				
		<i>Bacillus spp.</i>	<i>Staphylococcus spp</i>	<i>Coccobacillus spp</i>	<i>Klebsiella spp</i>	<i>Escherichia coli</i>
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.	CEP 10 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: Seprtrin, PN: Ampicillin, CEP: Ceporex, OFX: Tarivid, PEF: Reflacine, NB: Norfloxacin, NA: Nalidixic acid.

R= Resistant, S = Susceptible, ND = Not determined.

4.0 CONCLUSION

From the analysis carried out in this study on the toilets in the student hostels, it is evident that the toilets contain bacterial contamination, especially in all the toilets albeit higher in the male hostel toilets. Some of these bacteria may be pathogenic, this indicates that they are likely to cause diseases when a student gets infected. Therefore, proper sanitary measure should be taken in order to prevent the outbreak of infection.

The results also implicates the water in the WC (toilet seats) be of a higher bacteria mediated health hazard and therefore has the potentials of been 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 the area studied, and also the cleanliness of a toilets is partly dependent on the number of persons using the toilet due to differences in personal hygiene. Therefore, the safety of toilets, including public toilets 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 bacteria 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.

REFERENCES

1. Kolsky, P. Opening remarks for the Public and community toilets: Lessons and ideas from experience. Video conference. 2016
2. Greed, C. The role of public toilets: pathogen transmitter or health facilitator. *Journal of Building Engineering*. 2014; 27(2):127-139.
3. Gerhardt, A., Hummer, T., Balluff, R., Mucha, H. and Hofer, D. A model of the transmission of micro-organisms in a public setting and its correlation to pathogenic infection rises. *Journal of applied microbiology*. 2012; 122: 614-621
4. Maori L, Agbor V. and Ahmed W. The prevalence of bacterial organisms on toilet door handles in secondary schools in Bokkos L. G. A., Jos, Plateau state, Nigeria. *Journal of Pharmacy and Biological Sciences*. 2013; 8(4): 85-91
5. Cheesbrough, M. District laboratory practice in tropical countries part 2 (2nd Ed.). Capetown: Cambridge University press. 2006; 76: 181, 64.
6. Alves, J., Ferreira, I., Martins, A. and Pintado, M. Antimicrobial activity of wild mushroom extract against clinical isolates resistance to different antibiotics, *Journal of applied microbiology*. 2012; 11: 466-475.
7. Whittington, M. Epidemiology of infections with trichomonas vaginalis in the Light of improved diagnostic methods. *British journal of venereal diseases*. 1957; 33: 80-91.
8. Burgess, J. Trichomonas vaginalis infection from splashing in water closets. *British journal of venereal disease* 1963; 39: 248-50.
9. Amala, E. and Ade, J. Bacteria associated with toilets and offices lock. *International Journal of Epidemiology and Infection*. 2015; 3(1): 12-15.
10. Wiencek, M., and Fletcher M. Effects of substratum wettability and molecular topography on the initial adhesion of bacteria to chemically defined substrata. *Biofouling*. 1997; 11: 293–311.
11. Ista, I., Fan, Y., Baca, O and Lopez, P. Attachment of bacteria to model solid surfaces: oligo (ethylene glycol) surfaces inhibit bacterial attachment. *FEMS Microbiology Letters*. 1996; 142: 59–63.
12. Ngonda F. Assessment of bacterial contamination of toilets and bathroom doors handle/knobs at Daeyang Luke hospital. *Pharmaceutical and biological evolution*. 2017; 4: 193-197

13. Mendes M. and Lynch J. A bacteriological survey of washrooms and toilets. *Journal of hygiene*. 1976; 76(2):183-90
14. Cortney, M., Angela F. and Roman S. Cleaning and Disinfecting Bathrooms. Department of Food, Nutrition, and Packaging Sciences, Clemson University, Clemson, SC 29634. *W.w.w. FightBac.org*. Retrieved December 2019.
15. Stevens M., and Edmond M. Endocarditis due to vancomycin-resistant enterococci: case report and review of the literature. *Clinical Infectious Diseases*. 2005; 41(8):1134–1142.
16. Brenner, D., McWhorter, C., Knutson, K, and Steigerwalt G. *Escherichia vulneris*: a new species of Enterobacteriaceae associated with human wounds. *Journal of clinical microbiology*. 1982; 15: 1133-1140.
17. Edberg, C., Rice, W., Karlin J. and Allen J. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*. 2000; 88: 106-116.
18. Seladi-Schulman, J. Your Guide to Coccobacilli infections. www.healthline.com. Accessed July 11th 2019 at 12.00 GMT.
19. Ejim, I., Mary, A., and Egberongbe, H. Characterization of Micro-organisms Isolated from Bathroom Walls in a Nigerian University *Journal of Applied Life Sciences International*. 2016; 9(4): 1-11
20. WHO-World Health Organization. Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines. World Health Organization (WHO) and the United Nations Children's Fund (UNICEF); Geneva, Switzerland: 2017.
21. Lim, S., Vos, T., Flaxman, D., Danaei, G., Shibuya, K., Adair-Rohani, H., Amann, M., Anderson, R., Andrews, G., and Aryee, M. A systematic analysis for the Global Burden of Disease Study 2010. *Journal of lancet, London*. 2012; 380: 2224–2260.
22. Prüss-Üstün A., Bos R., Gore F. and Bartram J. Safer Water, Better Health: Costs, Benefits and Sustainability of Interventions to Protect and Promote Health. World Health Organization; Geneva, Switzerland. 2008
23. Flores, E., Bates, T., Knights, D., Lauber, L., Stombaugh, J., Knight, R., Fierer, N. Microbial biogeography of public restroom surfaces. *PLoS ONE*. 2011; 6 (11):1-7
24. Owuna G., Abimku, R., Nkene, I., Joseph, G. and Ijalana, O. Isolation and antibiotics susceptibility of *Staphylococcus Aureus* from fresh poultry meats sold in keffi metropolis Nigeria. *International Journal of Research Studies in Biosciences*. 2015; 11(3), 1-5

25. Boada, A, Pons-Vigues, M, Real, J, Grezne, E, Bolibar, B and Llor, C. Previous exposure and antibiotic resistance of commensal *Staphylococcus aureus* in Spanish primary care. *European Journal of General Practice*. 2018; 24 (1): 125 – 130.

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