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Characterization of Micro-organisms Isolated from Bathroom Walls in a Nigerian University

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Authors' contributions

This work was carried out in collaboration between all authors. Author NIE contributed to experimental analysis, design of study and preparation of manuscript. Author MAE prepared and edited the manuscript. Author HOE designed the study, managed the experimental work and prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Micro-organisms are found occurring in both indoor and outdoor environments. Restrooms and bathrooms are places where people go to relieve themselves as well as clean up, posing the possibility of these places being prone to microbial contamination.
Methods: A total of twenty public and private residence bathrooms used by students in a Nigerian university were screened for the occurrence of bacterial contamination. Bathroom walls were swabbed and samples analysed for microbial occurrence using different biological growth media. Organisms isolated were characterized morphologically and further identified using some biochemical tests including catalase, methyl red, Voges Proskauer, oxidase, citrate utilization and urease activity tests.
Results: Results from the study showed different gram-positive and gram-negative bacteria

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isolated including *Staphylococcus aureus*, *Escherichia coli*, *Clostridium difficle* and *Klebsiella pneumoniae* from both public and private bathrooms of students, with highest percentage incidence recorded for *Staphylococcus aureus* 15.6% and 12.9% in public and private bathrooms respectively.

Conclusion: The little difference in microbial contamination between private and public bathrooms observed in the study shows that maintaining microbial free bathrooms is not only dependent on the number of users but their personal hygiene practice meaning that proper and regular cleaning can ensure an almost microbial free bathrooms in public institutions.

Keywords: Bacteria; toilets; public health; hygiene; surfaces.

1. INTRODUCTION

Microorganisms are very diverse such that they can be found everywhere in the environment on living and non-living things with humans inclusive [1]. These organisms which can be found on the skin, mouth, gut, urine, intestine, reproductive organs of humans and even bathing water have been seen on the surfaces of toilets and bathrooms [2]. The bathroom which is a room for personal hygiene generally contains a bathtub or a shower, and in some instances contains toilets. Surfaces such as walls, sinks, doors, windows, flush handles and toilet seats in the bathroom can be sites for microbial contamination [3,4]. Microbes that are likely to be found in bathrooms can be divided into three groups; microorganisms that live on human skin, those from the outdoors that are carried in with foot-wears and bacteria that live inside humans which are passed out in urine or faeces [5,6].

Bathroom facilities are an important tool integrated in gastrointestinal health and associated with faecal transmission of diseases [7,8]. Large number of bacteria and viruses when seeded into house hold toilets may remain in the bowl even after continual flushing [9]), due to adsorption of the organisms to porcelain surfaces of the toilet bowl, with gradual elution occurring after each flush. Hence, there is the possibility of an individual contracting an infection from aerosols created in the bathroom and toilet [10-12]. Micro-organisms isolated from bathrooms include bacteria such as *Salmonella enterica*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* [13-15]; yeasts such as *Candida albicans* [16] and fungi such as *Aspergillus*, *Clostridium* and *Fusarium* [17,18]. These micro-organisms are associated with a range of infections including gastroenteritis [19], hepatitis [20] and urinary tract infection [21].

Typical of many higher institutions in Nigeria, inadequate accommodation force students to live in rented apartments with many individuals sharing bathroom and toilet facilities. This leads to deterioration of these facilities with lack of water and poor management as contributory factors. The possibility of contamination of surfaces of bathrooms shared by students is therefore inevitable in this scenario which could contribute to causing an epidemic. The study aims to screen walls of public and private bathrooms of Olabisi Onabanjo University, Ago-Iwoye students' residences for bacterial contamination in order to determine health risks the students are exposed to on a daily basis.

2. MATERIALS AND METHODS

2.1 Study Area and Collection of Samples

A total of 20 swab samples were taken from bathrooms in ten (10) self-contained and ten (10) populated bathrooms used by students who reside in Ago – Iwoye for the purpose of the experiment. Samples were taken from walls of bathrooms excluding washed bathrooms and parts of bathroom walls with soap lather because of the bactericidal effect of soaps and disinfectants on the microorganisms. Samples were collected using sterile swab sticks that had been placed in peptone water for 24 hours before collection. After each sample was collected, the swab sticks were reinserted in peptone water for some hours to resuscitate the organisms.

2.2 Isolation and Characterization of Organisms

All media used were prepared according to the different manufacturers' procedure. The following agar were used for the experiment; MacConkey, Nutrient and Eosin methylene blue (EMB). Pour plate method was used for the inoculation of the organisms. One millilitre of the peptone water containing the swab sticks was dispensed into sterile Petri dishes that were labelled and agar poured into the petri dishes after it had cooled to about 50℃. After solidifying, petri dishes

containing agar and specimen were incubated at 37℃ for 24 hours. Culture of samples collected was done in triplicates.

After 24 hours incubation, plates were observed and colonies of organisms observed were sub cultured using sterilized inoculating loops on freshly prepared agar plates. Plates containing sub cultured organisms were incubated for another 24 hours at 37℃. After incubation, plates were also observed for microbial growth. Following observation of organisms, gram staining was done and nutrient slants were made using bijou bottles and left to stand overnight. The following day, slants were checked for contaminants. Uncontaminated slants were inoculated with sub cultured organisms for further biochemical tests.

2.3 Biochemical Tests

Media used for biochemical tests are as follows; Starch agar, triple sugar iron agar, methyl red voges proskeur (MRVP) broth, phenol red agar, simmons citrate agar and urea broth. All media were prepared following manufacturers' procedure and sterilized using an autoclave at 121℃ for 15 minutes. The different broths were dispensed into bijou bottles while agar were poured into sterile Petri dishes. The following biochemical tests were carried out to identify and characterise organisms isolated from samples collected.

2.3.1 Spore formation test

Under sterile conditions, a thin smear was made from 24 hrs old culture by placing a drop of sterile saline on a clean grease free slide and a colony was picked from the culture plate with the aid of a sterile, cooled loop and emulsified. The smear was air dried and heat fixed with minimum amount of heat. Slides were then flooded with 5% malachite green solution and heat was applied gently until evaporation occurred. The slides were left to cool for 5 mins and then rinsed under slow running tap water. Afterwards, slides were counterstained with 0.5% safranin for 1-2 minutes and rinsed with water. The slide was left to drain and viewed afterwards, under a microscope using oil immersion objective (x100) [22].

2.3.2 Methyl red test

Wire loops full of isolates under investigation were inoculated onto MRVP broth in the bijou bottles and incubated for 24 hrs. Three drops of methyl red (MR) solution were added and colour change observed. A colour change from light yellow to pink indicates a methyl red positive reaction, meaning that acid is produced; while no change in colour (colour remains yellow) indicates a methyl red negative reaction [23-25].

2.3.3 Voges Proskauer test

To the other bijou bottles containing broth, using wire loop, isolates were inoculated and incubated for 24 hrs at 37℃. This was followed by addition of 0.5ml of 6% α-naphthol solution and 0.5 ml of 40% KOH which was allowed to stay for 30 minutes. A development of pink coloration, within 30 mins indicated a positive reaction [25].

2.3.4 Gram staining

Under sterile conditions, a smear was made from 24 hrs old culture by placing a drop of sterile saline on a clean grease free slide and a colony was picked from the culture plate with the aid of a sterile cooled loop and emulsified. The smear was air dried and heat fixed by passing the slides over a flame. Slides were flooded with crystal violet and the stain was allowed to act for 1 minute. It was then rinsed under slow running tap water and flooded with Lugol's iodine for another 1 minute, followed by rinsing with water and decolourizing with acetone for 30 seconds (care was taken to avoid over decolourization). The slide was then flushed with water allowed to drain. Slides were later counter stained with safranin for 1 minute, rinsed with water and viewed under a microscope using oil immersion objective (x100) [24,25].

2.3.5 Starch hydrolysis test

The surface of media incubated with isolates at 37℃ for 24 hrs was flooded with 5 ml of Lugol's iodine solution. Un-hydrolysed starch formed blue black colouration while hydrolysed starch appeared as a clear zone which results from α-amylase activity [24].

2.3.6 Indole production test

The indole test is used to distinguish among members of the family Enterobacteriaceae [25] by testing their ability to degrade an essential alpha amino acid, tryptophan to produce indole. The isolates were inoculated in nutrient broth and incubated at 37℃ for 16-24 hrs. After incubation, few drops of Kovac's reagent were added and the tube shaken gently and allowed to stand. The pinkish and ring-like colour at the upper layer

indicates indole production in the tubes, and if otherwise, no indole production [26].

2.3.7 Triple sugar iron

This test determines the ability of an organism to ferment sucrose, lactose and glucose. It also determines the production of hydrogen sulphide. Test organisms were inoculated on triple sugar iron agar and incubated for 24 hrs [24].

2.3.8 Sugar fermentation test

The inoculum is fixed with the sugar discs (glucose, sucrose, lactose, maltose and D-mannitol) and incubated for 4 hrs after which, a colour change around each disc is observed. Colour change around a sugar disc from red to yellow indicates a positive reaction meaning that acid is being produced and the sugar has fermented. No change of colour around the sugar disc indicates a negative reaction [24].

2.3.9 Coagulase test

For this analysis, a drop of sterile distilled water was added to both ends of a slide. A colony of the test organism was emulsified in each drop of water and a loop full of plasma added to one of the suspensions with thorough mixing. Clumping is observed within 10 seconds, to determine the identity of the organism [24].

2.3.10 Catalase test

Using a sterile dropping pipette, a drop of 3% Hydrogen peroxide solution was placed on a slide and a colony of the test organism was added to the drop of hydrogen peroxide solution. Formation of oxygen bubbles which is indicative of the presence of catalase was looked out for. This is done to differentiate between staphylococci and Streptococci. Effervescence of gas indicates the presence of gram positive organism whereby H_2O_2 is broken into O_2 and H_2O.

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$ [25]

2.3.11 Oxidase test

Oxidase test is done to determine the production of oxidase enzyme by an organism which is also indicative of the presence of cytochrome C [25]. For this test, a few drops of 1% aqueous solution of tetramethyl-p-phenylenediamine hydrogen chloride (oxidase reagent) was added to a piece of No 1 Whatman filter paper in a petri-dish with a glass slide and a few colonies smeared unto the filter paper. A development of purple colour within 5-10 seconds indicates a positive result [25].

2.3.12 Citrate utilization test

The test differentiates among enteric organisms by determining their ability to utilize citrate as their only source of carbon [25]. Test organisms were inoculated on simmon citrate agar slants streaking gently and incubated at 37℃ for 24 hrs. A colour change in the agar from green to blue indicates a positive reaction. No colour change indicates a negative reaction [24,25].

2.3.13 Urease activity test

In order to determine the ability of an organism under investigation to produce the urease, an enzyme which breaks down urea to release ammonia, the urease activity test is done. Organisms were inoculated into urea broth and incubated for 24 hrs. A colour change of the medium to pink indicates a positive reaction [24,25].

3. RESULTS AND DISCUSSION

Data obtained from microbial analysis of samples collected from the different bathrooms of public and private residences of university students in this study showed that different bacterial organisms were contaminating the walls. Colony formation units per gram of samples collected in Table 1 showed that the walls of public bathrooms were more contaminated than those of private bathrooms, with as much as 210 cfu/g and sometimes too numerous to count colonies observed on growth media.

Table 1. Average bacterial count in private and public bathrooms of university students

Sample code	Average colony count (cfu/g)	
	Private bathrooms	Public bathrooms
1	68	210
2	131	190
3	83	137
4	70	193
5	102	159
6	109	203
7	193	TMTC
8	115	TMTC
9	89	166
10	109	180

TMTC- too many to count. Sample code 1 to 10 indicates sampling from 10 bathrooms

Table 2. Morphological examination of organisms isolated from private and public bathrooms of university students' accommodation

Sample code	Private			Public		
	Gram stain reaction	Microscopic reaction	Spore formation	Gram stain reaction	Microscopic reaction	Spore formation
1	+	Coccus in clusters	No	-	Bacillus	
	-	Bacillus	No	-	Bacillus	No
	-	Bacillus		-	Bacillus	
	+	Bacillus	Yes			
2	+	Coccus in clusters	-	-	Bacillus	
	-	Bacillus		+	Coccobacillus	No
	-	Bacillus		+	Coccus in clusters	No
3	-	Bacillus	No	-	Bacillus	No
	+	Bacillus	Yes	-	Bacillus	
	-	Bacillus		+	Coccus in clusters	No
4	-	Bacillus		-	Bacillus	No
	+	Coccus in clusters	No	+	Coccobacillus	Yes
	-	Bacillus		-	Coccobacillus	No
				+	Coccus in chains and pairs	
5	+	Coccus in chains and pairs		+	Coccobacillus	No
	-	Bacillus		-	Bacillus	
	-	Bacillus	No	+	Coccus in clusters	No
				-	Bacillus	No
6	+	Coccus in clusters	No	-	Bacillus	No
	-	Bacillus		-	Bacillus	No
	+	Bacillus	Yes	-	Bacillus	
7	-	Bacillus		-	Bacillus	No
	-	Bacillus		+	Coccobacillus	Yes
	-	Coccusbacillus	No	+	Coccus in chains and pairs	
8	-	Bacillus	No	+	Coccus in clusters	No
	-	Bacillus		+	Bacillus	Yes
	+	Coccus in clusters	No	-	Bacillus	
9	+	Coccus in clusters	No	-	Bacillus	No
	-	Bacillus	No	+	Bacillus	Yes
	-	Bacillus		+	Coccus in chains and pairs	
10	-	Bacillus		-	Bacillus	
	+	Coccobacillus	No	+	Coccus in clusters	No
	-	Coccobacillus	No	-	Bacillus	No

Table 3. Incidence and percentage occurrence of bacteria isolates from private and public bathrooms of university students' accommodation

Isolated organisms	Private bathrooms		Public bathrooms	
	Incidence (n= 31)	Percentage (%)	Incidence (n=32)	Percentage (%)
Bacteriodes fragilis	2	6.45	1	3.13
Clostridium difficle	3	9.68	1	2.13
Bacteroides ovatus	-	-	2	6.25
Enterococcus faecium	1	3.23	1	3.13
Corynebacterium genitalium	1	3.23	1	3.13
Enterococcus faecalis	-	-	2	6.25
Citrobacter freundii	1	3.23	1	3.13
Pseudomonas aeruginosa	4	12.9	2	6.25
Staphylococcus aureus	4	12.9	5	15.6
Proteus mirabilis	3	9.68	2	6.25
Escherichia coli	5	16.1	3	9.38
Klebsiella pneumoniae	4	12.9	3	9.38
Staphylococcus epidermidis	3	9.68	1	3.13
Salmonella enterica	-	-	3	9.38
Enterobacter aerogenes	-	-	2	6.25
Bacillus subtilis	-	-	2	6.25

n= total number of isolates

Morphological examination of sample cultures from walls of the different sampling sites (bathrooms) showed many types of gram-positive and gram-negative bacteria species and strains which were both sporing and non-sporing. Some bacteria isolated from samples were rod shaped (*Bacillus*), spherically shaped in pairs and in chains (*Coccus*) and having intermediate shapes between spherical and rod shapes (*Coccobacillus*) (Table 2). There was not much difference between private and public bathrooms when considering the distribution of organisms isolated. There was also little difference observed for the number of isolates recorded for private and public bathrooms (Table 3). Most of the organisms isolated from walls of public bathrooms with the exception of *Bacteroides ovatus*, *Enterococcus faecalis, Salmonella enterica*, *Enterobacter aerogenes* and *Bacillus subtilis* were same as those isolated from walls of private bathrooms with percentage occurrence less in some instances.

Results obtained from biochemical tests to further confirm identity of organisms isolated from samples showed different gram-positive and gram-negative bacteria species present (Tables 4 and 5). Bacteria which belong to the genus *Staphylococcus*, *Escherichia*, *Clostridium*, *Enterococcus* and *Corynebacterium* were some of the organisms identified.

Microbial screening of swabs collected from public and private bathroom walls of university students showed that different bacterial species were present. Some of these bacteria isolated in the study are known to be significant human pathogens, causing different diseases such as infectious diarrhoea caused by *Clostridium difficle* [27], bacteremia caused by *Staphylococcus aureus* [28] and urinary tract infections associated with *Enterococcus faecalis* [29,30]. The different species of bacteria found in the bathrooms have also been reported to contaminate bathrooms and toilets in studies by Agbagwa and Nwechem [31], Mahdavinejad, Bemanian, Farahani and Tajik [32], Nworie, Ayeni, Eze and Azi [14] and Barker and Jones [10].

The distribution of bacterial species on walls of both private and public bathrooms could be attributed to splashing of water on the walls during body washes and flushing of toilets [9,12]. A build-up of bacteria will be expected on the walls with continuous body washes of different individuals and toilet flushes especially in public bathrooms if not cleaned frequently. Some other reason for the presence of bacteria in swabs from bathroom walls could be attributed to human vectors [6] bringing in these organisms on their skin with their hands touching the walls. The presence of *Staphylococcus aureus* and *E. coli* which were some of the most occurring bacteria isolated indicates the possibility of human vectors involved.

Table 4. Biochemical reaction of organisms isolated from private bathrooms

Sample	Oxidase	Catalase	Coagulase	Motility	Indole	M R	VP	Citrate	H_2S	Starch OH	Urease	Gas P	Glucose F	Maltose	Lactose	Sucrose	Mannitol	Organisms detected
1	-	+	-	-	+	-	+	-	-	-	+	-	+	+	+	+	-	*Staphylococcus epidermidis*
	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
	-	+		+	-	+	+	+	+	-	+	+	+		-	+	-	*Proteus mirabilis*
	-	-		-	-	-	+	-		-	-	-	+	-	-	-	+	*Clostridium difficle*
2	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
	-	+		-	-	-	-	-	-	+	-	-	+	+	+	+	-	*Bacteroides fragilis*
3	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
	-	-		-	-	-	+	-		-	-	-	+	-	-	-	+	*Clostridium difficle*
	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
4	-	+		+	-	+	+	+	+	-	+	+	+		-	+	-	*Proteus mirabilis*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
5	-	-		-	-	-	-	+	-	-	-	-	+	+	+	-	+	*Enterococcus faecium*
	-	+		-	-	-	-	-	-	+	-	-	+	+	+	+	-	*Bacteroides fragilis*
	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
6	-	+	-	-	+	-	+	-	-	-	+	-	+	+	+	+	-	*Staphylococcus epidermidis*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
	-	-		-	-	-	+	-		-	-	-	+	-	-	-	+	*Clostridiu difficle*
7	-	+		+	-	+	+	+	+	-	+	+	+		-	+	-	*Proteus mirabilis*
	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
	-	+		+	+	+	-	+	+	-	+	+	+	+	+	+	+	*Citrobacter freundii*
8	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Sample	Oxidase	Catalase	Coagulase	Motility	Indole	M R	VP	Citrate	H_2S	Starch OH	Urease	Gas P	Glucose F	Maltose	Lactose	Sucrose	Mannitol	Organisms detected
9	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
10	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
	-	+		-	+	+	+	+	-	+	-	-	+	+	-	-	-	*Corynebacterium genitalium*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Key: MR – methyl red, VP – Voges Proskauer, Citrate- citrate utilization, H_2S – hydrogen sulphide, Starch OH – Starch hydrolysis, Gas P – gas production from glucose, Glucose F – glucose fermentation

Table 5. Biochemical reaction of organisms isolated from public bathrooms

Sample	Oxidase	Catalase	Coagulase	Motility	Indole	M R	VP	Citrate	H_2S	Starch OH	Urease	Gas P	Glucose F	Maltose	Lactose	Sucrose	Mannitol	Organisms detected
1	-	+		-	+	-	-	-	+	+	-	-	+	+	+	+	+	*Bacteroides ovatus*
	-	+		+	-	+	-	+	+	+	-	+	+	+	-	-	+	*Salmonella enterica*
	-	+		+	-	+	+	+	+	-	+	+	+		-	+	-	*Proteus mirabilis*
2	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
	-	+		-	+	+	+	+	-	+	-	-	+	+	-	-	-	*Corynebacterium genitalium*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
3	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		+	-	+	-	+	+	+	-	+	+	+	-	-	+	*Salmonella enterica*
4	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	+	+		+	-	-	+	+	-	+	-	-	+	-	-	+	+	*Bacillus subtilis*
	-	+		+	+	+	-	+	+	-	+	+	+	+	+	+	+	*Citrobacter freundii*
	-	-		-	-	+	-	-	-	+	-	-	+	+	+	+	+	*Enterococcus faecalis*

Sample	Oxidase	Catalase	Coagulase	Motility	Indole	M R	VP	Citrate	H_2S	Starch OH	Urease	Gas P	Glucose F	Maltose	Lactose	Sucrose	Mannitol	Organisms detected
5	-	+		-	+	+	+	+	-	+	-	-	+	+	-	-	-	*Corynebacterium genitalium*
	-	+		-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
6	-	+		+	-	+	-	+	+	+	-	+	+	+	-	-	+	*Salmonella enterica*
	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
	-	+		-	-	-	-	-	-	+	-	-	+	+	+	+	-	*Bacteroides fragilis*
7	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*
	+	+		+	-	-	+	+	-	+	-	-	+	-	-	+	+	*Bacillus subtilis*
	-	-		-	-	-	-	+	-	-	-	-	+	+	+	-	+	*Enterococcusfaecium*
8	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		-	+	-	-	-	+	+	-	-	+	+	+	+	+	*Bacteroides ovatus*
	-	+		+	-	+	+	+	+	-	+	+	+		-	+	-	*Proteus mirabilis*
9	-	+		+	-	-	+	+	-	+	-	+	+	+	+	+	+	*Enterococcus aerogenes*
	-	-		-	-	-	+	-		-	-	-	+	-	-	-	+	*Citrobacter difficle*
	-	-		-	-	+	-	-	-	+	-	-	+	+	+	+	+	*Enterococcus faecalis*
10	+	+		+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Pseudomonas aeruginosa*
	-	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	-	+		+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Escherichia coli*

Key: MR – methyl red, VP – voges Proskauer, Citrate- citrate utilization, H_2S – hydrogen sulphide, Starch OH – Starch hydrolysis, Gas P – gas production from glucose, Glucose F – glucose fermentation

Comparing both private and public bathrooms, there is a little significant difference in bacterial contamination of walls indicating that sanitation practices of individuals is contributory to maintaining hygienically clean bathrooms and toilets in general. The ratio of bathroom users to number of bathroom is also very important because results from the study showed that swabs from walls of public bathrooms had more microbial load than those from walls of private bathrooms and organisms such as *Bacteroides ovatus*, *Enterococcus faecalis, Salmonella enterica*, *Enterobacter aerogenes* and *Bacillus subtilis* which were isolated from public bathrooms were not found in the private bathrooms.

4. CONCLUSION

Findings from the study, are indicative that microbial free bathrooms are dependent on individual hygiene practices as well as the number of users. The study showed that there is a possibility of contracting infections from bathrooms which are usually perceived to be a place for cleansing. Results from this study therefore serves as a means to proper public health education paving the way for investigations to reduce the occurrence of micro-organisms in bathrooms and toilets.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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