Bacteriological Quality Of Different Bathroom Wall Surface Biofilms

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Abstract: Bacteriological assessments of three different bathroom wall surface type biofilms were performed. Twenty eight biofilm samples scraped from tiled, wooden and concrete bathroom wall surfaces and three control samples from non bathroom surfaces of each surface material types were collected from ten different locations in Mile 2 Diobu.Port Harcourt. All the samples were highly contaminated. The mean aerobic and anaerobic bacterial load of the tiled, wooden and concrete bathroom wall surface samples on nutrient agar and thioglycollate agar were $1.32\pm 0.30 \times 10^5$ cfu/g, $8.17\pm 0.60 \times 10^4$ cfu/g, $9.27\pm 0.50 \times 10^5$cfu/g and $1.92\pm 0.13 \times 10^5$ cfu/g, $2.61\pm 0.10 \times 10^6$cfu/g and $2.64\pm 0.2 \times 10^5$ cfu/g respectively. The control samples had a mean of $2.82\pm 0.10 \times 10^5$cfu/g, $1.55\pm 0.30 \times 10^6$ cfu/g and $1.77\pm 0.10 \times 10^6$ cfu/g for the tiled, wooden and concrete surfaces respectively on nutrient agar. Also, the mean microbial counts of the control samples on thioglycollate agar were as follows, $1.38\pm 0.10 \times 10^5$cfu/g, $1.70\pm 0.12 \times 10^5$ cfu/g and $1.30\pm 0.20 \times 10^5$ cfu/g for the tiled, wooden and concrete surfaces respectively. Seventeen aerobic bacteria and twelve anaerobic bacteria genera were isolated with *Bacillus* and *Clostridium* being the most prevalent. Tile surfaces showed the least in bacterial density when compared to the other surface types and so does not support the growth of bacteria very well. This study has shown a high bacterial diversity in the bathroom wall surface type biofilms and so has served as a baseline in the development of strategies and safety plans for bathrooms, to reduce potential hazards to health for all its numerous users.
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1. Introduction

Many household locations are always vulnerable to microbial exposure, particularly in family dwellings where many facilities are shared. The bathroom is a good example of a place within a house that has a microbial presence. The microbial abundance found in toilet seats and bathtubs may not be sufficiently eliminated even if detergents are used to clean these places on a daily basis (Gajanan and Singh, 2013). Bathroom is any building or room made for people to have their bath usually with soap and water. Most bathrooms comprise of integrated toilet facilities and sinks for other related washings. Data on public washroom contamination shows how often and easily high-contact washroom surfaces can be contaminated although, people may claim their personal hygiene and clean toilet facilities, researches have shown a different picture (Ajayi and Ekozien, 2014).

In nature, microorganisms exist as both planktonic free floating cells or in a community referred to as biofilms (Mahami and Adu-Gyamfi, 2011). A biofilms is a community (population) of microorganisms that may include bacteria, fungi, yeasts and protozoa, attached to a solid surface. Biofilms generally form on any surface that is exposed to non sterile water or other liquids and are consequently found in many environmental, industrial and medical environments (Rao *et al.,* 2005).

Bacteria are microscopic organisms found everywhere in the universe as pathogenic or non pathogenic. They are found in the environments all around us and within each one of us, there are trillions and trillions of them. Majority of them are harmless to human and animals but those few which are harmful, can lead to death of affected individuals. Public restrooms may contain a variety of dangerous bacteria including those from genus *Escherichia*, *Salmonella*, Rotavirus, Cold virus and *Staphylococcus* (Chengula *et al*., 2014). Bacteria from public restrooms are of importance when they enter the body through hand or mouth contact or hand to food contact. (Sheriffa, 2013). Restroom is contaminated with microbes from human secretions as saliva, skin, urine and faecal origin. (Scott *et al*., 1982). Bacteria have been cultured from many environments in and around the homes, particularly in moist settings such as those involving water pipes, tooth brushes and spas. Several studies have also shown that domestic water supplies can be a source of opportunistic infectious agents, and household plumbing accumulates numerous organisms (Scott *et al*., 2004). Although, evidences of microbial growth and biofilm formation are ubiquitous in households, little is known about the diversity and complexity of the organisms that make up household microbial communities.

Sanitary conditions in places have always been a major problem, especially in bathrooms. Health departments are continually checking the cleanliness and safety of these bacterial breeding places to prevent the spread of sickness and diseases. The bathroom and toilet are communal areas of the home which are in constant use throughout the day. It thus provides an ideal environment for the spread of gut, respiratory and skin pathogens via hands and the surfaces from one family member to another if basic hygiene standards are not observed (Ajayi and Ekoden, 2014).

The aim of this study therefore, were to identify the kinds of organisms that colonize the different type of bathroom wall biofilms and also to determine which bathroom surface material is best in a normal environment. This in turn will serve in the development of strategies and safety plans for health and hygiene improvement of the numerous bathroom users.

2. Materials and Methods

2.1. Sample collection: A total of twenty eight (28) biofilm samples were scrapped from three bathroom wall surface types, ten (10) from concrete bathroom wall surfaces, ten (10) from wooden bathroom wall surfaces and five (5) from tiled bathroom wall surfaces. Three (3) control samples from non bathroom concrete, wooden and tiled wall surfaces respectively were also collected, using a sterile scapel knife, into sterile sample containers. They were collected from ten different locations in Mile 2 Diobu namely, Abel Jumbo1 and 2 (AJ1 and AJ2), Obidianso 1 and 2 (OB1 and OB2), Echue 1and 2 (EC1 and EC2), Timber 1 and 2(TIM1 and TIM2) and Akokwa 1 and 2(AK1and AK2) for the concrete and wooden surfaces five locations for the tiled surfaces. These areas (streets) were chosen because they are almost the longest streets and have a greater number of the people residing in them. The samples were sent to the laboratory immediately after collection for microbiological analysis.

2.2. Physicochemical Analysis

2.2.1. pH test: The measurements were done using a Metler Delta 340 pH meter. The pH meter was standardized with buffer solutions and the probe immersed into the sample solutions and the readings were obtained and recorded.

2.2.2. Temperature: The temperature was taken using mercury in glass thermometer. The thermometer was dipped into the scrapped biofilm samples and readings were taken.

2.2.3. Moisture content (ASTM D2216): Dry clean crucibles with its lid were weighed and the weight was recorded as (MC).A known weight of the biofilm sample was weighed into the crucible and it was covered with the lid. The crucible containing the sample with the lid (MCMS) was placed in the oven at a temperature of 105^{o}C for 24 hours.

-The crucible containing the dry sample with the lid was removed and placed in a desicator to cool and then weighed (MCDS) to a constant weight.% water content was then calculated as follows

$$\text{Moisture content \%} = \frac{\text{Mass of water loss} \times 100}{\text{Mass of dry sample}}$$

$$= \frac{\text{MCMS} - \text{MCDS} \times 100}{\text{MCDS-MC}}$$

2.3. Microbial Enumeration and Identification: Spread plate method, according to APHA, 1998 was used to isolate and enumerate the bacterial in the biofilm samples using the Nutrient agar (Fluka Biochemika) and thioglycolate agar (Brewer, Lab M) prepared plates, for the aerobic and anaerobic bacteria isolations respectively. Serial dilutions of the biofilm samples were made up to 10^{-5}. A 0.1ml portion of the 10^{-4} dilution was plated out on already prepared nutrient agar plates. Also, a 0.1ml portion of the 10^{-4} dilution was plated out on already prepared thioglycolate agar plates in an anaerobic chamber. The nutrient agar plates were incubated at 37^{0}C for 24 - 48 hours while the thioglycolate agar plates were incubated in an anaerobic incubator at 37^{0}C for 24 -48 hours. Colonies were counted using a colony counter (Scan Interscience, Scan 500).

2.4. Isolation and purification of colonies: The colonies on nutrient agar for heterotrophic bacteria were further purified by sub culturing on nutrient agar for pure culture and characterized on the basis of their colonial, cellular and biochemical characteristics. The identification of bacteria followed the scheme of Holt, (1994). Colonies on thioglycolate agar for anaerobic bacteria was also purified for pure culture by sub culturing on thioglycolate medium and identification of bacteria followed the scheme of Baron and Citron (1997) and PHE (2015).

2.5. Statistical Analysis:

Univariate Analysis of Variance (ANOVA) at 95% Confidence Interval, Games-Howell and Dunnet multiple pairwise comparisons were done to analyze the differences among the means, using SPSS statistical analysis package version 20. The result of the total aerobic and anaerobic bacteria counts of the different samples of the different surface types was not significantly different at this confidence interval.

3. Results

3.1. The physicochemical analysis: The temperature, pH and moisture contents of the different bathroom wall surface type biofilms are as shown in Table 1.

Table 1: The physicochemical parameters of the bathroom wall surface biofilms from the different material types

Physicochemical parameter	Bathroom surface type		
	Tile	Wood	Concrete
Mean temperature (^{0}C)(Range)	23.98 (24-27)	25.18 (24-27)	26.44 (24 -27)
Mean pH (Range)	8.55 (8.00 – 9.13)	4.70 (4.00-5.50)	7.47(8.00 – 9.13)
Mean Moisture content (%)(Range)	34.80 (33.20– 39.10)	39.45(31.40 - 48.40)	39.02(33.20 – 39.10)

3.2. Microbiological analyses:

The average aerobic bacterial count of the tiled surfaces was 1.32×10^5 ± 0.30 cfu/g ranging from1.00×10^5- 6.92 $\times10^5$cfu/g. The average anaerobic bacteria count was 8.17×10^4 ± 0.60 cfu/g ranging from 1.42×10^4cfu/g - 2.63×10^5cfu/g. The average aerobic bacteria count of the wooden surfaces was 9.27×10^5± 0.50 cfu/g ranging from 2.04×10^5cfu/g - 6.61×10^6cfu/g. The average anaerobic bacteria count was 1.92×10^5± 0.13 cfu/g ranging from 1.12×10^5cfu/g - 2.88×10^5cfu/g. The average aerobic bacterial count of the concrete wall surfaces was 2.61×10^6± 0.10 cfu/g ranging from1.30 $\times10^6$ -5.50×10^6 cfu/g. The average anaerobic bacteria count was 2.64×10^5 ± 0.2 cfu/g ranging from 1.38×10^5cfu/g - 7.50×10^5cfu/g. The mean microbial counts of the control samples on nutrient agar were as follows, 2.82± 0.10 $\times10^5$cfu/g, 1.55± 0.30 $\times10^6$ cfu/g and 1.77± 0.10 $\times10^6$ cfu/g for the tiled, wooden and concrete surfaces respectively. Also, the mean microbial counts of the control samples on thioglycollate agar were as follows, 1.38± 0.10 $\times10^5$cfu/g, 1.70± 0.12 $\times10^5$ cfu/g and 1.30± 0.20 $\times10^5$ cfu/g for the tiled, wooden and concrete surfaces respectively.

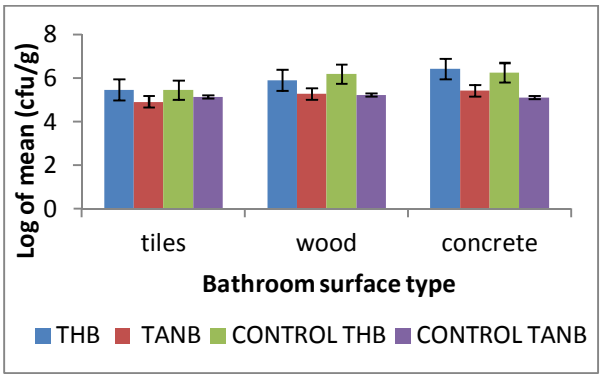

Figure 1: The mean microbial load on the different bathroom wall surface types

3.3. Characterization and identification of isolates:

Based on the colonial, cellular and biochemical characteristics, aerobic bacteria from seventeen (17) genera and anaerobic bacteria from twelve(12) genera were isolated respectively from the three material surface types as shown in Tables2 and 3.

Table 2: Relative abundances of the aerobic bacteria on the different surfaces

S/N	Isolate	Tiles %	Wood %	Concrete %
1	*Bacillus sp*	22.58	12.5	9.95
2	*Pseudomonas sp*	16.13	13.89	10.96
3	*Staphylococcus aureus*	9.68	15.28	9.59
4	*Flavobacteria sp*	9.68	4.17	2.74
5	*Burkholderia sp*	6.45	4.17	0.0
6	*Chromobacterium sp*	6.45	4.17	2.74
7	*Proteus sp*	6.45	2.78	9.59
8	*Staphylococcus sp*	6.45	11.11	12.33
9	*Micrococcus sp*	3.23	5.56	4.11
10	*E. coli*	3.23	5.56	8.22
11	*Enterobacter sp*	3.23	4.17	1.37
12	*Edwardsiella sp*	3.23	0.0	0.0
13	*Serratia sp*	3.23	5.56	1.37
14	*Salmonella sp*	0.0	5.56	2.74
15	*Klebsiell sp*	0.0	2.78	2.74
16	*Providencia sp*	0.0	1.39	5.48
17	*Citrobacter sp*	0.0	0.0	2.74

Table 3: Relative abundances of the anaerobic bacteria on the different surfaces

S/N	Isolate	Tiles %	Wood %	Concrete %
1	*Clostridium sp*	21.88	20.69	24.07
2	*Clostridium perferingens*	15.67	6.90	5.56
3	*Bacteroidesfragilis group*	15.67	17.24	18.52
4	*Prevotella sp*	12.50	13.49	9.26
5	*Veillonella sp*	9.30	3.45	0.0
6	*Porphyromonas sp*	0.0	10.35	5.56
7	*Peptostreptococcus sp*	9.30	6.90	5.56
8	*Bilophila sp*	0.0	0.0	7.41
9	*Desulfovibrio sp*	0.0	0.0	3.70
10	*Fusobacterium sp*	3.13	10.35	3.70
11	*Propionibacterium sp*	9.37	8.62	11.11
12	*Actinomyces sp*	3.13	1.72	5.56

4. Discussion

The present study results have revealed that the different bathroom wall surface types have marked differences in microbial population types and numbers in each community, even though these bathrooms are routinely cleaned. The occurrence of these microorganisms is assumed to be part of its normal microbial flora (Gajanan and Singh, 2013). These can be attributed to the four key factors that control the growth of all microorganisms: temperature, pH, water availability and oxygen. Table 1 shows the results of these parameters of the sampled biofilms of the different material surface kinds. Temperature is probably the most environmental factor affecting the growth and survival of microorganisms. Temperatures are divided into minimum, optimum and maximum limits at which growth may or may not be possible. The minimum and maximum limits may damage the organism's cytoplamic membrane that it no longer functions in nutrient transport (Madigan *et al*, 2009).

The temperature of the studied biofilm samples fell within the mesophilic range of between 20^{o}C – 45^{o}C which is the mesophilic range of temperature. Mesophiles are widespread in nature: in warm-blooded animals, terrestrial and aquatic environment in temperate and tropical latitudes and so are rightly found on these bathroom wall environments where they grew. Some studies have shown the effect of temperature on microbial communities. Frankel *et* al. (2012), showed a positive correlation between temperature and concentration of indoor fungi in Danish homes. The results also revealed that the temperatures of the control samples on the different surfaces (outdoor) are higher than those of the bathroom wall surface samples (indoor). This could be because architects and engineers design buildings for human comfort by controlling factors such as humidity, temperature and airflow (Kembel *et al*., 2012). These designs therefore, had affected these bathrooms used for the study as can be seen from the results, as the samples could be seen to have a lower temperature than the control samples.

Most natural environments have pH values between 4 and 9 and organisms with optima in this range are more commonly encountered (Madigan *et al,* 2009). Most bacteria prefer neutral pH. Thus, building materials with pH levels between 6 and 8 are more sensitive to microbial colonization (Verdier *et al.,* 2014). The pH values of the different surface types studied are shown to differ in their results. The tiled walled surface samples and its control is alkaline, having shown a pH range of 8.00 – 9.13. The concrete wall surface samples and its control showed a pH range of 7.47 – 7.83, lying between neutral to alkaline. This accounts for their higher bacterial populations. This neutral to alkaline pH range also supports the growth of pathogenic organisms. Most disease causing bacteria grows best at pH 5-8 (Linton and Dick, 1990). At pH of between 7 and 9, concrete starts to breakdown. This accounts for the visible cracks and crevices on the surfaces of the bathroom walls from where the samples were collected. The wooden surfaces showed a pH range of 4.00 – 5.50 which are acid tolerant. This leads to the breakdown of the wood material. This suggests why the wooden surfaces have a lower bacteria population than the concrete surfaces as this environment does not support the growth of most organisms.

Moisture is another key factor that controls the growth of all microorganisms (Madigan *et al.*, 2009). Most of the results had all shown a lower percentage moisture content than the control samples. This could be as a result of the control samples direct contact with water, as they were collected from areas close to public borehole water supply so, they have water always in constant supply. The results showed that the wooden bathroom wall surface biofilms had moisture contents that exceeded the fiber saturation point (FSP) of wood; the threshold of moisture in wood which is approximately 26%. This can lead to a quick deterioration of the wood material and the efficient growth of microorganisms in the presence of the moisture. Concrete walls depending on the coatings, manufacturer's and owner's specifications should not have moisture contents well above 5% (Cole, 2015).

The results have moisture contents well above this threshold and so can lead to water entering the walls and moldings thereby promoting the growth of fungi, bacteria and other microorganism types. This can lead to a high pH increase as the moisture can condense into water and leach calcium hydroxide which efflorescence on the wall surfaces. These suggests that the material types also affect the microbial growth and populations as seen in the work of Nielsen *et al*., (2004), Yli-pirila *et al*., (2004) which showed that the same water content for mineral insulation and pinewood, resulted in remarkable lower water activity for the later and hence the difference between the occurrence of amoeba in the different material types.

These results, having shown that the temperature, pH and moisture contents of the samples had positive effects on the survival of microorganisms on these bathroom surface types, supported wide varieties of these organisms in all the locations. In general, attachment of microorganisms to surfaces will occur most readily in surfaces that are rougher, more hydrophilic and coated by surface conditioning films (Donlan, 2002).

Figure1, Table 2 and 3 shows the population density and types associated with the different bathroom surface types Tiles are ceramic artifacts of

reasonable resistance and durability due to their nature and manufacture (Oliviera, 2001). This also accounts for their lowest population density. They do not support the growth of microorganisms so well. According to Aries (2009), bacteria need a food source to grow, which is usually carbohydrate. Ceramic tiles cannot provide this food source as it is inorganic and inert. Any bacteria present on ceramic tiles are usually just hanging out on the surface and will dry out or die when the surface dries out. Bacteria feed primarily on the cellulosic starches in the sapwood, travelling from cell to cell by destroying the pit membranes- the thin carbohydrate semi porous remnant of the primary wall of wood (Chris and Adrian, 2011) and this makes wood more porous. Due to the availability of nutrients, bacterial attachment, growth and colonization on concrete surfaces takes place (NPTEL). This accounts for the increase in trend in the bacterial population density of tile-wood- concrete as also supported by the work of Blanton (2007) who found out that the less porous the material, the longer it took for that material to obtain microbial growth. In his work he found an increasing trend in population growth of Stainless steel – Porcelain - Solid surface material – Plastic – Tiles - Varnished wood and marble.

Restroom surfaces host relatively diverse microbial communities dominated by human-associated bacteria with clear linkages between communities on or in different body sites and those found on restroom surfaces, relevant to the public health field that human-associated microbes are commonly found on restroom surfaces suggesting that bacterial pathogens could readily be transmitted between individuals by touching of surfaces (Sheriffa, 2013). This is confirmed by the different varieties of bacteria isolated from the different surface types. Chris *et al,* (2002), Ajayi and Ekozien (2014) also found some of the isolated bacteria in their work. Several of the organisms are known to be infectious. *Pseudomonas* sp, *Bacillus* sp and *E. coli* are common to the three different bathroom surface material types.

The variation in the organisms isolated from the different surface type materials could be from difference in personal microbial cloud of the environment or from the water distribution system in the area which deposits microorganisms on walls as biofilms according to Bonadonna *et al.* (2009), Meadow *et al* (2015). *Salmonella* and *Shigella* were not found as also confirmed by Lynch and Mendes (1976). Majority of the organisms were enterobacteriaceae. Possible diseases that can be caused by the isolated bacteria include food borne diseases (S.*aureus* and *E.coli*), urinary tract infections (*E. coli* and *Pseudomonas aeruginosa*), (*P. klebsiella*), sore throat (*Streptococcus. pyogens*) and diarrhea(*E. coli*). (Chengula*et al*, 2014).

The anaerobes isolated were the common elements of the mucous membrane flora (gastro intestinal tract) throughout the body. They often act as secondary pathogens. They are the most common anaerobes involved in infection and include some of the most antibiotic resistant species (Finegold, 1996). Obligate anaerobes and spore forming bacteria do not play a role in cross-infections in washrooms as they cannot survive in open surfaces (Mendes and Lynch, 1976), which is why they are secondary pathogens. They are harmless under normal conditions and can only lead to infection if anaerobic conditions are achieved in an immune compromised individual.

5. Conclusion

From this study, it can be noted that biofilms on the wall surfaces are reservoirs to different types of aerobic and anaerobic bacteria in high densities in bathrooms. This can act as a good source for the transmission of pathogenic diseases to humans through contact, airborne or inhalation of these organisms. Therefore a good hygiene plan need be adopted to reduce the diversity and microbial load of these organisms from reaching infectious threshold which could be harmful to the numerous users of these bathroom environments.

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