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Prevalence and Concentration of Airborne Microorganisms Isolated from Domestic Toilets in Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Microorganisms are ubiquitous in nature and transient airborne microorganisms have been shown to constitute major health hazards.
Aim: This study was carried out to assess the microbial air quality in ten (10) domestic toilets located in mile-3, Alakahia, Choba, Ada-George and Rumuokwuta, Port Harcourt, Rivers state, Nigeria.
Methodology: Microbial air quality was sampled using sedimentation techniques. Freshly prepared Nutrient Agar, MacConkey and Sabouraud Dextrose Agar (SDA) plates were placed one meter above the floor of the toilets for 1 hours at different sections of each toilet exposed to an open air. The agar plates were closed and transported to the Laboratory were incubation took place at 37°C for 24 hours and 25°C for 3-5 days for growth of bacteria and fungi respectively. Isolates were characterized and identified by standard microbiological methods.
Results: The bacteria isolated were *Staphylococcus species* 22(22.45%), *Bacillus species* 20

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(20.41%), *Enterococcus species* 20(20.41%), *Escherichia coli* 16(16.32%), *Micrococcus species* 15 (15.31%), *Klebsiella species* 3(3.06%), *and Proteus species* 2(2.04%) while *Aspergillus species* 14(27.45%), *Penicillium species* 16(23.53%), *Fusarium species* 10(19.60%), *Mucor species* 8(15.69%), and *Rhizopus species* 7(13.73%) were the fungi identified. The highest bacteria colony count before and after flushing were 52.380×10^3CFU/m^3/hr & 67.261×10^3CFU/m^3/ hrrespectively while the lowest bacteria counts observed before and after flushing 19.047×10^3CFU/m^3/hr & 39.286×10^3CFU/m^3/hr while the highest and lowest fungal count before and after flushing were 10.119×10^3CFU/m^3/hr & 13.690×10^3CFU/m^3/hr and 5.952×10^3CFU/m^3/hr & 5.953×10^3CFU/m^3/hr respectively.

Conclusion: This study shows that considerable numbers of both pathogenic bacteria and fungi particles were released into the air in higher quantity after flushing domestic toilets when compared to air quality before flushing. These organisms have been implicated in major and minor infectious diseases. Inhalation or contact may easily lead to infection especially in immunocompromised individuals and the older adults. To maintain the health of users, it is necessary to carefully manage the environmental factors that promote the growth and multiplication of microorganisms in domestic toilet environment.

Keywords: Airborne; microorganisms; domestic; toilet; health; hazards.

1. INTRODUCTION

"A toilet is a sanitation fixture used for disposal of human urine and feces" [1]. "In developed countries, different forms porcelain flush toilets are common. Seats are usually used in west while squat toilets are common in Africa and East Asia. These are connected to sewer system in most urban areas and to septic tanks in less built-up areas" [1].

"Toilet is also one of public facilities, which is frequently used by people and located indoor. In recent years, Scientist and public have put much concern about microbial air quality in toilet systems. Several studies have found out that indoor air pollution levels to be greater than outdoor levels" [2]. "Thus it is risky to health posed by indoor air pollution than those posed by outdoor air pollution. Therefore, maintaining good air quality in toilet is essential in order to keep it hygienic and sanitary. In order to create a healthier and safer indoor environment, the first step is to maintain a good indoor air in toilets and washrooms. In an environment, spores of molds and bacteria may become air borne and are therefore ubiquitous. They can enter indoor areas either by means of ventilation systems. The relative humidity and/or the moisture content of the materials determines that to what extent different micro-organism are able to grow on indoor" [3]. "These may cause destruction, adverse health effects and unpleasant odors. Therefore, the task of microbial examinations is to differentiate between normal indoor microorganisms which may cause adverse health effects" [4].

"Air sampling of microorganisms is a popular method of conducting microbial examinations. Specific activities like talking, sneezing, coughing and toilet flushing can generate air borne biological particulate matter and occasionally release spores of *Altenaria, Aspergillus, Botrytis, Cladosporium, Penicillium,* and *Scopulariopsis* into the air" [5]. "Indoor air pollution can be as much worse than that of outdoor air, it can cause wide range of health problems. Insufficient ventilation, high influx of people and improper management of public toilets, are main sources of indoor air contamination in public toilets" [6].

"Humans are an important source of indoor bacteria. The upmost layer of the normal human skin is continuously renewed, and skin scales containing bacteria are shed into the environment" [7].

"The presence of bacteria, either as viable bacteria or bacterial spores, mycotoxins, chemical markers like β-glucans and volatile organic compounds, and endotoxins, represents an indication of high humidity in the indoor environment" [8]. Mould specifically grows on all materials, including the dirty toilet bins and under the toilet seats; following the damp nature of most toilets.

"The most common fungal genera occurring in indoor environments are Penicillium, *Aspergillus, Cladosporium, Alternaria*, and yeasts" [9,10]. "These genera are also the most frequently occurring fungi in outdoor air. Spores of fungi are present everywhere, and they are able to germinate wherever there is water available and the ranges between 0.80 and 0.98. Fungi need

carbohydrates, proteins, and lipid to develop. They can find all these elements in house dust, construction materials like wallpaper or textiles, paint, glue, wood, paper and books, stored food, or deposit of cooking oil. Fungi can grow on inert materials like ceramic tiles" [9].

"A review made by the WHO on the number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effects on respiratory health, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze, and dyspnoea" [11]. This study provides clear data of microbial air quality and respective bacterial loads in indoor air of toilet environment.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in 10 selected domestic toilets all located in Mile-3, Alakahia, Choba, Ada-George and Rumuokwuta, Port Harcourt, Rivers State, Nigeria. Port Harcourt metropolis has a Coordinates 4.75°N 7°E and lies along the Bonny Stream and is arranged in the Niger Delta. Beginning at 2016, the Port Harcourt urban domain has a population of 1,865,000 inhabitants, up from 1,382,592 beginning at 2006 [12]. The 10 selected toilets where used by maximum of 5 persons. They were ventilated by natural ventilation through open windows.

2.2 Sampling Method

Bacteria and fungi measurement were made by passive air sampling technique: the settle plate method using 9 cm diameter Petri dishes. The sampling height was 1m above the floor and at the center of the toilet.

2.3 Sample Collection and Isolation of Microorganisms

Passive air sampling [13] was done to determine the index of microbial air contamination. Freshly prepared Nutrient Agar, MacConkey and Sabouraud Dextrose Agar (SDA) plates were placed one meter above the floor of the toilets for 1 hours at different sections of each toilet exposed to an open air after which incubation at 37°C for 24 hours and 25°C for 3-5 days for growth of bacteria and fungi took place respectively. Microbial growths were then isolated and identified morphologically and characterized biochemically using standard procedures and results expressed in CFU/m2/hour.

Fig. 1. Map of rivers state showing study area

2.4 Microbial Plate Count

The following formula used for estimating the CFU count in the air [14].

$$N=5a \times 10000/(bt)$$

where N is CFU/m3,

a is the number of colonies per Petri dish for the time t,
m is the diameter of Petri dish (cm^2),
B is the dish square centimeter
t is the exposure time (min).

2.5 Bacterial Identification

The cultural characteristics of the isolates were observed after 24 and 48 hours of incubation at about 37^oC on nutrient Agar and MacConkey Agar. Features such as nature and pattern of growth, shape of colony, colour of colony, elevation surface area, edge, odour, haemolysis on or in the media plates were carefully noted after which the colonies were sub-cultured into nutrient Agar to have a pure culture. Bacteria were identified using standard procudures.

2.6 Fungi Identification

2.6.1 Lacto phenol cotton blue staining preparation for fungi

Lacto phenol cotton blue preparation is used to stain fungi element, which aids in the microscopic identification of mycotic agents. The cotton blue dye stains the chitin, a nitrogenous substance present in the cell wall of most fungi agents. The phenol kills any organism and the lactic acid preserve fungal structures.

Fungi colonies were identified using standard microbiological procedures based on their colony appearance, microscopic examination of their spores and hyphal characteristics using lactophenol cotton blue preparation.

2.7 Determination of Percentage (%) of Occurrence

The occurrence of each bacterium isolated from the domestic Toilets were determined as percentage ratio of their prevalence rates and represented in tables.

3. RESULTS

3.1 Microorganisms Isolated from Domestic Toilets

The results show that 98 isolates of 7 bacteria species were identified from the 10 different domestic toilets. They include: *Bacillus species, Staphylococcus species, klebsiella species, Proteus species, Enterococcus species*, *Micrococci species* and *Escherichia coli* were identified from the various toilets. Fifty-one (51) isolates of 5 fungi species were also identified which includes *Aspergillus species, Penicillium species, Mucor species, Fusarium species* and *Rhizopus species.* The frequency of occurrence of bacteria and fungi species isolated from the Domestic toilets are shown in Table 1 and 2. It was found that the frequency of occurrence of the isolated bacterial colonies were 22(22.45%) *Staphylococcus species* which was the highest, 20(20.41%) *Bacillus species*, 16(16.32%) *Echerichia coli,* 20(20.41%) *Enterococcus species*, 15(15.31%) *Micrococcus species,*3 (3.06%) *Klebsiella species* and 2(2.04%) *Proteus species.* While the frequency of occurrence for the isolated fungal colonies were 14(*27.45%*) *Aspergillus species* which was also the highest occurring fungi followed clodely by 12(23.53%) *Penicillium species*, 10(19.60%) *Fusarium species*, 8(15.69%) *Mucor species* and 7(13.73%) were *Rhizopus* species which had the lowest fungi frequency of occurrence.

The frequency of Bacteria and Fungi loads before and after flushing the of the different domestic toilets are shown in Table 3 & 4. Toilet 7 had the highest bacteria colony count of $52.380\times10^3CFU/m^3/hr$ & $67.261\times10^3CFU/m^3/hr$ before and after flushing followed by Toilet 5 $47.023\times10^3CFU/m^3/hr$ & $60.119\times10^3CFU/m^3/hr$ while Toilet 1 and Toilet 3 least bacteria colony count of $19.047\times10^3CFU/m^3/hr$ & Toilet $3.38.691\times10^2CFU/m^3/hr$ before and after flushing. Toilet 3 had the highest fungal colony count of $10.119\times10CFU/m^3/hr$ before flushing while Toilet 7 had the least fungi colony count of $5.905\times10^3CFU/m^3/hr$ after flushing [15].

4. DISCUSSION

The result of this study revealed the presence of 7 bacteria species: *Bacillus species, Staphylococcus species, klebsiella species, Proteus species, Enterococcus species*, *Micrococci species* and *Escherichia coli* and 5 fungi species: *Aspergillus species, Penicillium*

species, Mucor species, Fusarium species and *Rhizopus species.* Most of these microorganisms isolated are pathogenic and have been reported to have caused various infections including opportunistic infections [16].

The objective of this study was to assess microbial air quality of domestic toilets and health burden on its users. "Findings of microbial bio aerosol population in indoor air can help to project health dangers and to standardize indoor air quality control" [17].

From the investigation carried across ten (10) selected domestic toilets, Toilet 7 had the highest bacteria count of 52.380×10^3CFU/m^3/hr & 67.261×10^3CFU/m$^{3/}$hr before and after flushing. Toilet 5 also had high count of 47.023×10^3CFU/m^3/hr & 60.119×10^3CFU/m^3/hr, while Toilet 3 had the highest fungal count of 10.119×10^3CFU/m^3/hr & 13.690×10^3CFU/m^3/hr followed by Toilet 5 9.524×10^3CFU/m^3/hr & 12.500×10^3CFU/m^3/hr before and after flushing. Showing that considerable numbers of both pathogenic bacteria and fungi particles were released into the air in higher quantity after flushing when compared to before flushing of the domestic toilets. This finding is in line with a similar study by Knowlton *et al*., [18] who measured "Particle and bio aerosol concentrations in hospital bathrooms across three sampling conditions; no waste no flush, no waste with flush, and fecal waste with flush". "Their results indicated that bio aerosols concentrations when flushing fecal waste were found to be significantly greater than before flushing" [17]. Similarly, Johnson *et al*., [19]. in a study, concentrated *Serratia marcescens* into the toilet before flushing; meanwhile the sedimentation plates were placed round the toilet at different distances to collect settled bio aerosols. Their results showed that the bacterial colonies grew onto the sedimentation plates placed on the ground after flushing. This observation shows that microorganisms could be dispersed into the air and settle in toilet surfaces. The possibility that aerosols containing enteric pathogens could cause infection after being swallowed following deposition in the nose or pharynx was suggested by Merino-Alado *et al*., [20]. It is also possible that these particles when released into the air by flushing of the toilet could impact on humans and toiletries such as toothbrush, bathing towels and sponge of which these items are not sterilized daily before use.

In this study, *Staphylococcus aureus* was found to be one the most isolated bacteria which is as a result of its ubiquitous nature in the body. This is similar to the results of a study in Benin on indoor assessment of air by Ekhaise *et al*., [21]. "It can survive for some hours on dry environmental surfaces. *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furuculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis and urinary trait infections; and deep-seated infections such as osteomyelitis and endocarditis. *Staphylococcus aureus* causes food poisoning by releasing enterotoxins into food, and shock syndrome by releasing of super antigens into the blood stream" [22].

"Staphylococcus aureus has also been associated with Community Acquired Methicillin Resistant *Staphylococcus aureus*, urinary tract infections, skin infections and food poisoning" [23]. "In the United Kingdom, for example, *Staphylococcus aureus* has been implicated in an increase in skin infections in children from 1997 to 2006" [23].

Bacillus species was also predominantly isolated from the 10 toilets. This is in accordance with Ohagim *et al*., [24] in "an assessment of indoor air in public Toilets. Common species of Bacillus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. *Bacillus sp* isolated from the toilet has been linked to food poisoning and can cause serious problems if toilet users do not maintain proper hygiene standards".

Micrococcus species was isolated from all the toilets. This might be due to their inhabitation in the skin, environment, including water, dust and soil. This study also agrees with Hayleeyesus & Manaye, [25] in a similar study. *Micrococci* may be involved in the other infections, including recurrent bacteraemia, septic shock and septic arthritis [26].

Enterococcus species had higher number of occurrence in this study which might be as a result of its occurring as normal flora in the intestine. This is however not in disagreement with Ohagim *et al.,* [24] in an assessment of indoor air in public Toilets. Its increase could also be due to force of the water running down the surfaces of the bowl and from the turbulence caused by mixing with water contained in the bowl. A member of the genus which has been identified in research, can cause infections, especially difficult-to-treat urinary tract infections.

Table 1. Frequency of occurrence of bacterial species isolated from the different domestic toilets

Organisms	No. of Isolates	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Total percentage
Staphylococcus sp	22	2(2.04)	3(3.06)	2(2.04)	3(3.06)	1(1.02)	2(2.04)	4(4.04)	1(1.02)	2(2.04)	2(2.04)	22.45
Escherichia coli	16	2(2.04)	1(1.02)	2(2.04)	1(1.02)	3(3.06)	1(1.02)	3(3.06)	1(1.02)	1(1.02)	1(1.02)	16.32
Bacillus sp	20	3(3.06)	1(1.02)	2(2.02)	1(1.02)	3(3.06)	1(1.02)	4(4.0)	2(2.04)	1(1.02)	2(2.04)	20.41
Enterococcus sp	20	3(3.06)	2(2.04)	1(1.02)	2(2.04)	3(3.06)	2(2.04)	4(4.08)	1(1.02)	3(3.06)	1(1.02)	20.41
Micrococcus sp	15	2(1.02)	1(1.02)	1(1.02)	2(2.04)	1(1.02)	2(2.04)	2(2.04)	1(1.02)	1(1.04)	2(2.04)	15.31
Klebsiella sp	3	1(1.02)	-	-	-	-	1(1.02)	-	-	1(1.0)	-	3.06
Proteus sp	2	-	-	-	-	1(1.02)	-	1(1.02)	-	-	-	2.04
	n=98											100

Key: T1= Toilet1, T2= Toilet2, T3 = Toilet3, T4 = Toilet4, T5 = Toilet5, T6 = Toilet6, T7 = Toilet7, T8 = Toilet8, T9 = Toilet9 and T10 = Toilet10

Table 2. Frequency of occurrence of fungi species isolated from the different domestic toilets

Organism	No of isolated	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Total percentage (%)
Aspergilus sp	14	1(1.96)	3(5.88)	1(1.96)	1(1.96)	2(3.92)	1(1.96)	1(1.92)	2(3.92)	1(1.96)	1(1.96)	27.45
Fusarium sp	10	2(3.92)	1 (1.96)	2(3.92)	-	1(1.96)	-	2(3.92)	1(1.96)	-	1(1.96)	19.60
Mucor sp	8	-	1(1.96)	2(3.92)	2(3.92)	1(1.96)	1(1.96)	1(1.96)	1(1.96)	-	-	15.69
Rhizopus sp	7	1(1.96)	1(1.96)	-	-	1(1.96)	-	2 (3.92)	2(3.92)	-	-	13.73
Penicillium sp	12	2(3.92)	2(3.92)	1(1.96)	1(1.96)	-	1(1.96)	1(1.96)	1(1.96)	1(1.96)	2(3.92)	23.53
	n=51											100%

Key: T1= Toilet1, T2= Toilet2, T3 = Toilet3, T4 = Toilet4, T5 = Toilet5, T6 = Toilet6, T7 = Toilet7, T8 = Toilet8, T9 = Toilet9 and T10 = Toilet10

Escherichia coli was isolated from all the toilets which could be as a result of it being a normal colonist of the human gastrointestinal tract. *Escherichia coli* are responsible for two types of infections in humans: urinary tract infections (UTI) and intestinal disease (gastroenteritis) [27]. "Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome; however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli"* [28].

Table 3. Colony Forming Unit (CFU/m^3) count of bacteria isolated from domestic toilets

Sample	Before flushing (CFU/m^3/hr)	After flushing CFU/m^3/hr
T1	19.047×10^3	42.857×10^3
T2	43.452×10^3	55.357×10^3
T3	28.571×10^3	38.691×10^3
T4	42.262×10^3	39.286×10^3
T5	47.023×10^3	60.119×10^3
T6	21.143×10^3	41.075×10^3
T7	52.380×10^3	67.261×10^3
T8	25.000×10^3	44.047×10^3
T9	34.523×10^3	41.072×10^3
T10	30.952×110^3	46.428×10^3

Key: T1= Toilet1, T2= Toilet2, T3 = Toilet3, T4 = Toilet4, T5 = Toilet5, T6 = Toilet6, T7 = Toilet7, T8 = Toilet8, T9 = Toilet9 and T10 = Toilet10

Table 4. Colony Forming Unit (CFU/m^3) count of fungi isolated from the different domestic toilets

Sample	Before flushing (CFU/m^3/hr)	After flushing CFU/m^3/hr
T1	8.333×10^3	9.524×10^3
T2	8.928×10^3	11.309×10^3
T3	10.119×10^3	13.690×10^3
T4	5.952×10^3	8.333×10^3
T5	9.524×10^3	12.500×10^3
T6	5.953×10^3	10.119×10^3
T7	8.309×10^3	5.905×10^3
T8	8.928×10^3	10.714×10^3
T9	7.738×10^3	5.953×10^3
T10	6.547×10^3	11.309×10^3

Key: T1= Toilet1, T2= Toilet2, T3 = Toilet3, T4 = Toilet4, T5 = Toilet5, T6 = Toilet6, T7 = Toilet7, T8 = Toilet8, T9 = Toilet9 and T10 = Toilet10

Proteus species was also isolated from only two toilets as shown in Table 1. *Proteus spp.* are most commonly found in the human intestinal tract as part of the normal flora. *Proteus* organisms are implicated in serious causes of infections in humans which include urinary tract infection and infection of the blood (bacteraemia). When these organisms invade the bloodstream, endotoxin, a component of Gram-negative bacteria cell walls, apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects [29].

*Klebsiella species w*as also isolated in 3 toilets. The usage of the toilet by an individuals infected with *Klebsiella spp*. within the toilet might have led to occurrence of *Klebsiella spp.* in our study. "*Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, sepsis, meningitis, diarrhea, peritonitis and soft tissue infections" [30]. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer.

"It would not be unreasonable to suggest that the persistence of enteric organisms within the air could be a potential infection risk via inhalation and swallowing. In normal use the toilet is unlikely to present a great risk to health as formed stool is quickly washed away and does not create large numbers of bacterial aerosols" [31].

Different fungal genera were isolated from this study. The prevailing fungal, *Aspergillus spp. and Penicillium spp.* were detected in 14(27%) and 12(23%), respectively. This is in agreement with Luksamijarulkul & Pipitsangjan [32] in a similar study. "*Aspergillus spp*. appears to be the most aggressive of these fungi, giving rise to infections also in patients with less severe airway disease, such as cystic fibrosis, asthma and chronic obstructive pulmonary disease. People who are atopic sometimes contract a severe infection in which aspergillosis causes an allergic reaction with the infection, giving the person wheeze, pulmonary infiltrates and eventually fibrosis" [33]. *Penicillium* can cause health problems to all occupants of the space it affects, including children and pets. It can cause inflammation in the lungs and asthma and can lead to serious illness in people with immune disorders.

Fusarium species 10(19.60%) were also found to be high in this study in this study. This is also in agreement with a similar study by *Ohagim et al.,*[24]. It increased might be due to the dampness of the toilets. *Fusarium spp.* frequently encountered in localized infections in immunocompetent and disseminated in severely

immunocompromised patients. Development of skin lesions is the most common infectious aspect of this genus and often used in diagnosis of the infection [34,35]. Development of skin lesions is the most common infectious aspect of this genus and often used in diagnosis of the infection [34].

In this study, *Rhizopus and Mucor species* where found to be the least occurring fungi species isolated. This is in accordance with a study carried out by Luksamijarulkul & Pipitsangjan [32] in air quality assessment. Exposure to *Mucor* can cause a range of health problems, mostly affecting the lungs. It can cause asthma symptoms, or can make existing asthma worse.

Epidemiological studies from recurrent outbreaks of norovirus infection in successive cohorts of guests in hotels and on cruise ships [13] suggests spread from infected persons after vomiting by settling of aerosol particles onto surfaces which are then touched by hands. In addition, this study suggests that spraying or aerosolizing during toilet flushing can deliver bio aerosol particles to contact surfaces such as toilet seats or water handles. Combined with experimental data, the possibility of spreading intestinal diseases through contact with pathogenic bathroom surfaces cannot be ignored and should be considered a serious risk of infection. The World Health Organisation (WHO) calculates a limit of 500 CFU/m3 for fungi, which are higher than bacteria because they are not associated with most infectious diseases. The number of fungi from this study is well below the limit in some areas during rainy and dry seasons. Such monitoring can be caused by low levels of mold and adverse environmental conditions that can mold from spreading in the air.

5. CONCLUSION

In this study, microbial air quality was measured to determine the level of aerosol found/exist in the air in the selected domestic toilets within Port Harcourt, Nigeria. Based on the results, it can be concluded that sampling after flushing showed an increase in the total concentration of heterotrophic bacteria and coliform bio aerosols as compared to before flushing. However, flushing the toilet can produce bio aerosols, and most importantly, some of these bio aerosols contain known intestinal pathogens. Thus, the microbial loads of the toilets were favored by the environmental conditions which enhance their development. And also it was stated by WHO that dampness situation has to be considered as the risk indicator for health risks of biological contaminants of indoor air [36]. Therefore, to maintain the health of users, it is necessary to carefully manage the environmental factors that promote the growth and multiplication of microorganisms in indoor environment.

CONSENT AND ETHICAL APPROVAL

Ethical approval for this study was obtained from the Rivers State Health Management Board and informed consent was obtained from the owners of the toilets used for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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