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Microbiological Indoor Quality Assessment of Public Toilets in Port Harcourt Metropolis, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author SID designed and supervised the study. Author JAL performed the work, statistical analysis, wrote the protocol, managed the analyses and literature searches of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Assessing the microbiological quality of indoor air in some public toilets of major motor parks in Port Harcourt metropolis

Study Design: Indoor air of public toilets in three major parks in Port Harcourt was sampled using the sedimentation technique. This was done in two periods (morning and evening).

Place and Duration of Study: This study, were carried out in the Mile 3, Waterlines and RTC motor parks public toilets. This was a three months study (March-May, 2018).

Methodology: The sedimentation technique was used in evaluating for microbial population of air samples. Freshly prepared Nutrient (NA), MacConkey and Sabouraud Dextrose agar (SDA) plates in duplicates were left open for 15minutes and 1m above the ground in the various study sites. Samples were later transported to the Microbiology Laboratory and incubated at 28ºC for 3-7 days for fungi and at 37ºC for 24 hours for bacteria and coliforms. After incubation, bacterial and fungal populations were enumerated and distinct isolates were purified by subculturing onto fresh NA and SDA plates. The purified isolates were used for characterization.

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Results: Bacterial genera belonging to; *Staphylococcus, Bacillus, Providencia, Pseudomonas, Escherichia, Enterobacter* and *Klebsiella* were isolated and fungal genera belonging to: *Aspergillus, Penicillium, Rhizopus, Candida*, and *Mucor* species were identified in this study.

Conclusion: The high microbial loads in this study indicate that the indoor airs of these public toilets were above the suggested WHO standard of 1000Cfu/m^3. Thus, this could pose serious health challenges to people who use and even those who work in the public toilets. Also, some of the bacterial and fungal genera identified could be pathogenic strains which may cause diseases or other allergic reactions.

Keywords: Motor park public toilets; bacteria and fungi contamination; indoor air; plate exposure.

1. INTRODUCTION

Indoor air is the air within an enclosed space or a building. Thus, indoor air quality of toilets refers to the quality of air within the toilet. In respect to the growing concern about possible biological effects of deposition of various pollutants in the atmospheric environment, air pollution and the health of the populace has become one of the most important environmental and public health issues [1]. Reason being that atmospheric pollution poses significant impact both to human health and the environment. Evidences from various governmental organisations and international bodies have proven that air pollution is a major risk to the environment, quality of life, and health of the population [2,3,4,5,6]. This is because the biological agents which are found in the air as a result of the pollution contaminates the air and could cause serious health challenges to people who inhale them or touch surfaces contaminated with these biological agents. Previous studies listed diseases such as carcinogenicity, pulmonary tuberculosis, cerebrospinal meningitis, pneumonia, whooping cough and measles as the health effects that could result due to air pollutants [7,8]. In a recent study by Wemedo and Robinson [9], it was reported that the presence of biological aerosol in the air which are pathogenic could cause serious health challenges and that these biological aerosols are discharged via talking, sweeping, sneezing, as well as flushing toilets. Furthermore, Douglas and Robinson [10] in the study of indoor air reported hypersensitivity reactions, pneumonia, and toxic reactions could arise when bye products of this biological aerosols are in contact with man.

Toilets are sanitation facilities at the user interface that allow the safe and convenient urination and defecation [11]. Thus, maintaining the indoor quality of toilets is important so as to keep it hygienic and sanitarily conducive for usage [12]. According to Mirbahar and Memon [13], maintaining good indoor air quality of toilets is one of the first step to create a healthier and safer indoor environment. Air is the easiest means by which agents of pathogenic microbes are disseminated, which can cause significant problems in the environment; especially, in public rooms such as toilets [13]. Insufficient ventilation, high influx of people and improper management of public toilets, are main sources of indoor air contamination in public toilets [10,14,9].

A motor park is an area of land where cars or vehicles of different sizes are parked. Travelers as well as those returning from journey get into the vehicle or alight from the vehicles. The park which houses vehicles also is inhabited by traders, and other activities such as areas for relaxation and facilities for convenience are made available. Thus, travelers as well as those returning from journey and others who partake in different activities within this area utilize the toilet facilities. The influx of persons as well as the unhygienic habits of toilet users no doubt leaves the place dirty thereby making it a comfortable environment of airborne pathogens [14]. Understanding the quality of the indoor air of the toilet is important so as to check the hygienic conditions. Thus, this study is aimed at assessing the quality of indoor air in some public toilets of major motor parks in Port Harcourt metropolis.

2. MATERIALS AND METHODS

2.1 Study Area/ Study Duration

The Mile 3, Waterlines and Rivers Transport Company (RTC) motor park toilets were the area of study. The three motor parks are in Port Harcourt, Rivers State, Nigeria. This is an urban setting. The control was a private toilet. This was a three months study (March - May, 2018). These motor parks, due to their locations enjoy high patronage, because of the different activities taking place around them. There are shops, markets, hawkers, passengers boarding and alighting from buses and taxi (both inter and intra passengers). The Mile 3 and RTC public toilets are water cistern which supports squatting while the control toilet and Waterlines are standard water cistern that supports sitting. All toilets use water for flushing urine and faeces. The images of the toilets are presented below. All toilets use water for flushing.

2.2 Sample Collection

Air samples of the various public toilets were collected using the direct sampling method (plate exposure technique) which has been described by previous studies [10,15,9]. In this technique, Petri dishes containing specific growth medium were exposed to the ambient air in the study sites for fifteen minutes. After which plates were aseptically covered and transported to the Microbiology laboratory and were incubated for 37ºC and 28ºC for bacteria and fungi respectively. Duration of incubation for bacteria was 24 hours while fungal plates were kept for 3-5 days. The samples were collected twice a day in all sampling locations, that is morning (8 A.M) and evening (7 PM).

2.3 Enumeration of Bacterial and Fungal Populations

Nutrient agar, MacConkey agar and Sabouraud Dextrose Agar (SDA) plates were exposed in the

Plate 1. The control toilet

Plate 2. The mile 3 public toilet

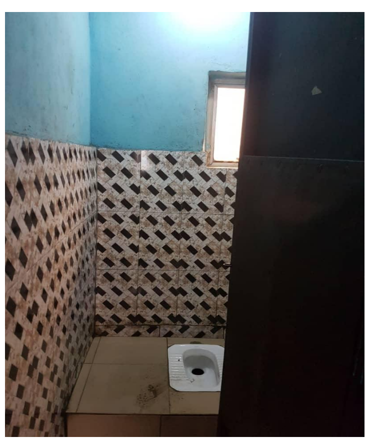

Plate 3. The RTC public toilet

Plate 4. The waterlines public toilet

respective studied sites to enumerate the total heterotrophic bacteria, Total coliform and fungal populations. Resulting bacterial and fungal colonies after incubation were enumerated using the sedimentation formulae as described by previous studies [10,15,9], and were represented as CFU/M^3 and SFU/M^3 for bacterial and fungal isolates. The formula is described below.

$$A = \frac{a \times 10^4}{0.2 \times \pi r^2 \times t}$$

a= number of colonies on plates
r = radius of the Petri dish
t = time of exposure of plate
Note:
SFU: Spore forming unit

2.4 Isolation and Identification of Bacterial Isolates

The distinct bacterial isolates were subcultured on freshly prepared nutrient agar plates until it was certain that colonies were pure (having no contaminants) and were identified as described by Cheesbrough [16]. Isolates were identified based on macroscopy (colour, shape, size, elevation and texture), microscopically and response to some biochemical tests (catalase, citrate, oxidase, motility, MR, VP, indole, and fermentation of glucose, mannitol, maltose and lactose).

2.5 Isolation and Identification of Fungal Isolates

After incubation, distinct fungal colonies were isolated and subcultured on freshly prepared SDA plates to obtain pure fungal isolates. Macroscopy and microscopic examination were used in identifying the fungal isolates before they were compared with fungal texts to aid proper identification [10].

2.6 Statistical Analysis

Two - Way ANOVA was adopted to check for significant difference in the microbial loads of the study sites.

3. RESULTS AND DISCUSSION

In this study, the mean microbial population of the various study sites is presented in Log$_{10}$Cfu/m^3. The mean microbial population of the various study sites for the morning sampling period is illustrated in Table 1. The total heterotrophic bacteria ranged from 3.08±0.15 to 3.83±0.17 Log$_{10}$Cfu/m^3, while the total coliform ranged from 2.82±0.00 to 3.73±0.27 Log$_{10}$Cfu/m^3. The mean fungal load ranged from 2.21±0.23 to 3.01±0.48 Log$_{10}$Sfu/m^3. The RTC and the Mile 3 motor park public toilet have the highest microbial load while the control had the least microbial loads. The Two-way analysis of variance revealed a significant difference between the control private toilet and the public toilets at $P \geq 0.05$. There was no significant difference in the microbial loads of the public toilets $P \geq 0.05$ even though the microbial loads in this study sites were very high. Also, there were no recorded significant differences in the total coliform bacterial and fungal loads of the various study sites (Table 1).

The mean microbial loads of the study sites in the evening sampling period are presented in Table 2. The result shows that the mean total heterotrophic bacterial load ranged from 3.03±0.16 to 3.83±0.14 Log$_{10}$Cfu/M^3 while the mean total coliform ranged from 2.82±0.00 to 3.81±0.19 Log$_{10}$Cfu/M^3. The mean total fungal load as shown in Table 2 ranged from 2.28±0.28 to 2.69±0.08 Log$_{10}$Sfu/M^3. The results revealed higher bacterial load in the Mile 3 public toilets while lower bacterial loads were recorded in the control (private toilet). Apart from the significant difference observed in the control of the total heterotrophic bacterial loads, all other studied sites despite the variations in bacterial load show no significant difference at P≥0.05. Also, the coliform and fungal load had no significant differences across the studied sites (Table 2). Furthermore, there was increase in the total heterotrophic bacteria loads of the evening sampling as compared to the morning sampling of the Mile 3 and Waterlines public toilets, while a slight decrease was recorded in the evening sampling of the control. Also, apart from the Mile 3 public toilet whose coliform loads increased in the evening period, other studied sites had higher coliform counts in the morning period. Despite the variations of microbial loads observed in the different sampling period, there were no significant difference between the microbial load of the morning and evening sampling period. In a recent study, it was reported that the microbial load increased in the evening sampling period and that increased microbial population is related to the influx of persons and activities going on indoor [14,9]. Thus, the high microbial load observed in the evening sampling period of the Mile 3 and

Waterlines Motor Park public toilet could be that the public toilet is frequently used more in the evening period than in the morning. In the RTC motor park public toilet, the heterotrophic bacterial load was almost the same for both sample periods. It could be asserted that the number of persons who used the toilet in the morning could be equal to the number of persons who used it in the evening. It may also be attributed to the regular cleaning of the toilets and ventilations. Cleaning of toilets could cause unfavourable conditions for the microbes thereby causing a reduction in the microbial load [14]. Furthermore, the microbial load in the public toilets are very high and exceeds the 1000Cfu/m^3 suggested standard of microbial loads in indoor air [9]. The results in this study revealed that the private toilet has the least microbial populations (both in total heterotrophic bacteria, coliform bacteria and fungal loads). This could be related to the continuous use by lots of persons in the motor parks as well as other activities taking place in these motor parks. Also, the attitude of the users such as indiscriminate disposal of wastes in the toilets, urinating on toilet floors instead of the sink, and littering the toilet environment with used toilet paper or tissues paper or not flushing of the toilets after use. This statement agreed with [14], who had reported that the indiscriminate disposal of wastes in toilets, urinating on the floor as well as littering the toilet provides the environment which favours the growth of microorganisms.

In this study, seven bacterial genera belonging to; *Bacillus, Escherichia, Staphylococcus, Providencia, Enterobacter, Pseudomonas* and *Klebsiella* species were identified. The frequency of occurrence of the bacterial isolates is presented in Table 3. The result revealed that *Escherichia, Staphylococcus, Klebsiella,* and *Bacillus* species were isolated from all the public toilets including the control (private toilet). *Providencia* species were only isolated in the Mile 3 and Waterlines public toilets, while *Enterobacter* and *Pseudomonas* species were isolated in all the public toilets but were not isolated in the control. Also, from the frequency of occurrence, *Staphylococcus* species had the highest in the Mile 3 toilet (33.3%), followed by the 25% in the Waterlines and RTC toilets while 16.7% was recorded in the private toilets. The frequency of occurrence revealed that *Bacillus, Providencia, Enterobacter, Escherichia* and *Klebsiella* were very high in the Waterlines toilet as compared to other locations and frequency of occurrence was observed as 34.8%, 55.6%, 54.6%, 40% and 40% respectively. *Bacillus* and *Staphylococcus* species which are present in the indoor air of this study have been isolated in the indoor air of some motor park public toilets in Imo State, Nigeria [14]. Similar bacterial genera have been isolated from indoor air in previous study [15,9]. The presence of *Staphylococcus* and *Bacillus* species could be attributed to their ubiquitous nature. Previous studies have reported infections from the bacteria in this study [9,17]. The presence of endospores in *Bacillus* sp confers them the ability to survive even in unfavourable environments [9]. *Escherichia coli* could cause urinary tract infections and gastro intestinal infections under favourable conditions [17].

Table 1. Mean counts of the microbial population of the morning sampling session of the various study locations in $Log_{10}Cfu/M^3$

Location	THB	TCC	TFC
Control	$3.08±0.15^a$	$2.82±0.00^c$	$2.21±0.23^d$
Mile 3	$3.83±0.03^b$	$3.70±0.26^c$	$2.88±0.53^d$
RTC	$3.83±0.17^b$	$3.73±0.27^c$	$3.01±0.48^d$
Waterlines	$3.54±0.21^b$	$3.37±0.43^c$	$2.91±0.57^d$

Means with the same alphabet across columns shows no significant difference (P≥0.05)

Table 2. Mean count of the microbial population of the evening sampling session of the various study locations in $Log_{10}Cfu/M^3$

Location	THB	TCC	TFC
Control	$3.03±0.16^a$	$2.82±0.00^c$	$2.28±0.28^{ab}$
Mile 3	$3.87±0.05^b$	$3.81±0.19^c$	$2.69±0.08^{ab}$
RTC	$3.83±0.12^b$	$3.60±0.38^c$	$2.36±0.17^{ab}$
Waterlines	$3.83±0.14^b$	$3.6±0.24^c$	$2.47±0.09^{ab}$

Means with the same alphabet across columns shows no significant difference (P≥0.05)
Note: Abbreviations: THB: Total Heterotrophic Bacteria, TCC: Total Coliform Count, TFC: Total Fungal Count

Table 3. Frequency of occurrence of bacteria in the different locations (%)

S/N	Bacterial isolate	Control	Mile 3	Waterline	RTC
1	*Staphylococcus* sp	16.7	33.3	25	25
2	*Bacillus* sp	13	21.7	34.8	30.4
3	*Providencia* sp	0	44.4	55.6	0
4	*Pseudomonas* sp	*0*	41.2	35.3	23.5
5	*Escherichia* sp	20	15	40	25
6	*Enterobacter* sp	0	36.4	54.6	9.1
7	*Klebsiella* sp	10	30	40	20

Table 4. Frequency of occurrence of fungi in the different locations (%)

S/N	Bacterial isolate	Control	Mile 3	Waterline	RTC
1	*Aspergillus* sp	14.8	25.9	25.9	33.3
2	*Candida* sp	23.1	46.2	30.8	0
3	*Rhizopus* sp	0	0	62.5	37.5
4	*Penicillium* sp	14.3	42.9	0	42.9
5	*Mucor* sp	0	66.7	33.3	0

Five fungal genera belonging to *Aspergillus, Candida, Rhizopus, Mucor* and *Penicillium* were isolated in this study (Table 4). The frequency of occurrence of fungal isolates as presented in Table 4 revealed that *Aspergillus* species were the most predominant fungi and was present in all study sites. *Candida* species were isolated from all the studied sites except from the RTC motor park public toilet. The RTC motor park public toilet recorded the highest frequency of *Aspergillus* species (33.3%) followed by the 25.9% recorded in the Mile 3 and Waterlines motor park public toilets. *Mucor* species was isolated in only the Mile 3 and Waterlines motor park public toilets, while *Rhizopus* species were only isolated from the Waterlines and RTC public toilets. The fungal isolates in this study have been isolated from public toilets in of some motor parks in Imo state, Nigeria. In a recent study, the fungal isolates in the indoor air of this study have been reported in the indoor air of some government health facilities [10]. The presence of these fungal spores in the indoor air could impact the health of individuals especially those with low immune response who may come in close contact with the spores [10,15].

4. CONCLUSION

This study has revealed that the microbial loads in these public toilets are higher than those suggested by WHO standard of 1000Cfu/m^3. Thus, this could pose serious health challenges to people who use these public toilets regularly. Also, the bacterial and fungal genera in this study could cause diseases or allergic reactions to users as studies have associated these microbes to cause related infections such as urinary tract infections to respiratory infections. It is therefore recommended that proper sanitary measures be put in place to enhance the cleanliness. Also, water should be made available both for flushing of toilets and hand washing. Waste bins should be provided and emptied regularly; people should be put in charge to ensure that users utilize the facilities effectively without littering the place.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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