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Assessment of Bacteriological Quality of Indoor Environment of Selected Toilets from a Male Hostel, University of Benin, Nigeria

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Abstract

Indoor air is a home to a plethora of bacterial isolates. Pollution of indoor environment is caused by lack of ventilation, chemicals, temperature and microbes. Unavoidable exposure to the indoor pollutant can cause diverse respiratory health challenges such as pneumonia, pulmonary tuberculosis, Whooping cough and measles. This study was aimed at assessing the bacteriological quality of the indoor environment of some selected male student's hostels toilets in University of Benin, Benin city. Settled plate method was used for the air sampling, at a sampling height of 1m above the floor level. Samples were collected in triplicates monthly between February, 2021 and May, 2021. Upon sampling, the samples were transported to the laboratory for immediate analysis using standard bacteriological methods. The bacterial isolates were subjected to antibiotic susceptibility test using standard discs and the multiple antibiotic index was determine in other to evaluate the public health importance. The concentration of bacterial aerosols in the indoor environment of the sampled hostel toilets ranged between 96.02± 71.00 cfu/m3 and 222.60± 187.33 cfu/m3. The highest airborne bacterial counts, 246.86± 67.44cfu/m3 was recorded in April and the least count, 76.62± 35.13cfu/m3 was recorded in March. Culturally and morphologically, eight (8) airborne bacterial isolates were isolated, they include Staphylococcus aereus, Serratia marcescens, Escherichia coli, shigella sonnei, Pseudomonas aeruginosa, Pseudomonas alcaligenes, Proteus mirabilis and Enterococcus faecalis.

1. Introduction

Humans spend a lot of their time in different indoor environments. Indoor air pollution is caused by a lack of ventilation, chemicals, high temperatures and microbes [1]. Bioaerosols are produced by cool mists, humidifiers, vaporizers, and flush toilets in the indoor environment [2, 3].

A main mechanism of respiratory illnesses is inhaling bioaerosols that are dispersed through human and animal activities [4]. Due to the growing concern about the biological effects of these pollutants in the atmosphere, pollution of the air and public health has emerged as one of the most pressing environmental and public health concerns, for the simple reason that these pollutants have a significant effect on both environment and health of humans [5]. One of the most serious

consequences of poor microbiological indoor air quality is an increased risk of respiratory infections and other health problems associated with hypersensitivity disease [6].

Pollution of the air is a severe threat to the surrounding, quality of life, and health of the public, according to evidence from many governmental organizations and international authorities [7]. This is because biological agents found in air due to pollution, contaminate the air and may pose substantial health risks to persons who breathe them or come into contact with biological agents-contaminated surfaces. Air pollution can cause diseases such as pneumonia, measles, pulmonary tuberculosis, whooping cough and cerebrospinal meningitis [8]. According to [9], pathogenic biological aerosols which are in the air are capable of causing major health problems and that these biological aerosols are released by talking, sweeping, sneezing and toilet flushing. Toilets are sanitation facilities at the user interface that allow the safe and convenient urination and defecation. Maintaining the indoor quality of toilets is one of the first step to create a healthier and safer indoor environment. Insufficient ventilation, high influx of people, poor sanitation and improper management of public toilets are the main sources of indoor air contamination in public toilets. It is critical to maintain the indoor quality of toilets in order to keep them clean and sanitarily suitable for use [10, 11]. According to [12], improving toilet indoor air quality is one of the first stages toward creating a better and safer environment. Pathogenic bacteria can easily spread through the air, causing substantial concerns in environments, particularly public spaces like restrooms. It therefore became importance to undertake the study of the bacteriological quality of the indoor environment of some randomly selected toilets of a male student hostel in University of Benin.

2.0. Materials and Methods

2.1. Sample collection

Airborne bacteria samples were collected by the passive air sampling technique (Settled plate method) by exposing 9 cm diameter petri dish size at a height of 1m above the floor level. Sampling height of 1m has been reported by several authors sampling indoor air quality of toilets and bathrooms. It is regarded as human breathing level in indoor air quality assessment. Samples were collected once monthly in triplicates for the period of four months from randomly selected toilets of a male hostel in University of Benin, Ugbowo Campus. The media used were all prepared following the manufacturer's guide. The media were sterilized by autoclaving at 121 ^{O}C for 15 mins [13,14].

2.2. Enumeration of the Bacterial Isolates

The colonies on each petri dish were counted, the average (mean) value from the triplicate plates was calculated and expressed in cfu/m^3 using Equation (1).

$$\text{cfu/m}^3 = \frac{5a * 10{,}000}{b*t} \quad (1)$$

Where;

a= Number of colonies counted in Petri dish

b= Surface area of the 9 cm diameter Petri dish (πr^2)

t= Time of exposure (10 min) [14, 15].

2.3. Identification of Bacterial isolates

The bacterial isolates were identified using cultural, morphological and biochemical identification methods which include Gram stain techniques, Catalase, Oxidase, Urease, Citrate, Coagulase, indole tests.

2.4. Detection of Phenotypic Virulence Factors

2.4.1 Protease Activity

Triptone Soy Agar (TSA) plates enriched with 1% casein (v/v) was used to test the isolates' extracellular protease activity. The colonies from the Tryptone Soy Agar colonies were suspended in 3 mL Mueller Hinton broth. This suspension's density was adjusted to 0.5 Mc Farland standard. The TSA plate was inoculated with 1 mL of the suspension and then enriched with 1 % casein, the plate is then incubated for 24 to 48 hours at 37°C. A positive result was defined as a zone of clearance due to casein hydrolysis, while a negative result was defined as no clearance.

2.4.2 Lipase Activity

Spirit blue agar plates were used to test the isolates' lipase activity (Himedia). Tryptone soy broth (TSB) agar colonies were suspended in 3 mL Mueller Hinton broth. This suspension's density was corrected to 0.5 McFarland standards, which equates to 1.5×10^8 cells/mL. 1 mL of this suspension was inoculated on Spirit blue plate is then incubated for a period of 24 to 48 hours at a degree of 37°C. Lipases are enzymes that break down lipids (fats), Lipase breaks down lipids into smaller bits in the organisms that make it. These breakdowns are transformed into a range of end products that the cell can use for energy production or other operations. Where the bacterium producing Lipase has grown on the agar is surrounded by a transparent halo. The cleared zone was measured and analyzed objectively.

2.4.3. Gelatinase Production

In a nutritional gelatin medium, the isolates' gelatinase production was measured (Micromaster) Tryptone soy agar (TSA) colonies were suspended in 3mL Mueller Hinton broth. This suspension's density was corrected to 0.5 McFarland standards, which equates to 1.5×10^8 cells/mL. Gelatin medium was inoculated with 1mL of the suspension and incubated for 24 to 48 hours at a degree of 37°C. Presence of gelatin-liquefying bacteria was indicated by zones of clearing in the medium. Absence of cleared zone connote negative result.

2.4.4. DNA Degrading Activity

The isolates were cultivated on DNase agar plates to see if they could degrade DNA (Himedia). TSA colonies were suspended in 3mL Mueller Hinton broth. This suspension's density was corrected to 0.5 McFarland standards, which equates to 1.5×10^8 cells/mL. DNase agar plates where inoculated by 1mL of the suspension and incubated for 24 to 48 hours at 37°C. Methyl green is emitted when DNA is hydrolyzed, rendering the liquid colorless around the test organism. The medium remains green when there is no DNA degradation.

2.4.5. Hamolysin Production

Tryptone soy broth (TSB) agar colonies were suspended in 3mL Mueller Hinton broth. This suspension's density was corrected to 0.5 McFarland standards, which equates to 10^8 cells/mL. Sheep blood agar plate with a 5mL sample were inoculated to the suspension and it was then incubated at 37°C for 24 to 48 hours. Following that, obvious colorless zones around the colonies revealed beta hemolysis, indicating that the red blood cells had been completely lysed. A tiny zone of greenish to brownish decolorization of the medium indicates alpha hemolysis. The conversion of haemoglobin to methaemoglobin and subsequent diffusion into the surrounding media causes this. No change in the medium indicates gamma hemolysis [16].

2.4.6. Antibiotic Susceptibility Testing

A fully grown bacterial culture (from 24 hours) was cultivated on MHA for this experiment. The inoculum corresponding to 1.5 x 108 cells/ml McFarland standard was sprinkled onto the MHA plates using sterile loops before the introduction of antibiotic discs, which were inserted to the plates with careful care using a sterile forcep. After 24 hours of incubation period at a degree of 37°C, the susceptibility results were interpreted and reported. Following the AST standard or regulations issued by CLSI in 2017. (Clinical Laboratory Standards Institute). zone of inhibition which is around each of the disc (measured in diameter using a meter rule) was evaluated according to CLSI standard 2017 as either Resistant (R), Intermediate Resistant (I), and Sensitive (S).

2.4.7 Multiple Antibiotic Resistance (MAR) Index

In this study, the Multiple Antibiotic Resistance index was obtained using Equation (2).

$$\text{MAR Index} = \frac{y}{n} \quad (2)$$

where y = number of resistant per isolates recorded
n= total number of antibiotics

MAR index greater than 0.2 is indictive from high-risk' contamination source or that the bacteria were of public health significance or worse still, where antibiotic have often been used or abused

2.4.8. Data Analysis

The results were compared and analyzed. The mean and standard deviation of the diameter of zones of inhibition were calculated. The statistical tests employed was one-way analysis of variance (ANOVA) [17].

3.0. Results

3.1. Bacterial Counts at Different Hostels Toilets and Months

Figure 1A showed the spatial variation of bacterial counts, it revealed that toilet 9 had the highest bacteriological count ($222.60 \pm 187.33 \times 10^3$ cfu/m^3) while the least count was obtained in toilet 4 ($96.02 \pm 71.00 \times 10^3$ cfu/m^3). Figure 1B showed the temporal variation of bacterial counts, it was revealed that the month of April had the highest with a count of $246.84 \pm 67.44 \times 10^3$ cfu/m^3 of air while the least count was observed in the month of March with a count of 76.62 ± 35.13 cfu/m^3. The bacterial count in May was recorded as $98.93 \pm 49.33 \times 10^3$ cfu/m^3.

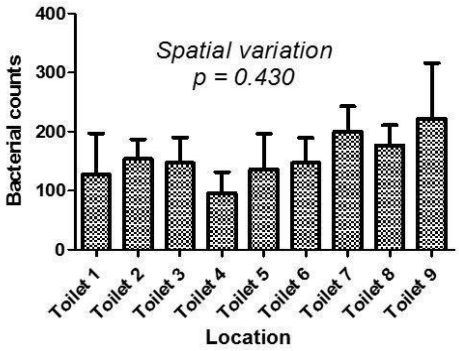

Figure 1A: Spatial variation of bacterial counts

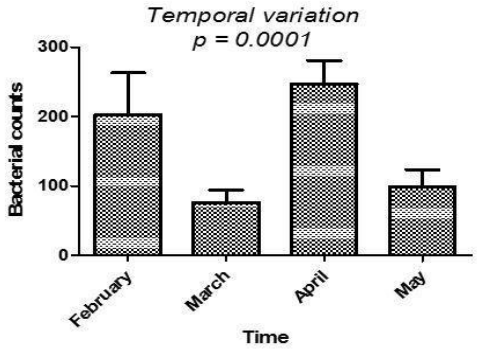

Figure 1B: Temporal variation of bacterial counts

3.2. **Frequency of Bacteria Occurrence from Selected Male Hostel Toilets**

Table 1 showed that in the month February *Staphylococcus* aureus had more frequency occurrence (43%) than other isolated bacteria in the study. *Enterococcus faecalis and Escherichia coli* being the least had 7% from the identified bacteria isolates in the month of February analysis. Following analysis for the month of March *Staphylococcus* aureus had the highest frequency of bacterial occurrence (65%) while Serratia marcescens had the least frequency of occurrence (4%). Escherichia coli and pseudomonas alcaligenes had a frequency of occurrence of 7% apiece. In the month of April, Pseudomonas aeruginosa had the highest frequency of bacterial occurrence (43%) while Escherichia coli had the least occurrence (7%). The percentage occurrence for Staphylococcus aureus was 21% while Enterococcus faecalis had 14% frequency of occurrence. *Staphylococcus aureus* had the highest frequency of bacteria occurrence (79%) in the month of May while *Escherichia coli* the least at 5%.

Table 1: Percentage frequency of bacteria occurrence from the month of February, 2021 to May, 2021

February	% occurance	March	% occurance	April	% occurance	May	% occurance
P.aeruginosa	28.57	*P.alcaligenes*	6.90	*S. aureus*	21.43	*E. coli*	5.26
S. aureus	42.86	*S. aureus*	65.52	*E. faecalis*	14.29	*Shigella* spp.	15.79
E. faecalis	7.14	*S. marcescens*	3.45	*P.aeruginosa*	42.86	*S. aureus*	78.95
E. coli	7.14	*P. mirabillis*	17.24	*E. coli*	7.14		
		E. coli	6.90				

3.3. **Antibiotics Susceptibility of the Bacterial Isolates**

Table 2 revealed that most of the isolates were susceptible to ceftriaxone, imipenem, ciprofloxacin and augmentin. The susceptibility for each isolate with reference to selected antibiotics include 100% for *Serratia marcescens, Pseudomonas alcaligenes* and *Proteus*

mirabilis to ciprofloxacin. All identified isolates were found to have greater than 50% susceptibility to ceftriaxone antibiotics.

Table 2: Antibiotic susceptibility results of the Bacterial Isolates

Bacterial Isolates	No.	CIP	IMP	CXM	AZN	AUG	CTX	CRO	ERY	GN	ZEM
P. aeruginosa	10	5(50)	0(0)	0(0)	5(50)	2(20)	0(0)	8(80)	6(60)	5(50)	0(0)
Proteus mirabilis	5	5(100)	3(60)	2(40)	5(100)	4(80)	2(40)	5(100)	1(20)	4(80)	0(0)
P. alcaligenes	2	2(100)	2(100)	1(50)	1(50)	2(100)	1(50)	2(100)	0(0)	1(50)	0(0)
S. aureus	62	58(94)	60(97)	25(40)	31(50)	40(65)	55(89)	61(98)	23(37)	50(81)	2(3)
Serratia marcescens	1	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)
Shigella sonnei	2	1(50)	2(100)	0(0)	1(50)	2(100)	1(50)	1(50)	0(0)	1(50)	0(0)
E. coli	5	5(100)	4(80)	3(60)	3(60)	3(60)	2(40)	4(80)	5(100)	1(20)	0(0)
S. faecalis	3	3(100)	3(100)	0(0)	1(33)	2(67)	0(0)	2(67)	1(33)	3(100)	0(0)

CIP=Ciprofloxacin, IMP=Imipenem, CXM=Cefuroxime, AZN=Azithromycin, AUG=Augumentin, CTX=Cefotaxime, CRO=Ceftriaxone, ERY=Erythromycin, GN=Gentamicin, ZEM= Zemdri

3.4. Multiple Antibiotic Resistance Pattern

Figure 2 revealed that all identified bacteria isolates had MAR index > 0.2 except *Staphylococcus aureus* that had less than the delineated limit of 0.2

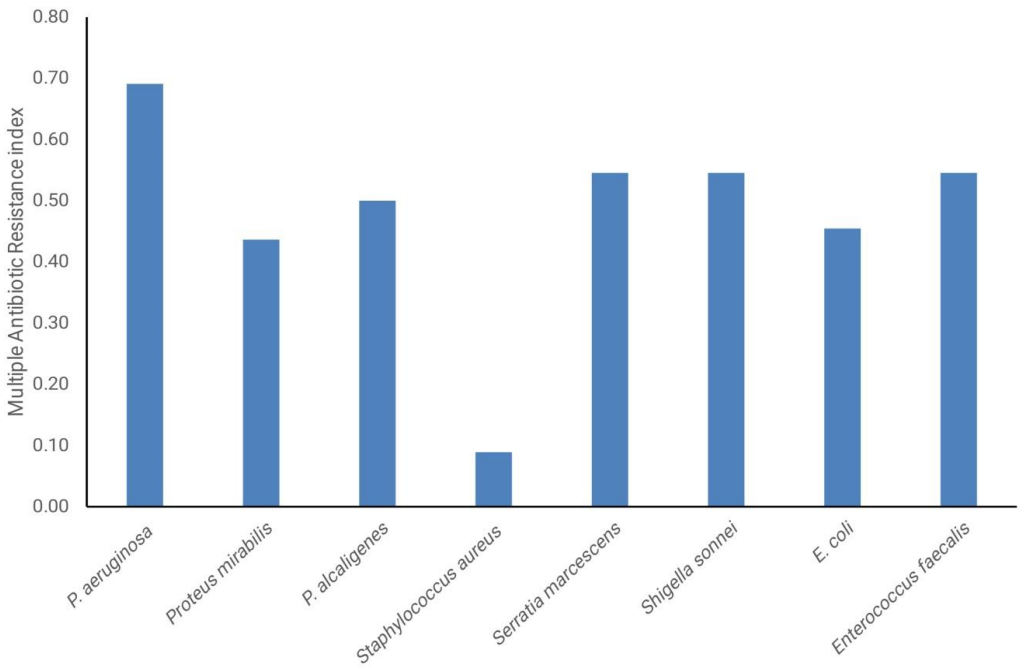

Figure 2: Multiple antibiotic resistance pattern exhibited by bacterial isolates

3.5. Discussion

The study of microbiological quality of indoor air is very important which enable the determination of the rate of pollution caused by microorganisms in indoor environment. Information about the concentration of microorganisms in an indoor environment is very important to determine the danger and health implications associated with poor indoor air as well as to develop control requirements for indoor air quality. A fundamental knowledge of the microbial community in the indoor air environment, which includes the estimates of diversity, function, and concentration, is necessary to develop an accurate portrayal of human exposure and to improve human health [18]. The sampled toilets have low sanitary management as the toilets are cleaned once per day with little or no disinfectants. Some Hostel occupants and toilet users lack the ability to manage and maintain the hostel toilets which have led to increase in microbial population in the indoor environment of public toilets.

The concentration of bioaerosols in the indoor air of nine toilets sampled in this study evaluated using the settle plate method for the period of 4 months ranged between $96.02 \pm 71.00\times10^3$ cfu/m^3 – $222.60 \pm 187.33\times10^3$ cfu/m^3. The highest bacterial counts were recorded in the month of April, 2021 and the counts of $246.84 \pm 67.44\times10^3$ cfu/m^3 while the least count was recorded in the month of March, 2021 and the counts of $76.62 \pm 35.13\times10^3$ cfu/m^3. No significant difference ($P>0.05$) in concentration or amount of bacterial isoaltes recovered from the sampled toilets in the study. From February, 2021 to May, 2021, there were obviously no significant differences between male toilets sampled in the hostels. However, the month of April, 2021 had a significant difference ($p<0.05$) for all the hostel toilets sampled in the study. Worthy of note, was that the range of values according to delineated standard for bacterial indoor air quality revealed that the month of February, 2021 and April, 2021 had intermediate level of contamination with bacterial counts ranging between 100 cfu/m^3 to 500 cfu/m^3, While the month of March, 2021 and May, 2021 had bacterial count between the ranges of 25 cfu/ m^3 to 100 cfu/m^3 thus having a low degree of pollution. A similar observation was recorded by [6] in their study (although with hospital environment) in Benin City and reported that most indoor environment assessed were found to have intermediate level of contamination.

The bacteria isolated from the sampled male hostel's toilets in this research work include *Serratia marcescens, Escherichia coli, Pseudomonas aeruginosa, Shigella sonnei, Pseudomonas alcaligenes, Proteus mirabilis, Enterococcus faecalis and Staphylococcus aureus.* The lack of sanitary management, inadequate ventilation, high influx of people and high humidity level are the contributing factors to the frequency of observed isolates as well as some major sources of pollution in indoor air of public toilets. *Escherichia coli* is likely to arise from fecal and water contamination. Spread of *Staphylococcus aureus* through air can be through skin scales which has been liberated into the air during daily activities.

The susceptibility for each isolate with reference to selected antibiotics includes 100% for *Serratia marcescens, Pseudomonas alcaligenes* and *Proteus mirabilis* to ciprofloxacin. All the identified isolates were found to have greater than 50% susceptibility to ceftriaxone antibiotics. Bacterial isolated from this study except *staphylococcus aureus* were found to have antibiotics resistance index higher than 0.2. Microbial isolates showing multiple antibiotic resistance higher than 0.2 indicates that the isolates have being previously exposed to antibiotics and pose as a concern to human health, mandating remedial measures [19]. Multi resistant isolates have been implicated in causing diverse diseases such as pulmonary tuberculosis, pneumonia, whooping cough and cerebrospinal meningitis especially in immuno-compromised system.

4.0. Conclusion

In conclusion, the indoor air quality was evaluated in this study. The isolated bacterial species are pathogens implicated to cause infections of public health importance. Their presence and

concentration in the indoor air environment is worrisome as it could pose a problem for the child whose immune system is underdeveloped or a child with compromised immune system.

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