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Microbiological Assessment of Secondary School Toilets Wall and Door Handles in Ondo, Ondo State

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Abstract

Toilet facilities among many other facilities ought to be present in secondary schools to promote hygiene and meet the physical and emotional needs of students. However, toilet wall and door handle could serve as fomite in the indirect transmission of infectious diseases, while toilet air microflora could enhance the direct transmission of infectious microorganisms. In this study, microbiological quality of secondary school toilets, types of microorganism and antibacterial susceptibility assay were carried out using standard microbiological methods. Swab sample were collected from toilet wall and door handles and agar plates were exposed to indoor and outdoor for the isolation of toilet air microflora. Total numbers of 160 samples were collected from six (6) secondary schools (A, B, C, D, E and F), A and B were boys school, C and D were girls only while E and F were co-educational schools. Microbiological assessment of toilet wall and door handle revealed zero staphylococcal and coliform counts in all the schools however, total bacterial counts of wall and door handle ranged from 1.03±0.11 to 4.06±0.02 and 1.04±0.06 to 7.04±0.05 cfu/10 cm^2 respectively. The highest fungal counts was observed in door handle, the total viable bacterial counts ranged from 0.00±0.0 to 12.51±0.03 and 1.24±0.03 $x10^2$ to 40.01±0.21 $x10^2$ cfu/m^3 in indoor and outdoor air microflora respectively. The fungal and staphylococcal counts of outdoor air microflora were significantly ($p<0.05$) higher than the indoor. The microorganisms isolated from toilet were; *Bacillus subtilis, Staphylococcus aureus, B. polymyxa, B. alvei, B. cereus, S. epidemidis, Seratia* sp. *Aspergillus niger, A. flavus* and *Rhizopus stolonifer* in which the most frequently occurred bacterial were *Bacillus subtilis* and *B. alvei* while that of fungus was *Rhizopus stolonifer. Staphylococcus aureus* were less susceptible to amoxicillin, augmentin, chloramphenicol and ciprofloxacin. The presence of these microorganisms could have impact on the health and well-being of secondary school students in Ondo, Ondo State.

Keywords

Microorganisms, Bacterial Counts, Fungal Counts, Air Microflora, Toilet

1. Introduction

Toilets and sanitation systems cater for one of the most basic human functions. The state of school's toilets is a matter of concern internationally, especially the impact on the health and well-being of children and young people. In many countries the sanitation, including that provided by schools, can be a matter of life and death [1-2].

Where hygiene is poor or nonexistent, the health risks to both children and adults are well documented. Girls in developing countries [3] report missing classes, particularly when they are menstruating, in order to ensure privacy in a communal toilet. Young people seeking privacy outside the school building may encounter snakes or other dangers [3]. Reluctance to use dirty, smelly, or inappropriately public facilities can lead to shared avoidance strategies that may have major short and long-term health implications. Both boys and girls may respond by limiting their intake of water during the day to reduce the need to use the toilet, or suppress any physical urge, contributing to physiological problems in eliminating waste effectively [4].

In some cases lack of cleanliness or poor toilet hygiene and usage represents a very specific risk of passing on

infection and disease which can cause short term illness and absence from school. In others it contributes to conditions that will persist beyond school and may be manifested in more serious forms in later life. Awareness and encouragement of good practice, sanitation and ensuring appropriate standards are maintained, can go a long way towards improving the health, confidence and self-esteem of children, young people and adults in education settings [3-4].

Facilities for academic and non-academic activities need to be properly put in place to provide an optimal sanitary environment which is safe and conducive for physical, mental and emotional health of the student in order to achieve maximum benefits from educational programmes [4-5]. Human excreta which form an important cause of environmental pollution need to be properly disposed through modern methods that are socially and culturally acceptable to the people [6]. Apart from its availability, the facilities should proportionately meet the demand of the population of both students and members of staff in such institutions.

In this century, almost all children spend a significant part of their time at school, where provision of toilets and hand washing facilities is the norm [7]. Provision of toilet facilities is considered a privilege rather than a necessity by most school authorities and this may affect their roles to address health issues of the students but rather concentrating on the academic pursuits of these students. Inadequate or lack of toilet facilities has its health implications, some of these may be bacterial, viral and parasitic in origin such as typhoid and paratyphoid fever, dysenteries, diarrhoeas, cholera, hookworm, ascariasis, viral hepatitis, schistosomiasis, guinea worm diseases, genito-urinary tract infections and a host of other intestinal and parasitic infections or even eye infections like trachoma (caused by *Chlamydia trachomatis* which is caused by flies that breed in dirty environment) and may lead to blindness, which can occur due to environmental pollution as a result of indiscriminate disposal of human excreta and inadequate sanitation [8]. This study was aimed at microbiological assessment of secondary school toilets in Ondo, Ondo State.

2. Materials and Methods

2.1. Description of Study Site

Ondo city is the second largest city in Ondo State, Nigeria. Ondo city is the trade center for the surrounding region. Yam, cassava, grain and tobacco are grown. Cotton is also grown, and is used to weave clothe called "Aso Oke" fabric. The city is the largest producer of cocoa products in the region. Ondo city is located on coordinates 7.088923^{o}N and 4.7990935 ^{o}E, there are two local governments in the city, namely; Ondo West and Ondo East Local Government Authority. The popular secondary schools in Ondo city include; Ondo Boys High School, St. Joseph's College, Ondo Grammar School, St. Helen's Unity Girls' Secondary School, St. Louis Catholic School, St. Monica's Grammar School, Hallmark Secondary School, Adeyemi College of Education Demonstration Secondary School. The Ondo Boys High School, founded in 1919, is one of the Africa's 50 oldest schools.

During this study, samples were collected from six different secondary schools across the two Local Governments. Total numbers of 160 samples were collected from six (6) secondary schools (A, B, C, D, E and F), school A and B were boys school, C and D were girls school while E and F were co-educational schools.

2.2. Sample Collection

All samples (toilet wall, door handle, Indoor and outdoor air microflora) were collected from school toilets by 8:30 am because it was established that the toilets were usually clean and sanitised in the morning, also many students do visit toilet in the morning to defecate or adjust their clothes just before or after the assembly; before the commencement of academic work.

2.3. Microbiological Assessment of Toilet Wall and Door Handle

A sterile swab stick was used for the collection of samples, prior the sample collection, all the swab stick were moistened with 1.0 ml of sterilized peptone water and covered. Swab sample from selected surfaces were collected according to the reference method [9]. Sterile swabs were removed from its coating, moistened tip and placed the tip of swab (s) on the surface to be investigated. The area was covered by a single swab was 10 cm^2 while rotating the swab clock and anti-clock wise against the selected area at right angles. Collected swabs were aseptically transferred in an ice pack to laboratory, Department of Microbiology, Federal University of Technology, Akure for microbiological analysis. The swab was thoroughly mixed with the peptone water and 1.0 ml was dispensed into petri dishes for the enumeration of total viable bacterial, coliform bacteria, *Staphylococcus* and fungi on NA, EMB and MSA respectively using pour plate method [10]. NA, EMB and MSA plates were incubated at 37°C for 24 hours while PDA plates were incubated at 25°C for 72 hours. The numbers of colony/spore were enumerated as cfu/10m^2 or sfu/10m^2 for bacteria and fungi respectively.

2.4. Indoor and Outdoor air Microflora

Indoor and outdoor air microfloras were determined using passive air sampling technique; the settle plate method using 9 cm diameter petri dishes. The sampling height which approximated to human breathing zone was 1.0 m above the floor. Bacterial, coliform bacteria, *Staphylococcus* and fungi were collected on freshly prepared double strength nutrient agar (NA), eosin methylene blue agar (EMB), manitol salt agar (MSA) and potato dextrose agar (PDA) plate respectively. The plates were exposed for 30 minutes and transported to laboratory, Department of Microbiology, Federal University of Technology, Akure for microbiological analysis. NA, EMB and MSA plates were incubated at 37°C for 24 hours while PDA plates were incubated at 25°C for 72

hours. The numbers of colony/spore were enumerated as cfu/m^3 or sfu/m^3 for bacteria and fungi respectively using the equation below [11 - 12];

$N = 5a \times 10^4 (bt)^{-1}$

Where N = microbial load cfu/m^3,

a = number of colonies per petri dish,

b = dish surface (cm^2);

t = exposure time (minutes)

2.5. Identification and Characterization of Bacterial Isolates

The identification of bacteria was based on morphological characteristics and biochemical tests. Morphological characteristics were observed for each bacteria colony after 24 hour of growth. Conventional biochemical characterization and identification were performed using standard microbiologcal methods [13 - 14].

2.6. Cultural Microscopic Examination of Fungi

Using visible observation and microscope at low power magnification (x40), the parameters such as colony color, characteristics of the submerged hyphae rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed [15]. Small piece of mycelium free of medium were picked using a sterile inoculating loop unto a clean glass slide containing a drop of cotton blue-in-lactophenol and the mycelium was spread properly. The preparation was covered with a clean grease free cover slip and observed under medium power (x100). The observations made were used in identifying the fungi organism [15].

2.7. Standardization of Inoculum (McFarland Turbidity Standard)

Method modified by [14], was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of bacterial cells. In this method, fifty milliliter (50ml) of a 1.175% (wt/vol) dehydrates Barium chloride ($BaCl_2.2H_2O$) solution was added to 99.4ml of 1% (vol/vol) sulfuric acid. McFarland standard tube was then sealed with Paraffin to prevent evaporation and stored in the dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1cm light path. The 0.5 McFarland standards were vigorously agitated before use.

2.8. Antimicrobial Susceptibility Test

Antibiotic susceptibility test of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method. The suspension of the test organism in nutrient broth was matched with 0.5 McFarland turbidity standards to give concentration of 1.5×10^8 CFU/ml, 0.5ml of the suspension was transferred to prepared Mueller-Hinton agar and spread with a sterilized glass spreader, excess suspension was drained. The surface of the agar was allowed to dry and antibiotic disc was aseptically picked and gently placed on top of agar plate by sterile forceps. The inoculated plates were incubated at 37°C for 18 hours, after incubation a clear zone of no growth in the immediate vicinity of an antibiotic disk was measured and recorded as zone of inhibition in millimeter (mm) and interpreted as resistant, susceptible or intermediate according to the method of Committee for Clinical Laboratory Standards [16].

2.9. Ethical Permit

Letter of introduction to school principals was obtained from the Head of Department, Department of Microbiology, Federal University of Technology, Akure, letter was submitted to school principal's office and the verbal consent of both principal and vice principal were sought for before sample collection. All schools requested that the name of the schools be kept confidential.

2.10. Data Analysis

Data was statistically analysed using SPSS version 20, mean microbial load and zones of inhibition were separated using new Duncan's Multiple Range test and significant difference was value at $p \leq 0.05$.

3. Results

3.1. Number and Types of Samples Collected from Toilet and Description of Secondary Schools Where Samples Were Collected in Ondo, Ondo State

Table 1 showed the number and types of swab samples collected from toilet and the description of secondary schools where samples were collected. Total numbers of 160 samples were collected from six (6) secondary schools (A, B, C, D, E and F), A and B are boys school, C and D are girls only while E and F are co-educational schools. Forty (40) samples were collected from each of toilet wall, toilet door handle, air microflora in and outside the toilet. The highest numbers of samples were collected from co-educational schools (E and F).

3.2. Microbiological Assessment of Toilet Wall in Secondary Schools in Ondo, Ondo State

Microbiological assessment of toilet wall in secondary schools in Ondo, Ondo State is shown in Figure 1. The result revealed that there were zero staphylococcal and coliform counts in all the schools however, there was high total bacterial and fungal counts in the school toilet wall compared to staphylococcal and coliform counts. Total bacterial counts ranged from 1.03±0.11 to 4.06±0.02 cfu/10 cm^2, the highest total bacterial counts of toilet wall was observed in school D (girls school) and there were no

significant ($p \leq 0.05$) difference in the bacterial counts of school B (boys school), C (girls school) and F (co-educational school) with the bacterial counts of 3.12±0.04, 3.09±0.21 and 3.16±0.02 cfu/10 cm^2 respectively. There was no significant ($p \leq 0.05$) difference in the fungal counts in the toilet wall of school A (boys school), C (girls school) and F (co-educational school) with the fungal count 7.22±0.06, 8.02±0.02 and 6.09±0.11 sfu/10 cm^2 respectively.

3.3. Microbiological Assessment of Toilet Door Handles in Secondary Schools in Ondo, Ondo State

The results of microbiological assessment of toilet door handles in secondary schools in Ondo, Ondo State is shown in Figure 2. The result revealed that there were significant ($p \leq 0.05$) difference in the total viable bacterial and fungal counts of the toilet door handles except the total bacterial counts of door handle in school C (girls school) and D (girls school). The total bacterial counts ranged from 1.04±0.06 (school E) to 7.04±0.05 (school B) cfu/10 cm^2 while the total fungal counts ranged from 1.02±0.07 to 10.00±0.06 sfu/10 cm^2.

3.4. Microbiological Assessment of Indoor air Microflora of Toilet in Secondary Schools in Ondo, Ondo State

The results of microbiological assessment of indoor air microflora of toilet in secondary schools in Ondo, Ondo State were shown in Figure 3. The result revealed that the total viable bacterial counts ranged from 0.00±0.0 (school E) to 12.51±0.03 $x10^2$ cfu/m^3 (school C) and there was no significant ($p<0.05$) different between the total viable bacteria counts in indoor air microflora of school A, B and C. Staphylococcal counts were observed in school B (2.94±0.41 $x10^2$ cfu/m^3), C (4.11±0.03 $x10^2$ cfu/m^3) and F (5.73±0.01 $x10^2$ cfu/m^3) only and there was no significant ($p<0.05$) different between the counts. The fungal counts were significantly ($p<0.05$) higher than bacteria and staphylococcal counts, the fungal counts ranged from 1.62±0.11 $x10^2$ (school E) to 21.52±0.21 $x10^2$ sfu/m^3 (school C). However, zero coliform counts were noted in indoor air microflora of all the schools.

3.5. Microbiological Assessment of Outdoor Air Microflora of Toilet in Secondary Schools in Ondo, Ondo State

The results of microbiological assessment of outdoor air microflora of toilet in secondary schools in Ondo, Ondo State were shown in Figure 4. Total viable bacterial counts ranged from 1.24±0.03 $x10^2$ (school E) to 40.01±0.21 $x10^2$ cfu/m^3 (school C). There was no significant ($p<0.05$) difference between the staphylococcal counts observed in school B, C and F while the staphylococcal counts of outdoor air microflora of toilets in other schools were zero. However, no coliform was observed but the fungal counts ranged from 3.26±0.12 $x10^2$ (school D) to 29.63±0.04 $x10^2$ sfu/m^3 (school C).

3.6. Occurrence of Microorganisms Isolated from Different Sources in Secondary Schools Toilets in Ondo, Ondo State

The results presented in Table 2 revealed the occurrence of microorganisms isolated from secondary schools toilets in Ondo, Ondo State. It was observed that total number of bacteria and fungi isolated from different sources were twenty nine (29) and twenty four (24) respectively. The most frequently occurred bacterial isolates were *Bacillus subtilis* and *B. alvei* 8 (27.60%) while the mostly occurred fungus was *Rhizopus stolonifer* 10 (41.66%). Also, *Bacillus subtilis* and *B. alvei* were isolated from toilet wall, door handle, indoor and outdoor air microflora, *Staphylococcus aureus* were isolated from indoor and outdoor air microflora only while *S. epidemidis* was isolated from outdoor air microflora only. Also, *Aspergillus niger* and *A. flavus* were mostly isolated from air microflora.

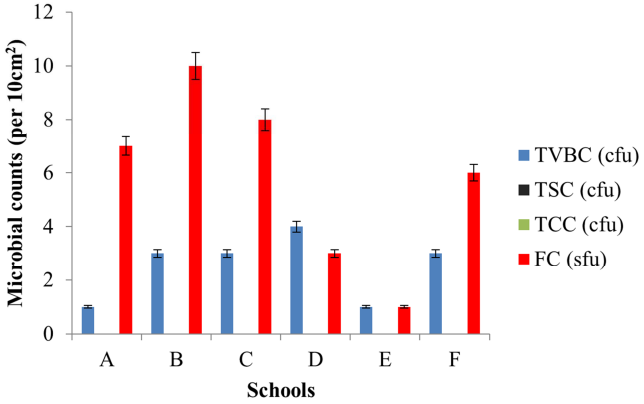

Figure 1. *Microbiological Assessment of Toilet Wall in Secondary Schools in Ondo, Ondo State.*

Keys: TVBC = total viable bacterial counts, TSC = total staphylococcal counts, TCC = total coliform counts, FC = fungal counts

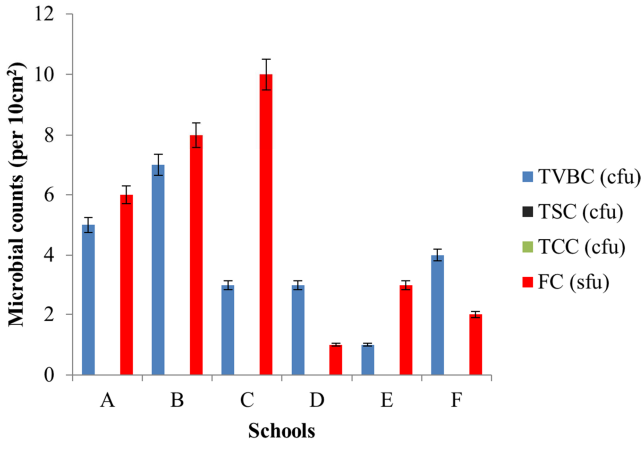

Figure 2. *Microbiological Assessment of Toilet Door Handle in Secondary Schools in Ondo, Ondo State.*

Keys: TVBC = total viable bacterial counts, TSC = total staphylococcal counts, TCC = total coliform counts, FC = fungal counts

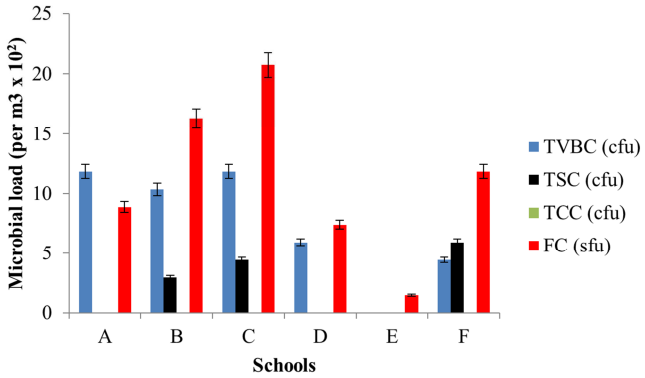

Figure 3. *Microbiological Assessment of Indoor Air Microflora of Toilets in Secondary Schools in Ondo, Ondo State.*

Keys: TVBC = total viable bacterial counts, TSC = total staphylococcal counts, TCC = total coliform counts, FC = fungal counts

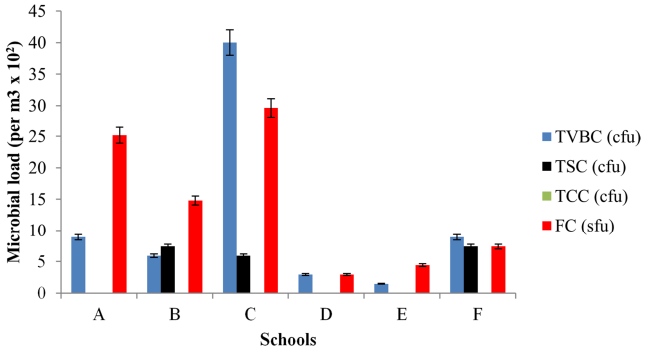

Figure 4. *Microbiological Assessment of Outdoor Air Microflora of Toilets in Secondary Schools in Ondo, Ondo State.*

Keys: TVBC = total viable bacterial counts, TSC = total staphylococcal counts, TCC = total coliform counts, FC = fungal counts

Table 1. *Number and Types of Samples Collected from Toilet and Description of Secondary Schools where Samples were collected in Ondo, Ondo State.*

Schools	Description of school	Types of samples collected				Total number of samples
		Air microflora inside toilet	Air microflora outside toilet	Toilet wall swab	Toilet door handle swab	
A	Boys only	8	8	8	8	32
B	Boys only	6	6	6	6	24
C	Girls only	6	6	6	6	24
D	Girls only	5	5	5	5	20
E	Co-educational	8	8	8	8	32
F	Co-educational	7	7	7	7	28
Total	6	40	40	40	40	160

Table 2. *Occurrence of Microorganisms Isolated from Different Sources in Secondary Schools Toilets in Ondo, Ondo State.*

Microorganisms	Air microflora inside toilet	Air microflora outside toilet	Toilet wall swab	Toilet door handle swab	Total (%)
Bacteria					
Bacillus subtilis	2	2	3	1	8 (27.60)
Staphylococcus aureus	3	1	0	0	4 (13.79)
B. polymyxa	1	2	0	0	3 (10.34)
B. alvei	2	1	3	2	8 (27.60)
B. cereus	1	0	1	1	3 (10.34)
S. epidemidis	0	2	0	0	2 (6.90)
Seratia sp.	0	0	1	0	1 (3.45)
Total	9	8	8	4	29
Fungi					
Aspergillus niger	3	2	0	2	7 (29.17)
A. flavus	3	3	1	0	7 (29.17)
Rhizopus stolonifer	1	4	3	2	10 (41.66)
Total	7	9	4	4	24

3.7. Antibiotics Susceptibility Patterns of Bacteria Isolated from Secondary Schools Toilets in Ondo, Ondo State

The result shown in Table 3 revealed the antibiotics susceptibility patterns of bacteria isolated from secondary schools toilets in Ondo, Ondo State. The result showed that there were significant ($p<0.05$) differences in the susceptibility of all bacterial isolates to antibiotic used. *B. subtilis* (15.03±0.21 mm) and *S. epidemidis* (15.02±0.01 mm) were more susceptible to chloramphenicol, *B. alvei* (12.00±0.00 mm) and *B. polymyxa* (12.10±0.12 mm) were more susceptible to sparfloxacin and amoxicillin respectively. There were no significant ($p<0.05$) differences in the susceptibility of all bacterial isolates to augmentin except *S. aureus* and *Seratia* sp. also, the susceptibility of all the bacterial isolates to septrin and gentamycin were generally low. In Table 4, the result showed that all the bacterial isolates were 100% resistant to sparfloxacin, ciprofloxacin, amoxicillin, augmentin, gentamycin, pefloxacin and ofloxacin.

Table 3. *Antibiotics Susceptibility Patterns of Bacteria Isolated from Secondary Schools Toilets in Ondo, Ondo State.*

Antibiotics	Mean zones of inhibition (mm)						
	B. subtilis	***S. aureus***	***B. polymyxa***	***B. alvei***	***B. cereus***	***S. epidemidis***	***Seratia* sp.**
Chloramphenicol (30µg)	15.03±0.21^{e}	0.00±0.00^{a}	10.00±0.00^{d}	10.01±0.09^{d}	3.00±0.00^{b}	15.02±0.01^{e}	4.01±0.21^{c}
Sparfloxacin (10µg)	5.11±0.14^{b}	10.03±0.41^{c}	10.02±0.10^{c}	12.00±0.00^{d}	10.02±0.02^{c}	6.11±0.11^{b}	3.02±0.22^{a}
Ciprofloxacin (10µg)	10.04±0.01^{c}	3.00±0.00^{a}	10.01±0.10^{c}	8.02±0.04^{c}	2.10±0.02^{a}	10.03±0.12^{c}	6.00±0.00^{b}
Amoxicillin (30µg)	9.10±0.01^{c}	7.02±0.12^{b}	12.10±0.12^{d}	10.10±0.03^{c}	7.01±0.12^{b}	5.00±0.00^{a}	4.10±0.03^{a}
Augmentin (30µg)	10.00±0.00^{b}	5.10±0.11^{a}	10.03±0.30^{b}	10.21±0.01^{b}	10.03±0.02^{b}	10.01±0.05^{b}	6.00±0.00^{a}
Gentamycin (10µg)	5.01±0.12^{a}	5.01±0.20^{a}	5.00±0.00^{a}	8.01±0.10^{b}	10.00±0.00^{c}	9.02±0.03^{c}	7.11±0.12^{b}
Pefloxacin (10µg)	5.00±0.00^{a}	10.00±0.00^{c}	9.00±0.00^{b}	6.00±0.00^{a}	12.00±0.00^{d}	12.31±0.15^{d}	10.00±0.00^{c}
Ofloxacin (10µg)	8.04±0.10^{a}	6.03±0.31^{a}	8.04±0.22^{a}	9.02±0.03^{ab}	8.00±0.00^{a}	10.11±0.02^{b}	7.00±0.00^{a}
Streptomycin (10µg)	12.21±0.01^{d}	9.02±0.10^{c}	2.00±0.00^{a}	10.10±0.11^{c}	2.01±0.02^{a}	10.00±0.00^{c}	4.12±0.02^{b}
Septrin (25µg)	5.03±0.31^{b}	8.20±0.21^{cd}	2.00±0.00^{a}	10.11±0.21^{d}	2.12±0.11^{a}	5.00±0.00^{b}	2.00±0.00^{a}

Values are presented as mean ± standard error, values carrying same superscript along same row are not different significantly different at $p<0.05$ according to new Duncan's Multiple Range test

Table 4. *Interpretation of zones of inhibition of Antibiotics used against Bacteria Isolated from Secondary Schools Toilets in Ondo, Ondo State.*

Antibiotics	***B. subtilis***	***S. aureus***	***B. polymyxa***	***B. alvei***	***B. cereus***	***S. epidemidis***	***Seratia* sp.**	**% Resistance**
Chloramphenicol	I	R	R	R	R	I	R	71.43
Sparfloxacin	R	R	R	R	R	R	R	100
Ciprofloxacin	R	R	R	R	R	R	R	100
Amoxicillin	R	R	R	R	R	R	R	100
Augmentin	R	R	R	R	R	R	R	100
Gentamycin	R	R	R	R	R	R	R	100
Pefloxacin	R	R	R	R	R	R	R	100
Ofloxacin	R	R	R	R	R	R	R	100
Streptomycin	I	R	R	R	R	R	R	85.71
Septrin	R	R	R	I	R	R	R	85.71

Key: I = intermediate, R = resistance

4. Discussion

Toilets are one of the school facilities frequently used by students and are located indoor. Therefore, maintaining clean toilets is essential in order to keep it hygienic and sanitarily conducive for usage [17]. During this study, samples were collected from toilet wall, toilet door handle, air microflora in and outside the toilet at six (6) secondary schools of which A and B are boys school, C and D are girls only while E and F are co-educational schools. All the schools visited had students' toilet, this could be because toilet facility is an essential part of learning. Transmission of infectious agent could be direct or indirect, indirect transmission can occur through contact with contaminated surfaces and objects or through insect vectors. Toilet wall and door handle could serve as fomite in the indirect transmission of infectious diseases, while toilet air could enhance the direct transmission of infectious microorganisms through droplets containing microbial agents from toilet and individuals, which when inhaled by another can lead to an infection [18].

The result of microbial assessment of secondary schools toilet in Ondo, Ondo State revealed that all the schools visited had students' toilet, toilet wall and door handle at secondary schools had zero staphylococcal and coliform counts in all the schools however, there was higher total bacterial and fungal counts in toilet door handle than wall per square meter. Total viable bacterial and staphylococcal counts of toilet outdoor air microflora were higher than the indoor as well as the air mycoflora.

Microbiological assessment of toilet wall and door handle at secondary schools in Ondo, Ondo State revealed that there were zero staphylococcal and coliform counts in all the schools however, there was higher total bacterial and fungal counts in toilet door handle than wall per square meter. Toilet wall and door handles are usually in direct contact with human skin, the high microbial load observed in door handle could be due to continual touch by the users and it could also be due to the type of the metal used for the door handle. Gilberto [19], reported high microbial diversity on the surface that were constantly in contact with human skin in public restroom which also correspond to the findings of this study.

One of the indoor and outdoor air pollutants are microorganisms. The presence of these in air is termed bioaerosols or microbial air pollutant; they decrease air quality and affect human health. The result obtained from this study revealed that the total viable bacterial and staphylococcal counts of toilet outdoor microflora were higher than the indoor microflora, as well as the air mycofloral. The air microflora could have originated from feacal droppings and discharged of men, water splash during washing and flourishing of toilet, and from contaminated environment. They can exist in air as an individual entity or create aggregates of biological structures. Some microbial cells produce pigments or mucous halo to protect them from harmful effect of ultraviolet radiation there by posing a great risk to public health. Spore formation is one of most widely used strategies adopted by many microbes to survive in air. Bioaerosol, are easily transferred by winds and air currents

from one ecosystem to another, making them an important vehicle for the spread of infectious microorganisms [20 - 21]. The bacterial counts observed in this study were lower compared with what was obtained by Ohagim [18], who reported microbial load of indoor air in public toilets at car parks, this differences could be attributed to the high influx of people at the car parks. Also, the higher microbial load observed in the outdoor than indoor air could be that the source of contamination of outdoor air microflora is not limited to only toilet but environment and animals that are around the toilet.

It has been documented that human population and activities affect the concentration of microoragnisms which are released through brisk movement, talking, coughing, sneezing and other actions such as cleaning of toilet [15, 18]. The microorganisms isolated from toilet were; *Bacillus subtilis, Staphylococcus aureus, B. polymyxa, B. alvei, B. cereus, S. epidemidis, Seratia* sp. *Aspergillus niger, A. flavus* and *Rhizopus stolonifer* in which the most frequently occurred bacterial were *Bacillus subtilis* and *B. alvei* while that of fungus was *Rhizopus stolonifer.* Also, *Bacillus subtilis* and *B. alvei* were isolated from toilet wall, door handle, indoor and outdoor air microflora, *Staphylococcus aureus* were isolated from indoor and outdoor air microflora only while *S. epidemidis* was isolated from outdoor air microflora only. Also, *Aspergillus niger* and *A. flavus* were mostly isolated from air microflora. The presences of these microorganisms as observed in this study have been reported in other studies [18, 22]. The presence of *Staphylococcus aureus* and *S. epidemidis* could be attributed to the ubiquitous nature of the organism in human skin. *Bacillus cereus* isolated from the toilets has also been linked to food poisoning which could cause a serious problem when proper hygienic standards are not maintained by toilet users and the presence of *Aspergillus* spp. could result in aspergilloma if the spores are inhaled by immunocompromised toilet user.

The low susceptibility of *Staphylococcus aureus* isolated from toilet to amoxicillin, augmentin, chloramphenicol and ciprofloxacin could be that the *Staphylococcus aureus* has developed resistance to these antibiotics and *Staphylococcus aureus* has been associated with Community Acquired Methicillin Resistant *Staphylococcus aureus* that can cause urinary tract infections, skin infections and food poisoning [18].

Bacteria high resistance to β-lactam antibiotic, tetracycline, cotrimoxazole and chloramphenicol observed in this study have been reported by [23 - 24]. In addition, the high frequency of resistance to many antibiotics especially β-lactam and β-lactam inhibitor antibiotics may suggest that the bacterial isolates were Extended-spectrum β-lactamases (ESBLs) producer because ESBL-producing bacteria often exhibit multidrug resistance [25]. However, an unfavorable factor in this study is to be seen in the increased frequency of ofloxacin and ciprofloxacin resistant bacteria, [24, 26, 27] described 100% susceptibility to fluoroquinolones. High resistance to fluoroquinilones drug may result to over use of the drug in treating infection, since the drug is highly toxic at high dose hence bacteria resistance may expose man to dose toxicity.

In conclusion, lack of cleanliness or poor toilet hygiene and usage represents a very specific risk of passing on infection and disease which can cause short term illness and absence from school. In others it contributes to conditions that will persist beyond school and may be manifested in more serious forms in later life.

5. Conclusion

The microbial assessment of secondary schools toilet in Ondo, Ondo State revealed that all the schools visited had students' toilet. Toilet wall and door handle at secondary schools had zero staphylococcal and coliform counts in all the schools however, there was higher total bacterial and fungal counts in toilet door handle than wall. Total viable bacterial and staphylococcal counts of toilet outdoor air microflora were higher than the indoor as well as the air mycoflora. The microorganisms isolated from toilet were; *Bacillus subtilis, Staphylococcus aureus, B. polymyxa, B. alvei, B. cereus, S. epidemidis, Seratia* sp. *Aspergillus niger, A. flavus* and *Rhizopus stolonifer* in which the most frequently occurred bacterial were *Bacillus subtilis* and *B. alvei* while that of fungus was *Rhizopus stolonifer. Aspergillus niger* and *A. flavus* were mostly isolated from air microflora. Also, *Staphylococcus aureus* isolated from toilet had low susceptibility to amoxicillin, augmentin, chloramphenicol and ciprofloxacin. The low susceptibility of *Staphylococcus aureus* to antibiotics could pose a serious threat to secondary school student therefore awareness and encouragement of good practice, sanitation and ensuring appropriate standards are maintained, can go a long way towards improving the health, confidence and self-esteem of students in education settings.

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