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EVALUATION OF BACTERIOLOGICAL CONTAMINATION OF PALMS OF PUPILS IN THE UNIVERSITY OF BENIN TEACHING HOSPITAL STAFF SCHOOL, BENIN CITY, NIGERIA

***Sagay, M. O.[1,3], Idemudia, I. B.[1,3], Imarhiagbe, E. E. [2,3] and Ekhaise, F. O.[1,3]**
[1]Department of Microbiology, University of Benin, Benin City, Nigeria
[2]Department of Environmental Management and Toxicology, University of Benin, Benin City, Nigeria
[3]Applied Environmental Bioscience and Public Health Research Group, Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin, Benin City, Nigeria
Corresponding mail: *marthasagay@gmail.com / +23407064239956 +2349053647512

ABSTRACT
Hands are the chief organs for physical manipulation of the environment. As a paired organ, the hand is controlled by the opposing brain hemisphere and enables one to all manner of conscious and unconscious activities. The hand serves as a medium for the propagation of microorganism from place to place and from one person to another. A well-structured questionnaire bothering on age, sex and parental social status of the pupils was administered to three hundred and sixty (360) respondents recruited for the purpose of this study in University of Benin Teaching Hospital Staff School (UBTHSS). The mean heterotrophic Bacterial Count ranged from 2.10±0.19 x 10^6 to 3.77±0.26 x 10^6 cfu/cm^2. Total coliform count ranged from 0.88±0.08 x 10^6 to 2.08±0.18 x 10^6. November recorded the highest heterotrophic and coliform counts ranging from 2.70±0.24 x 10^6 to 3.77±0.26 x 10^6 cfu/cm^2 and 1.14±0.11 x 10^6 to 2.08±0.18 x 10^6 cfu/cm^2. The male pupils had the highest Heterotrophic and coliform counts of 4.132 x10^6 cfu/cm^2 and 2.273x10^6 cfu/cm^2 against the female of 1.433x10^6 cfu/cm^2 and 0.667x10^6 cfu/cm^2. The mean heterotrophic bacterial counts of parental status ranged from 2.78±0.10 x 10^6 to 2.83±0.08 cfu/cm^2 and total coliform counts of 1.24±0.05 x 10^6 to1.35±0.15 x 10^6.The mean Heterotrophic barial and coliform counts of age ranged from 2.51±0.74 x 10^6 to 3.38±0.15 x 10^6 cfu/cm^2 and 0.59 -3.43 x 10^6 to 0.35 - 2.62 x 10^6 cfu/cm^2. A total of ten (10) Gram positive and negative bacterial were isolated in this study. The bacterial isolates with the highest frequency of occurrence were staphylococcus aureus and E.coli (12.00%), E.coli and the least frequency was salmonella enterica (7.3%). The antimicrobial sensitivity pattern showed that the bacteria isolates exhibits varying degree of resistance to the antibiotics before and after curing. The isolates were found to harbor plasmid of different sizes but below 1000bp.There was no plasmid after curing.
Keywords: Benin City, Hand swab, Microbial species, Pupils, University

INTRODUCTION

The normal flora of the human skin provides good examples of various microenvironments. The composition of the dermal micro flora varies from site to site according to the character of the microenvironment (Grice and Segre, 2011). A different bacterial flora characterizes each of the three regions of the skin: which are face, hands, and legs. The human skin inhabits different micro-organisms mostly bacteria (Todar, 2007; Grice *et al*., 2009).

The number of bacteria on an individual's hand colonization probably depends partly on the exposure of skin to a particular environment and partly on the innate and species-specific bactericidal activity in hands and skin. In addition to resident skin flora, the greatest influence on the density of microbial population on the skin and hand is humidity (Butmarc *et al.*, 2004). Human skin may be another potential reservoir for the microorganisms implicated in infectious diseases. All human beings, including healthy individuals, routinely touch their skin and if skin harbors different or more abundant pathogens, then hair may impact health risk and act as a reservoir for bacteria of public health significance (Roth and James, 1989).

Poor hygiene by handlers can also lead to transmission of bacteria (Todar, 2007). The number of bacteria on an individual's hand probably depends partly on the exposure of the hand to a particular environment and partly on the innate and specific bactericidal activity of the hand (Cole *et al*., 2001). Human hands can be contaminated by bacteria through contact with contaminated soil, vegetable and water. Also contaminated hands play a major role in faecal-oral transmission of diseases.

The pupils can be exposed to infection if they do not properly wash their hands before taking food as washing of hands is believed to reduce infection transmission by washing off potential microorganisms and also by removing dirt which could harbor the microorganisms and allow their survival for longer period of time. In the 1840s, Dr Semmelweis Ignaz gave the clear evidence of hand hygiene when working in the great hospital of Vienna, pointing the link between infection and unclean hands. Systemic reviews have pointed the effectiveness of hand washing in reduction of diarrhea and acute respiratory illness, which are the two major childhood killer diseases. Studies around the world including some parts of Nigeria has proved that the hand contamination of bacteria has health implications on human health such as allergies, infections and other disease conditions and sanitary practice has a contributing factor to transmission and proliferation of bacteria, especially amongst children who naturally are less aware of the dangers of zero hygiene (Dongre *et al.,* 2007).

In the indoor environment, we encounter microorganisms on virtually every surface we touch, and this frequent exposure to indoor microbes carries with it the potential for disease transmission, as well as interactions with our own commensal microbiome (Meadow *et al*., 2014). Culture-based studies of human indoor environments have shown that significant levels of bacteria are present in seemingly innocuous areas such as office buildings, residential homes, shopping centers, children's schools and daycare centers (Lee *et al*., 2007).

Disinfection of surfaces is also necessary to prevent infection from transient microbes especially surfaces that the hand comes in contact with frequently (Walsh, 2000). Promotion of improved hand hygiene has been recognized as an important public health measure but it is unclear how much hand hygiene is required to interrupt transmission of diarrhoea pathogens (John *et al*., 2009). In particular it has not been conclusively shown whether use of soap is essential to remove pathogens from hands. Recent hygiene promotion campaigns especially in low income settings have not been unanimous in recommending soap use (Ejemot *et al*., 2008).

MATERIALS AND METHODS

Study Site

This research was conducted in the University of Benin Teaching Hospital staff school, Benin City, Nigeria which lies on latitude $6^{o}20^{l}$ 1.32^{ll} N and Longitude $5^{o}36^{l}$ 0.53"E.

Sample Population

Samples were collected from the hands of three hundred and sixty primary school pupils between the ages of 2 and 12years in all classes and analyzed using standard microbiological methods.

Sample Collection

Samples were collected using sterile cotton swabs at noon. Each swab stick was returned into its tube-like container after use, labelled and immediately transported to the microbiology research laboratory (Cheesbrough, 2000.)

Preparation Of Samples And Culturing

Five millilitres (5mls) of cooled sterile peptone water was dispensed aseptically into swab stick container before allowing it to stand overnight $37^{o}C$. Thereafter 0.1ml of the samples was inoculated onto nutrient agar (Oxoid), MacConkey agar (Oxoid) Muller Hilton agar and Eosine methylene blue agar. Inoculated plates were incubated at $37^{o}C$ for 24hours

After incubation bacterial colonies were enumerated using the formulae:

$$\text{Total number of microorganisms} = \frac{\text{Number of microorganisms}}{\text{Area swabbed}}$$

Where;

$$\text{Number of microorganisms} = \frac{\text{number of colonies}}{\text{volume plated(ml)x dilution factor}}$$

Discrete colonies were sub-cultured on nutrient agar for proper preliminary identification as described by Gui *et al*. (2014); Cheesebrough, (2000).

Identification of the Bacterial Isolates

Pure bacterial isolates were characterized and identified based on cultural, morphological and biochemical tests following standard microbiology procedure as described by Collins *et al* (1989); Cheesbrough (2010).

Antibiotics Sensitivity Test of the Bacterial Isolates

Antibiotic sensitivity pattern of the identified isolate was carried out using disc diffusion method with standard antibiotic disc. Identified bacteria culture (0.1 mL) in nutrient broth was inoculated on solidified nutrient agar and allowed to dry for 5 mins, then antibiotic discs was placed. The plates was incubated at 37^{o}C for 24hrs and the zone of inhibition was measured to the nearest millimetre Bauer *et al.,* (1966); Cheesebrough (2000); CLSI (2012).

Plasmid Profile and Curing Of Bacterial Isolates

Bacteria that showed resistance to the test antibiotics were further subjected to plasmid extraction. An overnight cultured was of the isolates was prepared. The antibiotic resistance plasmids in the culture were isolated using alkali e lyses protocol. The resistant isolates were cured with acridine orange to ascertain the involvement of plasmids in antibiotics resistance determination. Small inoculums of 100 to 300 cfu/ml was added to acridine orange up to 0.25 mg/ml and incubated at 37^{0} C for 24 hours. Cultures containing the highest concentration of acridine orange in which growth was clearly visible were diluted and spread on nutrient agar plates with appropriate antibiotics for susceptibility testing. As described by Bimboim and Doly (1979): CLSI (2013): Raghada *et al* (2017).

Statistical Analysis

The data generated were analyzed by one –way ANOVA (analysis of variance) using Genstat 12th edition analytical package as well as Non-Parametric T.test. Differences in mean were compared by Duncan's multiple range tests. Tables and charts were drawn using Microsoft Excel version 2007 as described by Ogbeibu (2015).

RESULTS

The Total Heterotrophic Bacterial Counts (THBC) of the samples range from 2.07 x 10^6±0.20 to 3.77 x 10^6±0.26cfu/cm^2. Primary one having the highest counts and primary four having the least counts, also showing significant difference between means across the months and the class (Table 1).

The Total Coliform counts ranges between 0.88 x10^6±0.08 to 2.08x10^6±0.18cfu/cm^2. They were differences between means across the classes and months, there was no significant difference. ($p< 0.05$) (Table 2).

The influence of the age of the pupils shows that the Total Heterotrophic Bacteria Counts (THBC) and Total Coliform Counts of the School Pupils varied. Children within the age of 2-5 had the highest THBC (4.38x10^6±0.25cfu/cm^2) and TCC (1.99x10^6±0.12cfu/cm^2), while Children within age 9-12 had the least THBC (3.07x10^6±0.16cfu/cm^2) and total Coliform counts (1.26±0.07cfu/cm^2) respectively. There was significant difference between Total Heterotrophic Bacteria Counts (THBC) and Total Coliform Counts of hands of school pupil ($P<0.05$) (Table 3).

The Total Bacterial and coliform Counts of pupils based on gender is displayed in figures 1 and 2. The males were observed to have the highest counts of 4.132 x 10^6cfu.cm^2 and 2.27 x 10^6cfu.cm^2 respectively, while females had the least bacterial count of 1.433 x 10^6cfu.cm^2 and 0.875 x 10^6cfu.cm^2 respectively.

Figures 3 and 4 shows the total heterotrophic bacterial counts and total coliform counts of the Pupils based on their Parental Status shows that Children of civil servants had the highest THBC (91.75 x 10^6 cfu/cm^2, while children of politicians had the highest coliform counts of 94.55 x 10^6 cfu/cm^2).

Tables 4 and 5 shows the total bacterial and coliform counts between classes, while there was nosignificance difference recorded for the THBC they was a significant difference in the TCC.

Total Percentage frequency of Bacterial isolates in each month reveals that *Escherichia coli* was the most prevalent bacteria in the month October with a prevalence rate of 4.2%, *Salmonella enterica* was the least prevalent bacteria with prevalence rate of 2.4%, *Staphylococcus aureus* was the most prevalent bacteria in the month of November with a prevalence rate of 4.4%, while *Salmonella enterica* was the least prevalent bacteria with prevalence rate of 2.3% . The most prevalent bacteria isolate in the month of December was *E.coli* with prevalence rate of 3.9%, (Table 6)

Table 7 shows the cumulative frequency of occurrence of Bacterial Isolates shows that *Staphylococcus aureus* and *E. coli* were the most occurred bacteria with occurrence rate of 12%, while *Salmonella enterica* was the least occurred bacteria with occurrence rate of 7.3% respectively.

In the Antibiogram Profile of Bacterial Isolates. *Bacillus mycoides* was the most resistant bacteria to the tested antibiotics with multiple antibiotic resistance index of 0.6, followed by *Staphylococcus aureus* with multiple antibiotic resistance index of 0.5 respectively (Table 8).

The electrophoretic separation plasmid profile DNAs of isolates before curing revealed detectable plasmid profiles in the bacterial isolates with band size ranging from 100 to 1000 bp. Some isolates possessed single-sized plasmids while one isolate had multiple plasmids. High antibiotic resistance was detected in isolates with high molecular weight plasmids. This indicates that the multi-drug resistance exhibited by these isolates was plasmid-mediated while the rest are non-plasmid encoded isolates (Plate 1).

Zones of Inhibition for the sensitivity testing of bacterial isolates against antibiotics after curing. Amoxillin, septrin and Tarivid had the highest zone of inhibiton (23mm) followed by Sparfloxacin and Perfloxacin (22mm), Augumentin and Sparfloxacin against *Bacillus mycoides* (21mm), Sparfloxacin (21mm) against *Bacillus* sp and Streptomycin (21mm) against *Citrobacter Freundii* . Augumentin and Tarivid showed the least zone of inhibition against *Citrobacter freundii* (2mm) followed by Chloramphenical and Ciprofloxacin (3mm) against *Salmonella enteric and Corynebacterium Kutsceri. Pseudomonas, bacillus* and *staphylococcus* all had the highest multiple antibiotic index of 0.3 respectively

Plasmid profile of the bacterial isolates after curing, as seen the plasmids were totally cured, as they was no presence of any visible bands (Plate 2).

Table 1: Total Heterotrophic Bacterial count (x10^{6}cfu/cm^2) (UBTHSS)

Primary	October,2017	November,2017	December,2017
1	2.93 ± 0.20^{Aa}	3.77 ± 0.26^{Ab}	3.45 ± 0.27^{ab}
2	2.89 ± 0.19^{Aa}	3.72 ± 0.24^{Ab}	3.10 ± 0.20^{a}
3	2.33 ± 0.18^{Ba}	3.00 ± 0.24^{Bb}	2.73 ± 0.23^{ab}
4	2.07 ± 0.20^{Ba}	2.66 ± 0.26^{Ba}	2.33 ± 0.24^{a}
5	2.29 ± 0.16^{Ba}	2.94 ± 0.21^{Bb}	2.34 ± 0.20^{a}
6	2.10 ± 0.19^{Ba}	2.70 ± 0.24^{Ba}	2.47 ± 0.25^{a}
p-value	0.000	0.002	0.003

Table 2: Total coliform counts (x10^{6}cfu/cm^2) (UBTHSS)

Primary	October,2017	November,2017	December,2017
1	1.62 ± 0.14^{Aa}	2.08 ± 0.18^{Aa}	1.94 ± 0.18^{Aa}
2	1.26 ± 0.80^{Ba}	1.62 ± 0.10^{Bb}	1.36 ± 0.10^{Ba}
3	1.03 ± 0.08^{Ba}	1.33 ± 0.11^{Ba}	1.24 ± 0.12^{Ba}
4	0.91 ± 0.09^{Ca}	1.17 ± 0.11^{Ca}	1.03 ± 0.11^{Ba}
5	0.96 ± 0.07^{Ca}	1.23 ± 0.08^{Cb}	1.01 ± 0.10^{Ba}
6	0.88 ± 0.08^{Ca}	1.14 ± 0.11^{Ca}	1.05 ± 0.11^{Ba}
p-value	0.000	0.000	0.000

Table 3: Total Heterotrophic Bacterial Counts (x10^{6}cfu/m^2) and Total Coliform Counts (x10^{6}cfu/m^2) of School Pupils based on their age.

	Total Heterotrophic Bacterial Counts		Total Coliform Counts
Age (years)	Sample size(n)	Mean THBC (10^6 cfu/cm^2)	Mean TCC (10^6 cfu/cm^2)
2-5years	60	3.38 ± 0.15^{a}	1.89 ± 0.10^{a}
6-8 years	125	2.94 ± 0.09^{b}	1.27 ± 0.04^{b}
9-12 years	175	2.51 ± 0.74^{c}	1.09 ± 0.03^{c}

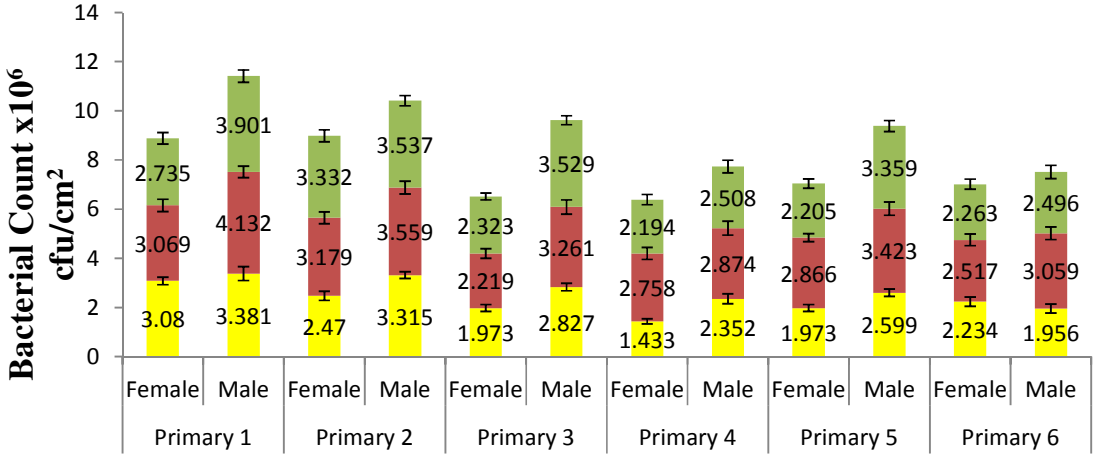

Fig 1: Influence of gender on the total heterotrophic bacterial counts between October, 2017 and December, 2017. (UBTHSS)

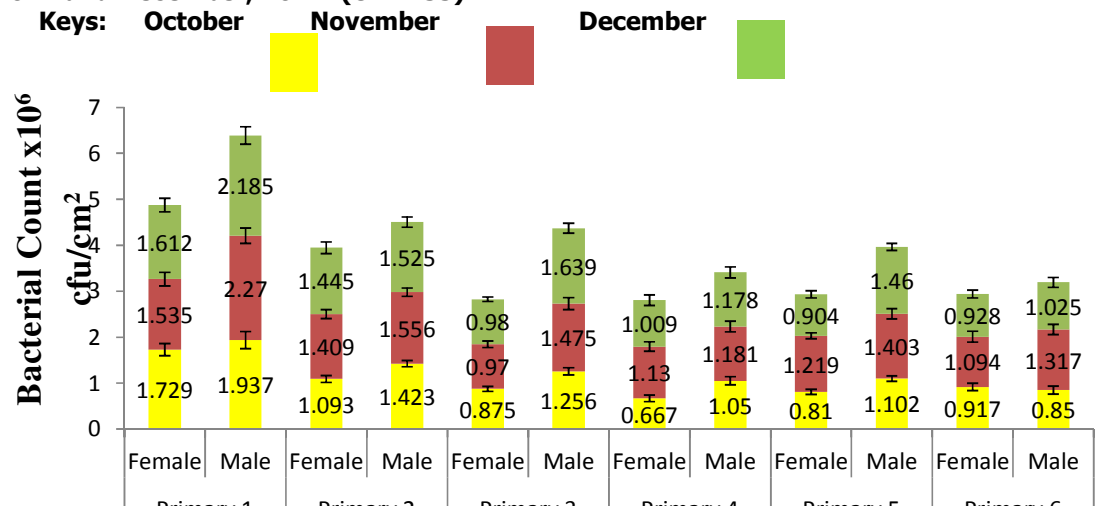

Figure 2: Influence of gender on the total coliform counts between October, 2017 and December, 2017. (UBTHSS).

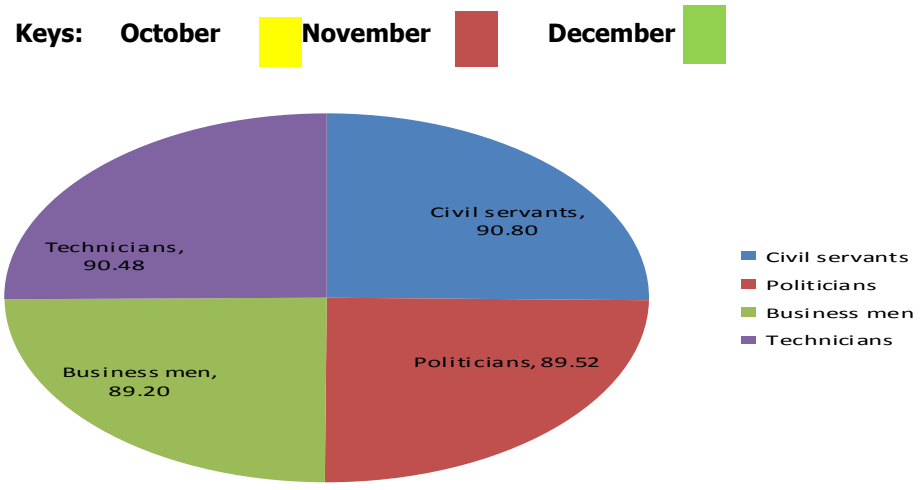

Figure 3: Total heterotrophic bacterial counts of the Influence of parental status in the hygiene state of the pupils in UBTHSS.

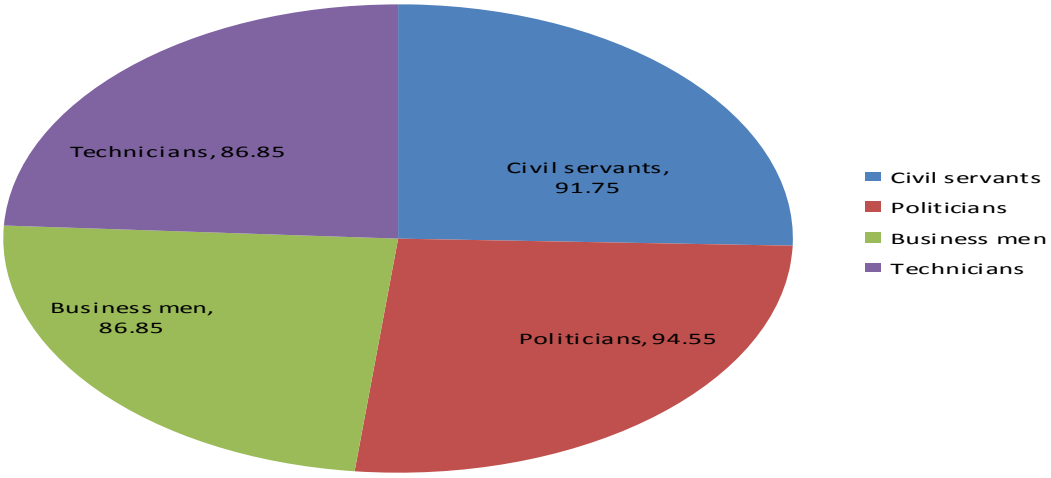

Figure 4: Total coliform counts of the Influence of parental status in the hygiene state of the pupils in UBTHSS.

Table 4: Class by class comparison of Total Heterotrophic Bacteria Count (x10^6 cfu/cm^2)

	University of Benin Teaching Hospital staff School (UBTHSS)					
Classes	Primary 1	Primary 2	Primary 3	Primary 4	Primary 5	Primary 6
Sample Size(n)	60	60	60	60	60	60
Range	0.96 - 5.62	1.52 - 5.59	1.20 - 4.95	0.85 - 4.94	0.96 - 5.26	1.07 - 5.36
$\bar{X}$ ± S.E	3.35±0.15	3.24±0.13	2.69±0.13	2.35±0.14	2.52±0.11	2.42±0.13
***p*-value**	**0.002**	**0.002**	**0.014**	**0.013**	**0.003**	**0.055**

Table 5: Class by class comparison of Total coliform counts (x 10^6 cfu/cm^2)

	University of Benin Teaching Hospital Staff School (UBTHSS)					
Classes	Primary 1	Primary 2	Primary 3	Primary 4	Primary 5	Primary 6
Sample Size(n)	60	60	60	60	60	60
Range	0.59 - 3.43	0.62 - 2.45	0.49 - 2.62	0.35 - 2.03	0.40 - 2.16	0.35 - 2.20
$\bar{X}$ ± S.E	1.88±0.10	1.41±0.06	1.19±0.06	1.04±0.06	1.07±0.05	1.02±0.06
***p*-value**	**0.124**	**0.006**	**0.059**	**0.067**	**0.005**	**0.100**

Table 6: Total Percentage frequency of Bacterial isolates sampled in each month.

MONTHS	**OCTOBER, 2017**	**NOVEMBER, 2017**	**DECEMBER, 2017**
Pseudomonas aureginosa	3.6	2.9	3.8
Klebsiella pneumoniae	3.8	3.2	3.3
Aeromonas verontii	3.2	3.1	3.1
Bacillus mycoides	3.6	3.4	3.8
Corynebacterium kutsceri	3.0	2.8	3.0
Escherichia coli	4.2	3.9	3.9
Citrobacter freundii	3.1	3.1	2.9
Micrococcus.	3.4	3.3	2.9
Salmonella enterica	2.4	2.3	2.5
Staphylococcus aureus	4.1	4.4	3.6
Total	34.4	32.4	32.8

Table 7: Cumulative frequency (%) of occurrence of Bacterial Isolates sampled throughout the duration.

Isolates	percentage
Pseudomonas aureginosa	10.4
Klebsiella pneumonia	10.3
Aeromonas verontii	9.5
Bacillus mycoides	10.7
Corynebacterium kutsceri	8.9
Escherichia coli	12
Citrobacter freundii	9.2
Micrococcus spp.	9.7
Salmonella enterica	7.3
Staphylococcus aureus	12
TOTAL	100

Table 8: Antibiogram Profile of Bacterial Isolates for University of Benin Teaching Hospital staff school (UBTHSS).

Microorganism	AU	GEN	PEF	TRV	S	SEP	CH	SP	CPX	AM	MARI
Pseudomonas aureginosa	R	R	R	S	I	S	S	R	I	S	0.4
Klebsiella pneumonia	R	I	S	I	R	S	R	S	S	R	0.4
Aeromonas verontii	R	R	S	S	S	S	S	I	S	S	0.2
Bacillus mycoides	I	R	R	I	R	R	I	R	R	S	0.6
Corynebacterium kutsceri	S	R	S	S	I	S	R	S	S	S	0.2
Escherichia coli	R	I	I	S	I	S	S	S	S	R	0.2
Citrobacter freundii	S	R	I	R	S	R	S	S	R	S	0.4
Micrococcus sp.	S	R	R	I	S	R	I	S	S	R	0.4
Salmonella enterica	I	S	R	S	I	S	R	R	I	S	0.3
Staphylococcus aureus	R	S	R	S	R	I	R	S	R	I	0.5

KEY: AU-Augumentin, GEN- Gentamycin, SP-Sparfloxacin, AM- Amoxillin, CH-Chloramphenicol, PEF-Perfloxacin, S-Streptomycin, CPX-Ciprofloxacin, TRV-Tarivid, SEP-Septrin. S = ≥ 18mm I = 15 – 17mm R = ≤ 14mm MARI = Multiple Antibiotic Resistance index. MAR index ≥ 0.2 (public health significance).

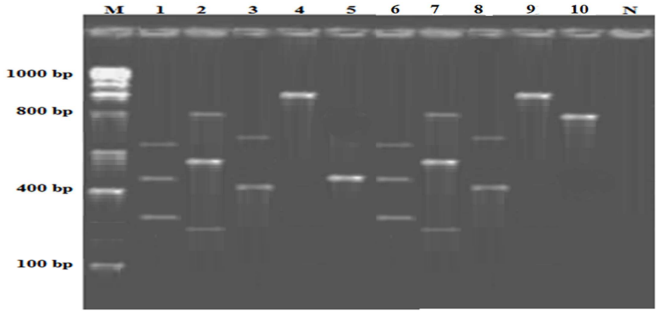

Plate 1: Electrophoretic separation plasmid profile DNAs of isolates before curing.
Lane 1: *Citrobacter freundii, Lane 2: Salmonella enterica, Lane 3: Micrococcus* sp.
Lane 4: Bacillus mycoides, Lane 5: Staphylococcus aureus. ,Lane 6: Klebsiella pnuemoniae,
Lane7: Corynebacterium kutsceri, Lane 8: Aeromonas verontii,
Lane 9: Pseudomonas aeruginosa, Lane 10: Escherichia coli
Lane M: Marker Lane N: Control

TABLE 9: **Inhibition zones (diameter in mm) for the sensitivity testing of bacterial isolates against antibiotics after curing. (UNIVERSITY OF BENIN TEACHING HOSPITAL STAFF SCHOOL)**

KEY: AU-Augumentin, GEN- Gentamycin, SP-Sparfloxacin, AM- Amoxillin, CH-Chloramphenicol, PEF-Perfloxacin, S-Streptomycin, CPX-Ciproflxacin, TRV-Tarivid, SEP-Septrin, **S = ≥** 18mm I = 15 – 17mm, R = ≤ 14mm

Microorganism	**AU**	**GEN**	**PEF**	**TRV**	**S**	**SEP**	**CH**	**SP**	**CPX**	**AM**	**MARI**
Pseudomonas aureginosa	I	R	I	R	S	S	R	S	I	S	0.3
Klebsiella pneumonia	R	S	S	S	I	S	R	S	S	S	0.2
Aeromonas verontii	S	R	S	S	S	I	S	S	R	S	0.1
Bacillus mycoides	S	S	I	S	S	S	S	S	R	S	0.3
Corynebacterium kutsceri	S	R	S	S	S	S	R	S	S	S	0.1
Escherichia coli Citrobacter	I	S	I	S	R	S	S	S	S	S	0.1
Freundii	R	R	S	S	R	S	I	S	R	S	0.2
Micrococcus	S	S	S	S	S	S	I	S	S	R	0.1

Plate 2: Electrophoretic separation plasmid profile DNAs of isolates after curing.
Lane 1: *Citrobacter freundii,* Lane 2: *Salmonella enterica,* Lane 3: *Micrococcus* sp.
Lane 4*: Bacillus mycoides,* Lane 5*: Staphylococcus aureus*, Lane 6*: Klebsiella pnuemoniae,*
Lane 7*: Corynebacterium kutsceri* Lane 8*: Aeromonas verontii,*
Lane 9*: Pseudomonas aeruginosa,* Lane 10*: Escherichia coli*
Lane M: Marker
Lane N: Control.

DISCUSSION

In this study three hundred and sixty (360) pupils were sampled and pathogenic microorganisms were found to be present on their hands. Findings from this study also showed the presence of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Aeromonas verontii, Bacillus mycoides, Corynebacterium kutsceri, Escherichia coli, Citrobacter freundii, Micrococcus* sp., *Salmonella enterica* and *Staphylococcus aureus*. These findings are in consonance with that of Bouffard *et al*. (2008) who isolated and identified *Escherichia coli, Citrobacter* sp, *Enterobacter* sp, *Klebsiella* sp, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus subtilis* from hands of various categories of individuals in the Federal University of Technology, Akure.

Leading to the conclusion that human hands can be contaminated with bacteria through contact with contaminated soil, food, water, handshake etc. Studies of the indoor environments have revealed the presence of a significant level of bacteria (Lee *et al*., 2007).Children within 6-12 of age really loves to play and to buy snacks at school and at the area around the school, therefore, there are so many health challenges that could affect elementary school aged children because they have immunity that are more vulnerable than adults (Bennik *et al.,* 2008).

The rate of coliform spread among the pupils could be as result of the location of the restroom as well as their hand washing habit after using the restroom, as the pupils were observed to wash their hands in a common bowl and as such encourages the transfer of pathogens as also reported by Ejemot *et al*., 2008. The highest counts recorded amongst younger children is also in line with the work of Oyibo (2012), who reported that kids between the ages of 2-6 years are more susceptible compared to older kids within four primary schools in Abraka, Delta state. The total heterotrophic bacterial and coliform counts were generally higher in males than in females. The work of Yalcin and Altin*.* (2004) indicates that girls take care of themselves, and care about their self-image more than boys. It was also noted that the knowledge and practices of personal hygiene was correlated to their academic achievements. This interesting observation goes with the notion that clean hands possess clean books that achieves better grades (Yalcin *and Altin* 2004).

The parental status influence on the hygiene of school pupils revealed that hands of children of civil servants and politicians were more contaminate, this could be as a result of the very busy schedule of these parents and as such they have little or no time to look after their wards leaving them the option of living them in the sole care of inexperienced nannies, this finding is in resonance with the work of Adams *et al*., 2015 who recorded that children with lesser care are prone to run into dangers of various degrees.

The month of November recorded the highest counts, thus could be because we experienced a perfect dry month, thus serving as a good vector and vehicle source of pathogen transfer. The high presence of *staphylococcus aureus* maybe as a result of contact with the genital areas or with already infected toilet surfaces. The resistance of in this study is in tune with the work of Mohanty and Pal (2017) who reported *S. aureus* to show resistant to a wide range of antibiotics to include azithromycin, doxycycline, ciprofloxacin, tetracycline, gentamycin, ofloxacin, chloramphenicol, ampicillin and oxacillin. Methicillin (MRSA) and vancomycin (VRSA) were 90.7% and 14.8 respectively. *Klebsiella pnuemoniae* has been reported to naturally occur in the soil, school pupil in the studied schools who play with soil especially pupil in the lower classes are predisposed to *Klebsiella pnuemoniae* contamination (Lee *et al*., 2017).

Variation of multiple antibiotic resistances of bacteria isolates in this study may be due to the multi-drug resistance to bacterial isolate strains. The plasmid profiles of the bacterial isolates from hands of pupil before curing revealed that the bacteria showed high level of multi-drug resistance. Plasmid profile is effective in tracing the epidemiology of antibiotic resistance (Yah *et al*., 2007). The detected plasmids indicates that it is efficient as a typing tool for *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Bacillus mycoides* since epidemiologically unrelated isolates could contain different plasmid profiles whereas related isolates could also display variation in plasmid profiles.

CONCLUSION

The study revealed that amongst the studied population of which 50% were male and 50% were female, a high level of contamination was recorded ranking a total of 87% across both gender. The male pupils were more heavily contaminated compared to the females recording 48% level of contamination. A high level of antibiotic multi-drug resistance was also observed as one isolates prove resistance to at least three antibiotics therefore zeros out the effectiveness of such antibiotics in the treatment of diseases caused by such bacteria. Various predisposing factors were also observed serving as a good vector and vehicle means for the distribution and proliferation of pathogenic microorganisms. A significant level of relatedness was also brought to play in the resistance pattern of these isolates and the resistivity posed by them to the various antibiotics. Thus proffering different options in the use of antibiotics.

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