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BACTERIOLOGICAL ASSESSMENTS OF INDOOR AIR IN THE STAFF QUARTERS OF A TERTIARY INSTITUTION IN BENIN CITY, NIGERIA

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ABSTRACT
Indoor air contains large number of airborne microorganisms such as bacteria and fungi and their estimation is important for use as index of cleanliness for any particular environment and to determine the relation they bear on human health. This study was aimed at determining the bacteriological air quality of the living rooms in some selected Staff Quarters in University of Benin and University of Benin Teaching Hospital. The airborne bacterial loads of indoor air in the living rooms of Twelve (12) Staff Quarters in University of Benin and University of Benin Teaching Hospital both in Benin City were determined using the Settle Plate methods. The houses were categorized as Apartment 1 which represents University of Benin Junior Staff Quarters (JSQ), Apartment 2 which represents University of Benin Teaching Hospital Quarters, and Apartment 3 which represents University of Benin Senior Staff Quarters (SSQ). The living rooms were sampled twice a month between April, 2017 and September, 2017. The airborne samples were studied using standard microbiological methods and Polymerase Chain Reaction and 16S rRNA techniques were used for the gene sequencing. The antibiotic susceptibility pattern and plasmid profile of the characterized airborne bacterial isolates were evaluated using spread plate and agarose gel electrophoresis methods. The temperature and relative humidity of the indoor air environment in the sampled areas in the living rooms were determined using the thermometer and hygrometer respectively. The mean indoor temperature and relative humidity for Apartments 1, 2 and 3 ranged from 26.10 ± 1.19^{0}C to 31.20 ± 0.87^{0}C and 81 ± 2.70% to 87 ± 1.89% respectively. The mean indoor airborne bacterial counts in Apartment 1 and Apartment 3 ranged between 1.00 x 10^3 ± 0cfu/m^3 to 4.42 x 10^3 ± 1.42cfu/m^3 and 1.09 x 10^3 ± 0.09cfu/m^3 to 5.17 x 10^3 ± 3.17cfu/m^3 respectively. In Apartment 2, the counts ranged from 1.00 x 10^3 ± 0.00cfu/m^3 to 6.99 x 10^3 ± 4.69cfu/m^3. The difference in the airborne bacterial counts obtained in the morning and afternoon period of study in Apartment 1 was statistically significant ($P<0.05$) while Apartments 2 and 3 showed no significant differences respectively ($P>0.05$). Ten airborne bacterial isolates were characterized, further characterization by molecular techniques, confirmed them to be identified as Staphylococcus aureus strain S33 R, Bacillus subtilis subsp. strain 168, Oceanobacillus manasiensis strain YD3-56, Streptomyces vietnamensis strain GIM4.0001, Actinosynnema pretiosum strain C-15003, Micrococcus caseolyticus strain 235, Ornithinibacillus composti strain GSS05 and Bacillus ectoiniformans strain NE-14. General and personal hygienic practices are important for healthy living, and living rooms used for close contact interactions and good ventilation.
Keywords: Air quality, Benin City, Staphylococcus aureus, plasmid profile, antibiotic susceptibility

INTRODUCTION

Indoor environments are fundamental environmental factors capable of impacting health. Air quality of indoor environments is one of the main factor affecting health, wellbeing and productivity of people. One of the problems of indoor air quality is influenced by the presence of microorganisms which include bacteria, moulds and viruses (Wamedo *et al*., 2012). People 80% - 90% of their time in indoor environments (Awad and Farag, 1999) by breathing on average 14 m^3 of air per day (Brochu *et al*., 2006), this makes people highly exposed to indoor air environments. Indoor air is important also because human populations are known to spend a substantial fraction of time within buildings (Heseltine and Rosen, 2009). The activity of people and equipment within the indoor environments is thought to be the principal factor contributing to the build-up and spread of airborne microbial contamination (Meadow *et al*., 2014). Particular activities like talking, sneezing, coughing, walking and washing have been implicated in the generation of airborne biological particulate matter. The number of people per room may likewise be sources of indoor infection (Jaffal *et al*., 1997; Ekhaise *et al.,* 2008; 2010). Food stuffs, house plants and flower pots, house dust, textiles, carpets, wood material and furniture stuffing, occasionally release various fungal spores into the air (Kalogerakis *et al*., 2005). Environmental factors such as temperature, humidity, air exchange rate, air movement, building structures and location, poor design, can enhance microbial growth and multiplication of the indoor atmosphere (Awad and Farag 1999).

A house is a building that functions as a home, ranging from simple dwellings such as rudimentary huts of nomadic tribes and complex, fixed structures of wood, brick, concrete or other materials containing plumbing, ventilation and electrical systems (Halttunen and Karen 1989). Many houses have several large rooms with specialized functions and several very small rooms for other purposes. These may include a living/eating area, a sleeping area, and (if suitable facilities and services exist) separate or combined washing and lavatory areas. The houses accommodated numerous people, including family, relatives, employees, servants and their guests. A living room, also called lounge or sitting room, or apartment for relaxing and socializing (Halttunen and Karen 1989). The living room may be a vision of neatness but it does not mean it is devoid of microorganisms. A microorganism or microbe is a microscopic living organism, which may be single-celled or multicellular (Madigan and Martinko, 2006). They are ubiquitous and sitting on every surface, just waiting to jump up and infect us and the majority of bacteria found are common, non-dangerous species. Studies have shown that of the bacteria found in indoor air, the most common four are: *Micrococcus, Staphylococcus, Bacillus, Pseudomonas* and also fungi such as Yeast and Mucor (Gorny and Dutkiewicz*,* 2002). *Bacillus* is a harmless saprophyte although some species of *Bacillus* and *Staphylococcus* can cause food poisoning, and some can cause illness or infection (Gorny and Dutkiewicz*,* 2002). It is therefore pertinent to study the airborne bacterial isolates which inhabits the living room in the homes and its public health consequence. The living room is the public room of a house. It is the first point of contact and most accessible for both the inhabitants and the visitors as compared to other rooms in the house, hence it is significant in assessing the air quality of a house.

The aim of this study therefore was to determine the bacteriological air quality in the living rooms of some selected apartments in the University of Benin and University of Benin Teaching Hospital, Benin City.

MATERIALS AND METHODS

Study Area

Airborne sampling was done in the living rooms of University of Benin Junior and Senior Staff Quarters and University of Benin Teaching Hospital Staff Quarters within Benin City. The study sites were the living rooms of 6 houses in University of Benin Staff Quarters and 6 houses in University of Benin Teaching Hospital Staff Quarters.

Sampling Procedure

Air samples were collected from the indoor air environments of the selected living homes twice a day in the morning (7am - 9am) and evening (4pm - 6pm) for six months (April, 2017 - September, 2017) using the settled plate method by exposing duplicate plates of freshly prepared nutrient agar in the living rooms at a height of 1.5 m from the floor for 10 mins (Ekhaise and Ogboghodo, 2011a). The plates were thereafter incubated at 37 ^{0}C for 24 h – 48 h. The temperature and relative humidity of the environment were obtained using thermometer and hygrometer respectively.

Enumeration of the Airborne Microbial Isolates

The isolates were enumerated and converted to colony forming units/ m^3 using the formular below as described by Idemudia and Ekhaise (2019)

$cfu/m^3 = \frac{a \times 10000}{p \times t \times 0.2}$ - - - - 1

Where, a: Number of colonies on the Petri dish, p: Surface area of the Petri dish, t : Time of exposure (10 min), 10,000 and 0.2 are constant values.

Characterization and Identification of Airborne Bacterial Isolates

The airborne bacterial isolates were characterized using cultural, morphological and biochemical/physiological examinations (Ekhaise *et al*., 2008). Further characterization and identification were done using the molecular methods according to Chen *et al.* (2001).

Chromosomal DNA Extraction:

DNA extraction was carried out directly from the samples by boiling as follows, 1.5 ml of the bacterial isolate in broth was centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and the pellet was washed twice with sterile water, 200 µl of sterile water was added to the pellet and vortexed to homogenize and boiled in a dry bath at 100°C for 10 min. This was followed by vortexing and centrifugation at 12,000 rpm for 5 min, the supernatant containing the DNA was transferred to another tube and stored at -20°C. The concentration and purity of the extracted DNA was estimated using a Nanodrop Spectrophotometer (manufactured by Analytik Jena, Jena, Germany).

PCR Amplification of the 16S rRNA Gene

Polymerase chain reaction was carried out to amplify the 16S rRNA gene of the bacterial isolates using the universal primer pair 27F- 5′-AGAGTTTGATCCTGGCT CAG -3′, and 1492R 5′-GGTTACCTTGTTACGACTT -3′. The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. The reaction was carried out in a 20 µl reaction mixture containing x1 PCR buffer (Solis Biodyne, Estonia), 2.5 mM Magnesium Chloride (Solis Biodyne), 200 µM of each dNTP (Solis Biodyne), 50 pmol of each primer, and 2U Taq DNA polymerase (Solis Biodyne). Amplification was carried out in an Eppendorf Thermal Cycler (Nexus Vapo protect series) using the following cycling parameters: An initial denaturation at 95ºC for 5 min and 40 cycles of 95ºC for 1 min, 30ºC for 1 min and 72ºC for 2 min. This was followed by a final extension of 72ºC for 10 min. The PCR products were separated on a 1.0 agarose gel and 1Kb DNA ladder was used as DNA molecular weight standard.

Detection of DNA by Agarose Gel Electrophoresis

DNA gel electrophoresis is a technique used for the detection and separation of DNA by applying an electrical field to move the charged molecules through an agarose matrix, and the DNA is separated by size in the gel matrix. Electrophoresis of the PCR products of the airborne bacteria isolates was carried out according to Chen *et al.* (2001). After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100 bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

Sequencing and Construction of Phylogenetic Tree

All PCR products were purified and sent to Epoch Life Science (USA) for Sanger sequencing. The corresponding sequences were identified using the online blast search at http://blast.ncbi.nlm.nih.gov/Blast.cgi. A phylogenetic tree was also constructed using BLAST to show strain relatedness of the bacteria (Sanger and Coulson, 1975).

Determination of Antibiotic Sensitivity

Agar diffusion technique was used for the antibiotic sensitivity test to determine if the airborne bacterial isolates were resistant or susceptible to antibiotics used such as gentamicin, erythromycin, Augmentin (amoxicillin/clavulanic acid), ofloxacin, cefuroxime, cloxacillin, ceftazidine, ciprofloxacin, ceftriazone and ranicef (Bauer *et al*., 1996). The airborne bacterial isolates were streaked on Mueller-Hinton agar plates and tested according to (Stryjakowska-Sekulska *et al*., 2007). The resultant visible zones of inhibition were measured.

Multiple Antibiotic Resistance (MAR) Index:

The MAR index identifies if isolates are from a region of high or low antibiotic use and is a good tool for public health risk assessment. An MAR index ≥ 0.2 indicates a high source of contamination (Rochell and Paul, 2016).

MAR Index was calculated as follows:

$MAR = \frac{a}{b}$ - - 2

Where; a, number of antibiotics to which isolate is resistant; b, total number of antibiotics tested (Adzitey, 2015).

Plasmid Isolation

Overnight freshly grown culture of the bacterial isolate (1.5 ml) was dispensed into a microfuge tube, spun for 1 min to pellet the cells, afterwards the supernatant was removed, while ~150 µl of media was kept in the tube. The cells were resuspended by vortexing and 300 µl of TENS buffer was added. The tubes were inverted 3 - 4 times gently, the cells lysed completely (the liquid turned from turbid to clear), 150µl of 3M NaOAc (pH 5.6) was added and inverted 3 - 4 times gently till white precipitate formed.

The white precipitate was spun for 5 min to pellet, the clear supernatant was pipetted to a clean tube, 900 µl of 95% ETOH was added and tube inverted to mix. The mixture was spun for 2 min to pellet DNA. The supernatant was decanted, 500 µl of 70% ETOH was added to wash the pellet by vortexing, spun for 1 min and 70% ETOH was decanted off. DNA pellet dried and dissolved in 50 µl 10mM Tris (pH 8) for further experimentation (Birmboin and Doly, 1979).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) Statistics 25.0 software was used to determine the means of temperature, relative humidity, airborne bacteria count and frequency of occurrence of airborne bacteria.

RESULTS

Table 1 shows the mean indoor temperature readings recorded for the Apartments which ranged from 26.10 ± 1.19^{0}C to 31.20 ± 0.87^{0}C, the highest temperature was recorded in Apartment 3 in the afternoon in the month of May and the lowest was recorded in the same apartment in the morning in the month of September. The relative humidity readings recorded for all the Apartments ranged from 81 ± 2.70% to 87 ± 1.89% and the highest humidity reading was recorded in Apartment 1 in the morning in the month of May while the lowest was recorded in the same Apartment the afternoon in the month of September. Apartment 3 is the living rooms in UBTH Quarters.

Table 1: Indoor air temperature and relative humidity in the living rooms studied.

Sampling points	Temperature (^{0}C)	Relative Humidity (%)	Temperature (IEE limit)	Relative Humidity (IEE limit)
1am	28.0 ± 0.58	85 ± 1.82	22.5 – 25.5	<70
1bm	28.0 ± 0.58	87 ± 1.89	22.5 – 25.5	<70
1cm	28.1 ± 0.59	86 ± 1.80	22.5 – 25.5	<70
1aa	30.4 ± 0.44	81 ± 2.70	22.5 – 25.5	<70
1ba	30.4 ± 0.48	82 ± 2.73	22.5 – 25.5	<70
1ca	29.9 ± 0.30	82 ± 2.92	22.5 – 25.5	<70
2am	26.4 ± 0.99	86 ± 1.35	22.5 – 25.5	<70
2bm	26.3 ± 0.99	86 ± 1.22	22.5 – 25.5	<70
2cm	26.4 ± 0.99	86 ± 1.31	22.5 – 25.5	<70
2aa	31.1 ± 0.78	83 ± 2.17	22.5 – 25.5	<70
2ba	31.1 ± 0.80	84 ± 2.36	22.5 – 25.5	<70
2ca	31.2 ± 0.87	83 ± 2.21	22.5 – 25.5	<70
3am	26.2 ± 1.16	87 ± 1.28	22.5 – 25.5	<70
3bm	26.1 ± 1.19	87 ± 1.49	22.5 – 25.5	<70
3cm	26.2 ± 0.16	87 ± 1.23	22.5 – 25.5	<70
3aa	30.4 ± 0.71	82 ± 2.60	22.5 – 25.5	<70
3ba	30.4 ± 0.71	83 ± 2.75	22.5 – 25.5	<70
3ca	30.6 ± 0.78	83 ± 2.83	22.5 – 25.5	<70

KEY:1—sampling locations in JSQ, 2--- sampling locations in SSQ, 3--- sampling locations in UBTH Quarters, am, bm and cm – apartment morning (triplicate sampling) aa, ba and ca – apartment afternoon (Triplicate sampling), IEE: Institute of Environmental Epidemiology, *Values are in mean ± standard error.

The mean indoor airborne bacterial counts presented in Table 2, ranged from 1.09 ± 0.09cfu/m^3 (all the apartments during the afternoon sessions in all the months except April.)- 6.99 x 10^{3}cfu/m^3 (Apartment 2, April morning sampling).

Table 2: Mean airborne bacterial counts in cfu/m^3 ($\times 10^3$) in in the Living homes of selected apartments.

SAMPLING LOCATION	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER
1am	4.42 ±1.42	4.33 ± 1.00	2.42 ± 0.09	2.25 ± 0.58	1.17 ± 0.17	1.25 ± 0.08
1bm	4.17 ±1.50	4.00 ± 0.17	2.09 ± 0.42	1.50 ± 0.17	1.25 ± 0.08	1.33 ± 0.00
1cm	4.17 ±1.50	3.59 ± 1.59	1.33 ± 0.00	1.25 ± 0.08	1.42 ± 0.25	1.17 ± 0.00
1aa	2.17 ±0.17	2.50 ± 0.83	2.92 ± 1.25	1.17 ± 0.00	1.17 ± 0.17	1.17 ± 0.17
1ba	1.59 ±0.09	1.92 ± 0.59	2.67 ± 1.17	1.34 ± 0.34	1.09 ± 0.09	1.17 ± 0.00
1ca	2.50 ±0.50	1.75 ± 0.75	2.42 ± 1.09	1.17 ± 0.17	1.09 ± 0.09	1.0 ± 0.00
2am	6.99 ± 4.69	3.09 ± 0.09	2.67 ± 1.34	1.09 ± 0.09	1.84 ± 0.17	1.09 ± 0.09
2bm	6.59 ± 4.42	2.92 ± 0.42	1.84 ± 0.17	1.17 ± 0.17	1.50 ± 0.17	1.17 ± 0.17
2cm	2.15 ± 0.15	2.67 ± 0.17	3.59 ± 1.42	1.42 ± 0.25	1.25 ± 0.25	1.17 ± 0.17
2aa	5.42 ± 4.09	1.17 ± 0.17	2.33 ± 1.0	1.33 ± 0.00	1.33 ± 0.00	1.09 ± 0.09
2ba	4.83 ± 3.50	1.17 ± 0.17	1.42 ± 0.09	1.09 ± 0.09	1.25 ± 0.08	1.17 ± 0.17
2ca	5.67 ± 4.0	1.17 ± 0.17	1.75 ± 0.25	1.00 ± 0.00	1.00 ± 0.00	1.09 ± 0.09
3am	2.34 ± 0.34	5.17 ± 3.17	1.84 ± 0.17	1.00 ± 0.00	1.59 ± 0.42	1.09 ± 0.09
3bm	2.08 ± 0.25	3.75 ± 1.92	2.75 ± 1.75	1.17 ± 0.00	1.50 ± 0.17	1.25 ± 0.08
3cm	2.25 ± 0.25	4.25 ± 1.92	2.34 ± 1.34	1.17 ± 0.17	1.67 ± 0.17	1.00 ± 0.00
3aa	1.92 ± 0.25	1.67 ± 0	1.5± 0.17	1.09 ± 0.09	1.84 ± 0.17	1.25 ± 0.08
3ba	1.83 ± 0.5	1.25 ± 0.08	1.17 ± 0.00	1.09 ± 0.09	1.09 ± 0.09	1.09 ± 0.09
3ca	1.58 ± 0.25	1.17 ± 0.17	1.25 ± 0.08	1.0 ± 0.00	1.09 ± 0.09	1.09 ± 0.09

*Mean± Standard error

1—sampling locations in JSQ, 2--- sampling locations in SSQ, 3--- sampling locations in UBTH Quarters, am, bm and cm – apartment morning (Triplicate sampling), aa, ba and ca – apartment afternoon (Triplicate sampling), *Values are in mean ± standard error.

The PCR Amplification results of all the airborne bacterial isolates having similar DNA bands of 1500bp with a universal primer (27F and 1492R) indicating that the isolates are bacteria are presented in Plate 1

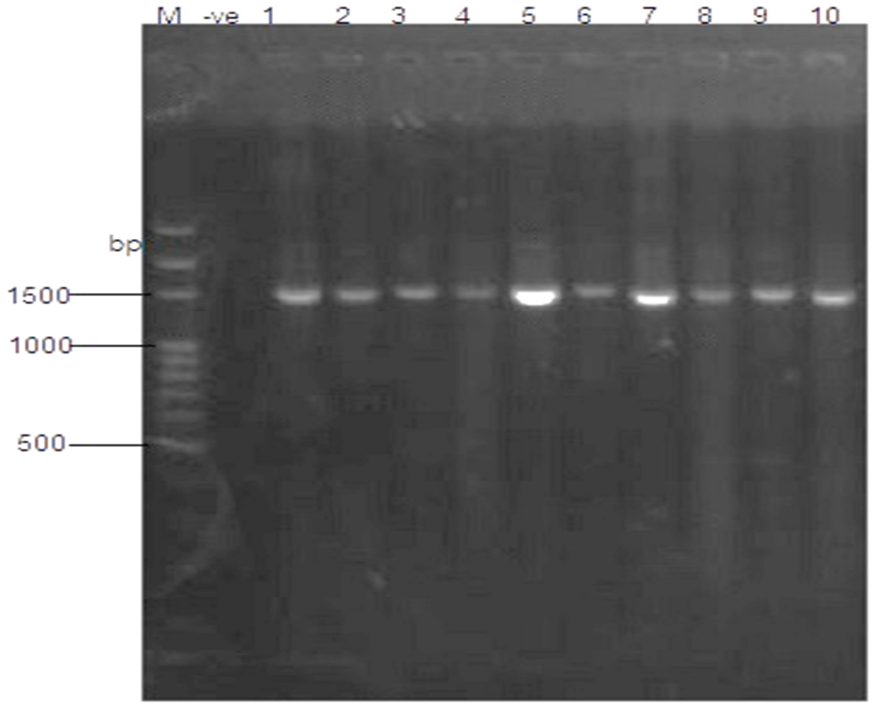

Plate 1: PCR amplification of the 16s rRNA gene of the extracted DNA using a universal gene marker (27F and 1492R).

Table 3: Identification of Nucleotide gene sequence of airborne bacterial isolate and their % identity.

Isolate	Sequence ID	Identity (%)	Closest Match
1	gi|1269859156|NR_037007.2	**77**	*Staphylococcus aureus* strain S33 R
2	gi|636560564|NR_116624.1	80	*Oceanobacillus manasiensis* strain YD3-56
3	gi|1269801457|NR_102783.2	77	*Bacillus subtilis subsp. subtilis* strain 168
4	gi|751387084|CP010407.1	77	*Streptomyces vietnamensis* strain GIM4.0001
5	gi|265678435|NR_028737.1	**91**	*Actinosynnema pretiosum* strain C-15003
6	gi|310975036|NR_036900.1	**73**	*Macrococcus caseolyticus* strain 235
7	gi|1212229226|NR_148296.1	71	*Ornithinibacillus composti* strain GSS05
8	gi|1227086103|NR_148614.1	80	*Bacillus ectoiniformans* strain NE-14

Figure 1 shows the phylogenetic tree obtained from the sequences of the PCR products of the sampled isolates using BLAST software. Bar, 0.01 substitutions per nucleotide position where *Staphylococcus aureus* strain S33 R was 100% similar to *Macrococcus caseolyticus* strain ATCC 13548.

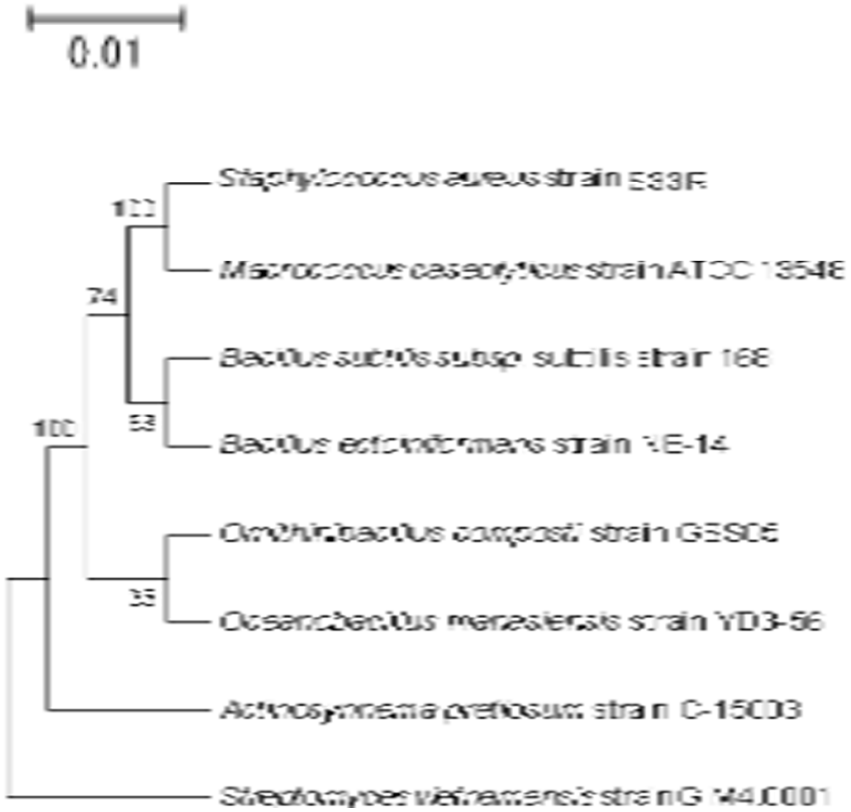

FIGURE 1: Phylogenetic tree of the sampled airborne bacterial isolates.

Figure 2 shows the phylogenetic tree obtained from the sequences of the PCR products using BLAST software. Bar, 0.01 substitutions per nucleotide position.

Actinosynnema pretiosum strain C-15003 genome was 100% similar to *Lechevalieria nigeriaca* strain NJ2035 both of which were 100% similar to *Streptomyces vietnamensis* strain GIM4.0001, *Staphylococcus aureus* strain S33 R was 100% similar to *Staphylococcus aureus* Subsp. anaerobius strain MVF-7 strain , *Bacillus subtilis* Subsp. *subtilis* strain 168 was 99% similar to *Bacillus aerius* strain 24K and *Bacillus pumilus* strain NRRL NRS-272, *Macrococcus caseolyticus* strain ATCC 13548 was 85% similar to *Macrococcus bovicus* strain C 2FA.

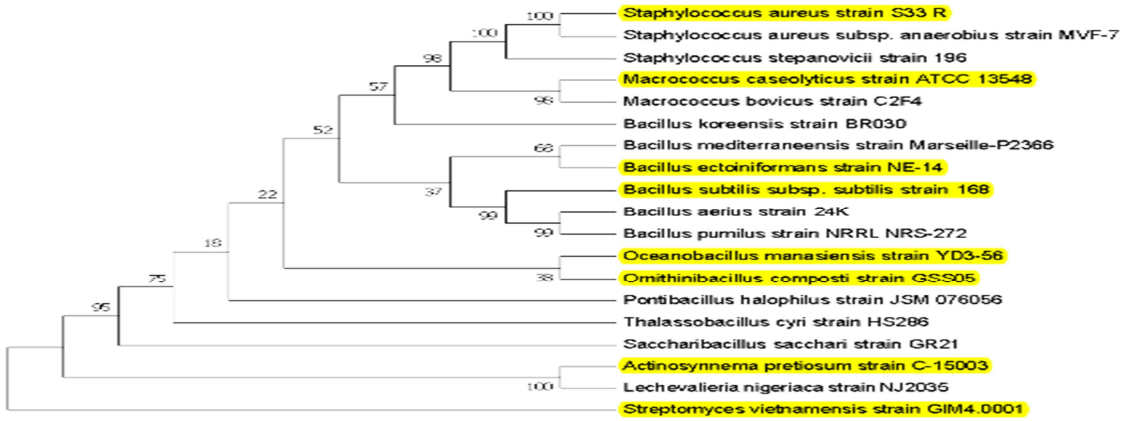

Fig 2: Phylogenetic tree based on 16S rRNA sequences showing relatedness of the bacteria isolates

Table 4 shows the percentage frequency of occurrence of the airborne bacterial isolates, in which the bacterial isolates *Staphylococcus aureus* and *Bacillus subtilis* recorded the highest percentage frequency of occurrence (87.5%) in Apartments 1 and 3 in the month of April, while *Bacillus ectoiniformans* had the lowest frequency of occurrence (0%) all the Apartments during the sampling periods.

Table 4: Percentage frequency of occurrence and distribution of airborne bacterial isolates in living homes of the selected apartments.

Bacterial isolates		**sampling months**					
	Sampling points	April	May	June	July	August	September
Staphylococcus aureus	A1	87.5	87.5	62.5	50.0	37.5	37.5
	A2	75.0	62.5	37.5	25.0	25.0	37.5
	A3	87.5	25.0	25.0	12.5	12.5	25.0
Oceanobacillus	A1	37.5	50.0	50.0	37.5	25.0	25.0
Manasiensis	A2	25.0	37.5	25.0	25.0	25.0	25.0
	A3	87.5	62.5	50.0	37.5	25.0	25.0
Streptomyces vietnamensis	A1	50.0	50.0	37.5	37.5	37.5	25.0
	A2	37.5	37.5	25.0	37.5	25.0	25.0
	A3	62.5	75.0	50.0	62.5	37.5	25.0
Actinosynnema pretiosum	A1	50.0	62.5	37.5	50.0	37.5	25.0
	A2	37.5	50.0	50.0	37.5	25.0	37.5
	A3	25.0	37.5	12.5	0.0	12.5	12.5
Bacillus subtilis	A1	75.0	50.0	37.5	25	12.5	0.0
	A2	87.5	87.5	62.5	0.0	12.5	0.0
	A3	75.0	37.5	50.0	12.5	0.0	0.0
Macrococcus caseolyticus	A1	37.5	25.0	0.0	12.5	0.0	0.0
	A2	12.5	25.0	25.0	12.5	0.0	0.0
	A3	0.0	25.0	12.5	0.0	0.0	0.0
Ornithinibacillus composti	A1	12.5	0.0	12.5	37.5	0.0	0.0
	A2	12.5	0.0	25.0	0.0	0.0	0.0
Bacillus	A3	12.5	0.0	0.0	12.5	0.0	0.0
Ectoiniformans	A1	12.5	0.0	12.5	0.0	0.0	0.0
	A2	0.0	0.0	0.0	0.0	0.0	0.0
	A3	0.0	0.0	0.0	0.0	0.0	0.0

Table 5 shows the antibiotic resistance susceptibility of the airborne bacteria isolates which were resistant to all the antibiotics used such as gentamicin, erythromycin, ciprofloxacin, ceftriazone, augmentin, cefuroxime and ofloxacin.

Table 5: ANTIBIOTICS SUSCEPTIBILITY PATTERNS OF THE AIRBORNE BACTERIAL ISOLATES

Bacterial isolates	GEN	ERY	AUG	OFL	CRX	CXC	CAZ	CPT	CTR	RNF	MARI
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	R	1.0
Oceanobacillus manasiensis	R	R	R	R	R	R	R	R	R	R	1.0
Streptomyces vietnamensis	R	R	R	R	R	R	R	R	R	R	1.0
Actinosynnema pretiosum	R	R	R	R	R	R	R	R	R	R	1.0
Macrococcus caseolyticus	R	R	R	R	R	R	R	R	R	R	1.0
Ornithinibacillus composti	R	R	R	R	R	R	R	R	R	R	1.0
Bacillus ectoiniformans	R	R	R	R	R	R	R	R	R	R	1.0
Bacillus subtilis	R	R	R	R	R	R	R	R	R	R	1.0

KEY:R = RESISTANT, S = SENSITIVE, I = INTERMEDIATE, GEN – GENTAMICIN, ERY- ERYTHROMYCIN, AUG- AUGMENTIN (amoxicillin/clavulanic acid), OFL-OFLOXACIN, CRX – CEFUROXIME, CXC – CLOXACILLIN, CAZ – CEFTAZIDINE, CPT – CIPROTAB, (CIPROFLOXACIN), CTR – CEFTRIAZONE, RNF – RANICEF, Sensitivity standard for disc (mm): <14 resistant, 14-17 intermediate, >17 susceptible. MARI- Multiple antibiotics resistant index, MARI ≥ 0.2 (public health significance).

DISCUSSION

The bacteriological air quality of living rooms in some houses in the University of Benin and University of Benin Teaching Hospital Staff Quarters was carried out to determine the public health consequence of the airborne bacteria on the health of the occupants. In this study, it was observed that living rooms contain different groups of bacteria, some of which are the normal flora of the air while others pose great danger to the health of the individuals who live in these homes as well as those who visit. This may occur due to number of people per room (Ekhaise *et al*., 2008), environmental factors such as temperature, humidity, air exchange rate, air movement, building structures and location, poor design, (Awad and Farag, 1999). These factors have been reported to influence microbial growth and multiplication in the indoor atmosphere (Awad and Farag, 1999). The qualitative and qualitative study of the living rooms were categorized as low class (A1), middle class (A2) and upper class (A3). These categories where based on the type of building structures, the number of persons living in the houses and the hygienic status. The low class members include non-academic staff, tailors, cleaners, drivers; the middle class members include medical personnel while the upper class include Professors and senior lecturers. The results of the mean indoor temperature and relative humidity of the indoor air environment in the living rooms of the sampled apartments were reported to be higher compared to the acceptable limits (22.5 – 25.5^{0}C and < 70%) by the Institute of Environmental Epidemiology (IEE, 1996). Although, the mean relative humidity values were above the IEE permissible limits, Stetzenbach *et al*. (2004) reported that the maintenance of indoor relative humidity values below 60 per cent within buildings such as offices and residential homes, can be a way of mitigating some infections associated with indoor air microbial contamination. In this study, the highest mean indoor airborne bacterial count was recorded in Apartment 2 (6.99 x 10^3cfu/m^3) in the morning in the month of April, 2017, this was observed to have occurred either as a result of poor ventilation, untidy environment or increased rainfalls. This is similar to the reports of Awad and Farag, (1999) and Ekhaise *et al*., (2008), thus an obvious practice to improve a more healthy quality of indoor air in the building would be to avoid overcrowding and to design good ventilation systems. The lowest mean indoor bacterial count (1.00 ± 0.00cfu/m^{3}cfu/m^3) was recorded in Apartments 1, 2 and 3 during the afternoon sessions in all the months except April.

This could be attributed to the availability of good ventilation system, low influx of people and regular cleaning of the living homes. High airborne bacterial counts were recorded in June, 2017 while September recorded the least counts which shows a gradual decline during months with lower temperature and higher rainfall. Information about the seasonal variation is important for any exposure assessment (Rintala *et al.,* 2008). Studies on the effect of temperature and relative humidity on microbial flora in indoor environments have mostly concentrated on viable counts of fungi not bacteria (Pitkäranta *et al*. 2008). Nevertheless, Rintala *et al*. (2008) reported that seasonal differences between bacterial aeroflora were not statistically significant during the course of their investigation. The study from Ekhaise and Ogboghodo (2011) reported that regardless of season, indoor environment allows aerosols build up, which could potentially lead to infections. Rintala *et al*. (2008) reported that bacterial counts showed a gradual decline during the rainy season. Kiekhaefer *et al*. (1995) also reported higher value for the number of microorganisms during the warm season, and these results to difficulties in the interpretation of results in respect to the season. The difference in the microbial counts obtained in the morning and afternoon period of study in Apartment 1 was statistically significant ($P<0.05$) while Apartments 2 and 3 showed no significant differences respectively ($P>0.05$). The airborne bacterial isolates phenotypically and molecularly characterized and identified to include *Staphylococcus aureus* strain S33 R*, Bacillus subtilis* subsp. strain 168*, Oceanobacillus manasiensis* strain YD3-56*, Streptomyces vietnamensis* strain GIM4.0001*, Actinosynnema pretiosum* strain C-15003*, Macrococcus caseolyticus* strain ATCC 13548*, Ornithinibacillus composti* strain GSS05 and *Bacillus ectoiniformans* strain NE-14. *Staphylococcus aureus* and *Bacillus subtilis* (87.5%) were reported to record the highest frequency of occurrence in Apartments 1 and 3 in April. *Bacillus ectoiniformans* had the lowest frequency of occurrence (12.5%) in all the Apartments studied during the sampling period. *Staphylococcus aureus* is generally associated to human skin and mucosa, which suggests that the main bacterial contamination present in the indoor air emerged from human presence. *Bacillus subtilis* is commonly found in the upper layers of the soil and in almost every environment. Possession of spores enables them survive long periods in the environment and although they are not known to cause infectious disease, they cause food spoilage. Some of the bacteria identified such as *Bacillus ectoiniformans, Oceanobacillus manasiensis*, *Macrococcus caseolyticus* are novel organisms in the environment and are not known to cause infectious disease except in immuno-compromised individuals. *Actinosynnema pretiosum* strain C-15003 genome was shown to be 100% closely related to *Lechevalieria nigeriaca* strain NJ2035 both of which were 100% closely related to *Streptomyces vietnamensis* strain GIM4.0001, *Staphylococcus aureus* strain S33 R was 100% closely related to *Staphylococcus aureus* strain MVF-7 strain , *Bacillus subtilis* Subsp. strain 168 was 99% closely related to *Bacillus aerius* strain 24K and *Bacillus pumilus* strain NRRL NRS-272, *Macrococcus caseolyticus* strain ATCC 13548 was 85% closely related to *Macrococcus bovicus* strain C 2FA, *Oceanobacillus manasiensis* strain YD3-56 was 38% closely related to *Ornithinibacillus composti* strain GSS05. The antibiotic susceptibility pattern of the airborne bacterial isolates revealed that their resistance to the antibiotics used, suggesting that they were multidrug resistant with a multiple antibiotics resistant index of 1.0, greater than 0.2, an indication of public health consequence. Resistance of microorganism have been shown to be attributed to the misuse of antibiotics and also the presence of resistant genes.

CONCLUSION

In conclusion, this study has shown that the living room is not devoid of airborne bacterial isolates and it does not matter if it is a low income class or middle income class or high income class homes. Good hygienic practice, proper ventilation and regular cleaning of these houses and their surroundings should be of utmost importance as this would help reduce exposure and transmission of these bacteria and infections.

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REFERENCES

Adzitey, F. (2015). Antibiotic Resistance of *Escherichia coli* Isolated from Beef and its Related Samples in Techiman Municipality of Ghana. *Asian Journal of Animal Sciences* **9**:233-240.

Awad, A.H. and Farag, S.A. (1999). An indoor bio-contaminants air quality. *International Journal for Environmental Health Research* **9**:313–319.

Birmboin, H.C. and Doly, J.A (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**:1513-1523.

Brochu, P., Ducré-Robitaille, J.F. and Brodeur, J. (2006). Physiological daily inhalation rates for free-living individuals aged 1 month to 96 years, using data from doubly labeled water measurements: a proposal for air quality criteria, standard calculations and health risk assessment. *Human and Ecological Risk Assessment* **12**:675- 701.

Chen, W.M., Laevens, S., Lee, T.M., Coenye, T., De Vos, P., Mergeay, M. and Vandamme, P. (2001). Ralstoniataiwanensis sp. nov., isolated from root nodules of Mimosa species and sputum of a cystic fibrosispatient. *International Journal on Systemic and Evolutionary Microbiology* **51**:1729–1735.

Ekhaise, F.O., Ighosewe, O.U. and Ajakpori, O.D. (2008). Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria. *World Journal of Medical Sciences* **3**(1):34-38

Ekhaise, F. O. and Ogboghodo, B. I. (2011a). Diversity of the airborne microflora in two private hospitals in Benin City, Nigeria. *Italian Journal of Occupational and Environmental Hygiene 2*(4):190-195.

Ekhaise, F. O. and Ogboghodo, B. I. (2011b). Microbiological Indoor and Outdoor Air Quality of Two Major Hospitals in Benin City, Nigeria. *Sierra Leone Journal of Biomedical Research* **3**(3):169-174.

Gorny, R.L. and Dutkiewicz, J. (2002). Bacterial and fungal aerosols in indoor environment in Central and Eastern Countries. *Annals of Agriculture and Environmental Medicine* **9**:17-23.

Halttunen, and Karen (1989). "From Parlor to Living Room: Domestic Space, Interior Decoration, and the Culture of Personality". Consumingvisions: Accumulation and Display of Goods in Ameri*ca* (1st edition). Norton, New York. 1920pp.

Heseltine, E. and Rosen, J. (2009). *WHO guidelines for indoor air quality: dampness and mould*. WHO Regional Office for Europe, Copenhagen. 248 pp.

Idemudia, I.B. and Ekhaise, F.O. (2019). Bacteriological enumeration and air quality of three major Hospitals in Benin City, Nigeria. *Journal of Science and Technology Research* **1**(2):77-86.

Institute of Environmental Epidemiology (IEE) (1996). *Guidelines for Good Indoor air quality in Office Premises*. IEE, Ministry of Environment. Singapore. 49 pp.

Jaffal, A.A., Banat, I.M., El Mogheth, A.A., Nsanze, H., Benar, A. and Ameen, A.S. (1997). Residential indoor airborne microbial populations in the United Arab Emirates. *Environment International* **23**(4):529-533.

Kalogerakis, N., Paschali, D., Lekaditis, V., Pantidou, A., Eleftheriadis, K. and Lazaridis, M. (2005). Indoor air quality-bioaerosol measurements in domestic and office premises. *Journal of Aerosol Science***36**: 751-761.

Kiekhaefer, M.S., Donham, K.J., Whitten, P. and Thorne, P.S. (1995). Cross seasonal studies of airborne microbial populations and environment in swine buildings: implications for worker and animal health. *Annals of Agricultural and Environmental Medicine*. **2**:37-44.

Madigan, M. and Martinko, J. (2006). *Brock Biology of Microorganisms* (13th edition.). Pearson Education. 1096pp.

Meadow, J.F., Altrichter, A.E, Kembel, S.W., Kline, J., Mhuireach, G. and Moriyama, M. (2014). Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* **24**(1):41-48.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Paulin, L., Auvinen, P., Nevalainen, A. and Rintala, H. (2008). Analysis of Fungal Flora in Indoor Dust by Ribosomal DNA Sequence Analysis, Quantitative PCR, and Culture. *Applied and Environmental Microbiology* **74**:233-244

Rintala, H., Pitkaranta, M., Toivola, M. Paulin, L. and Newalainen, A. (2008). Diversity and Seasonal Dynamics of Bacterial Community in Indoor Environment. *BMC Microbiology.* **8**(56).

Rochell, D. and Paul, D.B. (2016). Multiple antibiotic resistance index, fitness and virulence potential in respiratory *Pseudomonas aeruginosa* from Jamaica. Journal of Medical Microbiology 65:261-271.

Sanger, F. and Coulson, A. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* **94**:441-448.

Stetzenbach, L.D., Amman, H., Johanning, E., King, G. and Shaughnessy, R.J. (2004). *Microorganisms, Mold and Indoor Air Quality*. ASM, Washington DC, 20 pp.

Stryjakowska-Sekulska, M., Piotraszewska-Pajak, A., Szyszka, A., Norwicki, M. and Filipiak, M. (2007). Microbiological quality of indoor air in University rooms. *Poland Journal of Environmental Studies* **16**(4):623-632.