

## ASSESSMENT OF AIRBORNE BACTERIA IN RESIDENTIAL BUILDINGS IN BENIN CITY, NIGERIA

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### ABSTRACT

Exposure to bioaerosols poses significant health risks to occupants of buildings, especially in tropical areas due to its climatic characteristics that favour airborne bacterial growth. This study investigated the levels and composition of indoor and outdoor airborne bacteria in residential buildings in Benin Metropolis. Air samples were collected from forty-five randomly selected houses across the five local government areas in Benin City using the passive sampling technique. The culture-dependent 16S rRNA gene technique was utilized in the identification of the bacterial isolates. The results revealed that the mean concentrations of the indoor and outdoor total bacteria varied from 476.1 to 939.3 CFU/m<sup>3</sup> and 181.1 to 373.2 CFU/m<sup>3</sup> in the wet season, 335.0 to 457.2 CFU/m<sup>3</sup> and 387.2 to 627.8 CFU/m<sup>3</sup> in the dry season, respectively. The concentration of indoor and outdoor airborne bacteria varied significantly (P=0.001) during the wet and dry seasons. Basic Local Alignment Search Tool homology showed that the 16S rRNA gene sequences of isolates were related to *Proteus mirabilis*, *Pectobacterium brasiliense*, and *Enterobacter sichuanensis*. The bacterial communities identified are associated with humans and other outdoor environmental media and could lead to critical health outcomes among exposed vulnerable occupants of the buildings. This study, therefore, provides a database for airborne bacteria in residential houses in the study area and could assist in the implementation of effective interventions that reduce the risks and provide a safe environment.

**Keywords:** Anthropogenic pollution, Culture-dependent, Residential microbiome, Public health, 16S rRNA.

### INTRODUCTION

Bioaerosols consist of aerosol particles containing microorganisms or their organic compounds derivatives such as endotoxins, metabolites, and toxins. A significant portion of aerosols in the atmosphere is of biological origin (Fröhlich-Nowoisky *et al.*, 2016). The size and composition of bioaerosols vary (20 to >100 nm) and they are determined by the source, and the prevailing environmental conditions (Pillai and Ricke, 2002).

Exposure to environmental bioaerosols has become a serious public health issue in and around different microenvironments (Sadigh *et al.*, 2021). One of the major factors affecting the productivity and health of individuals, especially in developing countries, is an unhealthy and unsafe environment. Although the green building concept in creating a healthier indoor building environment is being adopted in developed nations, pollution from various sources has continued to be a problem in developing

countries. Airborne bacteria are one of the determinants of poor indoor air quality in buildings and inhalation of a high load of airborne bacteria might pose health risks in indoor environments as the causative agent of several infectious diseases (Falkinham, 1999).

Respiratory symptoms mainly result from inflammation of the airways caused by toxins and allergens, which are the predominant health outcomes linked to exposure to non-infectious bioaerosol (Marri *et al.*, 2013; Green *et al.*, 2014). Many of the health outcomes, symptoms, or conditions have also been associated with exposure to many air pollutants such as environmental tobacco smoke, gaseous pollutants, particulate matter, and volatile organic chemicals (Oguntoke *et al.*, 2010). The sources of indoor bioaerosols are building materials, microbial contamination of interiors including walls, ceilings, and re-suspension of dust on the floor, furnishings, and edibles. Others include

house plants and flower vases, house dust, pet bedding, clothing, floor coverings, and infiltration of air pollutants from outdoor sources (Kalogerakis *et al.*, 2005; Nazaroff, 2016). Various human activities can also give rise to airborne bacteria in the indoor environment (Chen and Hildemann, 2009). The presence of airborne bacteria is very important in different microenvironments, hence, previous studies have reported on environmental exposures to bioaerosols in developed countries (Hildemann, 2009; Chen *et al.*, 2010; Wu *et al.*, 2015).

In developing countries such as Nigeria, environmental health status is poorly defined due to a lack of empirical databases and guidelines for assessing indoor air quality. Although there are studies on levels of bioaerosols in occupational and institutional settings (Ekhaise and Ogboghodo, 2011; Makut *et al.*, 2014; Ana *et al.*, 2015; Ilondu and Nweke, 2016; Senyene *et al.*, 2018), there is a paucity of data for residential buildings. This might be partly attributed to the challenge of gaining access to the apartments in the area due to lack of proper education and awareness among residents. Studies on airborne bioaerosols in Nigeria utilized traditional biochemical tests as methods of identification of airborne bacteria (Makut *et al.*, 2014; Ana *et al.*, 2015; Ilondu and Nweke, 2016; Senyene *et al.*, 2018). The inaccuracy of this method which includes a lack of specificity and time wastage in bacterial characterization underscores the need for a rapid and reliable way of detecting and identifying airborne bacterial isolates. This research was designed to determine the indoor and ambient airborne bacterial concentrations and

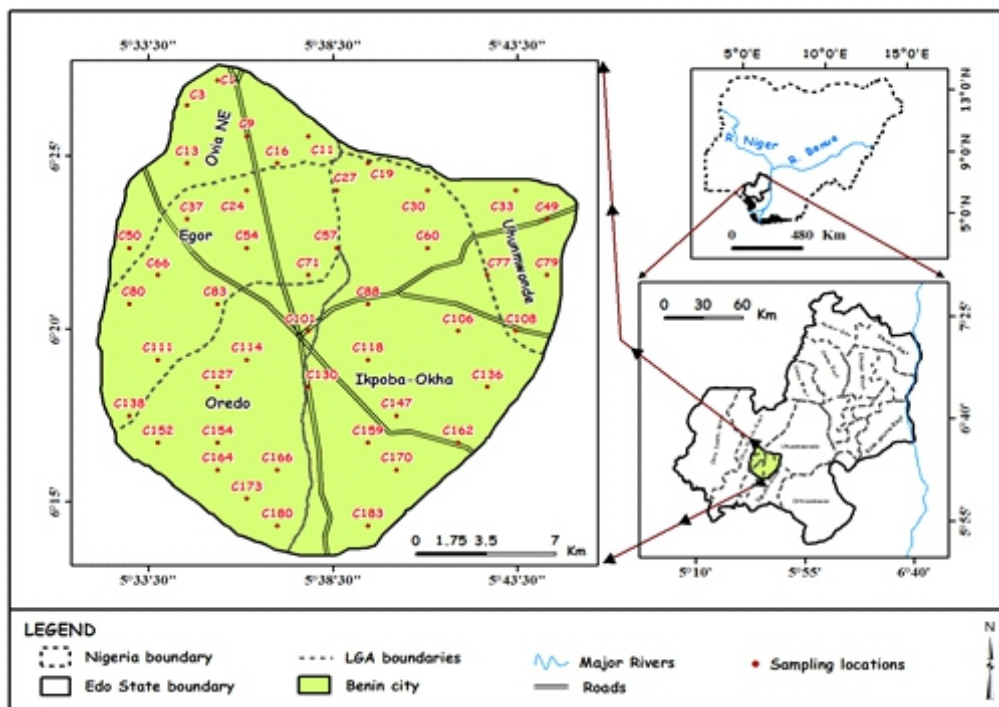
the identity of the airborne bacterial populations in residential buildings in a humid tropical urban area. This would assist in the intervention actions to enforce adequate preventive measures as well as help in policy guidelines for public health programmes.

## MATERIALS AND METHODS

### Selection of sampling areas

The map of Benin City (Figure 1) was obtained and gridded to create cells of 1.5 by 1.5 km with the aid of Arc Map 10.1. The locations that fell within each of the cells were extracted and documented. Twenty-five per cent (25%) of cells (45 of 181) were randomly selected from the gridded map of the study area for airborne bacterial sampling in each cell (Eghomwanre and Oguntoke, 2022). The houses were predominantly detached flat house types with three to five rooms of about 12ft by 12ft in size and an average of four occupants in a room. The wall and floor materials were mostly cemented and tiled, and roofs reportedly have leakages in many of the houses. The average daily temperature was 28 °C in the dry season and approximately 24 °C in the dry season. Also, the relative humidity of about 80% and 70% was observed in the wet and dry seasons, respectively. (UNOCHA, 2013).

The airborne microbial sampling was performed in both indoor and outdoor environments in the selected locations. The locations were grouped according to the local area councils for ease of presentation of data. Consent was obtained from the Landlords of each selected home.



**Figure 1:** Map of Benin City showing the selected sampling locations.

### Procedure for Airborne microbial sampling

Airborne bacterial sampling was conducted indoors in the living rooms and nearby the outdoor environment of the buildings. Sampling was carried out between 9:00 am to 1.00 pm in triplicates for one year covering wet and dry seasons between 2020 and 2021 using the settle plate method. Petri dishes of 9 cm in size containing nutrient agar (NA) were used for sampling. Agar plates were positioned and exposed for sampling at a height of 2 m above the floor level and with a distance of more than 1 m from the walls, doors, windows, and obstructions, for 30 min. After the exposure, plates were covered with their lids and transported to the laboratory, and incubated at 37°C for 48 h (Nasir and Colbeck, 2010). The culture plates that showed discrete macroscopic colonies were counted using a plate colony counter. Mean values of triplicate airborne concentration were expressed as colony-forming units per cubic meter (CFU/m<sup>3</sup>) using the empirical conversion formula as previously stated (Gizaw *et al.*, 2016).

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

a = number of colonies on the Petri dish, b = Surface area of the Petri dish (cm<sup>2</sup>), t = Time of

exposure (min.). The qualitative analysis of bacterial isolates was performed by obtaining the pure isolates from the mixed culture using the streak plate method. The cultured plates were incubated at 32°C for 24 to 48 h. The colonies were initially identified through standard microbiological methods (WHO, 1991). The presumptively identified isolates were subsequently sub-cultured to NA slants for molecular characterization.

### DNA extraction, polymerase chain reaction, and 16s rRNA sequence analysis

DNA extraction was performed from each airborne bacterial isolate using ZR Bacterial DNA miniprep. Extracted DNA templates were subjected to PCR using a set (Forward and Reverse) of primers targeting the 16S rRNA gene of isolates. The primers used for the amplification of 16S rRNA genes of the bacteria isolates were 27F-AGAGTTTGTATCMTGGCTCAG and 1492R-AAGGAGGTGWTCARCCGCA. PCR was performed with the following conditions: Initial denaturation at 96°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 s, annealing temperature at 56°C for 30 s, elongation at 72°C for 45 s, and final elongation at 72°C for 7

min. The products of the amplification process were purified using a resin column. DNA sequencing was performed using the standard shotgun sequencing reagents according to the manufacturer's instructions (Chun *et al.*, 2009).

### Processing of sequencing data

The 16S rRNA gene data were processed using MEGA6 software. This was done after a pairwise alignment was performed on the forward and reverse sequence. A consensus sequence was obtained from the aligned sequence. Identification of the 16S rRNA gene sequence was performed using GenBank/Basic Local Alignment Search Tool (BLASTn) algorithm on National Centre for Biotechnology and Information website (<http://blast.ncbi.nlm.nih.gov>). The phylogenetic relatedness of the 16S rRNA genes of the isolates was compared with the downloaded sequences of other species from the GenBank. The sequences of the isolates were submitted to GenBank, NCBI (National Centre for Biotechnology Information) and the following accession numbers: MT587574 (N1), MT587575 (N3), MT587576 (N3), MT587577 (N4), MT587578 (N5), and MT587579 (N6) were assigned. The evolutionary history of the bacterial strains was deduced using the Maximum Likelihood method based on the Jukes-Cantor model (Kumar *et al.*, 2016). The phylogenetic tree revealed the relationship of the bacterial isolates with the closest reference bacterial species. The 16S rRNA sequences of the bacterial isolates were aligned to twenty-four reference sequences of the 16S rRNA gene of closely related taxa as retrieved from the GenBank data library and *Pantoea agglomerans* (MT507236) was used as the out-group. The isolates were assembled with their corresponding genus in the tree with bootstrap values ranging from 69 to 98%.

### Data Analysis

The measured concentration of airborne bacteria in CFU/m<sup>3</sup> was subjected to descriptive and inferential statistics (the analysis of variance, ANOVA) to determine the mean concentrations of indoor and outdoor airborne bacteria and the variations in bacterial concentrations across the sampling locations. A paired t-test was done to determine the differences in bacterial levels during the wet and dry seasons.

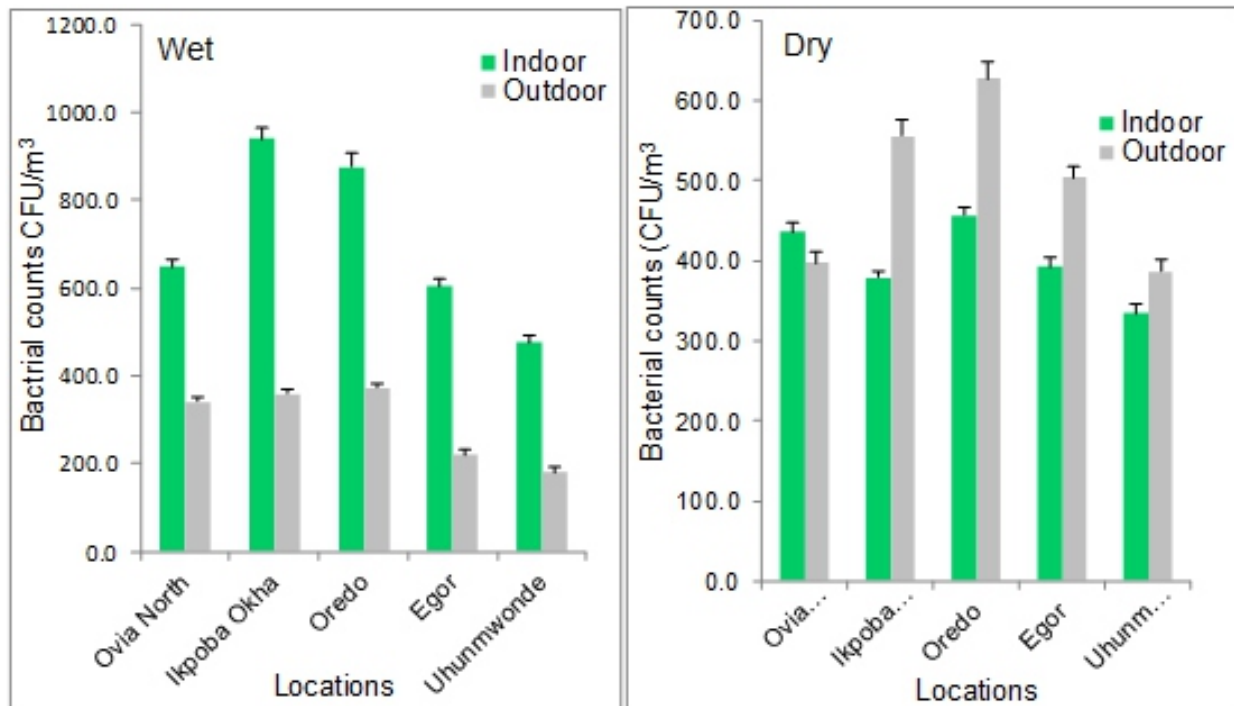
## RESULTS

### Mean Concentrations of indoor and outdoor airborne bacteria

The variation in concentrations of the indoor and outdoor airborne bacteria at the monitoring locations in the wet and dry seasons are presented in Figure 2. The mean indoor airborne bacteria ranged from 476.1 to 939.3 CFU/m<sup>3</sup> and 335.0 to 457.2 CFU/m<sup>3</sup> in the wet and dry seasons. The maximum indoor airborne bacterial count (939.3 CFU/m<sup>3</sup>) was observed in Ikpoba Okha, while the lowest (335.0 CFU/m<sup>3</sup>) was observed in Ovia North. The indoor bacterial concentrations varied significantly throughout the sampling locations ( $F = 26.4, 47.8$ ;  $P < 0.005$ ) in both the dry and wet seasons.

The mean count of the total outdoor airborne bacteria ranged between 181.1 to 373.2 CFU/m<sup>3</sup> and 387.2 to 627.8 CFU/m<sup>3</sup> in the dry season, respectively (Figure 2). The highest outdoor bacterial count (627.8 CFU/m<sup>3</sup>) was determined in Oredo, while the minimum count (181.1 CFU/m<sup>3</sup>) was observed in Uhunmwonde. There were significant variations ( $F = 67.5, 17.1$ ;  $P < 0.05$ ) in the concentrations of observed outdoor bacterial counts during the wet and dry seasons.





**Figure 2:** Total indoor and outdoor bacteria (CFU/m<sup>3</sup>) in the wet and dry seasons. Values are means  $\pm$  standard deviation of triplicates.

#### Seasonal variations of airborne bacteria

The t-test showed that the average indoor and outdoor concentrations of the bacterial population during the wet season were higher ( $P > 0.01$ ) than in the dry season for all the sampling sites (Table 1). The concentrations of

indoor airborne bacterial were significantly higher in the wet than in the dry season ( $t = 20.88$ ,  $P < 0.05$ ) but in the outdoor environment, there were higher concentrations of airborne bacteria in the dry season ( $t = -13.62$ ,  $P < 0.05$ ).

**Table 1:** Seasonal variation of airborne bacterial concentrations.

		Mean difference	t	p-value
Indoor	TBCwet – TBCdry	234.2021	20.888	0.000
Outdoor	TBCwet – TBCdry	-95.4733	-13.620	0.000

TBC: Total bacterial counts

#### Composition of airborne bacterial composition

The 16S rRNA gene sequencing and BLASTn (Basic local alignment search tool) analysis revealed that all the bacterial isolates belonged to the phylum of proteobacteria and the GC-rich *Enterobacteriales* family (Table 2). The study showed that the 16S rRNA gene sequences of four bacterial isolates (N4, N5, N6, and N7) were

identified as *Proteus spp*, while isolates N1 and N3 were confirmed as *Pectobacterium* and *Enterobacter spp*. Isolates N4, N5, N6, and N7 had a 100, 97.29, 90.23, and 100% similarity with *Proteus mirabilis* in the indoor and outdoor air samples. Isolates N1 and N3 had 99.29 and 99.34% similarity with *Pectobacterium brasiliense* and *Enterobacter sichuanensis*, respectively (Table 2).

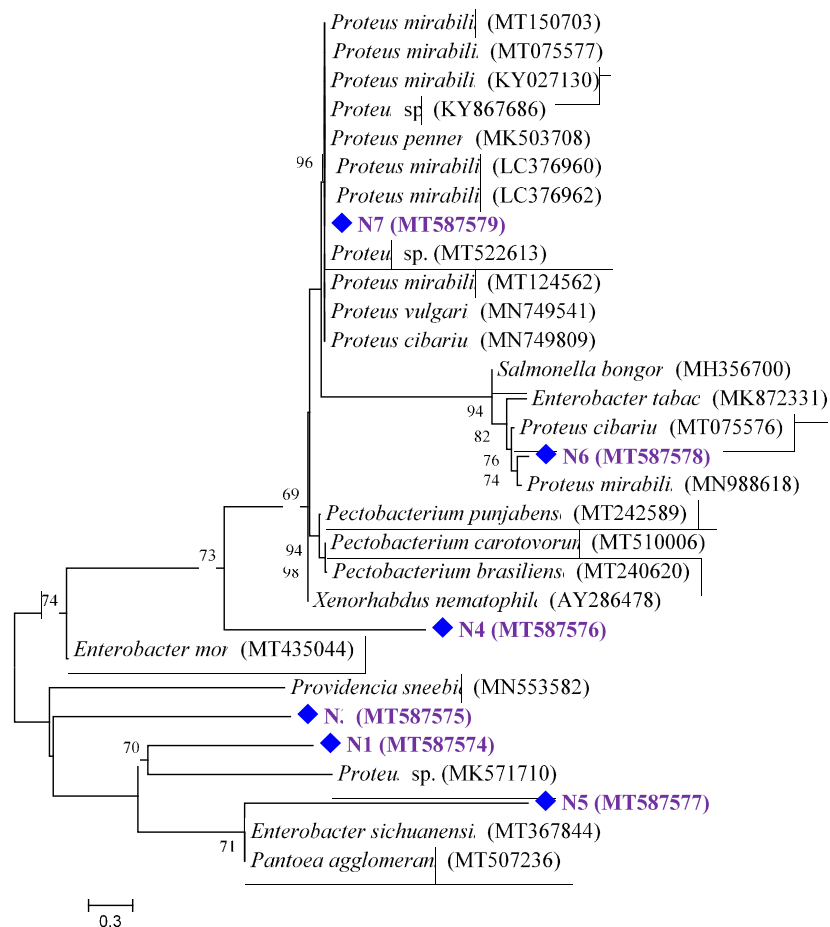
**Table 2:** Bacterial isolates identified in the indoor and outdoor air.

Sequence Id	Accession No	Organism	% Similarity	strain
SeqN1	MT587574	* <i>Pectobacterium brasiliense</i>	99.29	BN1
SeqN3	MT587575	+* <i>Enterobacter sichuanensis</i>	99.34	BN3
SeqN4	MT587576	+ <i>Proteus mirabilis</i>	100.00	BN4
SeqN5	MT587577	+ <i>Proteus mirabilis</i>	97.29	BN5
SeqN6	MT587578	+ <i>Proteus mirabilis</i>	90.23	BN6
SeqN7	MT587579	* <i>Proteus mirabilis</i>	100.00	BN7

\*Outdoor airborne bacteria, +Indoor airborne bacterial isolate

The phylogenetic tree showed that N6 had a close relationship with *Proteus mirabilis* having an accession number (MN988618) and *Proteus cibarius* (MT075576) and (MH130322). Isolate N4 was remarkably related to *Proteus cibarius* (MT075576) and *Proteus mirabilis* (MN988618). Isolate N4 had a close relatedness to three species of *Pectobacterium* with a 94%, 82%, and 76% bootstrap value for *Pectobacterium carotovorum* (MT510006), *Pectobacterium carotovorum* (MT510006), and

*Pectobacterium brasiliense* (MT240620), respectively. Isolates N3 and N5 and N1 are closely related to *Enterobacter sichuanensis* (MT367844) and *Proteus* sp. (MK571710) with bootstrap values of 73 and 71%, respectively. Also, isolate N7 with a 96% bootstrap value was closely related to *Proteus mirabilis* (MT150703), *Proteus* sp. (KY867686) *Proteus penneri* (MK503708), *Proteus vulgaris* (MN749541) and *Proteus cibarius* (MN749809) (Figure 3).



**Figure 3:** A maximum likelihood Tree showing phylogenetic affiliation of 16S rRNA Sequences of isolated indoor and outdoor bacterial species.

## DISCUSSION

The European Commission's sanitary standards for non-industrial environments suggested that airborne microbial particles between the range of 500–2000 CFU/m<sup>3</sup> are considered a 'high' load (CEC, 2003). Based on this recommendation, the bacterial load recorded in most of the sampling locations in this study is considered intermediate or above the recommended minimum threshold and could pose health effects to the residents of the study area. High concentrations of indoor and outdoor airborne bacteria have been previously reported in Ibadan, Nigeria (Alli *et al.*, 2017). Several factors that are likely to determine the increased microbial population in the study area are poor ventilation rates occasioned by the closure of windows, tight apartments, and overcrowding of rooms which was widely observed during the sampling regime. The high indoor airborne bacterial load may have also been strongly influenced by the re-suspension of dust from the floor and room surfaces due to various human activities (such as washing, flushing of toilet, talking, walking, and sweeping and cleaning of floors), which agitated previously settled microbial particles on the surfaces (Chen *et al.*, 2010). In addition, the direct shedding of resident bacteria on the skin, nostrils, hair of humans, and clothing found in indoor air could have contributed to the high bacterial load (Milstone *et al.*, 2004).

The mean counts of bacterial concentration in the sampling locations differed significantly from one sampling area to another. These variations in total bacterial counts across the locations could be attributed to varying environmental conditions, availability of substrates, and level of human occupancy in the sampling sites (Eghomwanre and Oguntoke, 2022). The significantly increased indoor bacterial count during the wet season could be attributed to leakages in the buildings which result in moist walls and floors that favour the growth of bacterial communities in the wet season. This finding is consistent with a previous study (Kang *et al.*, 2015) that observed that the level of culturable airborne bioaerosols in the wet season was higher when compared with the dry season due to increased relative humidity. Rainfall aid in the distribution of microbial particles from

higher elevations to ground-level environments, while cloud acts as a cover to prevent the penetration of ultraviolet irradiation, which inactivates culturable microorganisms (Kang *et al.*, 2015). However, this study is at variance with the reports of studies that opined that increased temperature and relative humidity of the environment favour the growth of microorganisms (Ren *et al.*, 2001; Ambrose *et al.*, 2015).

This study also revealed that the indoor and outdoor bacterial isolates expressed a very high similarity value (90.23 - 100%). This is above the borderline of 70% degree of identity as previously reported (Aremu and Bankole, 2005). Therefore, it indicates that the organisms share a more recent common ancestor, hence they are closely related. Furthermore, the similarities expressed by most of these isolates with the reference taxa belonging to different species are because of the high similarity value exhibited in the values of DNA re-association (Aremu and Bankole, 2015). Several studies have reported species of the genus *Proteus* and *Enterobacter* in the air. These bacterial isolates have been associated with indoor and outdoor environments in previous studies (Wemedo *et al.*, 2012; Ambrose *et al.*, 2015; Al-Mayahi, 2017; Agwaranze *et al.*, 2020). *Proteus mirabilis* is part of the normal flora of the human gastrointestinal tract. It is an opportunistic pathogen that can cause nosocomial infections, urinary tract respiratory, ear, digestive, and wound infections, burns, and otitis media (Al-Mayahi, 2017). *Enterobacter* sp can be found on the human skin, plants, soil, water, sewage, and intestinal tracts of man and animals. They are considered opportunistic pathogens, rarely causing disease in healthy individuals, but associated with infections in immune-compromised individuals. Uchida *et al.* (2020) also reported *Enterobacter sichuanensis* strain SGAir0282 from outdoor air by the playground where there is intense human activity in Singapore. They suggested that even though the species is predominantly found in soil, it may have been agitated from the floor and disseminated into the air before it was deposited on the agar plate. This indicates that environmental bacteria are transported by the wind in the form of aerosols (Milstone *et al.*, 2004).

## CONCLUSION

This study assessed the level of airborne bacteria in and around selected households in Benin City, a humid tropical region characterized by high relative humidity. The indoor and outdoor bacterial counts in most of the sampling locations were above the recommended limits for minimum concentrations of airborne bacteria that can cause significant human health effects. This study provided an understanding of the bacterial community in the indoor and outdoor environments in the study area. It also identified airborne bacteria such as *Pectobacterium brasiliense* and *Enterobacter sichuanensis* which have not been identified by traditional culture-based studies. Further studies on airborne bacteria in the study area using non-culture-dependent 16S rRNA techniques would be required for a comprehensive understanding of airborne bacterial communities in and around residential buildings.

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