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Original Article

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Multidrug resistance in bacteria isolated from indoor air of female hostels in a tertiary institution

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ABSTRACT

Objective: To determine the occurrence of multidrug resistant bacteria from the indoor environment of female hostels in a tertiary institution in order to provide epidemiological data.

Methods: The bacterial quality of the three female hostels was evaluated using the settle plate method, where Petri dishes containing different types of culture media were opened in the rooms. Isolated bacteria were identified using standard microbiological procedures. Using disc diffusion method, the antibiogram of the isolates was determined and based on this, the multiple antibiotics resistance index was also determined.

Results: The total heterotrophic colony forming units (CFU) for Hall A ranged from 2.09 x10^2 to 1.73 x10^3 CFU/m^3 while that of Hall B ranged from 4.71 x10^2 to 1.10 x10^3 CFU/m^3 and a statistically significant difference between the counts of both halls was observed (P=0.04). Microorganisms isolated included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Corynebacterium* sp., *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. All the isolates exhibited multidrug resistance to amoxycillin-clavulanic acid, cefuroxime and ceftriaxone. *Proteus mirabilis*, *Klebsiella pneumoniae* and *Corynebacterium* sp. had the least multiple antibiotic resistance index with 0.2 while *Staphylococcus aureus* had the highest with 0.8.

Conclusions: Female university hostels may become sources of exchange of microorganisms, especially in overcrowded rooms. A large percentage of isolates were multidrug resistant which could pose difficulty and increased cost of treatment of their resulting infections. Regular surveillance and control of the agents that encourage the growth of these bacteria present in indoor air is needed.

KEYWORDS: Indoor air; Multiple antibiotic resistance index; Multidrug antibiotics resistance; Settle plate technique; Bacteria

1. Introduction

Many factors affect indoor air quality which is important to people's comfort and health. The quality of air in a room can rarely be controlled which poses a health risk to the occupants[1]. Indoor air quality refers to the quality of air in a closed setting which is influenced by the concentration of pollutants, relative humidity and temperature[2]. These pollutants impart the health, performance and comfort of the individuals inhabiting such rooms.

Significance

Previous studies have shown the presence of bacteria in indoor air of environments such as hospitals and university administration buildings. However, there is paucity of information on their presence in university hostels in Nigeria. Our study has provided information on the presence of multiple drug resistant bacteria from female hostels of a tertiary institution in Nigeria. The presence of these microbes may be associated with the number of students per room and consumption of antibiotics without conducting appropriate tests and doctor's prescription.

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Microorganisms are present in every environment including air. Bioaerosols made up of moulds, yeasts and bacteria populates the air inhaled by individuals indoors[3]. Hypersensitivity, rhinitis, asthma and pneumonitis infections are some of the results of exposure to air harbouring microorganisms[4]. According to the World Health Organization (WHO), a basic necessity for life is clean air[5] and an average of 80-95% of time is spent in enclosed environments by most individuals[6]. Environmental conditions such moisture in walls and ceilings are common but neglected in buildings[7]. Consequently, a WHO review on some studies have shown a strong association between several respiratory illnesses and dampness of indoor air[8]. This is because air is a major vehicle for the transportation of microbes from outdoor to rooms through wind, walking and sweeping[9].

There has been increased cases of multiple antibiotics resistance especially among pathogenic bacterial isolates from environmental sources[10,11]. However, there is paucity of information on the antibiotics sensitivity profile of these isolates, especially those from university hostels. Methods of quantitatively determining the microorganisms in air include filtration, thermal and electrostatic precipitation, settle plate and centrifugation[8]. This study was aimed at evaluating the presence of multidrug resistant bacteria from the indoor air of female hostels in Caleb University, Lagos, using the settle plate method.

2. Materials and Methods

2.1. Study area

Caleb University is a private tertiary institution located in Imota axis of Lagos State in Nigeria. It began operating in January 2008 and is located in a 110 hectares land at Imota, Lagos. Presently, the student population is over 8 500 with the females making up more than 50% of the total population. It has male and female hostels situated in different buildings. The females occupy three buildings identified by different names. This study was conducted between February and April, 2022.

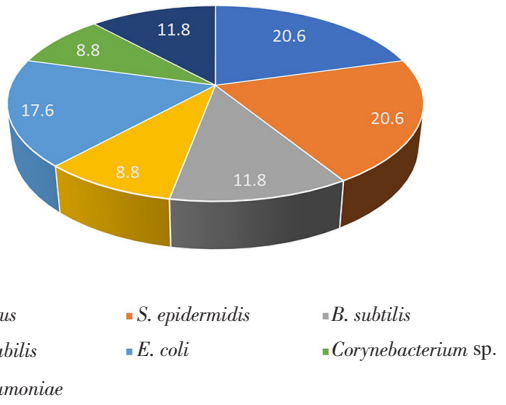

Figure 1. Percentage occurrence of the bacterial isolates (%).

2.2. Sampling procedure

Measurement of the bacteria in the rooms was done using 9 cm Petri dishes kept at the centre of the rooms using the settle plate method also called sedimentation method. The height for sampling was 2 m above the ground. Nutrient agar (TM media, India) prepared according to manufacturer's instruction, was used to isolate bacteria. The agar plates were exposed for 15 minutes and the samples were collected between 8 and 9 am when students were still in their rooms. Samples were immediately transferred to the Laboratory of the University (Department of Biological Sciences and Biotechnology, Caleb University) and incubation was done at 37 ℃ for 24 h. The CFU seen on dishes were used to determine the CFU per cubic meter (CFU/m^3) using the Omeliansky formular below[6].

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

Where "N" is CFU/m^3 of the indoor air, "a" is number of colonies in a Petri dish, "b" is surface area of the Petri dish, "t" is time of exposure in minutes. Identification of isolates was done using standard microbiological methods including microscopy and biochemical tests.

2.3. Antibiotics testing

The antibiotics sensitivity pattern of the isolates was done as described by the Clinical and Laboratory Standards Institute using the disk diffusion method[12]. The isolates were grown in Nutrient Broth (TM media) for 24 h at 37 ℃. The concentration of the isolates in the broth was adjusted to 0.5 MacFarland standard. Using sterile cotton swabs, the isolates were streaked on Mueller-Hinton agar plates (TM media, India). Using forceps, the antibiotics disks were aseptically placed on the surface of the agar. Antibiotics used included erythromycin (15 μg), gentamicin (15 μg), amoxycillin-clavulanic acid (30 μg), ofloxacin (5 μg), cefuroxime (30 μg), ceftriaxone (30 μg) and oxacillin (5 μg)

Table1. Colony forming units in both Halls.

Rooms	Colony forming units (CFU/m^3)	
	Hall A	Hall B
1	$1.05x10^3$	$7.34x10^2$
2	$1.31x10^3$	$1.10 x10^3$
3	$1.10x10^3$	$4.71x10^2$
4	$8.90x10^2$	$7.34x10^2$
5	$1.73x10^3$	$4.71x10^2$
6	$1.57x10^3$	$8.91x10^2$
7	$2.09x10^2$	$4.71x10^2$
8	$1.15x10^3$	$6.81x10^2$
9	$6.29x10^2$	$6.81x10^2$
Mean±SD	$1.07±0.46 x10^2$	$6.93±0.21 x10^2$
Range	$2.09x10^2$-$1.73 x10^3$	$4.71 x10^2$-$1.10 x10^3$
P value	0.04	

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(Abtek Biologicals, Liverpool UK and Oxoid Ltd, England). Oxacillin was used for *S. aureus* isolates alone. The inoculated agar plates were incubated for 24 h at 37 ℃.

2.4. Multi–antibiotic resistance index

The multiple antibiotic resistance (MAR) index of the isolates were determined using the formula a/b, where "a" is the number of antibiotics which a microbe is resistant to and b the number of antibiotics the microbe was exposed to[13].

2.5. Analysis of results

Testing of significance difference between the bacterial concentrations in the rooms was done using Analysis of Variance on the SPSS 24.0. The mean and standard deviation was also calculated.

3. Results

Samples were collected from 18 rooms in the hostels with varied bacterial counts obtained (Table 1). The colony counts in Hall A ranged from 2.09 x10^2 to 1.73 x10^3 CFU/m^3 while that of Hall B ranged from 4.71 x10^2 to 1.10 x10^3 CFU/m^3. The mean value for Hall A was 1.07 x 10^2 CFU/m^3 while Hall B had a higher mean value with 6.93 x 10^2 CFU/m^3. The *P* value calculated was 0.04 indicating a significant difference between the colony counts obtained from both halls. *Staphylococcus (S.) aureus* (20.6 %), *S. epidermidis* (20.6 %), *Bacillus (B.) subtilis* (11.8 %), *Proteus (P.) mirabilis* (8.8 %), *Corynebacterium* sp. (8.8 %), *Escherichia (E.) coli* (17.6 %), and *Klebsiella (K.) pneumoniae* (11.8 %) were isolated from the samples at varied percentages (Figure 1).

The disk content of each antibiotic tested, the corresponding class and the sensitivity pattern of the isolated bacteria is presented (Table 2). Ofloxacin and oxacillin had the least concentrations with 5 µg while amoxycillin-clavulanic acid, cefuroxime and ceftriaxone have higher concentrations with 30 µg. Ofloxacin recorded a higher sensitivity percentage with 100% while amoxycillin-clavulanic acid recorded the least with 0%.

All the isolates were resistant to amoxycillin-clavulanic acid and ceftriaxone while they exhibited a high percentage of susceptibility to ofloxacin (Table 3). The multiple antibiotics resistance index of the isolates is presented in Table 4 with *S. aureus* having MAR index of 0.8 and *P. mirabilis*, *K. pneumoniae* and *Corynebacterium* sp. having the least with 0.2; *E. coli* recording 0.6 and *B. subtilis* and *S. epidermidis* having MAR of 0.3 and 0.7, respectively.

Table 2. Percentage sensitivity of the isolates to multiple antibiotics.

Antibiotic	Disk content (µg)	Antibiotic class	Number of isolates	Resistant (%)	Sensitivity (%)
Amoxycillin-clavulanic acid	30	Penicillin	34	34(100)	0(0)
Erythromycin	15	Macrolide	34	20(58.8)	14(41.2)
Ofloxacin	5	Fluoroquinolone	34	0	34(100)
Gentamicin	15	Aminoglycoside	34	8(23.5)	26(76.5)
Cefuroxime	30	Cephalosporin	34	24(70.6)	10(29.4)
Ceftriaxone	30	Cephalosporin	34	34(100)	0(0)
Oxacillin	5	Penicillin	7	3(42.9)	4(57.1)

Table 3. Percentage resistance of the isolates to multiple antibiotics (%).

Antibiotic	*S. aureus*	*S. epidermidis*	*B. subtilis*	*P. mirabilis*	*E. coli*	*Corynebacterium* sp.	*K. pneumoniae*
Amoxycillin-clavulanic acid	100	100	100	100	100	100	100
Erythromycin	28.6	57.1	25	100	83.3	33.3	100
Ofloxacin	0	0	0	0	0	0	0
Gentamicin	28.6	14.3	0	0	33.3	0	75
Cefuroxime	85.7	100	25	33.3	66.7	33.3	100
Ceftriaxone	100	100	100	100	100	100	100
Oxacillin	42.9	0	0	0	0	0	0

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Table 4: Multiple antibiotics resistance index of the isolated bacteria.

Bacteria	Number of isolates	Average MAR* index
S.aureus	7	0.8
S. epidermidis	7	0.7
B. subtilis	4	0.3
P. mirabilis	3	0.2
E. coli	6	0.6
Corynebacterium sp.	3	0.2
K. pneumoniae	4	0.2

*: Multiple antibiotic resistance

4. Discussion

A necessity for life is air, it is needed at about 10-20 m^3 per day and is seen as a basic human right as well[3,14]. The highest bacterial count recorded from indoor air in this study was from Hall A (Room 5) with 1.73 x10^3 CFU/m^3. This range of colony counts have been reported by previous studies[7, 15]. Although there are no widely accepted standards with regards to microbial counts of indoor air, a group of experts from the WHO have suggested a limit of $\leqslant$1 000 CFU/m^3[16]; another group of researchers had suggested $\leqslant$750 CFU/m^3[17]. Also, the European sanitary standards says that the bacterial colony count for non-industrial buildings should not exceed 500 CFU/m^3[18]. The National Ambient Air Quality Standard set its limit at >400[19]. Comparing all four standards, the counts recorded in this study were above this limit which makes the rooms below standard. This could be a result of increased activities within the rooms as they prepare for classes for the day. Concentration of particulate matter, temperature, ventilation and humidity has been found to either decrease or increase the microbial count of indoor air[20]. The size of rooms and the number of occupants are also factors that could cause variation in microbial counts as more microorganisms from the human body will be shed[3]. Statistically, the concentration of bacteria in both halls were significantly different (P<0.05). This difference could be explained by the number of persons in the rooms in relationship to the size of the rooms at the time of sampling[6].

The presence of *K. pneumoniae*, *S. epidermidis*, *E.coli* and *B. subtilis* in this study which are often associated with patients with respiratory illnesses and potential causes of sick building syndromes is in consonance with previous studies[7,21–24]. *S.* spp. which occurred most are saprophytic microflora and often found on the mucosa and human skin and this according to Sa'id and Salihu suggests that humans are the main source of bacterial contamination of indoor air[9]. Preventing overcrowding, good ventilation system and preventing the walls and room surfaces from being moist are some ways of improving indoor air quality[25,26]. The cleanliness of the rooms may also have contributed to this variance as some occupants may be neater than others and would ensure that the room is kept clean. Moist surfaces are known to encourage the growth and proliferation of fungal and bacterial spores and an example of a spore-forming bacteria is *B. subtilis*.

Cephalosporin and Penicillin resistance exhibited by these isolates have been reported in previous studies[27,28]. These drugs are also called "over the counter drugs" as they are affordable, easily accessible and widely used in most developing countries such as Nigeria even without doctor's prescription[27]. This has promoted the rise and spread of antibiotics resistance and consequently, difficulty and increased cost of treatment of infectious diseases as these drugs are often the first line drug administered to patients. These bacteria may also serve as reservoirs of plasmids and resistance genes that could be horizontally or vertically transferred to other bacteria[29,30].

MAR is used to analyse the level of resistance to antibiotics as well as health risk[31]. It is a method for tracking bacteria source that is easy to carry out, requires no specific training, low cost and fast tracking. A value greater than 0.2 shows the microbes were isolated from a source where there is frequent use of antibiotics[32]. *S.* spp., *B. subtilis* and *E. coli* recorded MAR index values ranging from 0.3 to 0.8. This points to the frequent use of antibiotics by these female students which should be discouraged, except when prescribed by a physician.

5. Conclusions

The indoor air of Hall B was less contaminated with bacteria compared with Hall A. However, according to the National Ambient Air Quality Standard and the European Sanitary Standard, both hostels were highly contaminated as the bacterial counts exceeded the limits. Bacteria isolated have been linked with cases of sick building syndromes and ailments such as conjunctivitis, asthma and rhinitis, which calls for regular monitoring and control of the factors that promote the

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propagation of bacteria in the indoor air of these hostels, in order to promote the health of the occupants.

Conflict of interest statement

We declare that we have no conflict of interest.

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Authors' contributions

Olorunfunmi MO was responsible for literature collection, writing the draft of the article, methodology planning, local ethical application, data collection and data management. As the corresponding author, Adebayo-Olajide TC carried out data analysis, controlled the content of the whole review, serves as the content matter expert and supervisor for the entire study.

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