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MICROBIOLOGICAL ASSESSMENT OF INDOOR AIR QUALITY OF STUDENT HOSTELS AND CANTEENS IN A NIGERIAN UNIVERSITY CAMPUS: RISKS ON STUDENTS' HEALTH

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ABSTRACT

Microbial air contaminants affect the quality of air we breathe and these microbial air contaminants can settle on body parts, clothes, food etc. which poses a serious threat to human health. Therefore, the level of bacterial contamination in various in-door air of the students' hostels and canteens of Kaduna State University (KASU) and their antibiotics susceptibility patterns was studied. A total of 44 samples were collected from the rooms (18), toilets (18) of the students hostels and canteen (8) of KASU; using the settle plate method. Gram staining and standard biochemical methods were used to identify the bacterial isolates and antibiotic susceptibility testing was determined by disc diffusion method. The bacteria load was between 1.2 x 10^3 to 6.7 x 10^3 cfu/ml^3. A total of 30 bacteria isolates were identified, *Staphylococcus aureus* had the highest (9, 30%) occurrence, followed by *Streptococcus* spp (6, 20%), *Bacillus subtilis* and *Clostridium* spp had similar (4, 13.2%) occurrence, The antibiotic susceptibility testing carried out revealed that the Gram negative and Gram positive bacteria isolates were more susceptible to vancomycin (75%, 43.5%), tetracycline (75%, 47.8%) and gentamicin (50%, 43.5%) while high resistance was observed with chloramphenicol (100%, 73.9%), ceftriaxone (100%, 100%), cefoxitin (100%, 73.9%), amoxicillin/clavulanic acid (100%, 87%), and trimethoprim/sulphamethoxazole (100%, 78%). All the indoor samples were contaminated with pathogenic bacteria and there was high level of multidrug resistance. This is very dangerous to the students' health because it can be a source of transmission infections and antibiotic resistant bacterial strains.

Keywords: Indoor air, Assessment, Microorganisms, Quality

INTRODUCTION

Buildings and indoor air quality can affect human health and overall well-being because most people spend most of their time inside, in built environments such as homes, schools, workplaces, gym, and places of worship. Previous study had shown about 90% of people spend almost 22 hours indoor every day (Klepeis *et al.*, 2001)

Microorganisms and toxins are examples of biological materials which can be found in air, and the study of this area is termed aeromicrobiology (Pepper and Gerba, 2015). These microbes are referred to as bio-aerosols (Brooks *et al.*, 2004). Air also serves as transport or dispersal medium for microorganism, they occur in relatively small number in air when compared with soil or water. (Ghosh *et al.*, 2015). Once suspended in the air, microbes have the opportunity to travel long distance with the help of wind and precipitation increasing the occurrence of wide spread diseases by these microorganisms (Obayori, 2023).

The commonest genera of fungi found in indoor air are *Penicillium, Aspergillus, Cladosporium, Alternaria,* and yeast while the commonest genera of bacteria found in indoor air are *Staphylococci, Bacillus* and *Clostridium* (Nevalainen, 2009; Shiva, 2009). 'Legionnaires disease', a form of pneumonia caused by exposure to the *Legionella* bacterium has been associated with buildings with poorly maintained air conditioning or heating systems (Fields *et al*., 2002, NASEM, 2020).

In the dust and air of schools and hospital wards or the rooms of persons suffering from infectious disease, microbe such as *Tubercle bacilli, Streptococci, Pneumococci* and *Staphylococci* have been isolated. These respiratory bacteria are dispersed in air in droplets of saliva and mucus produced by coughing, sneezing, talking and laughing (Wang *et al*., 2021).

A review made by World Health Organization (WHO) on a number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effect on respiratory health, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and dyspnea. (WHO, 2009).

Air is a micro flora for different types of microorganisms which affects the quality of air we breathe and these microbial air contaminants can settle on body parts, clothes, desk, books, bags and food (too much exposure to air) etc., which may cause food poisoning and other infectious diseases. Short time exposure to poor air quality can result in illness and microbial infections, while long-term exposure can lead to serious complications (Mandal *et al.*, 2011; Sharma *et al* 2011a and Sharma *et al* 2011b). Chance for the spread of infectious diseases through indoor air is more especially in areas where people gather in large numbers, for example schools' lecture theatres, hostels and canteens. Examples of air borne bacterial diseases include pulmonary anthrax, diseases caused by *Streptococcus pyogenes*, rheumatic fever, *Streptococcal pneumonia*, meningitis, diphtheria, tuberculosis, and legionellosis (Karottki *et al.*, 2015; Mack *et al.*, 2019; Nevalainen, 2009). Prussin and Marr (2015) identified eight major sources of airborne microorganisms in the built environment, these include humans, pets, plumbing systems, plants, heating, ventilation, and air conditioning systems, mould, dust re-suspension and outdoor environment. Kaduna State University (KASU) is one of the fastest growing university in Nigeria with about 50,000 students across

different states for different programmes. Good accommodation that poses no threat to human health is part of factors that can promote good academic performance of students on campus. In view of this, this study was undertaken to assess indoor air bacteriological qualities of KASU female students' hostel, medical students' hostel and canteens; and to carry out the antibiotic susceptibility testing of the isolated bacteria, knowing fully well that antimicrobial resistance (AMR) now poses a serious global threat of growing concern to human, animal, and environmental health (Davies and Davies, 2010).

MATERIALS AND METHODS

Study area and sample collection

A total of 44 indoor air samples were collected from the following locations in Kaduna State University: toilets (18), rooms of the university female hostel (6) and medical hostel (male (6) and female (6), and canteens (8). Each sample was collected in duplicate, they were taken in the morning before the students left the hostels and the sampling time was 10 minutes.

Air sampling technique and determination of total bacterial count

Air sampling was performed using the settle plate method, 9 cm-diameter petri dishes containing sterile nutrient agar were used. The petri dishes were placed at two different places in the chosen locations. All the plates were placed at about 1 m height above the floor and were exposed for ten (10) minutes. After exposure, the plates were covered with their lids and were taken to the laboratory and incubated at 37°C for 24 hours in an incubator. The number of microorganisms were expressed as cfu/m^3 was estimated using the following equation described by Omeliansky (Borrego *et al.*, 2010; Gutarowska, 2010).

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

Where N = microbial cfu/m^3 of indoor air

a = number of colonies per petri dish

p = the surface measurement of the petri dish used in cm^2

t = the time of exposure of the petri dish (min)

Identification and Isolation of Bacteria

Gram staining was carried out on each isolate according to Cheesbrough, (2002) to classify the organism to either Gram-positive or Gram-negative. The organisms were also cultured onto selective media such as cetrimide agar, mannitol salt agar, macConkay agar for further identification. The following biochemical tests were carried out to further identify the organisms into specific bacterial isolates: coagulase test, methyl red, Voges Proknauer test, citrate, oxidase, indole, catalase and urease tests (Cheesbrough, 2002).

Antibiotics Susceptibility Test

Antibiotics susceptibility test was performed on each isolate using agar disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) recommendation (CLSI, 2018). The following antibiotic discs were tested: erythromycin (15µg**),** gentamicin (10µg**), c**hloramphenicol (30µg**), v**ancomycin (30µg**),** ceftriaxone (30µg**),** cefoxitin (30µg**),** amoxicillin/clavulanic acid (30µg**), c**iprofloxacin (5µg**),** trimethoprim/sulphamethoxazole (25µg**), t**etracycline (30µg**).**

A standard inoculum (approx. 10^8 cfu/ml) was prepared by making a turbid suspension of the isolates in sterile normal saline and compared with 0.5 McFarland Standard. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar plates. Sterile forceps were then used to place the antibiotic discs in a circular pattern on the media. The process was carried out for all the identified isolates, the plates were kept on the bench for about 30 minutes to allow the antibiotics to diffuse into the agar, the plates were, thereafter, incubated at 37°C for 24 hours in an incubator. After incubation, the zone of inhibition in diameter for each antibiotic was measured and interpreted as either sensitive, intermediate or resistant according to CLSI guidelines.

RESULTS AND DISCUSSION

The mean bacterial colony count of the indoor air of the samples ranged from 1.2×10^3 to 6.7×10^3 cfu/m^3. The details are presented in Table 1 below. The Gram staining results showed 26 (86.7%) and 4 (13.3%) to be Gram positive and Gram negative respectively. A total of 30 bacteria were isolated: *Staphylococcus aureus* had the highest (9, 30.0%) occurrence, followed by *Streptococcus* spp (6, 20.0%). (Figure 1).

Table 1: In-door bacteria mean colony count in Canteens, Female hostel and Medical hostel of Kaduna State University

S/N	Sampling site/mean bacteria colony count (cfu/m^3)					
	Female hostel		Medical hostel		Canteen	
			Male	Female		
1.	Room 1	1.2×10^3	1.9×10^3	1.9×10^3	Canteen 1	1.8×10^3
2.	Room 2	2.7×10^3	3.5×10^3	2.2×10^3	Canteen 2	2.4×10^3
3.	Room 3	1.9×10^3	2.4×10^3	2.1×10^3	Canteen 3	4.7×10^3
4.	Toilet 1	3.8×10^3	3.6×10^3	3.6×10^3	Canteen 4	2.2×10^3
5.	Toilet 2	6.7×10^3	3.4×10^3	3.2×10^3		
6.	Toilet 3	5.5×10^3	4.2×10^3	4.9×10^3		

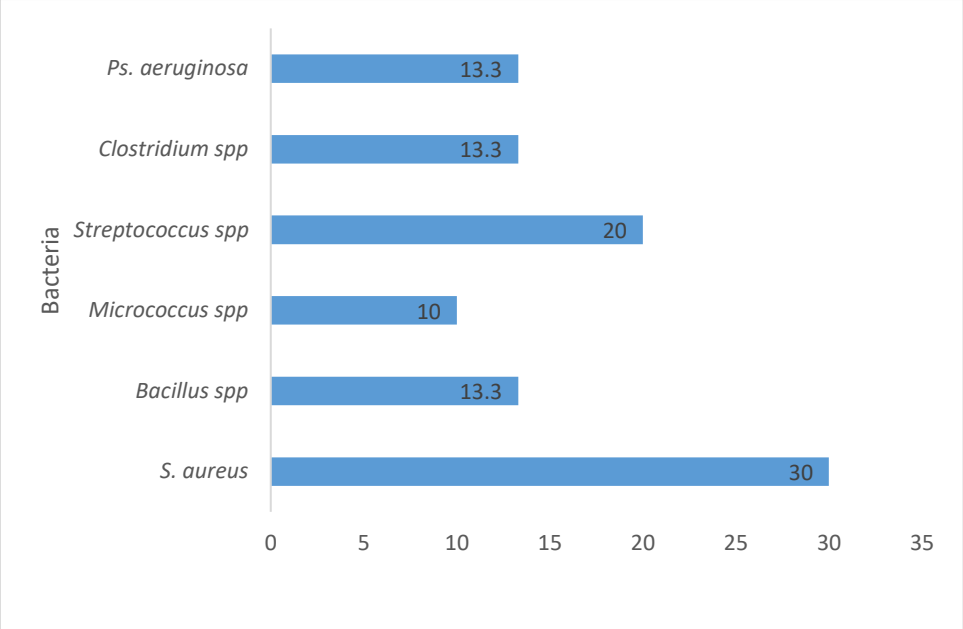

Figure 1: Percentage occurrence of bacterial isolates from the students' hostels and canteens of Kaduna State University

Antibiotics Susceptibility Test of bacteria from Canteens, Female hostel and Medical hostel of Kaduna State University

The Gram positive isolates were highly resistant to most of the antibiotics tested especially ceftriaxone (100%). This is followed by amoxicillin-clavulanic acid (87%), ciprofloxacin (82.6%), trimethoprim-sulphamethoxazole (78.3%), cefoxitin (73.9%) and chloramphenicol (73.9%). The most active antibiotics were erythromycin (47.8%) and tetracycline (47.8%), followed by gentamicin (43.5%) and vancomycin (43.5%) as shown in Table 2.

Table 2: Percentage Antibiotics Susceptibility of Gram positive Isolates from canteens and students hostels

Antibiotics	No of isolates (n) = 23 n (%)		
	Sensitive	**Intermediate**	**Resistant**
Erythromycin	11 (47.8)	2 (8.7)	10 (43.5)
Gentamicin	10 (43.5)	0	13 (56.5)
Chloramphenicol	6 (26.1)	0	17 (73.9)
Vancomycin	10 (43.5)	0	13 (56.5)
Ceftriaxone	0	0	23 (100)
Cefoxitin	6 (26.1)	0	17 (73.9)
Amoxicillin clavulanic acid	3 (13.0)	0	20 (87.0)
Ciprofloxacin	2 (8.7)	2 (8.7)	19 (82.6)
Trimethoprim sulphamethoxazole	3 (13.0)	2 (8.7)	18 (78.3)
Tetracycline	11 (47.8)	0	12 (52.2)

The Gram negative isolates were 100% resistant to five antibiotics namely chloramphenicol, ceftriaxone, cefoxitin, amoxicillin-clavulanic acid, and trimethoprim-sulphamethoxazole. Tetracycline (75%), and vancomycin (75%) were the most active antibiotics against the Gram negative isolates (Figure 2).

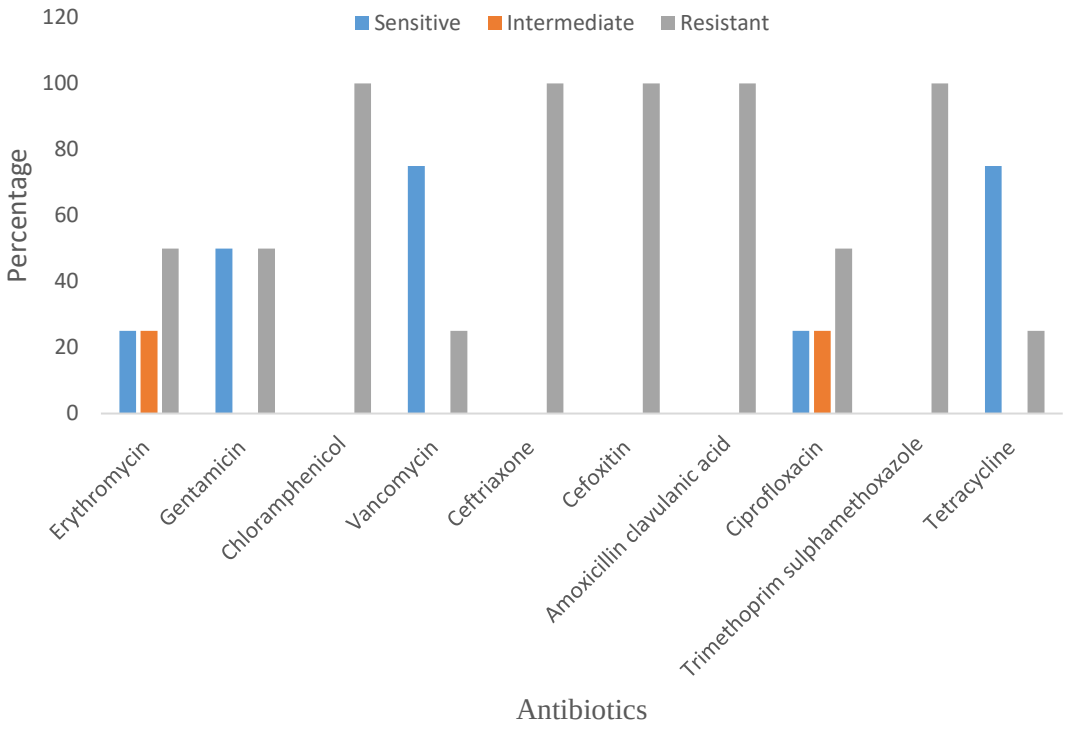

Figure 2: Percentage antibiotics susceptibility of Gram negative isolates from canteens and students hostels

Discussion

The mean bacterial colony count of the indoor air of the sampled canteens in the Kaduna State University ranged from 1.8 x 10^3 to 4.7 x 10^3 cfu/m^3 while that of the students' hostels ranged from 1.2 x 10^3 to 6.7 x 10^3 cfu/m^3. The highest bacterial load was observed in one of the female students' toilets. The difference in the bacterial load observed from the various sample site may be due to difference in the number of students staying in each room and those accessing the various canteens. The total bacterial counts obtained in this study was higher than of some previous studies: Stefanovic *et al.* (2021) reported 35 to 828 cfu/m^3 from indoor air of Faculty of Science rooms of University of Kragujevac, Serbia; bacteria load of 9.63 x 10^1 to 6.89 x 10^2 cfu/m^3 was reported by Shittu *et al.* (2019) from indoor air quality and microbial assessment of a Nigerian University campus in Lagos. However, the bacterial load obtained in this study was lower than that reported by Adetitun and Oladele. (2016) who reported a range of 0 to 24,735 cfu/ml from offices in University of Ilorin; but similar to that reported by Ekhaise and Erhunmwunsee, (2013) from indoor air of male student hostels in University of Benin, Ugbowo Campus, Benin city, Nigeria.

The possible reason for the difference in bacterial load may be attributed to the difference in the population of people inhabiting the various study areas, sampling time, and the sampling techniques used. During air sample collection in passive sampling, some microorganisms impact on petri dishes, while others are still suspended in the air due to heterogeneous spread of bacteria and their different size.

World Health Organization expert group in a research on the assessment of health risks of biological agents in indoor environment has set a guideline of bioaerosol count of 1,000 CFU/m^3, a higher value implies that the environment is contaminated (Shiferaw *et al.* 2016; WHO 2009). The mean indoor air bacterial load results in this study can also be evaluated based on the sanitary standard for non-industrial premises formulated by European Commission which considers <50 cfu/m^3 as 'very low, bacterial load, 50-100 cfu/m^3 as 'low' bacterial load, 100-500 cfu/m^3 as 'intermediate' bacterial load, 500-2,000 cfu/m^3 as 'high' bacterial load and above 2,000 cfu/m^3 as 'very high' bacterial load (Wanner and Gravesen, 1993). All the indoor air sampled in this study can therefore be evaluated to have very high bacterial load.

In this study, Gram positive isolates were more abundant than the Gram negative isolates. This is comparable with a study by Stefanovic *et al.* (2021), where Gram positive bacteria were higher (72.7%) than Gram negative bacteria (27.3%). Predominance of Gram positive bacteria was reported in some other studies (Ataylay *et a.l,* 2023; Enitan *et al.*, (2017). The possible reason for the predominance of Gram positive may be due the fact that Gram positive bacterial cell wall has a high content of peptidoglycan, which resists dryness; and the inability of Gram negative bacteria to survive for long period in the aerosolized state together with their inability to resist harsh drying conditions as reported by other studies (Luksamijarulkul *et al.*, 2014; Gizaw *et al.*, 2016).

In the present study, *S. aureus* (30%) and *Streptococcus* spp (20%) were the predominant isolated pathogens. Other Gram positive bacteria isolated were *Clostridium* spp (13.3%), *Bacillus* spp (13.3%) and *Micrococcus* spp (10%). *Pseudomonas aeruginosa* (13.3%) was the only Gram negative bacteria isolated. Similar bacteria were isolated by previous studies on microbiological assessment of indoor air from university campuses (Ilusanya *et al.*, 2020; Obajuluwa *et al.*, 2021; Ozougwu *et al.*, 2021). These pathogens can pose a state of health risk to students living in the various hostels especially the immunocompromised ones, and those accessing the canteens for their food.

Comparing the antibiotics susceptibility of the Gram positive with the Gram negative isolates: the Gram negative isolates demonstrated a higher level of resistance to the antibiotics used as compared with the Gram positive isolates. Both the

Gram positive and gram negative isolates were highly resistant to the beta lactam antibiotics used.
Gentamicin (43.5%, 50%), an aminoglycoside, tetracycline (47.8%, 75%), and vancomycin (43.5%, 75%), a glycopeptide, were the most active antibiotics against both the Gram positive and Gram negative isolates. Erythromycin demonstrated a better activity against Gram positive isolates (47.8%) as compared with the Gram negative (25%).
Increasing rate of antimicrobial resistant bacteria is of great concern. The World Health Organization (WHO) and many other stakeholders and researchers agree that the spread of AMR is an urgent clinical and social challenge that requires a global, coordinated action plan to address, with the WHO designating it among the top 10 global public health threats facing humanity (WHO, 2021). The Gram negative isolates were 100% resistant to five antibiotics (namely chloramphenicol, ceftriaxone, cefoxitin, amoxicillin-clavulanic acid, and trimethoprim-sulphamethoxazole) from three antibiotics classes. This is a high level of multidrug resistance, multidrug resistance was also observed with the Gram positive isolates though at a lower percentage as compared with the Gram negative. The continuous exposure of these students to the contaminated indoor air especially multidrug-resistant bacteria strains poses a lot of danger to the students' health and can be a medium for the transmission of antibiotic resistant strains. This high level of AMR observed in this study may also be an indication of possible misuse and/or abuse of antibiotics among the students living in the hostels or staff and students using the canteens.
The following are the recommendations based on the findings of this study: that the university management should pay close attention to the indoor air quality of Kaduna State University for the good health, well-being and overall performance of the students. There should be improvement in the cleanliness of the students' hostels and toilets by increasing the frequency of the cleaning including the canteens. Advocacy against misuse and abuse of antibiotics should be carried out in the campus.

CONCLUSION

The degree of indoor air bacterial load observed in all the students' hostels and canteens in Kaduna State Hospital, Nigeria was higher than the standard. The highest mean bacterial load was observed in the female hostels' toilet while the least was observed in one of the female hostel rooms. The predominant pathogenic bacteria was *S. aureus, Streptococcus* spp, *Bacillus subtilis, Pseudomonas aeruginosa* and *Micrococcus* spp. Tetracycline, gentamicin and vancomycin were the most active antibiotics observed in this study, while high level of multidrug resistance was observed. This is very dangerous to the students' health because it can be a source of transmission infections and antibiotic resistant bacterial strains.

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