Microbiological Indoor Air Quality of Classrooms in Rivers State University

***C.J Ugboma, G.C. Disegha and C.B Ojukwu**
[1]Department of Microbiology Rivers State University Nkpolu Oroworukwo

ABSTRACT

The presence of bacteria in the indoor air poses a serious problem from the health and environmental point of view. Precise determination of various groups of indoor microbes is necessary in estimating the health hazard and to create standards for indoor air quality control. This is especially important in such densely populated facilities like educational institutions. The indoor air of different classrooms in Rivers state university was investigated using the Koch sedimentation method with a view of determining the air quality and to determine the susceptibility of bacterial isolates to different antibiotics. The Kirby-Bauer disc diffusion method was used in determining the susceptibility of the bacterial isolates. The microbial load of the respective classrooms in the morning and peak of class activities showed that faculty of law class room had the highest aerobic bacteria load of 1.180×10^4 and the chemistry class room had the second highest aerobic bacterial load of 9.440×10^3 while the class room with the least bacterial load was the Psychology classroom (1.280×10^3). The microbial load of the respective classrooms in the evening period after class activities showed that the classroom with the highest bacterial load was the Chemistry classroom (4.903×10^3) followed by the Business Education (G law) class room (3.103×10^3). The Psychology classroom had the least bacterial load (9.17×10^2).There were significant differences in the total heterotrophic bacterial load of the morning period ($P \leq 0.05$) with the Chemistry laboratory having the highest microbial load. Bacillus, Staphylococcus and Micrococcus sp were the bacterial isolates. The antibiotic susceptibility result showed that the staphylococci isolates were highly resistant to Ampiclox but were very sensitive to Gentamycin, Chloramphenicol and Ciprofloxacin. Bacillus species were very susceptible to Ampiclox, Rifampicin, Ciprofloxacin and Erythromycin. Micrococcus sp showed no resistance to any of the antibiotics.

INTRODUCTION

The air in our atmosphere is composed of molecules of different gases. The most common gases are nitrogen (78%), oxygen (about 21%), and argon (almost 1%). Other molecules are present in the atmosphere as well, but in very small quantities (Meadow *et al.,* 2014). Air quality is the degree to which air in a particular place is free from pollutants. The air in our atmosphere is composed of molecules of different gases. The most common gases are nitrogen (78%), oxygen (about 21%), and argon (almost 1%). Other molecules are present in the atmosphere as well, but in very small quantities. Good air quality pertains to the degree which the air is clean, clear and free from pollutants such as smoke, dust and smog among other gaseous impurities in the air. Poor air quality can affect or harm human health and the environment. One of the problem of air quality is that it is affected by the presence of microorganisms which include bacteria, moulds and viruses and people spends 80%-90% of their time in breathing on average 14 m^3 of air per day (Wemedo *et al.,* 2012). These sources can seriously affect the overall air quality and can lead to severe health problems for humans.

Air pollutants are substances present in the atmosphere at concentrations above their normal background level which can have a measurable effect on humans, animals and vegetation.

Air pollution is the contamination of air we breathe, indoors or outdoors, by any chemical, physical, or biological agent that modifies the natural characteristics of the atmosphere.

Air quality of the environments is one of the main factors affecting health, wellbeing and productivity of people. One of the problems of indoor air quality is the presence of microorganisms which include bacteria, moulds and viruses and people spend 80%-90% of their time in indoors environments by breathing on average 14 m^3 of air per day. These make people highly exposed to indoor air environments. As of these, in recent years there has been a growing interest in indoor microbe studies (Wemedo *et al.*, 2012).

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. Particular activities like talking, sneezing, coughing, walking and washing can generate airborne biological particulate matter. Food stuffs, house plants and flower pots, house dust, textiles, carpets, wood material and furniture stuffing, occasionally release various fungal spores into the air (Brochu *et al.*, 2006). Moreover, the environmental factors mainly include temperature, humidity, air exchange rate, air movement, building structures and location, poor design, ventilation system as well as interior or redesign which enhance microorganism's growth and multiplication in the indoor atmosphere (Meadow *et al.*, 2014).

A review made by WHO on the number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effects on respiratory health, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and dyspnoea (Bonetta *et al.*, 2010). Thus, microbiological air quality is an important criterion that must be taken into account when indoor workplaces are designed to provide a safe environment.

Air pollution risk is a function of the hazard of the pollutant and the exposure to that pollutant. Air pollution exposure can be expressed for an individual, for certain groups (e.g. neighborhoods or children living in a country), or for entire populations. For example, one may want to calculate the exposure to a hazardous air pollutant for a geographic area, which includes the various microenvironments and age groups. This can be calculated as an inhalation exposure. This world account for daily exposure in various settings (e.g. different indoor micro-environments and outdoor locations). The exposure needs to include different age and other demographic groups, especially infants, children, pregnant women and other sensitive subpopulations (Tamberka *et al.*, 2007).

The exposure to an air pollutant must integrate the concentrations of the air pollutant with respect to the time spent in each setting and the respective inhalation rates for each subgroup for each specific time that the subgroup is in the setting and engaged in particular activities (playing, cooking, reading, working, etc.). For example, a small child's inhalation rate will be less than that of an adult. A child engaged in vigorous exercise will have a higher respiration rate than the same child in a sedentary activity (Wemedo *et al.*, 2012). The daily exposure, then, needs to reflect the time spent in each micro-environmental setting and the type of activities in these settings. The air pollutant concentration in each microactivity/micro-environmental setting is summed to indicate the exposure.

MATERIALS AND METHODS

Description of the study area

The study area is some of the frequently used classrooms in the faculty of science, Law and Engineering of the Rivers State University, Port Harcourt. The classrooms sampled include; the Business Education (G law), Chemistry lab, Computer (Sc7), Economics, Engineering (EDH), Engineering (EN2), Faculty of law (LHC1) and Psychology.

Media

Nutrient agar (NA), and Mannitol salt agar (MSA) were used to isolate the total heterotrophic bacteria and *Staphylococcus*, respectively in the various classrooms.The nutrient agar plates were prepared according to the manufacturer's instruction. This was autoclaved at 121^{o}C for 15 minutes. The medium was later dispensed into sterile Petri dishes having allowed the temperature to drop at 45^{o}C (Prescott *et al.*, 2011). Mannitol agar plates were prepared according to the manufacturer's instruction. The MSA was sterilized by boiling at 100^{o}C as instructed by the manufacturer.

Indoor Air Sampling

The Koch's sedimentation method also known as the "settling plate" or "passive" air technique described by Latika and Ritu (2011) was employed. In this method, plates containing sterile media were exposed to the atmosphere of the classrooms. This was to allow microbial flora within the classrooms to settle on the exposed plates. Plates were kept one meter above the ground. The air was sampled for two durations of the day (i.e. morning and evening). The plates were exposed for 10 minutes at each sampling sites.

Enumeration and Isolation of bacteria

Freshly prepared sterile nutrient agar (NA) and Mannitol Salt Agar (MSA) plates in duplicate were exposed to the atmosphere of the different sampling sites for about 10 minutes to allow air microflora within the wards to settle on the surface of the medium by gravity. The plates were kept about 1m above ground level to eliminate possible contamination and aid quick settling of microbial particles. These plates were transported to the microbiology laboratory and incubated for 24-48 hours at 37^{o}C. Counts were made for plates that showed significant growth at the end of incubation Discrete colonies on the different media plates were picked and inoculated onto freshly prepared nutrient agar and mannitol salt agar plates for bacteria and *Staphylococcus*, respectively. Pure cultures of the isolates were obtained by streaking the isolates on freshly prepared medium until it was ascertained that there were no contaminants.

Characterization and Identification of Bacterial Isolates

Cultural methods of characterizations employed were colour, shape, texture, odour, and microscopy under an oil emersion light microscope. Biochemical tests adopted include motility, catalase test, growth on blood agar, haemolysis test and coagulase test, citrate utilization and sugar fermentation tests.

Antibiotics Susceptibility Test

A pure single colony grown overnight (18-24 hours) on nutrient broth and was used for the antibiotic susceptibility. The antibiotic susceptibility test for the isolates was performed using the disk diffusion method on Mueller-Hilton agar plates. Petri dishes with Mueller-

Hiltonplates were flooded with inoculums of the test isolates using sterile swabs as described by Cheesbrough (2000), antibiotic disks were placed on the surface of inoculated agar plates using sterilized forceps. Finally, the plates were incubated overnight for 24hours at 37 ºC. Data were recorded based on the clear zones of inhibition. Zones were measured with a ruler in millimeters (mm) and compared with a standard interpretation chart based on performance standards for testing antimicrobial susceptibility (CLSI, 2013) used to categorize the isolates as susceptible, intermediate or resistant.

RESULTS

Microbial Load

The microbial load of the respective classrooms in the morning and peak of class activities is presented in Table 4.1. The result showed that faculty of law (LHC) class room had the highest aerobic bacteria load (1.180×10^{4}) and the chemistry class room had the second highest aerobic bacterial load (9.440×10^{3}) while the class room with the least bacterial load was the Psychology classroom (1.280×10^{3}).

The microbial load of the respective classrooms in the evening period after class activities is presented in Table 4.2. The classroom with the highest bacterial load was the Chemistry classroom (4.903×10^{3}) followed by the Business Education (G law) class room (3.103×10^{3}). The Psychology classroom had the least bacterial load (9.17×10^{2}).

Table 4.1: Microbial load of the different classrooms in the Morning hours

Source	Heterotrophic bacteria	Total staphylococci
Business Education (G law)	3.263×10^{3b}	1.516×10^{3b}
Chemistry	9.440×10^{3d}	1.930×10^{3b}
Computer	2.920×10^{3b}	2.79×10^{2a}
Economics	5.540×10^{3c}	4.67×10^{2a}
Engineering (EDH)	2.312×10^{3ab}	9.00×10^{2a}
Engineering (EN2)	2.459×10^{3ab}	1.604×10^{3b}
Faculty of law (LHC)	1.180×10^{4f}	3.381×10^{3c}
Psychology	1.280×10^{3a}	3.28×10^{2a}

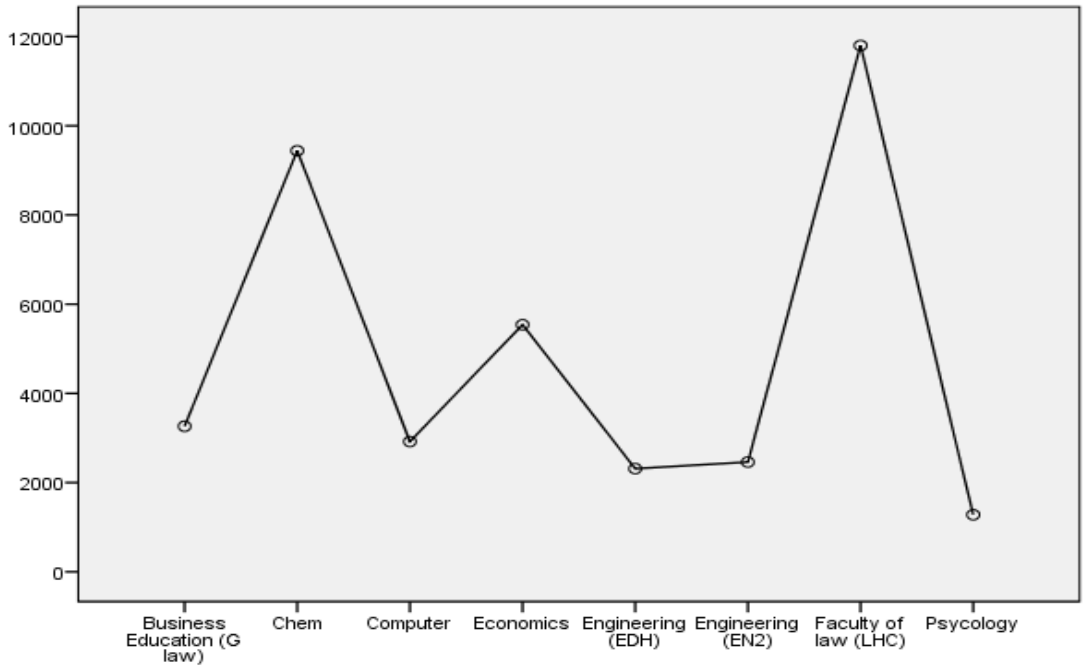

Fig. 4.1: The trend in the Heterotrophic bacterial load in the morning hours of the respective classrooms

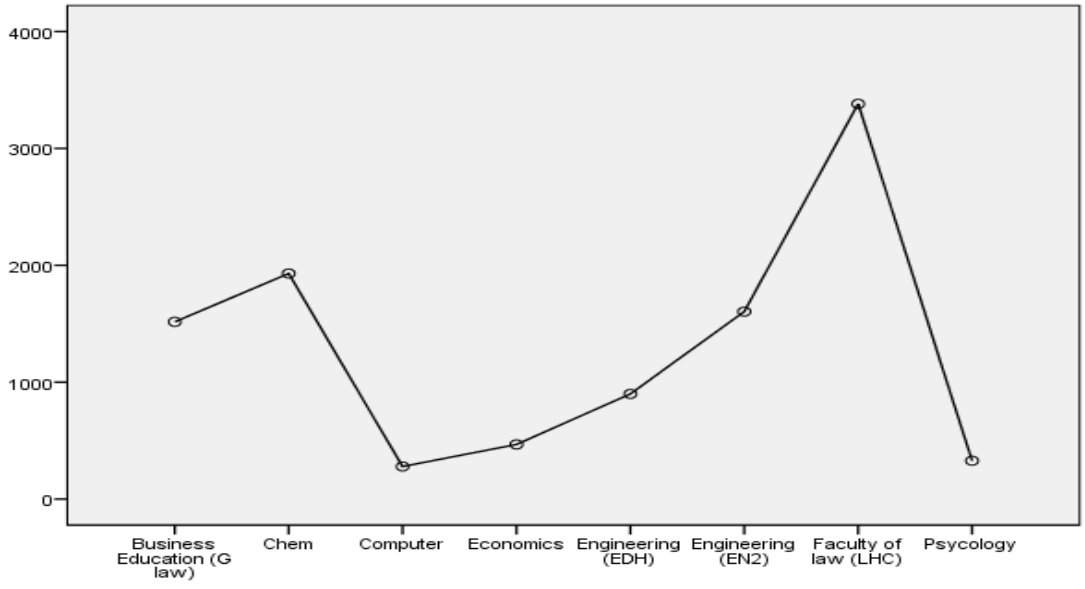

Fig. 4.2: The trend in the Staphylococcal load in the morning hours of the respective classrooms

Table 4.2: Microbial load of the different classrooms in the Evening / After school activities

Source	Heterotrophic bacteria	Staphylococci count
Business Education (G law)	3.103×10^{3c}	1.356×10^{3}
Chemistry	4.903×10^{3d}	7.15×10^{2b}
Computer	1.673×10^{3ab}	8.2×10^{a}
Economics	2.350×10^{3bc}	2.59×10^{2a}
Engineering (EDH)	2.300×10^{3bc}	7.50×10^{2b}
Engineering (EN2)	2.404×10^{3bc}	1.354×10^{3c}
Faculty of law (LHC)	1.050×10^{3ab}	1.430×10^{3c}
Psychology	9.17×10^{2a}	2.48×10^{2a}

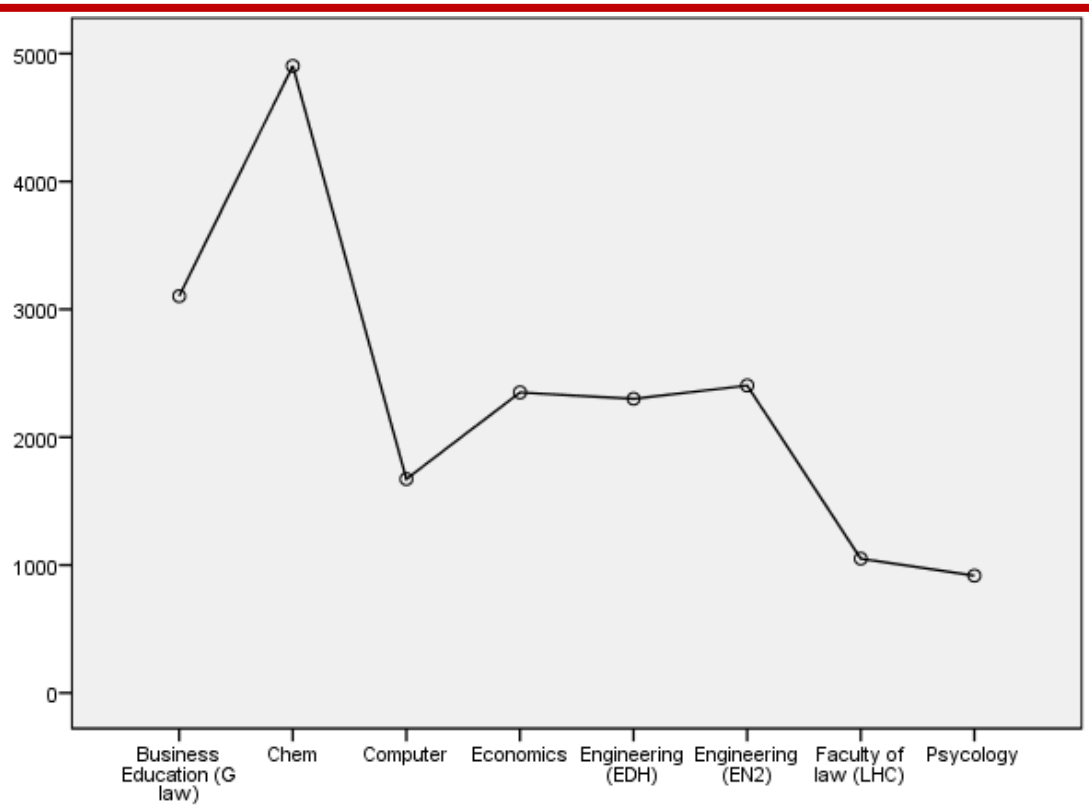

Fig 4.5The trend in the Heterotrophic load in the evening of the respective classrooms

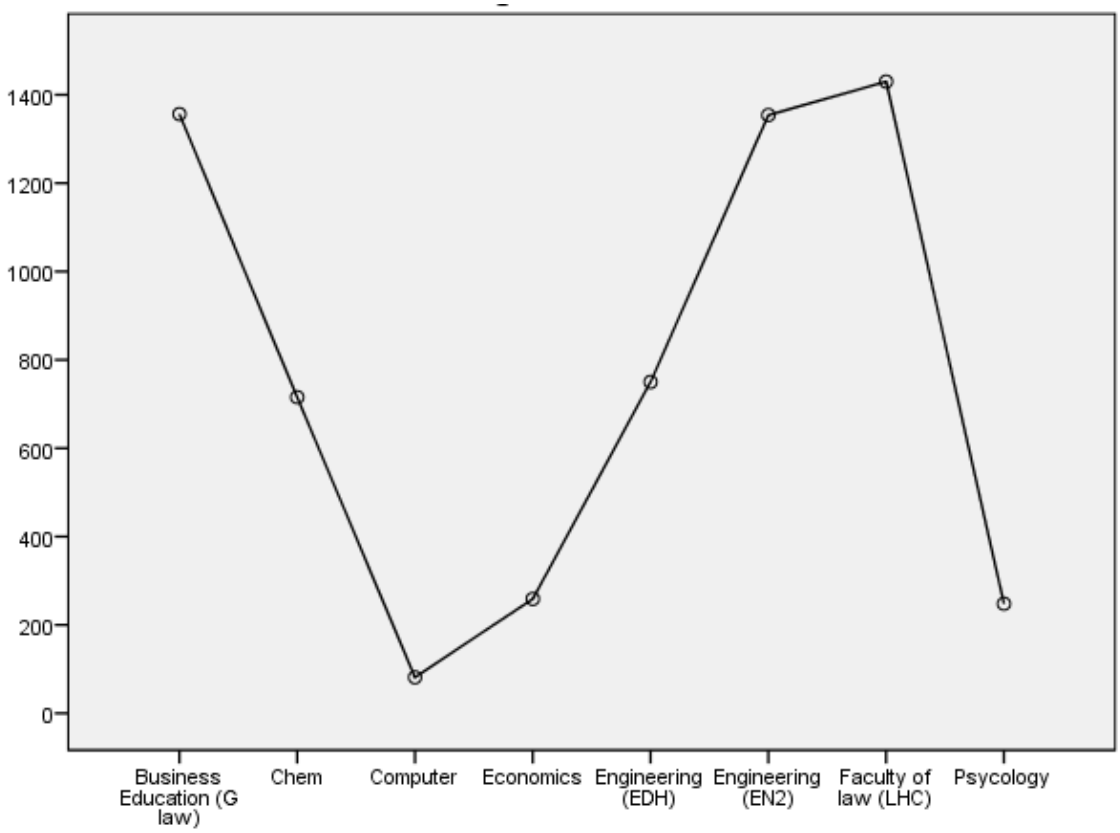

Fig. 4.6: The trend in the Staphylococcal load in the evening of the respective classrooms

Antimicrobial Susceptibility

The result for the antibiotic susceptibility of *Staphylococcus, Bacillus* and *Micrococcus* isolates are presented in Table 4.7, Table 4.8 and Table 4.9, respectively.

The result for the *Staphylococcus* isolates showed very high resistant to Ampiclox and Amoxicillin antibiotics. The isolates were very susceptible to Gentamycin, Chloramphenicol, Ciprofloxacin and Levofloxacin in Table 4.7.

In the result presented in Table 4.8, the *Bacillus* isolates were highly resistant to Amoxicillin. They were totally susceptible to Ampiclox, Rifampicin, Ciprofloxacin, Erythromycin and Levofloxacin. Furthermore, there was no form of resistance observed amongst the *Micrococcus* isolates in Table 4.9.

Table 4.7: Antibiotic Susceptibility Pattern of *Staphylococcus* isolates (n = 7)

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Gentamicin (CN)10 µg	7 (100)	0	0
Ampiclox (APX)20 µg	0	0	7 (100)
Rifampicin (RD) 20 µg	5 (71.4)	2 (28.6)	0
Amoxicillin (AMX) 20 µg	0	0	7 (100)
Streptomycin (S) 30µg	4 (57.1)	3 (42.9)	0
Norfloxacin (NB)10 µg	5 (71.4)	2 (28.6)	
Chloramphenicol (CH)30 µg	7 (100)	0	0
Ciprofloxacin (CPX)10 µg	7 (100)	0	0
Erythromycin (E)30 µg	6 (85.7)	1 (14.3)	0
Levofloxacin (LEV)20 µg	7 (100)	0	0

Table 4.8: Antibiotic Susceptibility Pattern of *Bacillus* (n =13)

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Gentamicin (CN)10 µg	10 (76.9)	3 (23.1)	0
Ampiclox (APX)20 µg	13 (100)	0	0
Rifampicin (RD) 20 µg	13 (100)	0	0
Amoxicillin (AMX) 20 µg	3 (23.1)	0	10 (76.9)
Streptomycin (S) 30µg	10 (76.9)	3 (23.1)	0
Norfloxacin (NB)10 µg	10 (76.9)	0	3 (23.1)
Chloramphenicol (CH)30 µg	10 (76.9)	3 (23.1)	0
Ciprofloxacin (CPX)10 µg	13 (100)	0	0
Erythromycin (E)30 µg	13 (100)	0	0
Levofloxacin (LEV)20 µg	13 (100)	0	0

Table 4.9: Antibiotic Susceptibility Pattern of *Micrococcus* (n = 2)

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Gentamicin (CN)10 µg	2 (100)	0	0
Ampiclox (APX)20 µg	2 (100)	0	0
Rifampicin (RD) 20 µg	2 (100)	0	0
Amoxicillin (AMX) 20 µg	2 (100)	0	0
Streptomycin (S) 30µg	2 (100)	0	0
Norfloxacin (NB)10 µg	2 (100)	0	0
Chloramphenicol (CH)30 µg	2 (100)	0	0
Ciprofloxacin (CPX)10 µg	2 (100)	0	0
Erythromycin (E)30 µg	2 (100)	0	0
Levofloxacin (LEV)20 µg	2 (100)	0	0

Discussion

As a result of industrialization, the atmosphere is constantly polluted with different chemicals as well as particles which could in one way or the other cause serious health problems especially respiratory and allergenic problems. Classrooms which are enclosed places where students and lecturers meet for the purpose of taking lectures are most times populated by students who come in to receive lectures. Thus, investigating the microbial types within this environment would be of public health benefit.

The indoor air of randomly selected classrooms in the Rivers State University was evaluated to ascertain the level of microbial contamination as well as the determining the antibiotic susceptibility pattern of the bacterial isolates identified within the classrooms.

The mean total heterotrophic bacteria in the morning sampling period was higher compared to the evening period in all the classrooms as revealed in the result (Table 4.1 and 4.2). More so, the counts recorded for the total heterotrophic bacterial population in this study is higher than those reported by Udochukwu *et al.,* (2015) who evaluated the microbiome of enclosed air in selected dormitories in University of Port Harcourt. Thus, the studied sites in this study are areas which are frequently used. For instance, the classrooms are used for lectures with the population of students in these classrooms exceeding 40 persons, and the Chemistry laboratory is frequently used for lecture; practical and some students relax in them or engage in other activities such as talking, etc. Thus, the high microbial population could be attributed to the different types of persons within the building. Wemedo and Robinson (2018) in a study of indoor air has reported that the influx of persons as well as the type of persons and the activities taking place within such enclosed space could increase the microbial loads as well as the diversity of the microorganisms. Other studies have suggested that poor ventilation as well as the exchange of microbial flora during talking, sneezing, and sweeping by persons within the enclosed space leads to the release of microbes into the atmosphere (Wemedo and Robinson, 2018; Latika and Ritu, 2011; Udochukwu *et al.,* 2015). The Two-way ANOVA showed significant differences in the total heterotrophic bacterial load of the morning period ($P \leq 0.05$) with the Chemistry laboratory having the highest microbial load (Table 4.1). Spatial control of 1000cfu/m^3 was used to check the level of contamination in the respective study sites. During the evaluation of indoor air in respect to the hazards caused by biological agents, some experts of the WHO posited that the total microbial load in indoor air should not exceed 1000Cfu/M^3 although other researchers posited that 750cfu/m^3 should be the limit for bacteria and fungi in indoor environment while others considered that the limits for ubiquitous bacterial aerosols of airborne microbial concentrations should have its range from 4500 to 10000 Cfu/m^3 (Zemichael *et al.,* 2016). Thus, the microbial load from this current study is high and considered to be unsafe for human health.

Three bacterial isolates belonging to *Bacillus* sp, *Staphylococcus* sp, and *Micrococcus* sp, were isolated and identified in the various studied sites. The bacteria genera were not evenly distributed in all the studied sites. *Bacillus* sp and *Staphylococcal sp* were identified in all the respective study sites while *Micrococcus* sp was only identified in the chemistry laboratory and LHC classroom in the faculty of law. *Enterobacteriacae* were not identified in these study sites. *Bacillus* species are known to be distributed everywhere due to the possession of endospores which helps them to withstand different environmental conditions (Wemedo and Robinson, 2018). The isolates in this study have been reported by previous studies to cause diseases especially in immune compromised persons. Allergic reactions have also been reported to arise

from inhalation of particles from these biological agents (Latika and Ritu, 2011; Udochukwu *et al.*, 2015; Wemedo and Robinson, 2018).

A total of twenty-two bacteria belonging to *Bacillus* sp (13), *Staphylococcus* sp (7) and *Micrococcus* sp (2), were subjected to eleven commercially prepared antibiotics (Optu disc) to determine their susceptibility pattern. The result in Table 4.7 showed that out of the seven staphylococcal isolates subjected to the antibiotics test, only Gentamycin, Chloramphenicol and Ciprofloxacin were the most sensitive antibiotics. *Bacillus* species were very susceptible to Ampiclox, Rifampicin, Ciprofloxacin and Erythromycin (Table 4.8). In a recent study by Wemedo and Robinson (2018), resistance of *Staphylococcus* species to amoxicillin was reported. Thus, their report agreed with findings in this current study which have reported high level of resistance of *Staphylococcus* sp to amoxicillin. Also, Caroline *et al.*, (2013) in a study of methicillin resistant bacterial isolates from toilet and class room handles reported that 93% of *Staphylococcus* species were susceptible to ampicillin were as only 7% was resistant. In this current study, the *Staphylococcal* isolate was resistant to ampicillin. *Micrococcus* species in this current study were highly susceptible to the antibiotic agents (Table 4.9). this report does not agree with a work reported by Wemedo and Robinson (2018) who reported that ampicillin was not very effective against *Micrococcus* species. Antimicrobial resistance is generally a global problem. It has been reported that microorganisms have acquired measures either through the possession of efflux pumps that helps them flush the drugs or acquisition of plasmid resistant genes. This enables them to be resistant to antimicrobial drugs (Prescott *et al.*, 2011).

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