BACTERIAL EMPIRE
2019, VOL. 2, NO. 4, 86-90

OPEN ACCESS
SCICELL

REGULAR ARTICLE

CORRELATION BETWEEN CLASSROOM POPULATION, VENTILATION BACTERIAL LOADS AND THIER ANTIMICROBIAL PATTERNS IN SCHOOLS WITHIN IKOT EKPENE, NIGERIA

James, I. I.,[a] Ibuot, A. A.,[a] Udoekong, N. S.,[a] Ben, M.G.,[a] Etuk, C.U.,[a] Charlie, C. A., Josiah, A., James, I. I.[b]

Address (es):
[a] Department of Science Technology, Akwa Ibom Sate Polytechnic, Ikot Osurua, Akwa Ibom State, Nigeria.
[b] Ministry of Agriculture, Uyo, Akwa Ibom State, Nigeria.

*Corresponding author: anialphonsus@yahoo.com

ABSTRACT

Indoor air of classroom in eight schools (4 nursery; NS1, NS2, NS3 and NS4, and 4 secondaries; SS1, SS2, SS3 and SS4) within Ikot Ekpene, Akwa Ibom State, Nigeria, were analyzed at ambient and populated sampling conditions using natural sedimentation on nutrient agar medium. The results revealed varying ventilation patterns in each of the classrooms, and the following airborne bacterial counts; NS1 (16.6 cfu/m^3), NS2 (13.3 cfu/m^3), NS3 (23.3 cfu/m^3), NS4 (33.3 cfu/m^3), SS1 (6.6 cfu/m^3), SS2 (6.6 cfu/m^3), SS3(28.3 cfu/m^3) and SS4 (15 cfu/m^3) at ambient sampling and 40 cfu/m^3, 41.6 cfu/m^3, 58.3 cfu/m^3, 68.3 cfu/m^3, 6.6 cfu/m^3, 31.6 cfu/m^3, 56.6 cfu/m^3 and 25 cfu/m^3 respectively at populated sampling. Bacterial isolates identified were *Lactobacillus, Staphylococcus, Bacillus, Rothia, Kurthia, Corynebacterium, Pseudomonas, Brevibacterium,* and *Flavobacterium.* Statistical analysis of the results revealed negative relationships between class area and aerobic plate counts ($p>0.05$), class population and aerobic plate count ($p>0.805$), and significant increase in aerobic plate counts at populated conditions over that at ambient conditions ($p<0.05$). The results therefore point to the dimensions of classrooms, ventilation and population of the classrooms as important factors in determining the bacterial air quality, and invariably affecting the health condition of students.

Keywords: indoor air, bacteria, contamination, health, antibiotic susceptibility

INTRODUCTION

Air serves as a key route and major source of human microbial exposure **(Jones, 1999)**. The knowledge of microbial air contamination is therefore an important criterion for the assessment of hygiene conditions of indoor air quality **(Nandasena *et al.*, (2010)**. In modern societies, people spend over ninety per cent of their time indoors such as in schools and classrooms, constantly exposing them to contents present in this primary habitat which include inhalable microbes **(Flynn *et al.*, 2000).** Microbial causative agents of adverse health conditions have been documented in aerosols of different indoor built environment such as schools and such agents can be transmitted between individuals in close proximity **(Wargocki and Wyon, 2006)**, including methicillin-resistant *Staphylococcus aureus* (MRSA) **(Gehring *et al.*, 2010).**
Researchers such as **Dunn *et al.* (2013)** have observed that microbial communities are vastly different between different types of indoor environments such as schools, houses and hospitals, even different rooms within the same building (e.g. bedroom vs. bathroom) exhibit distinct microbiomes. Air quality in classrooms is of special concern, since students spend a lot of time indoors, which potentially exposes them to many contaminants present in the air **(Jones, 1999).** Studies done on school indoor environments when compared to that of other building, show heightened health risks due to low funding for operation and maintenance of facilities **(Wargocki and Wyon, 2006; Zhao *et al.*, 2008)**. Environmental problems may be more pronounced in school buildings in poor and developing countries due to the perennial problems of overcrowding, low funding, age of buildings, materials employed in building construction (e.g. asbestos and lead), as well as ventilation patterns **(Espejord, 2000).**
The main concern about microbial growth in classroom indoor environments is related to the strong link to the adverse health effects on the occupants **(Douwes *et al.*, 2003)**. Indoor air pollutants might increase the chance of both long and short-term health problems among pupils and staff, reduce the productivity of teachers and degrade the pupils learning environment and comfort **(Shaughnessy *et al.*, 2006).** Hence there is also need to further characterize microbes in classroom indoor environment, and assess their antimicrobial sensitivity. This work is focused on studying the correlation between bacteriological air quality in schools within Ikot Ekpene and classroom population and ventilation pattern.

MATERIALS AND METHODS

Sampling location

Classroom atmosphere in four (4) nursery schools (labeled NS1, NS2, NS3 and NS4) and four (4) secondary schools (labeled SS1, SS2, SS3 and SS4) within Ikot Ekpene metropolis were sampled. A single classroom was sampled in all schools.
Table 1 and Figure 1 show the characteristics of the eight school buildings. All buildings were single floor buildings except NS2 and SS2 which were located in a four-storey building with classrooms sampled located on the ground floor. Classroom sampled in NS1 was located on the upper floor of a two-storey building, and classroom at NS3 was located on the ground floor of a two-storey building
All schools were brick with mortar-surfaced walls. Most schools had wooden windows which could be opened; NS2 and SS2 were in a building installed with sliding aluminum windows. SS1 and SS3 had no windows in place, although window frames had been installed. Additionally, SS1 classroom had no ceiling boards in place. None of the schools had any form of air handling system.

Sample collection

The air of classroom atmosphere of seven selected schools was sampled at different time intervals; ambient samples were obtained between 6-7.30 am, while samples under populated conditions were taken between 10 and 12 pm (while students were in class). Plates were set up at a height of 1.5 m above floor level, representing the normal human breathing zone **(Obbard and Fang, 2003).** Sampling was done by natural sedimentation; nutrient agar plates prepared and preserved overnight in the refrigerator at 4 ^{o}C were exposed in air in each of the classroom for 5 minutes (to avoid drying of the agar surface and overloading of the collection plate **(Stetzenbach *et al.*, 2004)** and then covered, labeled.

Bacteriological analysis of classroom atmosphere

Replica plates of nutrient agar plates were incubated at 37 °C for 48 hours to allow the growth of aerobic bacteria. Emerging visible discrete colonies of bacteria were enumerated and subcultured in fresh nutrient agar medium and incubated. Pure colonies so obtained were stocked on nutrient agar slants and incubated at 30 ^{o}C for 24 hours and were then preserved in the refrigerator at 4 ^{o}C for further tests. Bacterial colonies were initially characterized by morphology and microscopic appearance, and identified further by biochemical tests. Isolates were identified based on comparison of biochemical and physiological characteristics with that of known taxa in the Bergey's Manual of Systematic Bacteriology **(Holt *et al.*, 1994).**

Statistical analysis

The total number of colony forming units (cfu) enumerated was converted to organisms per cubic meter of air (cfu/m^3). The data were processed with SPSS and statistically significant differences were determined by one-way and two-way analysis of variance (ANOVA). P-value less than 0.05 was considered statistically significant.

Antibiotic susceptibility testing

The antibiotic susceptibility profile of the bacterial isolates was determined using the disk diffusion method on Mueller-Hinton agar according to the methods of Chessbrough, (1984). Bacterial isolates were tested against seven OPTUN disc antibiotics comprising Ceporex (CPX 10 µg), Norbactin (NB 10 µg), Gentamycin (GN 10 µg), Amoxil (AML 20 µg), Streptomycin (S 30 µg), Augmentin (AUG 30 µg), Rifampicin (R 20µg), Erythromycin (E 30 µg), Chloramphenicol (CH 30 µg), Ampiclox (APX 20 µg), and Levofloxacin (LEV 20 µg). The inoculum was standardized by adjusting its density to 0.5 McFarland turbidity standard (equal the turbidity of a barium sulphate ($BaSO_4$)). Examination of the cultures for zones of clearing was done after 24 hours of incubation at 37 ºC. Diameters of zones of inhibition observed were measured in millimeters (mm), and interpreted according to CLSI (2017) standards.

RESULTS

Table 1 shows the measurements of classrooms sampled., SS4 classroom had the largest space 19200 m^2 with 3 doors and eight windows, followed by SS3 classroom with 6750 m^2 with one (1) door and 7 windows, SS1 had an area of 6732 m^2 with one (1) door and 4 windows, NS1 had the smallest space: 712.5 m^2 with 2 doors and 4 windows.

Airborne bacterial counts of classroom atmosphere were as follows; NS4 (33.3 cfu/m^3), SS3 (28.3 cfu/m^3) and SS1 and SS2 were both observed to have lower plate counts of 6.6 cfu/m^3 at ambient sampling. Under populated conditions, NS4 had the highest plate count 68.3 cfu/m^3, NS3 (58.3 cfu/m^3), and SS4 classroom had the lowest count of 25 cfu/m^3.

Correlation coefficient between area and aerobic plate count was -0.417. Relationship between class size and aerobic plate count was negative (r = 0.015), and p-value of 0.805 (p>0.805). A t-calculated value of 5.16, and a t-critical of 2.36 at 0.05 level of significance and p-value of 0.001 (p<0.05) was obtained.

A total of 9 bacterial genera were identified from the classroom atmosphere and identified as *Rothia* sp, *Bacillus* sp, *Corynebacterium* sp, *Lactobacillus* sp, *Flavobacterium* sp, *Staphylococcus* sp, *Brevibacterium* sp, *Pseudomonas* sp, *Kurthia* sp and both in ambient and populated sampling respectively.

According to the bacterial distribution in the air, 28 % were *Lactobacillus* sp, 21 % were *Pseudomonas* sp, 18 % were *Staphylococcus* sp. *Bacillus* sp, *Flavobacterium* sp *and Brevibacterium* sp were 4% respectively, *Corynebacterium* sp, *Rothia* sp *and Kurthia* sp 7 % respectively.

The pattern of resistance of the bacterial isolates were Ceporex (11.1 %), Norbactin (66.7 %), Gentamicin (22.2 %), Amoxicillin (44.4 %), Streptomycin (22.2 %), Rifampicin (33.3 %), Erythromycin (33.3 %), Chloramphenicol (22.2 %), Ampiclox (44.4 %), Levofloxacin (33.3 %).

Table 1 Classroom area and populations in selected schools in Ikot Ekpene

Schools	Class sampled	Classroom Area	Number of Students	Number of staff
NS1	Nursery 2	712.5m^2	15	2
NS2	Nursery 1	7225 m^2	20	2
NS3	Primary 4	2500 m^2	26	1
NS4	Nursery 1	2400 m^2	26	2
SS1	JSS 3	6732 m^2	17	1
SS2	SS 3	4800 m^2	10	1
SS3	JSS 2	6750 m^2	36	1
SS4	SS 3	19200 m^2	95	0

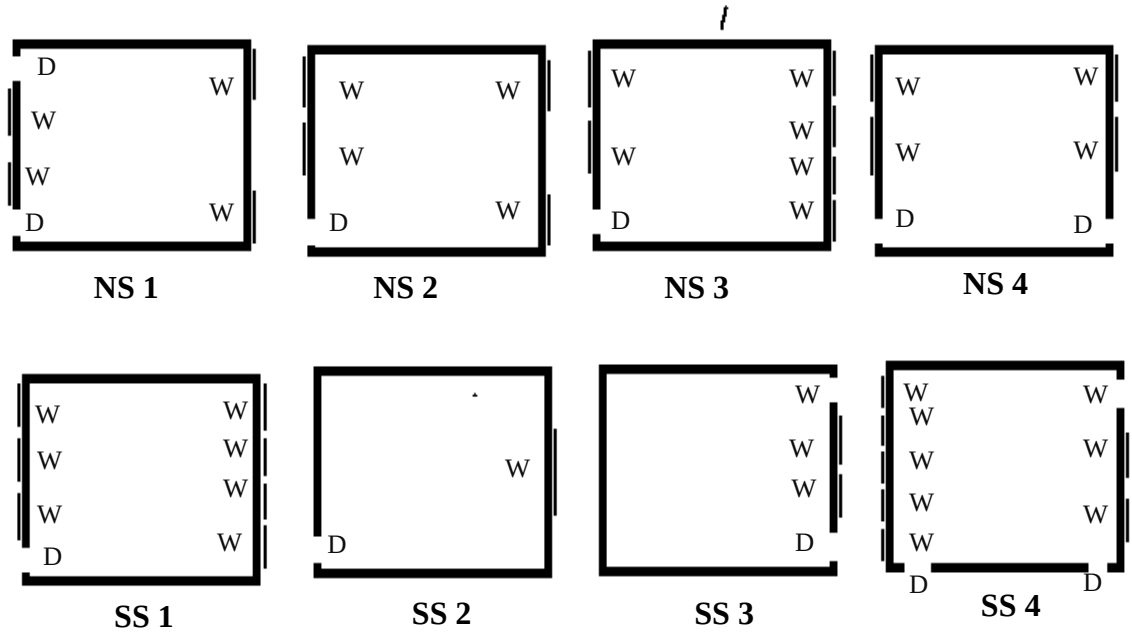

Figure 1 Ventilation patterns in school classrooms Key; W=Window, D = Door

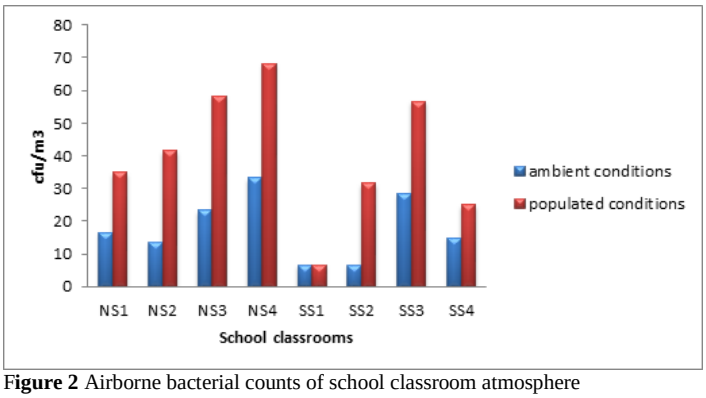

Figure 2 Airborne bacterial counts of school classroom atmosphere

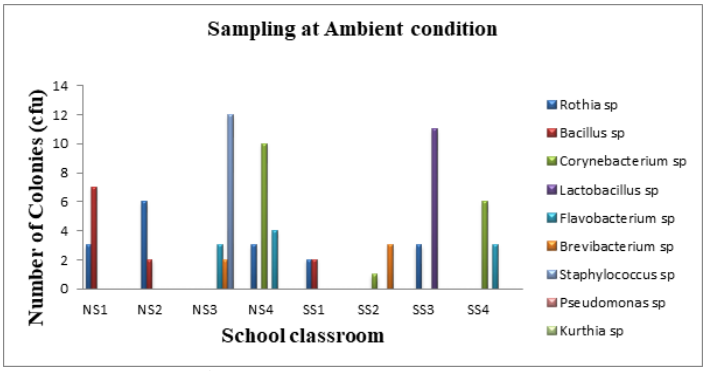

Figure 3 Distribution of bacteria in classroom atmosphere at ambient condition

Table 2 Biochemical characteristics of bacterial isolates from classroom atmosphere

Isolates	1	2	3	4	5	6	7	8	9
Spore	-	+	-	-	-	-	-	-	-
Motility	-	+	-	+	-	-	-	+	-
Coagulase	+	+	-	+	-	-	+	+	+
Oxidase	+	-	+	+	+	+	-	+	-
Urease	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
Sucrose	OO	AO	OO	AG	AO	OO	AO	OO	AO
Glucose	AO	AO	AG	AG	AO	OO	AO	OO	AO
Lactose	AO	AG	AO	AO	AO	AO	AO	OO	AO
Mannitol	OO	AO	OO	AO	AO	OO	AO	OO	AO

Legend: AG = acid and gas production; A O = acid production only; OO = no reaction; + = positive reaction; - = negative

Table 3 Phenotypic dentification of bacterial isolates from classroom atmosphere

Isolates	Cultural features	Gram/ shape	Probable isolate
1	Milky, rhizoid, viscous, translucent	+ rods	*Rothia* sp
2	Milky, circular, curled, viscous, opaque	+ rods in chains	*Bacillus* sp
3	Milky, circular, butyric, dull, opaque	+ rods in pairs	*Corynebacterium* sp
4	Brown, circular, viscous, shiny, translucent	+ rods in pairs	*Lactobacillus* sp
5	Yellow, circular, viscous, raised, translucent	+ rods in pairs	*Flavobacterium* sp
6	Pink, circular, viscous, raised, opaque	+ single short rods	*Brevibacterium* sp
7	Milky, circular, viscous, shiny, opaque	+ cocci in clusters	*Staphylococcus* sp
8	Milky, circular, viscous, shiny, opaque	- single rods	*Pseudomonas* sp
9	Milky, filamentous, viscous, shiny	+ cocci	*Kurthia* sp

Table 4 Antibiotic susceptibility patterns of bacterial isolates from classroom atmosphere

	(CPX)	(NB)	(GN)	(AML)	(S)	(R)	(E)	(CH)	(APX)	(LEV)
***Rothia* spp**	S	S	R	S	S	S	I	R	S	I
***Bacillus* spp**	S	R	I	R	R	I	I	I	I	R
***Corynebacterium* spp**	S	I	S	R	S	S	R	S	R	S
***Lactobacillus* spp**	S	R	R	I	S	R	R	S	S	R
***Flavobacterium* spp**	S	R	S	S	S	S	S	I	S	S
***Brevibacterium* spp**	R	R	I	R	I	R	R	R	R	R
***Staphylococcus* spp**	S	R	S	R	R	I	S	S	R	S
***Pseudomonas* spp**	S	S	S	I	S	S	S	I	R	S
***Kurthia* spp**	S	R	S	I	S	R	S	S	I	S
Percentage of resistance	11.1%	66.7%	22.2%	44.4%	22.2%	33.3%	33.3%	22.2%	44.4%	33.3%

Legend: CPX=Ceporex, GN= Gentamycin, S= Streptomycin, E=Erythromycin, APX= Ampiclox, NB=Norbactin, AML=Amoxicillin, R=Rifampicin, CH=Chloramphenicol, LEV=Levofloxacin

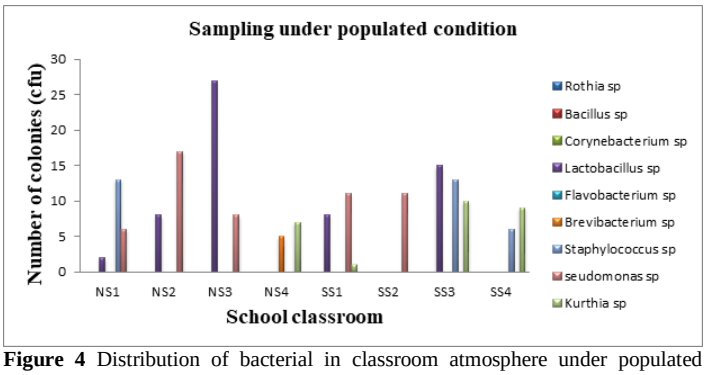

Figure 4 Distribution of bacterial in classroom atmosphere under populated condition

Table 5 Correlation between class area and airborne bacterial count

	Area	Populated aerobic plate count
Area	1	
Populated aerobic plate count	-0.417	1
	(0.304)	

Table 6 Correlation between class size and airborne bacterial count

	Class size	Aerobic plate count
Class size	1	
Aerobic plate count	-0.105	1
	(0.805)	

Table 7 Correlation between class size and airborne bacterial coun

	Class size	Aerobic plate count
Class size	1	
Aerobic plate count	-0.105	1
	(0.805)	

DISCUSSION

Microbial concentration of indoor air of schools is affected by many factors including human activity, the age of the school building, ventilation conditions, outdoor air and season (primarily temperature and humidity). In this study, classroom atmosphere of seven schools within Ikot Ekpene metropolis were sampled using natural sedimentation techniques.

Results obtained revealed high aerobic plate counts in classroom atmosphere of two schools during ambient sampling; NS4 and SS3 with (33.3 cfu/m^3) and (28.3 cfu/m^3) respectively. The absence of proper ventilation in SS3 classroom (the windows and doors were on only one side of the room, with no windows or doors on the three adjoining walls) is suggested to be a major factor. During populated sampling under population, four schools; NS4, NS3, SS3 and NS2 and SS2 section were revealed as having high aerobic plate counts (63.3 cfu/m^3, 58.3 cfu/m^3, 56.6 cfu/$m^3$and 41.6 cfu/m^3) respectively. Sampling under populated conditions revealed higher aerobic plate counts compared to sampling at ambient conditions in all the schools, correlating the findings of **Tham and Zurami, (2005)** who observed that human activities including movement, rafting, desquamated skin scales, sneezing, and coughing are main contributors of elevated viable microbial concentration in indoor air. The lower aerobic plate counts in ambient samples are suggested to be due to particle settling at night when the classrooms are empty.

Organisms that are capable of causing airborne diseases must be airborne, this means that environmental conditions of the sites as related to building design must be conducive for microorganisms to be airborne and cause pollution **(Wemedo *et al*., 2012)**. In this study, NS4 recorded the highest bacteria count suggested as due to overcrowding; 26 students occupying a small space of (92 cfu/m^2 per student). In contrast, SS2 classroom had a lower bacteria count than most classrooms in this study due to adequate ventilation and low occupancy (only 10 students occupied the classroom with an average space of 480 m^2 per student), as well as the use of quality building materials. This may have assisted in minimization of potential dispersion of dusts, mites or spores resulting in low bacterial counts. Classroom of NS3 may have encouraged the high aerobic plate count reported (58.3 cfu/m^3) due to poor ventilation, lack of windows and doors and also large number of students; 26 occupying a space of 96 m^2 per student.

Stryjakowska *et al*. (2007) and **Soto *et al.* (2009)** in their work have also observed a significant increase in concentration of bacteria in afternoon air sampling during lesson hours compared to ambient sampling at morning hours in various classes of schools, and concluded that bacterial contamination in indoor air derives from human presence. **Karwowsk, (2003)** also reported higher levels of bacterial contamination in schools during school hours (50-100 cfu/m^3 and 200 cfu/m^3). **Nandasena *et al.* (2010)** concluded in their study that as the number of people living in a space rises, so does the counts of organisms in the air.

Building design has been shown to determine the microbial quality of various schools **(Badir *et al*., 2016).** The building conditions of other schools like NS1, NS2, and SS4 though with higher number of students had larger spaces or well positioned windows and doors for proper ventilation and free flow of air. Schools where building design did not allow well positioned windows and doors, and gave high bacteria counts. This include; NS3, NS4 and SS3.

Prevalent bacterial genera isolated from the classroom atmosphere were; gram positive including: *Rothia, Bacillus, Corynebacterium,, Lactobacillus, Brevibacterium, Staphylococcus* and *Kurthia*, and gram negative bacteria including; *Pseudomonas* and *Flavobacterium*. This is also similar to previous reports by **Badir *et al.* (2016)** who isolated *Staphylococcus*, *Bacillus* and *E. coli* as main Gram-positive bacteria belonging to saprophytic microflora generally correlating to human skin and mucosa which can be dispersed through droplets or skin peeling and maintained in the air.

Correlation between area and aerobic plate count implies a negative relationship between the two variables, indicating that as the area increases, aerobic plate count decreases. The p-value of 0.304 ($p>0.05$) implies that this relationship is not significant ($p>0.05$). A negative relationship also exists between class size and aerobic plate count.

A significant difference however exists between aerobic plate count at ambient and population condition. Based on the means, it is clear that aerobic plate count at population condition is significantly higher than aerobic plate count at ambient. The levels of bacteria in classroom sample did not exceed standards (<500 cfu/m^3) **(EPA, 2000)**, but show the presence of potential bacterial pathogens in air.

Bacterial isolates obtained from these classrooms atmosphere are implicated in infectious disease commonly transmitted through air, e.g. *Staphylococcus aureus* which is an airborne bacteria dispersed into the air from human skin, oral and nasal surfaces and hair, and able to cause impetigo which is commonly seen among children. *Pseudomonas* causes pneumonia which affects the lungs. *Bacillus* especially *Bacillus cereus* causes conjunctivitis or orbital abscess which can be transmitted among children in the classroom through air. *Corynebacterium* are bacteria widely distributed in nature including air, causing diphtheria and transmitted through respiratory droplets part of human flora.

CONCLUSION

This study concludes that bacteria are present in, and can be isolated from the atmosphere such as in classroom atmosphere. Airborne bacterial counts of classroom atmosphere can be affected by major factors including classroom population, classroom dimensions, as well as the ventilation patterns. Since poor air quality can pose great risk to the health of students in a classroom, it is therefore important to manage and design classrooms, the class population size, and make adequate provisions for ventilation to maximize the air quality of such environments which will enhance learning, eliminate the risks of airborne transmissions of pathogens and infections among student and staff populations.

REFERENCES

Badri, R. M., Alani, R. R. and Hassan, S. S. (2016). Identification and characterization of air bacteria from some schools of Baghdad City. *Mesopotamia Environ, Jour.*, 2(4): 9-13.

Cheesbrough, M. (1984). *District Laboratory Practice in Tropical Countries*. Cambridge University Press, UK. Pp. 422-425.
CLSI (2017). *Performance standards for antimicrobial susceptibility testing*, 27th edition. Clinical and Laboratory Standard Institute Wayne, Pa. Pp. 33- 70.
Douwes, J., Thorne, P., Pearce, N. and Hendrik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects. *Annals of Occup. Hyg.*, 47(3): 187-200. https://doi.org/ 10.1093/annhyg/meg032
Dunn, R. R., Fierer, N., Henley, J. B., Leff, J.W. and Menninger, H. L. (2013). Home life; factors structuring the bacterial diversity found within and between homes. *PLOS one*, 8(5).
EPA (2000). *Indoor Air Quality and Student Performance*; U.S. Public Health Service, National Environmental Health Association. EPA 402-F-00-009; United States Environmental Protection Agency, Washington DC.
Espejord, I. (2000). Thermal factors-indoor climate. *International. Journal of Circumpolar Health*, 59(3-4):240-245.
Gehring, U., Wijga, A. H., Brauer, M., Fischer, P., De Jongste, J. C., Kerkhof, M. and Brunekreef, B. (2010). Traffic-related air pollution and the development of asthma and allergies during the first 8 years of life. *Am. Jour. Respir. Critical Care Med.*, 181 (6): 596-603. https://doi.org/ 10.1164/rccm.200906-0858OC
Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams A. T. (1994). *Bergey's manual of determinative bacteriology* (9th edition). Williams and Wilkins, Baltimore.
Jones, A. P. (1999). Indoor air quality and health. *Atmos. Environ*., 33(28):4535-4564. https://doi.org /10.1016/S1352-2310(99)00272-1
Karwowsk, E. (2003). Microbiological air contamination in some educational setting. *Polish Journal of Environmental Studies*, 12(2): 181-185.
Nandasena, Y. L. S., Wickremasinghe, A. R. and Sathiakumar, N. (2010). Air pollution and health in Sri Lanka: a review of epidemiologic studies. *BMC Public Health* 10:300. https://doi.org/10.1186/1471-2458-10-300
Obbard, J. P. and Fang, L. S. (2003). Airborne concentrations of bacteria in a hospital environment in Singapore. *Water, Air, and Soil Pollution*,144:333-341. https://doi.org/10.1023/A:102297340
Sharma, K. (2002). *Manual of Microbiology: Tools and Techniques* (2nd edition). Ane Books Pvt Limited, New Delhi, Pp. 100-150.
Shaughnessy, R. J., Haverinen-Shaughnessy, U., Nevalainen, A. and Moschandreas, D. A. (2006). Preliminary study on the association between ventilation rates in classrooms and student performance. *Indoor Air*, 16: 465–468. https://doi.org/ 10.1111/j.1600-0668.2006.00440.x
Soto, T. M., Rosa, G., Murcia, A., Franco, J., Vincente-Solen, J., Cansack, K. and Gacto, M. (2009). Indoor airborne microbial load in a Spanish university (University Of Marcia, Spain). *Anales De Biologica*, 31: 109-115.
Stetzenbach LD, Buttner MP, Cruz P. (2004). Detection and enumeration of airborne biocontaminants. Current Opin. Biotechnol. 15:170-174. https://doi.org 10.1016/j.copbio.2004.04.009
Styjakowska-Sekulska, M. A., Piotraszewska-Pajak, A., Szyszka, M., Nowicki and Filipiak, M. (2007). Microbiological quality of air in university rooms. *Polish Jour. of Environ. Studies*, 16(4): 623-632.
Tham, K. W. and Zuraimi, M. S. (2005). Size relationship between airborne viable bacteria and particles in a controlled indoor environment study. *Indoor Air*, 15(Suppl.9): 48-57. https://doi.org/ 10.1111/j.1600-0668.2005.00303.x
Thorsten, E. C., Hansen, J., Pejtersen, G. H., Clause, E. and Fanger, P. O. (1990). Air pollution sources and indoor air quality in schools. In: Proceedings of the 5th International Conference on Air Quality and Climate, July 29th to August 3rd, Toronto, Canada. Pp. 531-536.
Wargocki, P. and Wyon, D. P. (2006). Research report on effects HVAC on student performance. *ASHRAE Journal*, 48: 112-134.
Wemedeo, S. A., Edo, P. N. and Chuku, A. (2012). Interaction between building design and indoor airborne microbial load in Nigeria. *Asian Jour. of Biologic Sci.*, 5: 183-191. https://doi.org/ 10.3923/ajbs.2012.183.191
Zhao, Z., Zhang, Z., Wang, Z., Ferm, M., Liavcg, Y. and Norback, D. (2008). Asthmatic symptoms among pupils in relation to winter indoor and outdoor air pollution in schools in TaiYuan, China. *Environmental Health Perspectives*, 116:90-97. https://doi.org/ 10.1289/ehp.10576.