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Microbial Population, Physicochemical Parameters and Antibiotic Susceptibility of Classroom Indoor Air in Selected Primary Schools in Port Harcourt, Nigeria

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ABSTRACT

Clean air is necessary for healthy living. Indoor air is prone to bioaerosols, particulate matter (PM) and airborne chemicals. This study evaluated the microbial population and antibiogram of classroom indoor air in three public and three private primary schools in Port Harcourt metropolis. Standard aeromicrobiological methods were employed. Impactation method using Eco MBV-MAS 100 Microbiologic Air Sampler with medium to culture and enumerate specific bacteria and fungi, FC. Temperature, relative humidity (RH), carbon monoxide (CO), carbon dioxide (CO_2), PM, total volatile organic compounds (TVOCs), formaldehyde (HCHO), air quality index (AQI) and light intensity (LI) were measured with a multidigital analyser. Public and private primary school counts revealed THBC ($1.70–3.55 \times 10^3$ CFU/m^3;$1.21–6.41 \times 10^2$ CFU/m^3); TFC ($2.26–8.86 \times 10^2$ SFU/m^3;$1.36–3.97 \times 10^2$ SFU/m^3); TCC ($7.04–20.8 \times 10^2$ CFU/m^3; $1.72–5.78 \times 10^2$ SFU/m^3) and TSC ($8.03–53.1 \times 10^2$ CFU/m^3; $2.53–9.21 \times 10^2$ CFU/m^3). Frequency (%) of occurrence of bacteria were *S. aureus* (13%), *Escherichia coli* (12%), *Klebsiella* (12%), *Bacillus amyloliquefaciens* (10%), *Enterobacter* (9%), *Proteus* (9%), *Micrococcus* (8%), *Pseudomonas aeruginosa* (8%), *Streptococcus* (7%), *Serratia marcescens* (6%) and *Acinetobacter* spp. (6%); fungi were *Aspergillus* spp. (21%), *A. niger* (18%), *Candida* (16%), *Fusarium* (14%), *Penicillium* (13%), *Mucor* (10%) and *Trichoderma* (8%). Physicochemical ranges were; Temperature 25.0–32.0°C, RH 72.0–90.0%, CO 1.0–3.0 ppm, CO_2 404.33–504.67 ppm, PM10 28.0–38.33 µg/m^3, PM2.5 5.0–36.0 µg/m^3, TVOC 0.007–0.15 mg/m^3, HCHO 0.001–0.01 mg/m^3, AQI 30.33–40.67 and LI 4.33–526.67 lux. Microbial counts in public schools exceeded the acceptable standard limits of NESREA, whereas those recorded in private schools were within the acceptable limits. Temperature, RH, CO and PM2.5 exceeded NESREA limits and the USEPA PM2.5 standard. Light intensity was below European Norm 12464-1 except in three public-school classrooms; CO_2, PM10, TVOC, HCHO and AQI were within NESREA limits. Microbial load was likely influenced by each school's environment, while physicochemical parameters reflected the metropolis's conditions. *Proteus* was the most antibiotic susceptible and *Klebsiella* the most resistant. Presence of high *Staphylococcus aureus* population in aerosol of classrooms is of public health concern. Therefore, improved classroom hygiene, adequate ventilation and regular environmental sanitation are recommended to maintain healthy indoor air quality.

Keywords: Classrooms, indoor air Quality, Microbial Population, Bioaerosols, Antibiotic resistance, Primary schools.

Introduction

Indoor air quality is an important component of environmental health, particularly in schools where children spend substantial periods of the day within enclosed classroom environments. Classroom air may contain bacteria, fungi, particulate matter, volatile organic compounds and other pollutants originating from occupants, outdoor air, building materials, dust, cleaning activities and inadequate ventilation. Indoor microbial communities are influenced by occupancy, ventilation, temperature, relative humidity, building Characteristics and human activities (Rai *et al*., 2021; Madsen *et al*., 2023).

Children may be particularly vulnerable to poor indoor environmental conditions because of their developing respiratory systems and relatively high ventilation rates. Exposure to dampness and microbial contamination in indoor environments has been associated with respiratory symptoms, allergic manifestations and asthma-related outcomes (World Health Organization Regional Office for Europe, 2009).

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Recent evidence also indicates that school microbial communities interact with particulate matter and other environmental conditions, highlighting the need for integrated assessment of biological and physicochemical components of indoor air (Ferraz *et al*., 2026).

Bacteria commonly recovered from indoor air include members of the genera *Staphylococcus*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Enterobacter* and *Serratia*. Their presence may reflect human occupancy, resuspension of settled dust, outdoor air intrusion and sanitary conditions. Fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Trichoderma* are also commonly detected in indoor environments and may be associated with dampness, organic materials and outdoor fungal sources.

Environmental conditions can influence the abundance and composition of airborne microorganisms. Temperature and relative humidity are particularly important because they influence microbial survival, growth and aerosol behaviour. Ventilation additionally determines the removal or accumulation of airborne particles and microorganisms (Qiu *et al*., 2022; Madsen *et al*., 2023).

Antimicrobial resistance adds another dimension to the public-health importance of indoor microbial contamination. Airborne bacteria may constitute an environmental reservoir for antimicrobial-resistant organisms, and studies have demonstrated antibiotic-resistant bacteria in indoor bioaerosols (Khan *et al*., 2024; Sah & Khanal, 2025). Schools may therefore represent an important setting for environmental surveillance because large numbers of children interact within relatively confined spaces.

Previous Nigerian studies have investigated indoor microbial air quality in schools and other public environments, but data specifically integrating microbial population, physicochemical parameters, molecular identification and antibiotic susceptibility in primary-school classrooms in Port Harcourt remain limited.

Therefore, this study investigated the microbial population, physicochemical characteristics and antibiotic susceptibility of bacterial isolates recovered from classroom air in selected public and private primary schools in Port Harcourt metropolis, Nigeria.

Materials and Methods

Study Area

The study was conducted in six selected primary schools in Port Harcourt metropolis, Rivers State, Nigeria. The public schools were Alpha Unity primary school (A), Best Brains primary school (B) and Crownworh primary school (C). The private schools were Destiny Target primary school (D), Excellent primary school (E) and Faith international school (F).

Port Harcourt is the capital of Rivers State and is located in the South-South geopolitical zone of Nigeria within the Niger Delta region. The city has substantial oil and gas-related economic activities and contains numerous public and private educational institutions.

The selected schools differed in several observable environmental and infrastructural characteristics. This study documented differences in classroom population, building condition, sanitation facilities, water supply, electricity and ventilation between public and private schools.

Public schools generally had greater classroom occupancy and poorer building and sanitary conditions, whereas the selected private schools had comparatively better-maintained facilities.

Study design and classroom sampling

A modified impaction method as demonstrated by Brągoszewska et al. (2020) was adopted. Classroom air samples were collected from five classrooms in each of the six selected primary schools, comprising three public and three private schools, making a total of 30 classrooms using the MAS-100 ECO active microbial air sampler.

The air sampler collects airborne microorganisms by drawing a measured volume of air through its perforated sampling head onto prepared agar plates.

Four different culture media were used for the collection and isolation of airborne microorganisms: Nutrient Agar (NA) for total heterotrophic bacterial count (THBC), MacConkey Agar (MCA) for total coliform count (TCC), Mannitol Salt Agar (MSA) for total staphylococcal count (TSC) and Sabouraud Dextrose Agar (SDA) for total fungal count (TFC).

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Enumeration and characterization of air microbial isolates

Following incubation, microbial colonies were enumerated and expressed as air concentrations. Representative bacterial colonies were purified and characterized using cultural morphology, Gram staining and standard biochemical tests, including catalase, oxidase, citrate utilization, methyl red, Voges-Proskauer, indole and sugar (glucose, lactose, mannitol, sucrose) fermentation tests.

Fungal isolates were characterized using macroscopic and microscopic morphological features according to the methods of Disegha *et al.* (2024). Unique fungal colonies were isolated and subcultured to obtain pure cultures and identified by observing their colonial characteristics by macroscopy and microscopy.

Examination by macroscopy included observing morphological features such as surface topography, surface texture, pigmentation, and type of mycelium, medium of growth and pace of growth. Microscopy included observing distinctive microscopic structures after staining with lactophenol blue and viewing under light microscope using 40x objective magnification (Disegha *et al*., 2024).

The isolates were identified using. colour atlas and were verified by using reverse image (yandex.com)(Disegha *et al*., 2024).

Molecular identification of air microbial isolates

Selected dominant bacterial and fungal isolates were subjected to molecular characterization. Genomic DNA was extracted from the bacterium using ZR FUNGAL/BACTERIAL DNA MINIPREP (Zymo Research, USA.) extraction kit.

Bacterial isolates were characterized using amplification of the 16S rRNA gene, while fungal characterization involved amplification of the internal transcribed spacer (ITS) region (Akanazu *et al*, 2026).

The molecular analysis confirmed five dominant isolates as *Staphylococcus aureus* strain JMUB7521, *Escherichia coli* strain JML047, *Serratia marcescens* strain P8, *Bacillus amyloliquefaciens* strain SGD, and *Aspergillus niger* strain An14 Pairwise sequence identities ranged from 98–100%.

Evaluation of the physicochemical parameters of classroom air

Classroom air was evaluated for temperature, relative humidity, carbon monoxide, carbon dioxide, particulate matter (PM10 and PM2.5), total volatile organic compounds, formaldehyde, air quality index and light intensity using air quality sampler model ECO MBV-MARS 100 manufactured in China by EMS Industries Ltd.

These parameters were measured using a 9-in-1 air-quality monitor and digital lux meter (ECO MBV-MARS 100). The measurements were obtained for the sampled classrooms and compared with the standards adopted in the thesis. This equipment was manufactured by EMS Industries in China. The MBV MAS-100 Eco is a compact, portable air sampler engineered for air quality monitoring and microbial contamination assessment. It utilizes an impaction method with an adjustable suction and flow rate system compatible with standard agar plates. The device features an intuitive LCD interface for real-time parameter tracking and supports both mains power and battery operation (Erik *et al*., 2023).

Determination of antibiotic sensitivity of classroom air bacterial isolates

Antibiotic susceptibility testing was performed on bacterial isolates using the disk-diffusion approach on Mueller-Hinton agar, using a bacterial suspension standardized to 0.5 McFarland turbidity before inoculation, after which About 0.5 McFarland turbidity standard (equal the turbidity of a barium sulphate, $BaSO_4$) of each bacterial isolates was inoculated on sterile Mueller-Hinton agar plates by spread plate technique. Then ten antibiotic discs were aseptically placed on the plates, pressed a little to make firm contact with the surface of inoculated media. The plates were then incubated at 37℃ for 24 h. Thereafter, the cultures were examined for zones of clearing, diameters of zones of inhibition observed were measured in millimeters (mm) and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2012)) standards. The antibiotics tested against Gram negative bacteria include ofloxacin (OFX), augmentin (AUG), peflacine (PEF), ceftazidime (CTZ), gentamycin (CN), ciprofloxacin (CPX), ceporex (CEP), ceftriaxone (TRX), streptomycin (S), and cefuroxime (CEF).

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While the antibiotics tested against Gram positive bacteria include rifampicin (RD), ceftazidime (CTZ), streptomycin (S), azithromycin (AZM), ciprofloxacin (CPX), erythromycin (E), levofloxacin (LEV), gentamycin (CN), cefuroxime (CEF), and amoxil (amx).

The Gram-negative isolates tested included *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *Proteus* spp., *Acinetobacter* spp., *Enterobacter* spp. and *S. marcescens*. Gram-positive isolates included *Bacillus* spp., *Micrococcus* spp., *S. aureus* and *Streptococcus* spp.

Results

The result of Aeromicrobial Population is as presented in following figures. Figure 1 displays the aeromicrobial population of air sampled from School A. As revealed by the result, the highest THBC was in Class 1 (3.55 $\times 10^3$ CFU/m^3) and the least in Class 3 (2.03 $\times 10^3$ CFU/m^3), highest TFC was in Class 5 (4.25 $\times 10^2$ SFU/m^3) and the least in Class 4 (2.26 $\times 10^2$ SFU/m^3), highest TCC was in Class 4 (2.08 $\times 10^3$ CFU/m^3) and the least was in Class 3 (8.40 $\times 10^2$ CFU/m^3), highest TSC was in Class 1 (3.34 $\times 10^3$ CFU/m^3) and the least was in Class 4 (1.87 $\times 10^3$ CFU/m^3).

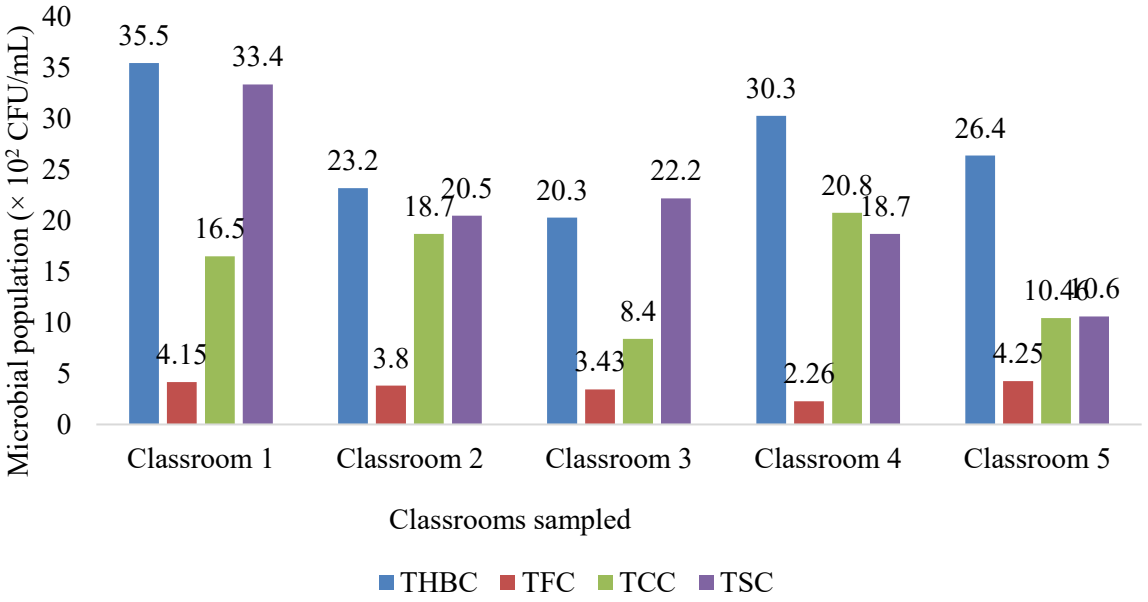


Figure 1: Changes in mean Aeromicrobial load (× 10² CFU/mL) of air sampled from School A

Key: THBC – Total Heterotrophic Bacterial Count; TFC – Total Fungal Count; TCC – Total Coliform Count; TSC – Total Staphylococcal Count; CFU/m^3 - Colony forming unit per cubic meter of air sample; SFU/m^3 - Spore forming unit per cubic meter of air sample

The results as presented in Figure 2. shows that School B with the highest (5.08 × 10^3 CFU/m^3) and least (1.98 × 10^3 CFU/m^3) THBC were recorded in Class 1 and 2 respectively, the highest (6.23 × 10^2 SFU/m^3) and least (2.35 × 10^2 SFU/m^3) TFC were in Class 5 and 3 respectively, the highest (1.82 × 10^3 CFU/m^3) and least (7.68 × 10^2 CFU/m^3) TCC were recorded in Class 1 and 2 respectively, the highest (5.31 × 10^3 CFU/m^3) and least (8.03 × 10^2 CFU/m^3) TSC were recorded in Class 1 and 4 respectively.

The results as presented in Figure 3 shows that School C with the highest (2.64 × 10^3 CFU/m^3) and least (1.70 × 10^3 CFU/m^3) THBC were recorded in Class 3 and 2 respectively, the highest (8.86 × 10^2 SFU/m^3) and least (4.15 × 10^2 SFU/m^3) TFC were in Class 2 and 1 respectively, the highest (2.05 × 10^3 CFU/m^3) and least (7.04 × 10^2 CFU/m^3) TCC were recorded in Class 1 and 4 respectively, the highest (2.16 × 10^3 CFU/m^3) and least (1.15 × 10^3 CFU/m^3) TSC were recorded in Class 5 and 4 respectively.

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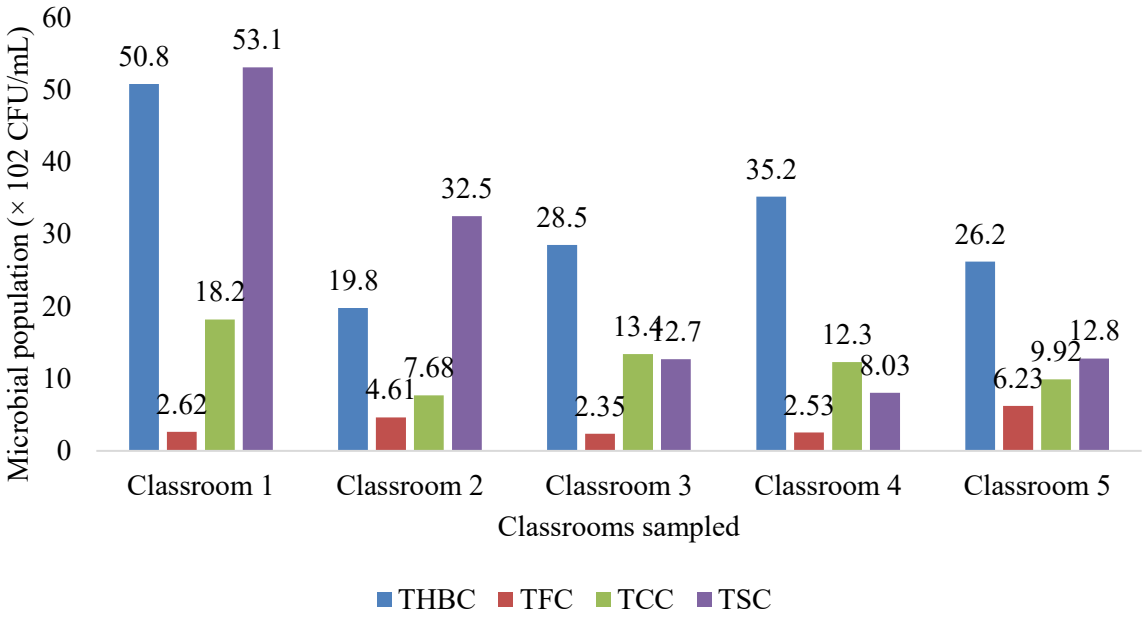


Figure 2: Changes in aeromicrobial load (× 10^2 CFU/mL) of sampled air in School B classrooms

Key: THBC – Total Heterotrophic Bacterial Count; TFC – Total Fungal Count; TCC – Total Coliform Count; TSC – Total Staphylococcal Count; CFU/m^3 - Colony forming unit per cubic meter of air sample; SFU/m^3 - Spore forming unit per cubic meter of air sample.

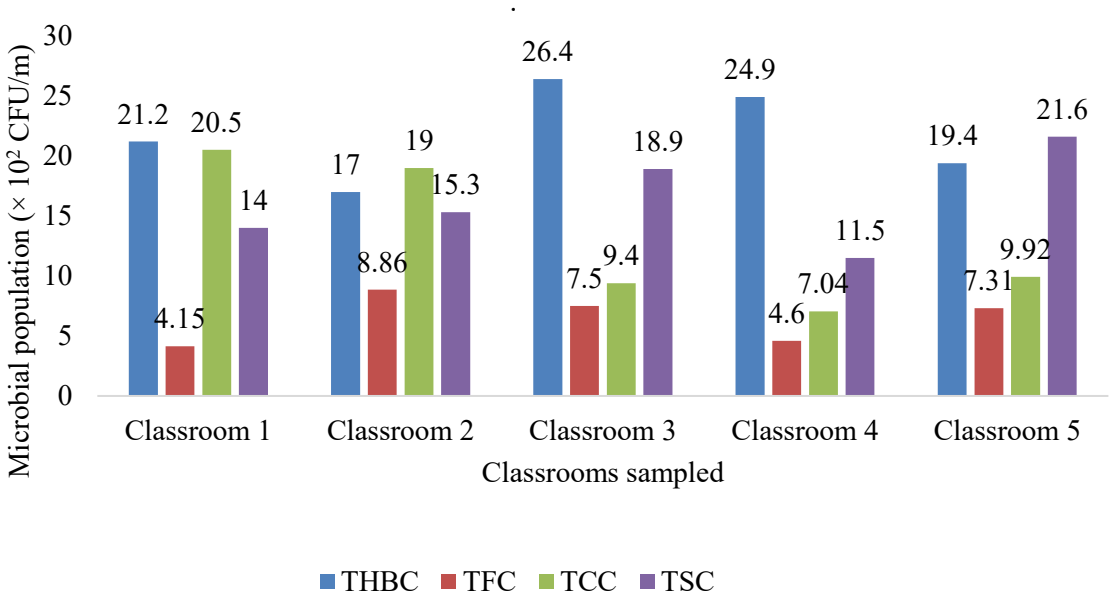


Figure 3: Changes in aeromicrobial load (× 10^2 CFU/mL) of sampled air in School C classrooms

Key: THBC – Total Heterotrophic Bacterial Count; TFC – Total Fungal Count; TCC – Total Coliform Count; TSC – Total Staphylococcal Count; CFU/m^3 - Colony forming unit per cubic meter of air sample; SFU/m^3 - Spore forming unit per cubic meter of air sample.

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Aeromicrobial plate counts of air sampled from School D classrooms' result is as presented in Figure 4. The result shows that the highest (5.69×10^2 CFU/m^3) and least (3.25×10^2 CFU/m^3) THBC were recorded in Class 3 and 4 respectively, the highest (3.25×10^2 SFU/m^3) and least (1.81×10^2 SFU/m^3) TFC were in Class 2 and 1 respectively, the highest (5.78×10^2 CFU/m^3) and least (3.88×10^2 CFU/m^3) TCC were recorded in Class 5 and 3 respectively, the highest (5.33×10^2 CFU/m^3) and least (2.53×10^2 CFU/m^3) TSC were recorded in Class 2 and 3 respectively.

Aeromicrobial plate counts of air sampled from School E classrooms' result is as presented in Figure 5. The result shows that the THBC highest (4.42×10^2 CFU/m^3) and least (1.21×10^2 CFU/m^3) were recorded in Class 2 and 1 respectively, the TFC highest (3.97×10^2 SFU/m^3) and least (2.08×10^2 SFU/m^3) were recorded in Class 5 and 4 respectively, the TCC highest (4.70×10^2 CFU/m^3) and least (1.72×10^2 CFU/m^3) were recorded in Class 1 and 4 respectively, the TSC highest (9.21×10^2 CFU/m^3) and least (3.52×10^2 CFU/m^3) were recorded in class 1.

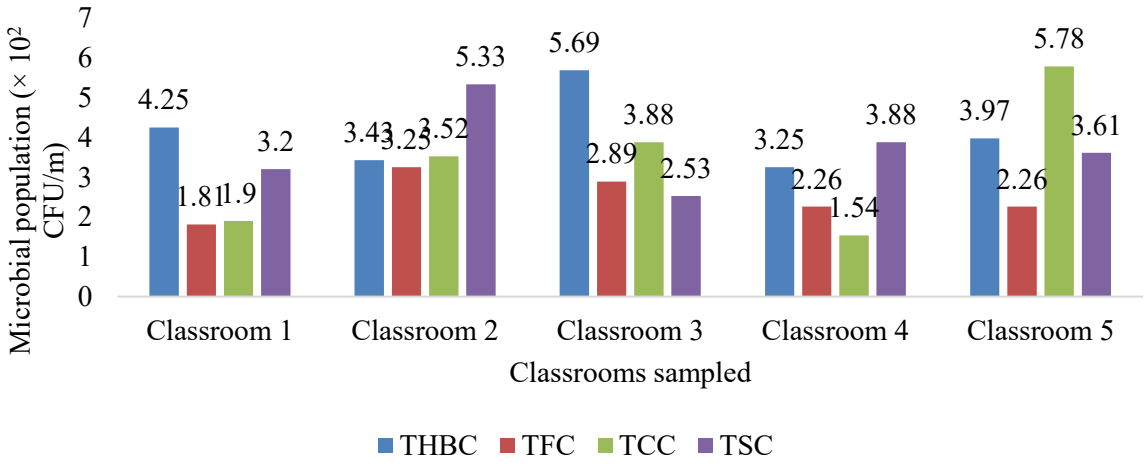


Figure 4: Changes in aeromicrobial load (× 10^2 CFU/mL) of sampled air in School D

Key: THBC – Total Heterotrophic Bacterial Count; TFC – Total Fungal Count; TCC – Total Coliform Count; TSC – Total Staphylococcal Count; CFU/m^3 - Colony forming unit per cubic meter of air sample; SFU/m^3 - Spore forming unit per cubic meter of air sample.

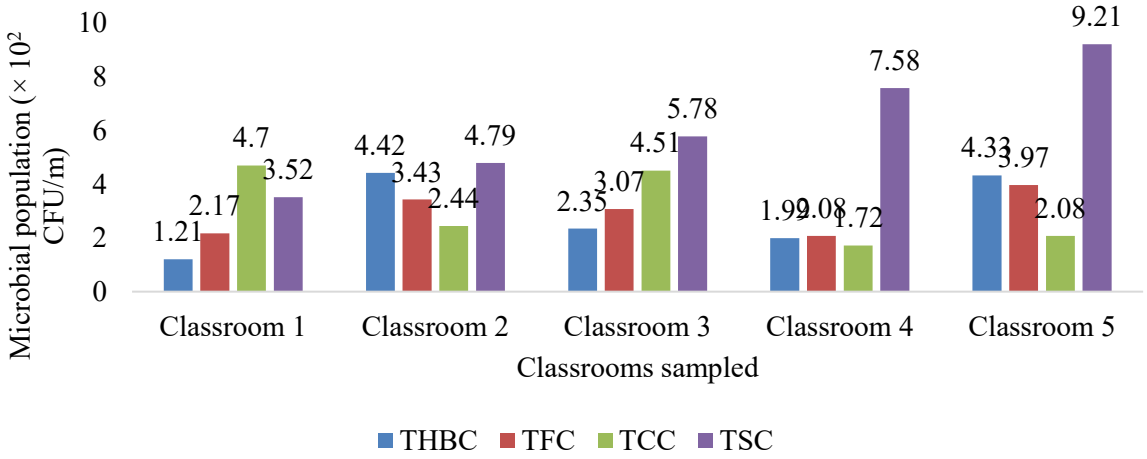


Figure 5: Changes in aeromicrobial load (× 10^2 CFU/mL) of sampled air in School E classrooms

Key: THBC – Total Heterotrophic Bacterial Count; TFC – Total Fungal Count; TCC – Total Coliform Count; TSC – Total Staphylococcal Count; CFU/m^3 - Colony forming unit per cubic meter of air sample; SFU/m^3 - Spore forming unit per cubic meter of air sample.

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The result of aeromicrobial plate counts of air sampled from School F classrooms is as presented in Figure 6. The result reveals that the THBC highest (6.41×10^2 CFU/m^3) and least (2.26×10^2 CFU/m^3) were recorded in Class 1 and 3 respectively, the TFC highest (3.97×10^2 SFU/m^3) and least (1.36×10^2 SFU/m^3) were Recorded in Class 1 and 5 respectively, the TCC highest (5.69×10^2 CFU/m^3) and least (3.88×10^2 CFU/m^3) were recorded in Class 4 and 2 respectively, the TSC highest (7.13×10^2 CFU/m^3) were recorded in classes 4 and 5, while least (5.15×10^2 CFU/m^3) was recorded in Class 3.

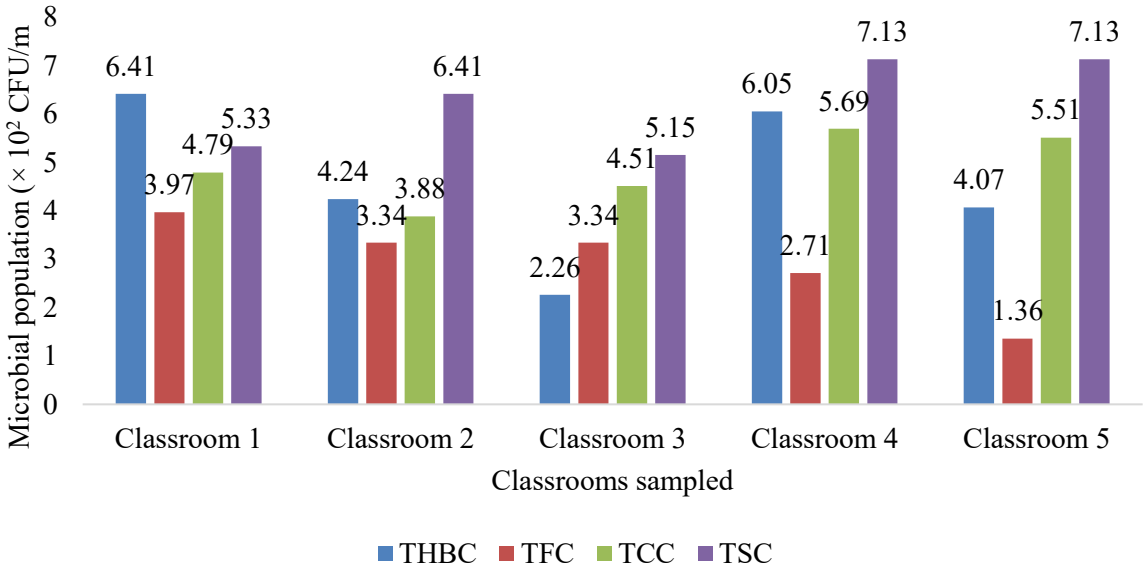


Figure 6: Changes in aeromicrobial load (× 10^2 CFU/mL) of sampled air in School F classrooms

Total heterotrophic bacteria counts (THBCs), Total fungal counts (TFCs), Total coliform counts (TCCs), Total staphylococcal counts (TSCs), Colony forming unit per cubic meter of air sample = CFU/m^3, Spore forming unit per cubic meter of air sample = SFU/m^3

A total of 11 bacterial groups were identified. *Staphylococcus aureus* was the most frequently recovered bacterial isolate, with 43 occurrences (13%), followed by *E. coli* and *Klebsiella* spp., with 40 (12%) and 38 (12%) occurrences, respectively.

Other isolates included *Bacillus* spp. (32; 10%), *Enterobacter* spp. (30; 9%), *Proteus* spp. (28; 9%), *Micrococcus* spp. (26; 8%), *P. aeruginosa* (25; 8%), *Streptococcus* spp. (22; 7%), *S. marcescens* (20; 6%) and *Acinetobacter* spp. (18; 6%).

Seven fungal groups were recovered. *Aspergillus* spp. was the most frequently occurring fungal group, with 39 occurrences (21%), followed by *A. niger* (35; 18%) and *Candida* spp. (30; 16%).

Fusarium spp. occurred 25 times (14%), *Penicillium* spp. 24 times (13%), *Mucor* spp. 18 times (10%) and *Trichoderma* spp. 15 times (8%).

Molecular characterization confirmed the identity of selected dominant isolates. *Staphylococcus aureus* strain JMUB7521 showed 98% pairwise identity, *Escherichia coli* strain JML047 99%, *Serratia marcescens* strain P8, 100%, *Bacillus amyloliquefaciens* strain SGD, 100% and *Aspergillus niger* strain An14 98%.

Results of the aerophysicochemical characteristics of air sample collected from classrooms of the schools are presented in Table 1. Aerophysicochemical characteristics of School A reveals that the temperature range in the classrooms was 29.0±0.00-29.33±0.58℃, relative humidity ranged 78.0±5.19-83.0±000, particulate matter (PM10) ranged 31.67±6.35-35.67±4.93 µg/m^3 and total volatile organic compounds (TVOC) ranged 0.007±0.00-0.016±0.00 mg/m^3.

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Table 1: Mean Aero-physicochemical parameters of public and private primary schools

Primary Class	Temperature (℃)		Relative humidity (%)		Carbon monoxide (ppm)		Carbon dioxide (ppm)	
	A, B, C	D, E, F	A, B, C	D, E, F	A, B, C	D, E, F	A, B, C	D, E, F
1	27.9± 2.54	29.3±1.53	84.8±4.60	82.9±3.42	1.22±0.39	1.22±0.19	424.1±14.36	456.2±40.46
2	28.8±2.00	31.6±0.77	82.0±5.89	77.3±5.51	1.33±0.34	1.33±0.34	409.6±7.08	451.2±41.08
3	29.3 ± 2.19	29.1±2.17	79.6±6.55	82.3±4.73	1.44±0.38	1.44±0.20	414.2±5.67	442.2±54.50
4	29.1 ±2.01	31.3±1.54	81.3±5.36	78.3±5.13	1.89±0.84	1.11±0.19	415.1±6.05	445.2±31.87
5	29.4 ± 1.50	29±2.65	79.6±2.89	79.3±3.30	2.33±0.88	1.11±0.38	418.2±6.05	445.8±34.23

Note: A, B, C = Public school; D, E, F = Private

The aerophysicochemical characteristics of air sample collected from classrooms of School B reveals the temperature range of 29.7±0.58-31.0±0.00, relative humidity ranged 78.0±5.19- 81.33±2.88, the carbon monoxide (CO) ranged 1.0±1.00-3.0±0.00%, particulate matter (PM2.5) ranged 25.0±4.36-29.67±0.58 µg/m^3 and Formaldehyde (HCHO) ranged 0.005±0.00-0.008±0.00 mg/m^3.

The result of aerophysicochemical characteristics of air sample collected from classrooms of School C reveals that the temperature ranged 25.0±0.00-28.0±0.00, the relative humidity (RH) ranged 83.0±0.00-90.0±0.00%, carbon dioxide (CO_2) ranged 417.67±7.23-422.67±5.13 ppm and air quality index (AQI) ranged 34.0±5.19-40.67±1.15.

The result of aerophysicochemical characteristics of air sample collected from classrooms of School D reveals that, the relative humidity (RH) ranged 77.0±0.00-82.0±0.00%, temperature ranged 30.67±0.58- 32.0±0.00, particulate matter (PM10) ranged 28.67±1.15-34±5.57 µg/m^3 and light intensity (LI) ranged 28.67±4.16-250.67±2.08 Lux.

The aerophysicochemical characteristics of air sample collected from classrooms of School E reveals that the temperature range in the classrooms was 27.67±0.58-32.0±0.00℃, total volatile organic compounds (TVOC) ranged 0.013±0.00-0.014±0.00 mg/m^3 and air quality index (AQI) ranged 30.67±0.58-38.33±6.35, relative humidity ranged 78.0±0.00- 86.67±3.21. The aerophysicochemical characteristics of air sample collected from classrooms of School F reveals that the relative humidity (RH) ranged 80.0±0.00-86.0±0.00%, formaldehyde (HCHO) ranged 0.002±0.00-0.007±0.00 mg/m^3 and light intensity (LI) ranged 67.0±27.73-88.33±6.66 Lux, temperature ranged 27.0±0.00-32.0±0.00.

The bacterial isolates demonstrated heterogeneous antibiotic susceptibility patterns. The result as presented in Table 4 is the antibiotic susceptibility result for Gram negative bacterial isolates. The result shows that *E. coli* was susceptible to 5 antibiotics, showed intermediate sensitivity to 2 antibiotics and resistant to 3 antibiotics, and *Klebsiella* spp. were susceptible to 3 antibiotics, intermediately sensitive to 1 antibiotic and resistant to 6 antibiotics.

Table 2: Mean physicochemical parameters of public and private primary schools

Primary Class	Total Volatile Organic Compounds (mg/m3)		Formaldehyde (mg/m3)		Air quality index (AQI)		Light intensity (LI) (Lux)	
	A, B, C	D, E, F	A, B, C	D, E, F	A, B, C	D, E, F	A, B, C	D, E, F
1	0.01±0.00	0.02±0.00	0.005±0.00	0.004±0.00	36.3±4.71	34.11±3.83	261.2±249.93	65.8±18.17
2	0.01±0.00	0.06±0.08	0.006±0.00	0.005±0.00	37.6±3.02	35.7±3.33	170.8±234.36	67.6±4.86
3	0.01±0.00	0.02±0.00	0.005±0.00	0.006±0.00	35.0±2.18	34.9±3.72	309.2±114.47	140.3±95.61
4	0.01±0.00	0.01±0.00	0.006±0.00	0.006±0.00	36.7±2.33	37.8±2.67	180.9±207.57	46.6±34.50
5	0.01±0.00	0.01±0.00	0.005±0.00	0.005±0.00	37.4±3.01	36.1±4.86	248.7±262.59	67.7±20.70

Note: A, B, C = Public school; D, E, F = Private

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Table 3: Mean (± SD) concentrations of PM 10 and PM 2.5 (µg/m^3) measured in public and private primary school classrooms (Primary Class 1–5).

Primary Class	Particulate matter (PM 10) (µg/m3)		Particulate matter (PM 2.5) (µg/m3)	
	A, B, C	D, E, F	A, B, C	D, E, F
1	35.4±2.69	30.7±4.05	27.3±2.19	23.8±1.68
2	35.0±2.85	33.1±1.95	26.3±1.15	28.1±1.35
3	32.9±1.83	32.7±3.52	26.2±1.65	29.4±5.74
4	34.2±1.65	31.4±2.01	26.4±1.26	27.3±0.34
5	34.3±2.03	32.0±3.33	26.8±1.71	30.8±4.02

Table 4: Antibiotic susceptibility result for Gram negative bacterial isolates.

Bacteria Isolate	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF
E. coli	R	S	S	I	S	S	S	I	R	R
P. aeruginosa	S	R	R	R	S	S	S	I	I	R
Klebsiella spp.	S	R	I	R	R	S	R	R	S	R
Proteus spp.	I	S	S	R	S	S	R	S	S	S
Acinetobacter spp.	I	S	S	R	R	S	R	R	I	S
Enterobacter spp.	S	R	R	R	I	S	S	S	I	I
S. marcescens	S	R	I	R	I	S	S	I	I	R

Note: Susceptible (S) ≥ 17mm, Intermediate = 14-16mm, Resistant ≤ 13mm; OFX- Ofloxacin, AU- Augmentin, PEF-Peflacin, CTZ- Ceftazidime, CN- Gentamycin, CPX- Ciprofloxacin, CEP- Ceporex, TRX- Ceftriaxone, S- Streptomycin, CEF- Cefuroxime

Table 5: Antibiotic susceptibility result for Gram positive bacterial isolates

Isolates	RD	CTZ	S	AZM	CPX	E	LEV	CN	CEF	AMX
Bacillus spp.	I	I	R	R	S	S	S	S	R	R
Micrococcus spp.	S	I	R	I	S	S	S	R	R	S
Staphylococcus aureus	I	R	R	R	S	S	S	R	I	I
Streptococcus spp.	S	S	R	I	S	I	R	R	R	R

Note: Susceptible (S) ≥ 17mm, Intermediate = 14-16mm, Resistant ≤ 13mm; RD- Rifampicin, CTZ- Ceftazidime, S- Streptomycin, AZM -Azithromycin, CPX- Ciprofloxacin, E-Erythromycin, LEV- Levofloxacin, CN- Gentamycin, CEF= Cefuroxime, AMX- Amoxil.

The result as presented in Table 5 is the antibiotic susceptibility result for Gram positive bacterial isolates. The result shows that *Bacillus* spp. was susceptible to 4 antibiotics, intermediately sensitive to 2 antibiotics and resistant to 4 antibiotics, *S. aureus* was susceptible to 3 antibiotics, intermediately sensitive to 3 antibiotics and resistant to 4 antibiotics, while *Streptococcus* spp. was susceptible to 3 antibiotics, intermediately sensitive to 2 antibiotics and resistant to 5 antibiotics. The population and percentage abundance of the identified bacteria isolates from school A-F classrooms is as presented in Figure 1. The result shows that the most abundant isolate was *S. aureus* (13%) followed by *E. coli* and *Klebsiella* spp. (12% each) and the least abundant isolate were *Acinetobacter* spp. (6%).

The population and percentage abundance of airborne fungal isolates from schools A-F classrooms in Port Harcourt are as presented in Figure 2. The result shows that the most abundant isolate was *Aspergillus* spp. (21%) and the least abundant isolate were *Mucor* spp. (10%).

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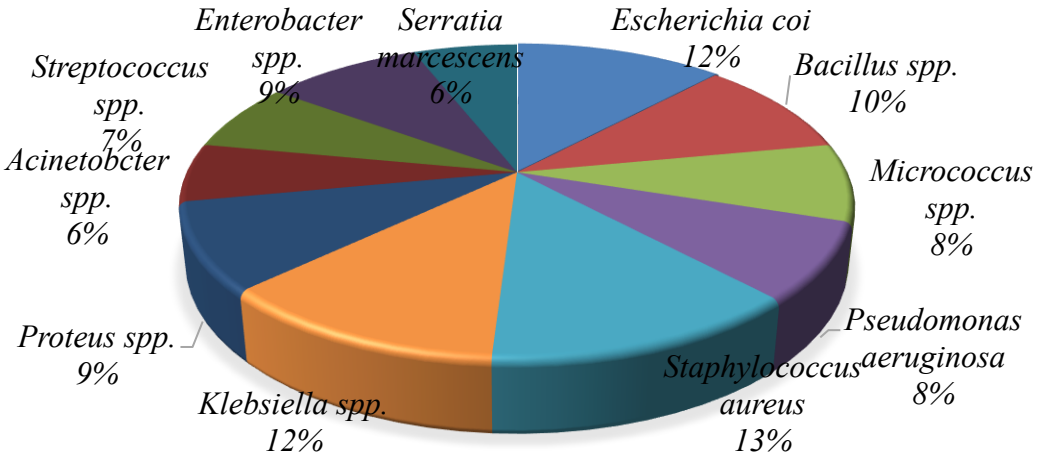


Figure 1: Population and percentage abundance of identified bacteria isolates from school A-F classrooms.

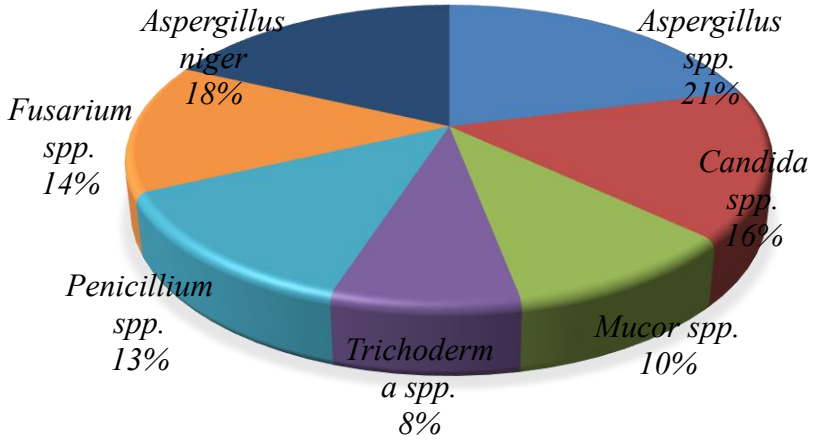


Figure 2: Population of airborne fungal isolates from school A-F classrooms in Port Harcourt

Discussion

The investigation across Schools A to F revealed substantial aeromicrobial contamination, with Total Heterotrophic Bacterial Counts (THBC) reaching up to 5.08×10^3 CFU/m^3, Total Staphylococcal Counts (TSC) peaking at 5.31×10^3 CFU/m^3, and Total Fungal Counts (TFC) reaching 6.23×10^2 SFU/m^3.

These findings strongly align with prior indoor air quality (IAQ) assessments conducted in tropical and densely populated school environments (e.g., studies in urban Nigerian and similar developing-region classrooms), which consistently report high bioaerosol concentrations exceeding recommended indoor microbiological limits (Yusof *et al.,* (2025).

In contrast, findings from studies in mechanically ventilated or climate-controlled modern schools in temperate regions report significantly lower microbial and fungal counts per cubic meter. The high microbial counts are primarily driven by high student occupancy density, restricted spatial volumes per student, and inadequate natural cross-ventilation.

Classrooms act as enclosed reservoirs where human activity (talking, coughing, walking, and shedding of skin scales/respiratory droplets) continuously aerosolizes microbes. Furthermore, high relative humidity levels (77% - 90%) create an optimal microclimate for microbial suspension, survival, and proliferation (Ferraz *et al*. 2026).

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Microbiological and molecular characterization identified *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., and *Bacillus* spp. as predominant bacterial groups, alongside *Aspergillus* spp., *A. niger,* and *Candida* spp. as dominant airborne fungi (Lubukola *et al*., 2022).

Dominance of *Staphylococcus* and *Streptococcus* species aligns with well-documented aeromicrobiological profiles of human-occupied indoor spaces, where occupants serve as the primary shedding source. Similarly, the prevalence of *Aspergillus* and *Penicillium* spores is a universally reported phenomenon in tropical indoor environments. While some researchers emphasize outdoor soil-borne actinomycetes or specific agricultural molds as primary contributors in rural schools, urban school profiles (such as those in Port Harcourt) show a heavier skew toward human-associated opportunistic pathogens and enteric bacteria (*E. coli*, *Klebsiella*) (Ogbonna and Ogbuleka, 2020).

The high incidence of *Staphylococcus aureus* stems from direct shedding from human skin, hair, and nasal passages. The presence of *E. coli* and *Klebsiella spp.* indicates fecal-oral or fomite-to-air cross-contamination, often exacerbated by poor hand hygiene practices among young primary school children, crowded seating arrangements, and dust resuspension. Fungal abundance (*Aspergillus* and *Mucor*) is directly promoted by high ambient humidity and damp structural surfaces within school buildings (Ogbonna and Ogbuleka, 2020).

The physical parameters and air quality dynamics recorded high ambient temperatures (25°C-32°C), elevated relative humidity (77%--90%), and varying concentrations of particulate matter (PM_{10} and $PM_{2.5}$), volatile organic compounds (TVOCs), and carbon monoxide (CO). These results corroborate findings from tropical school air quality assessments, which observe that natural ventilation systems frequently fail to filter out fine particulate matter ($PM_{2.5}$ and PM_{10}) originating from nearby vehicular traffic and outdoor dust resuspension (Kevin *et al.*, 2020).

Disagreements occur when comparing these data with studies from rural or strictly zoned educational complexes where ambient PM and CO levels remain minimal.

Elevated particulate matter results from dust entering open windows from unpaved or busy schoolyards combined with student movement indoors. High indoor humidity coupled with elevated temperatures accelerates biochemical reactions and microbial metabolic activity, facilitating spore germination and microbial aerosol stability (Marcovecchio and Perrino, 2021).

The antibiotic susceptibility patterns of isolates revealed that, both Gram-negative and Gram-positive isolates demonstrated alarming multiple-antibiotic resistance (MAR) profiles, with strains of *Klebsiella* spp., *S. aureus*, and *Pseudomonas aeruginosa* displaying resistance to several frontline antibiotics (e.g., Augmentin, Ceftazidime, Cefuroxime). These observations mirror a growing body of global environmental microbiology literature warning that indoor air acts as a reservoir for antimicrobial-resistant (AMR) pathogens (Atalay *et al.,* 2023).

Older environmental reports from decades past typically showed higher baseline susceptibility among airborne isolates, highlighting a modern escalation in environmental drug resistance. The widespread resistance pattern reflects the environmental dissemination of resistant strains originating from community and clinical settings. The selective pressure from indiscriminate antibiotic use in the broader community leads to the persistence and airborne dissemination of resistant genetic determinants among opportunistic pathogens inhabiting school microenvironments (Zhang *et al.,* 2024).

Conclusion

The physicochemical analysis revealed similar patterns in both public and private schools. Temperature, relative humidity, carbon monoxide (CO) and PM2.5 exceeded the recommended limits, while light intensity was below the recommended classroom standard, except in classrooms 1 of School B and 2–4 of School C.

However, CO2, PM10, TVOCs, HCHO and AQI remained within the acceptable limits specified by NESREA. Public schools recorded microbial counts exceeding the acceptable limits, whereas private schools had counts within the acceptable limits.

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The differences in microbial contamination may be associated with the immediate environmental conditions of individual schools, while the physicochemical parameters may reflect the prevailing environmental conditions of Port Harcourt metropolis. Also, the bacterial isolates showed varying levels of susceptibility and resistance to the antibiotics tested.

The findings highlight the need to improve classroom environmental conditions and indoor air quality, particularly in public schools. Regular cleaning, adequate ventilation, proper classroom maintenance and periodic monitoring of air quality are recommended to reduce microbial contamination and provide a healthier learning environment for pupils and teachers.

Recommendations

Schools should:

1. Schools should ensure adequate natural or mechanical ventilation to promote air exchange and reduce the accumulation of airborne microorganisms.
2. Classrooms should be cleaned regularly, with particular attention to floors, windows, desks, ceilings and other areas where dust can accumulate.
3. Adequate sanitation facilities, handwashing facilities and appropriate waste-management practices should be maintained to minimize environmental microbial contamination.
4. School authority should promptly address leaking roofs, water seepage, damp walls and other sources of persistent moisture because damp conditions can support fungal growth and microbial contamination.
5. Overpopulated classrooms should be avoided. Morning and afternoon sessions could be considered in overpopulated schools.
6. Good lighting and air-conditioning equipment should be installed in classrooms.
7. and functional ventilation systems.
8. Consider periodic monitoring of classroom microbial air quality.
9. Strengthen environmental antimicrobial-resistance surveillance where resources permit.

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