RESEARCH ARTICLE

Characterization of culturable airborne bacteria and antibiotic susceptibility profiles of indoor and immediate-outdoor environments of a research institute [version 1; referees: 2 approved with reservations]

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Abstract

Background: The study was conducted to determine the bacterial composition and antibiotic susceptibility profiles of a research institute at the University of Ghana where workers and students spend about 70-85% of their lives in indoor and immediate-outdoor environments. This is imperative as one-third of the recognized infectious diseases are transmitted through airborne-route. Furthermore, the increasing rate of bacterial antimicrobial resistance associated with such environments poses serious public health challenges.

Methods: A total of 42 airborne samples were collected from eight major sites at the Department of Biochemistry, Cell and Molecular Biology (BCMB), using passive bacterial sampling techniques. Standard phenotypic microbiological procedures were used to characterize the isolates. Antibiotic susceptibility profiles were determined using standard disk diffusion method and guidelines of Clinical and Laboratory Standards Institute (CLSI).

Results: Four groups of bacterial isolates were identified from the total samples collected with Gram positive bacilli as the most common. All the isolates showed resistance to beta lactam and sulfonamide classes of antibiotics with full resistance (100%) to ampicillin and penicillin. In total, seven different anti-biotypes were observed with the highest susceptibility displayed towards tetracycline and gentamycin. Significantly, the various air sampling sites of the institute indicated the presence of bacteria with the majority showing multiple antibiotics resistance.

Conclusions: Although the recovery of bacteria from supposed sterile environments calls for attention, the observed low contamination rate as compared to the WHO standard suggests a minimum risk of exposure of students and workers to airborne microbial contamination.

Keywords

Airborne Bacteria, Antibiotic resistance, Indoor Air, Bacteriological profile
Introduction
Quality of air, especially in indoor environments where people spend 80–95% of their lives is of significant health importance. Microorganisms are ubiquitous; they normally inhabit indoor and outdoor environments. The inhaled air in the indoor environment is dominated by a number of microorganisms, with consequent effects on the health those indoors. Little is known about the diverse communities of bacteria shared by indoor environments such as houses, offices, laboratories, schools, hospitals, and other indoor environments where people work, relax or find solace. The diversity of these microbes in the indoor environment is influenced by several factors such as water, temperature, moisturized surfaces or worktops, the rate of particle deposition, and other parameters like indoor pollutants, especially those generated by various human activities.

Bioaerosols, mostly bacterial and fungal spores are actively living complex particles that have been associated with contamination of indoor air. The presence of these biological contaminants has been reported in the air of hospitals, but little is known about their impact on a typical research environment in Ghana. As dangerous as bioaerosols are by themselves, they also secrete toxins that are transmitted by the airborne route through the nasal airways making indoor environment a potential source of human pathogens. Microorganisms gain access to different indoor compartments of buildings through openings like doors, windows, blowing fan blades, air conditioners and the immediate-outdoor environments. Immediate-outdoors areas, usual described as 0.9m to 3.5 away from the main building, includes foyers, such as the corridor, lobby or hallway. In addition the indoor air microbiomes originating from outdoor air-space drifts have influencing factors which account for diverse microbial distribution. In a typical research environment of academic training and learning, a series of movements do occur from the outdoors through the immediate-outdoors to indoors. This facilitates the movement of microorganisms, especially bacteria to different compartments of the building.

Several species of microorganisms have been isolated across indoor environments in previous studies. Although most of these microbes have been reported as opportunistic pathogens, they are not necessarily associated with severe infections. However, they can pose significant challenges to immune-compromised individuals. Sterile conditions, especially in biological laboratories control microbial growth. Interestingly, the microbes are able to survive using the air routes to other favourable environments within indoor environments. The laboratory hoods, although they are meant to provide a sterile environment for designated experiments, could serve as potential site for bacterial contaminations when sterility is compromised.

An increase in bacterial antimicrobial resistance and emergence of new strains associated with academic research environments is a serious public health challenge and has become increasingly important in recent years. In an environment where inter-personal and research activities are so diverse, bacteria resistance to antimicrobials is a possibility. Studies of indoor air qualities and antibiotic susceptibility patterns of bacterial isolates present in most public institutes in developing countries have not been reported so far. This study was designed to characterize the bacterial composition and antibiotic susceptibility patterns of isolates recovered from indoor and immediate-outdoor air of a tertiary research institute in Ghana.

Methods
Sampling sites
This study was conducted between January and May 2017. The study involved determination of bacterial loads and antibiotic susceptibility profiles of the air in selected study sites within the indoors and immediate-outdoors (foyers) of a research institute at the Department of Cell and Molecular Biology (BCMB). Sampling was conducted at different times within the day in duplicates for a period of eight weeks. Sampling sites included teaching laboratories, classrooms, experimental laboratories, laboratory biosafety hoods, foyers, toilets and the library.

Cultivation of samples
All samples were collected using the open plate passive sampling technique and processed under aseptic conditions following standard microbiological methods. Nutrient agar (Oxoid, England, CM0003), MacConkey agar (Oxoid, England, CM0007B), Blood agar (Oxoid, England, CM0055) and Mannitol salt agar (Oxoid, England, CM0085) plates were exposed for 60 minutes during daily active working hours (8am – 5pm) at different sites. The plates were incubated at 37°C under aerobic conditions for 24–48 hours.

Isolation and identification of isolates
Bacteria isolates were identified using phenotypic microbiological methods described by Aguilera-Arreola et al. (2016). Microscopy (Gram’s staining) and biochemical reactions were performed. Standard plate count was performed to determine the bacterial loads across the sampled sites.

Frequency of outdoor-indoor movements
The frequency of movements from outdoor to indoor environments was determined for a period of one month using manual counting and closed circuit television camera monitoring system.

Antimicrobial susceptibility testing
Clinical Laboratory Standards Institute (CLSI 12th Edition) guidelines were followed to carry out the Antimicrobial susceptibility testing using disk diffusion method. Commonly used antibiotics prescribed by clinicians were selected, based on their general known effectiveness against bacterial infections. The discs used for screening Gram positive and negative bacteria contained the following antibiotics with the respective concentrations: ampicillin (10 μg), cefotaxime (30μg), chloramphenicol (30μg), ciprofloxacin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), nitrofurantoin (200μg), tetracycline (30μg).
penicillin (15 μg), flucloxacillin (5 μg), cloxacillin (10 μg), erythromycin (5 μg), ceftriaxone (30 μg) and cotrimoxazole (25 μg) (Mast Diagnostics, Mast Group Ltd., Merseyside, U.K.).

Statistical analysis
All statistical analyses were performed using SPSS package version 17.0 and Graphpad prism version 6 software. P-values less than 0.05 were considered statistically significant.

Results
Total isolated bacteria and diversity across sites
Of the 42 total samples collected 87% of the isolates recovered were identified as Gram positive bacilli, Staphylococcus sp., Gram positive cocci and Gram negative bacilli, (Table 1 and Figure 1).

Staphylococci were isolated on Mannitol salt and nutrient agar media. Staphylococcus aureus was identified as mannitol fermenting colonies. Catalase positive isolates were classified as Staphylococcus species differentiating them from Streptococcus which are negative for catalase activities (Table 1). Haemolysis was used to further confirm the Streptococcus species by

| Biochemical Tests          | Probable Bacteria sp. | \( \text{GPB} \) | \( \text{GPC} \) | \( \text{GNB} \) |
|----------------------------|-----------------------|-----------------|----------------|-----------------|
| Cell shape                 | Rod                   | coccI           | cocci          | Rod             |
| Gram                       | +                     | +               | +              | -               |
| Catalase                   | +                     | +               | -              | +/-             |
| Motility                   | +                     | -               | +              | +/-             |
| Starch Hydrolysis          | +                     | -               | +              | -               |
| Hemolysis                  | +                     | -               | +              | +               |
| Acid Production from Mannitol | +                 | +               | -              | +               |
| Citrate                    | +                     | +               | +/-            | +               |
| Nitrate reduction          | +                     | -               | -              | +/-             |
| \( \text{H}_{2}\text{S} \) | +                     | -               | -              | +/-             |

+: Positive, - : Negative (\( \text{GPB} \): Gram positive bacilli, \( \text{GPC} \): Gram positive cocci, \( \text{GNB} \): Gram negative bacilli, Staph – Staphylococcus spp., Strep - Streptococcus spp.)

Figure 1. Distribution of bacterial isolates (\( \text{GPB} \): Gram positive bacilli, \( \text{GPC} \): Gram positive cocci, \( \text{GNC} \): Gram negative cocci, \( \text{Staph} \): Staphylococcus spp.)
checking their activities on Blood agar (Table 1). The identification was further confirmed as positive cocci with Gram’s reaction and microscopic examination, signifying the trapping of the staining dye in the peptidoglycan layer of the organism cell wall (Figure 1).

Gram positive bacilli were isolated from blood and nutrient agar media after an overnight growth (Table 1). Microscopy further confirmed the identification as Gram positive bacilli, mostly in chains (Figure 1). Gram negative bacilli were isolated from MacConkey agar medium after an overnight incubation period. The rose-pink colouration of the medium differentiates the lactose fermenters from the non-lactose fermenters (Table 1). The identifications were confirmed with characteristic appearance as pink rods after Gram staining (Figure 1).

Diversity and predominant isolated bacteria across sites
The percentage of bacteria diversity isolated across the different sites is presented in (Table 2), with a total of 54 isolates belonging to three the genera identified. The highest number of isolates was obtained from the foyers (n=13), followed by the toilets (n=11), then the classrooms (n=9) and finally from the library (n=7). The lowest number of isolates was from the railings of the stairways (n=2). A significant number of diverse bacteria was identified from the samples collected across the sampled sites (Figure 3). The most commonly isolated bacteria across the sampling sites are Gram positive bacilli, with highest percentage in the foyers and toilets as compared to the classrooms and library (Figure 4).

To compare the average percentage of the bacterial composition of both indoor and immediate-outdoor air, the results were also reported as the number of coliform forming unit (CFU m⁻³) (Table 3). The bacterial concentration is within the range 54 – 249 CFU m⁻³ with the foyers having the highest and the railings the lowest. In consideration of the total bacteria concentrations across the sites, indoors had the higher bacteria representation as compared to the outdoors. Frequent movements of students and workers from immediate-outdoor to indoor environments were determined with a daily minimum of 210 and maximum of 315 people (Table 4).

Table 2. Diversity of bacteria isolated across the sites.

| Sampling Sites    | Number of Samples (per site) | Number of Isolates (per site) | Genera            |
|-------------------|------------------------------|-------------------------------|-------------------|
| Railings          | 2                            | 3                             | GPB, GPC          |
| Library           | 3                            | 7                             | GPB, GPC, GNB     |
| Toilet            | 6                            | 11                            | GPB, GPC, GNB     |
| Teaching Lab      | 4                            | 5                             | GPB, GPC          |
| Classrooms        | 4                            | 9                             | GPB, GPC, GNB     |
| Foyers            | 6                            | 13                            | GPB, GPC, GNB     |
| Experimental Lab  | 13                           | 6                             | GPB, GPC          |
| Lab Biosafety Hood| 4                            | 5                             | GPB, GPC          |

GPB: Gram positive bacilli, GPC: Gram positive cocci, GNB: Gram negative bacilli, Staph – Staphylococcus sp., Strep - Streptococcus

Figure 2. Microscopy Results of Representative Isolates. A, E – Gram positive bacilli, B – Gram positive bacilli with spores unable to pick the staining dye, D, F – Gram positive cocci, C – Gram negative short rods (a representation of three different replicates).
Figure 3. Significance (p < 0.01) of the bacterial isolates with respect to sampling across the site.

Figure 4. Most common bacteria appearance across the sampling sites (GPB: Gram positive bacilli, GPC: Gram positive cocci, GNB: Gram negative bacilli, Staph – Staphylococcus spp.).
Antimicrobial susceptibility patterns

All the 54 isolates were tested against fourteen different selected antibiotic discs, belonging to eight different classes of antibiotics (Table 5). The antimicrobial resistance profile and susceptibility patterns showed that 87.7% and 76.7% of the Gram positive cocci were resistant to Beta Lactam and sulfonamides, 57.1%, 72.4% and 100% of Gram positive bacilli were resistant to Beta Lactam, macrolides and sulfonamides. Resistance to Nitrofurans by Gram negative bacilli was 80.4%, while 84.2% and 87.7% of *Staphylococcus* species showed resistance to sulfonamides and Beta Lactam respectively. Resistance to flucloxacillin across the isolates was observed; highest with *Staphylococcus* sp. Susceptibility of the isolates to tetracycline and gentamycin were observed especially with some Gram positive isolates (Figure 5). All the isolates showed resistance to at least 2-classes of the 8 different classes of antibiotics tested. Seven different anti-biotypes (multiple antibiotic resistance patterns) were observed across the isolates with a minimum of resistance to two different antibiotics and maximum of nine different antibiotics (Table 6).

### Table 3. Total number of bacteria in cfum⁻³.

| Sample Grade | Sampling Sites     | Total number of bacteria (cfum⁻³) |
|--------------|--------------------|-----------------------------------|
| 1            | Railings           | 0.54×10⁷                          |
| 2            | Library            | 1.34×10⁷                          |
| 3            | Toilet             | 2.06×10⁷                          |
| 4            | Teaching Lab       | 1.21×10⁷                          |
| 5            | Classrooms         | 1.76×10⁷                          |
| 6            | Foyers             | 2.49×10⁷                          |
| 7            | Experimental Lab   | 1.02×10⁷                          |
| 8            | Lab Fume Hood      | 0.89×10⁷                          |

### Table 4. Frequency of Outdoor-Indoor Movements.

| Day  | Active Working Hours | Day | manual count | cctv camera count |
|------|----------------------|-----|--------------|------------------|
|      |                      |     | wk1          | wk2  | wk3  | wk4  | Average | wk1  | wk2  | wk3  | wk4  | Average |
| wk1  | 201                  | Day 1 | 201          | 186  | 243  | 211  | 210     | 321  | 207  | 243  | 401  | 293      |
| wk2  | 281                  | Day 2 | 281          | 142  | 179  | 292  | 224     | 181  | 242  | 449  | 378  | 315      |
| wk3  | 181                  | Day 3 | 181          | 253  | 129  | 307  | 218     | 281  | 253  | 329  | 307  | 293      |
| wk4  | 282                  | Day 4 | 282          | 142  | 201  | 262  | 221     | 382  | 164  | 206  | 282  | 259      |
| Day 5 | 196                  | Day 5 | 196          | 263  | 187  | 289  | 234     | 196  | 286  | 281  | 248  | 252      |

wk – week, cctv – closed circuit television

### Table 5. Percentage Frequency of Isolates to Antibiotics.

| Antibiotics Tested | Disc Potency (µg) | GPC | GPB | GNB | Staph. spp. |
|--------------------|-------------------|-----|-----|-----|-------------|
|                    | S     | R     | S     | R     | S     | R     | S     | R     |
| Flucloxacillin     | 5     | 0     | 85.7  | 11.2  | 80.0  | 10.5  | 71.3  | 0     | 88.7  |
| Erythromycin       | 5     | 20.7  | 57.1  | 0     | 83.3  | NT    | NT    | 5.5   | 74.3  |
| Cloxacillin        | 5     | 3.6   | 64.3  | 0     | 50.0  | NT    | NT    | 3.6   | 64.3  |
| Ceftriaxone        | 30    | 15.3  | 58.6  | 12.5  | 68.3  | 2.7   | 95.0  | 15.3  | 58.6  |
| Cotrimoxazole      | 25    | 11.2  | 87.7  | 0     | 100.0 | 53.4  | 34.4  | 11.2  | 87.7  |
| Nitrofuratoin      | 200   | NT    | NT    | NT    | NT    | 80.4  | NT    | NT    | NT    |
| Chloramphenicol    | 30    | 85.7  | 4.2   | 66.6  | 6.7   | 81.4  | 11.6  | 92.6  | 0     |
| Tetracycline       | 10    | 96.0  | 2.5   | 97.0  | 0     | 87.6  | 12.1  | 85.7  | 7.1   |
| Cefotaxime         | 10    | 12.1  | 57.1  | 9.5   | 67.0  | NT    | NT    | 13.8  | 77.6  |
| Cefuroxime         | 30    | 22.6  | 71.4  | 21.1  | 41.6  | 0     | 100.0 | 0     | 100.0 |
| Penicillin         | 15    | 0     | 100.0 | 0     | 100.0 | 0     | 100.0 | 0     | 100.0 |
| Ampicillin         | 10    | 0     | 100.0 | 0     | 100.0 | 0     | 100.0 | 0     | 100.0 |
| Nalidixic Acid     | 30    | NT    | NT    | NT    | NT    | 60.0  | 24.3  | NT    | NT    |
| Gentamicin         | 10    | 97.0  | 2.0   | 95.5  | 2.6   | 66.7  | 13.2  | 97.0  | 2.0   |

R – Resistance, S – Susceptible, NT – Not Tested (Antibiotics were not available at the time of this experiment)

GPB: Gram positive bacilli, GPC: Gram positive cocci, GNB: Gram negative bacilli
Table 6. Multiple Antibiotic Resistance Patterns of the bacterial Isolates.

| Isolates          | Level | Type of Antibiotics       | Antibiotic Classes | N° Isolates |
|-------------------|-------|---------------------------|--------------------|-------------|
| Gram Positive Cocci | Max. (9) | FLX, ERY, CX, CTX, COT, CFX, CXM, PEN, AMP | B-Lac, Mac, Sun | 11          |
|                   | Min. (3) | C, TET, GEN | CH, TE, AMIN | 6           |
| Gram Positive Bacilli | Max. (8) | FLX, ERY, CX, COT, CFX, PEN, AMP, CRX | B-Lac, Mac, Sun | 7           |
|                   | Min. (2) | GEN, C | AMIN, CH | 3           |
| Gram Negative Bacilli | Max. (8) | FLX, CFX, NIT, CTX, PEN, AMP, NAL, COT | B-Lac, Nitro, Qui, Sun | 3          |
|                   | Min. (3) | C, TET, GEN | CH, TE, AMIN | 3           |
| Staph sp.         | Min. (9) | FLX, ERY, CX, CFX, COT, CXM, CTX, PEN, AMP | B-Lac, Mac, Sun | 9           |
|                   | Max. (2) | TET, GEN | TE, AMIN | 5           |

FLX – flucloxacillin, ERY – erythromycin, CX – cloxacillin, CTX – ceftriazone, NIT – nitrofuratoin, PEN – penicillin, AMP – ampicillin, NAL – nalidixic-acid, C – chloramphenicol, TET – tetracycline, GEN – gentamycin, COT – cotrimoxazole, CRX – cefuroxime, CFX - cefotaxime

Discussion
The study considered bacterial isolates in the indoor and immediate-outdoor air environments of a research institute in Ghana. It was observed that all the sections sampled showed diverse bacterial loads similar to other studies conducted elsewhere\(^7\),\(^15\). In accordance with this study, frequent movements of students and workers from immediate-outdoor to the indoor environments decisively influenced the diversity and abundance of the isolated bacteria. In this context, samples collected from different sections were significantly matched to the bacteria isolated.

The inflow of air through the immediate-outdoors and other openings, like the doors which have been engaged daily and almost every minute by the students and workers alike contributed to the high frequency of bacteria obtained in this study. This data supports a study conducted on understanding airborne microbial dynamics in built environments, which indicated that indoor airborne bacterial communities are influenced by outdoor air source and ventilation\(^26\). Classrooms and laboratories sampled were air-conditioned; therefore bacterial contamination of air reported in this study is inevitable, especially when the air conditioner blades are not properly or frequently cleaned.
Similar results and observation have been reported, which emphasized blowing-air blades as potential microbial sources. The foyers, regarded as an immediate-outdoor environment had a low bacteria representation as compared to the indoor environment. Although outdoor air has been reported as a major driver of the indoor air microbiome, our data suggests higher bacterial concentrations in the indoor environments. It could be that in addition to human occupancy and activities, the outdoor-indoor bacterial penetration were effective, thereby contributing to the high indoor bacterial loads.

The toilet is a small area of the building but visited by almost all the students and workers. Small areas with a lot of people have been reported to influence the concentration of bacteria, with high potentials to be propagated into the air wave. The library and classrooms had a higher percentage of contamination with indoor bacteria when compared to teaching and experimental laboratories. The results are similar to a study conducted on the assessment of bacteria in indoor air of a medical college. It is also interesting to mention that the experimental laboratories were more contaminated than the teaching laboratories. This may be due to diverse research activities in the experimental laboratories which suggest a need for more cautionary measures in basic routine laboratory operations. A study conducted on the analysis of variation in total airborne bacteria concentration in microbial laboratories reported improper disinfection practice and handling of specimens without following the basic rules of sterility as a possible contributing factor. Moreover, the population of students in the classrooms and library is also a possible contributing factor to the higher bacteria concentrations in these environments.

As expected, the laboratory biosafety hood had a relatively low percentage of bacterial concentration. Isolation of bacteria from the hoods appears inappropriate as the UV light shield and creates sterile conditions. However, the reasons for the presence of bacteria, albeit at low numbers, might be due to improper disinfection practice, dilution factors of the disinfectants used or/and cross-contamination. In the study, the staircase railings had the lowest percentage of bacteria isolates, contrary to studies conducted elsewhere. Although the reason for this is clearly unknown, it could be attributed to the low samples collected (n=2).

The resistance of the bacterial isolates to most of the antibiotics tested in this study calls for serious attention. Both Gram positive and Gram negative bacteria had higher rates of resistance to different classes of antibiotics. Most of the antibiotic classes were used as treatment options by clinicians in case of an infection in the study area. This might limit the antibiotic choice for the treatment of infections associated with these bacteria in the study area. Interestingly, gentamicin and tetracycline showed a level of effectiveness especially against some of the Gram positive bacteria, and these antibiotics might be considered as parts of the treatment regimen in the study area.

In conclusion, the various air sampling sites of the institute showed the presence of bacteria, though with low levels of contamination within the range (54 – 249 CFU/m³) as compared to the World Health Organization standard. Thus, students and workers are at low risk of exposure to airborne bacteria. Isolation of bacteria from the laboratory biosafety hood is of great health concern. Although the majority seems opportunistic, they may have pathogenic potentials with significant consequences. The strength of this study is that it unravels the level of bacterial contamination and subsequent antibiotic susceptibility profiles of a typical working and learning research environments. The antibiotic profiles of the bacterial isolates from the study centre have not been conducted before; the data presented only suggest possible exposure to resistant bacteria strains.

Overall, proper disinfection practice, working under a standard sterile condition, quality monitoring of air and maintenance of devices that can transmit bioaerosol across different locations are highly recommended; for this will safeguard the health of students, staff, and workers.

Data availability
The data underlying this study is presented in the tables with additional data available from Figshare. Dataset 1: S1_Isawumi Abiola et al. 2018.pdf. https://doi.org/10.6084/m9.figshare.6241829.v1
This dataset is available under a CC BY 4.0 license

Competing interests
No competing interests were disclosed.

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Suggested title: Characterization of culturable airborne bacteria and antibiotic susceptibility profiles of indoor and immediate-outdoor environments of a research institute in Ghana.

Introduction: this is well written and covered all the different aspects such as spaces, contamination, environment, antibiotics and antibiotic resistance.

Methods:

Sampling sites: This require more information and possibly pictures. This is because they affect sources, distribution and numbers of bacterial that will be sampled. Sizes of foyer and lecture theatres would have helped as well.

Control: Authors indicated incubating plates up to 48 h. Incubating a non-sampled plate for similar period will be a good control.

Data:
Authors could perform correlational analysis of location against cfu. Additionally, a correlation of frequency of sampling sites against cfu would also have strengthened the data.

Conclusion:
The above suggestions will greatly improve the data and strengthened the discussion.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Pramod Kumar**

Applied Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

**Title:** Characterization of culturable airborne bacteria and antibiotic susceptibility profiles of indoor and immediate-outdoor environments of a research institute

It need to be improved as (mention city and country in the title) - "Characterization of culturable airborne bacteria of indoor and immediate-outdoor environments of a research institute, Accra, Ghana".

**Material methods:**

Mention detailed of sampling sites including a graphical diagram. Describe basis of number of samples collected from each site. The manuscript is very basic and need significant enrichment. Mention climatic condition or meteorological factors during the sampling period.

Improve methodologies for bacterial identification with special emphasis on identification of *Staphylococcus aureus*. All the mannitol fermenters on MSA agar plates are not *S. aureus* as some of the other Staph sps also ferment mannitol. Authors need to identify *S. aureus* by using coagulase and thermonuclease tests or by PCR. Authors may refer a paper entitled “Prevalence of Methicillin Resistant Staphylococcal Bioaerosols in and around Residential Houses in an Urban Area in Central India” (Journal of Pathogen doi.org/10.1155/2016/7163615) for identification of airborne *S. aureus*. Mention formula for quantitative measurement of bacteria (cfu/m$^3$).

**Results:**

Major comments:
• Serious concern is lack of quality control strains antimicrobial susceptibility testing (AST). Authors should revise AST using *E. coli* ATCC25922 and *S. aureus* ATCC25923) as quality control strains.

• It would be better if authors mention statistical confidence/significance to represent comparative bacterial count in different sites.

• Authors are also advised to do statistical correlation analyses of outdoor-indoor movements with bacterial counts to represent effect of movements on indoor bacterial prevalence.

Minor comments: CFU is “colony forming unit” not “coliform forming unit”.

**Discussion:** needs more focus.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.