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Assessment of bacterial aeroflora in urban settings at different times of a day

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Abstract

Aerobiology explores the origin, dispersion and impact of organisms and materials released into the atmosphere on plant, animal and human life. With this objective in mind, the present study was carried at three different times of the day (morning, afternoon and evening) to investigate the diversity and distribution of airborne bacteria in three major marketplaces of Shillong i.e. Polo Bazar, Police Bazar, and Bara Bazar by petri plate exposure method. The isolates were subjected to biochemical characterisation such as catalase, oxidase, indole, methyl red, citrate, Voges-Proskauer, triple sugar iron tests and gram staining. Bara Bazar exhibited the highest bacterial count in the morning and evening while Police Bazar consistently showed the lowest count. Afternoon samples showed elevated counts in Polo Bazar, correlating with reduced foot traffic. Across all samples, eight bacterial genera were identified, with Bacillus and Staplylococcus present at all sites and time, highlighting their environmental resilience. Morning samples exhibited the highest bacterial diversity, aligning with diurnal patterns. All isolates were Gram-positive, consistent with atmospheric bacterial studies emphasizing their dominance in airborne environments. These findings underscore the dynamic nature of urban microbial communities and emphasize the need for effective monitoring and hygiene practices in public marketplaces, to mitigate potential health risks. This investigation sheds light on the abundance and diversity of airborne bacteria in these marketplaces, raising concern about potential health risk. The findings emphasize the need for effective monitoring and control measures to manage airborne microorganisms in urban environments.

Keywords: Aeroflora, Airborne, Bacteria, Market settings, Risk.

Introduction

Airborne particles of biological origin are called bioaerosols. Bacteria constitute part of the airborne aerosol and are widely encountered in the lower layer of the troposphere (Fahlgren *et al.* 2010). Bioaerosols can be viable or non-viable, examples include bacteria, fungal spores, pollens, mites, death tissue etc. Air serves as a mode of transport for the dispersal of bioaerosols from one location to another, required for the reproduction and for the colonization of new sites. The presence of bacteria in the air can significantly influence the ecology, climate and public health at both local and global scale (Nowoisky *et al.* 2016). Therefore, without understanding the bacterial aerosol diversity it is difficult to determine their functional importance to atmospheric chemistry and cloud formation, or the risk of infection (Brodie *et al.* 2007; Morris *et al.* 2014; Amato *et al.* 2017). Atmospheric transport of bacteria provides an essential redistribution mechanism for viable microbes and also for genetic potential transfer between distinct regions and essentially different habitats (Polis *et al.* 1997; Säwström *et al.* 2016; Mayol *et al.* 2017) making the bacterial aerosols a critical factor for understanding the connections driving diversity (Prospero *et al.* 2005). Different types of bacteria are present in air which can be pathogenic and non-pathogenic. The pathogenic bacteria can be dispersed in the air from natural events or human activity. Some environments and human-related activities can lead to the generation of large numbers of bioaerosols and numerous airborne pathogenic agents (Nehme *et al.* 2008; Oppliger *et al.* 2008; Lecours *et al.* 2012). They are of important concern as it affects public health, agriculture, ecology, health and international security.

Bioaerosols that consist of fungi, bacteria, plant material (their associated cell wall material and toxins) are capable of causing allergies and respiratory problems. The aerobiological dispersal of pathogens such as species in the *Escherichia, Salmonella, Legionella, Neisseria, Bacillus, Francisella, Burkholderia, Clostridium, Brucella* and *Yersinia* genera pose important health and ecological issues (Kuske *et al.* 2006). Some of the researchers have found that the pathogenic bacteria can be transported in massive dust clouds from one place to another (Sultan *et al.* 2005). Recently atmospheric microbiology studies (Kuske *et al.* 2006) have contributed to our ability to detect bacterial pathogens in air samples; these studies increase our knowledge of the spatial and temporal dispersal of aerosolized bacteria and also increase our understanding of natural bacterial diversity and composition of air. Bacterial community fingerprints have been used to broadly assess the composition of bacteria in urban aerosols (Kuske *et al.* 2006). Thus, there is a need for knowing about the normal abundance, distribution and composition of bacteria in the atmosphere. This type of study poses challenges due to its broad diversity and tremendous variability (both locally and regionally), in terms of microbial load and composition owing to seasonal effects, local climatic conditions, etc. Despite these important aspects, the diversity of bacteria inhabiting atmospheric ecosystems remains poorly constrained in terms of biogeography (Dueker *et al.* 2018). Several studies have also found increased diversity in microbial aerosol samples with low humidity conditions and increased wind speeds (Jones *et al.* 2004; Lee *et al.* 2017).

The study and characterization of these bioaerosols is necessary for several reasons such as to understand the diversity of bacteria in air; increasingly comprehensive taxonomic surveys of bacterial diversity in the outdoor air, helps in explaining the bio geographical distribution of bacteria (Hagstro¨m *et al.* 2000; Kellogg *et al.* 2006; Hervas *et al.* 2009). This further aids in understanding pattern of aerosol dispersal of bacterial pathogens, monitoring aerosol pathogen surveillance, providing clues for the spread of disease (Brown *et al.* 2002) and understand alteration in meteorological processes such as ice nucleation (Mo¨hler *et al.* 2007).

These studies represent a significant effort in understanding the composition, transport, survival and viability of bacteria in the air. However, many questions remain unanswered about patterns and factors affecting the aforementioned components, and the overall abundance, composition and consequences of bacteria in air (Kuske *et al.*2006). Microorganisms can be transported within and between continents on upper air currents(Brown *et al*. 2002). The threat of biological warfare and the need to develop early warning systems also add to the importance of knowing the identity of microorganisms commonly dispersed in the atmosphere (Fahlgren *et al.* 2010). The present-day study was carried out to identify the bacterial forms in three different market places in the Shillong area. Thus, this study will provide more information regarding the microbes present in air and how they vary from location to location.

Materials and methods

Study area

Shillong is the capital city of Meghalaya and is situated in the North-eastern part of the country situated at 25.5788° North(N), 91.8933° East(E) at an altitude of 1,491m above sea level. Three markets namely, Polo Bazar, Police Bazar and Bara Bazar were selected for the present study.

Polo Bazar situated at 25.5823° N; 91.8830° E extended to an area of about 3000 sq. m. The different types of stalls in the market are vegetable shops, fish stalls, pork stalls, beef stalls, chicken stalls, tea stalls, grocery, kwai (betelnut) shops, mutton stalls, pharmacies etc.

Police Bazar is another crowded market of Shillong famous for its shopping haunt, which remains on the priority list of locals as well as tourists. It is a modern market that has a number of hotels, shops and eating joints, catering to varied needs of every visitor. It is situated at 25.5779° N, 91.8837° E.

Bara Bazar is a popular market in Shillong for household items like fruits, vegetables, clothes and other items and is situated at 25.5724° N, 91.8745° E.

Sample Collection

Airborne microbial sampling was carried out by the petri plate exposure method (passive method) in morning, afternoon and evening. The air samples were collected aseptically from Polo Bazar, Police Bazar and Bara Bazar three times a day i.e. early in the morning from 6:00am-7:00pm, in afternoon from 12:00pm-2:00pm and in evening from 5:30pm-6:30pm. The sample was collected in triplicates using the petri plate exposure method. During the exposure the number of people around was also counted. Nutrient agar served as a culture media for the growth of bacteria. After collecting the sample, the plate was sealed with paraffin and transferred to the laboratory. Thermometric as well as hygrometric readings were also taken during the time of sample collection.

Isolation of bacteria

The bacterial samples collected from air were incubated at 37°C for 24 h. After the growth of the microorganism on the plate, colonies that developed were counted and recorded. Based on the colony morphology, representative colonies were picked for further characterization. To isolate the pure colonies of bacteria, streak plate technique was used and incubated at 37°C for 24 h. The pure cultures of bacterial samples were maintained in a 2 ml glycerol stock for further use.

Characterization of bacterial culture

Morphological characterization: Shape, size and colour of bacterial colonies

Morphological characteristics of the colonies such as shape, size and colour were observed for presumptive identification. The opacity of the bacterial colonies was noted by observing whether the colonies were translucent, transparent or opaque. Their margin and elevation were also observed.

Gram staining

For differentiating Gram positive bacteria from Gram negative bacteria Gram's staining was performed. A thin smear of culture on glass slides was made with a loop full of bacteria. The smear was air dried and then heat fixed, and then stained with crystal violet (Himedia, India) for 60 s, Gram's iodine (Himedia, India) for 60 s, followed by Gram's decolourizer (Himedia, India) for 30 s and safranin (Himedia, India) for 60 s. The smear was then washed with distilled water and air dried. The slide was then observed under the microscope in 100x magnification (Aneja *et al*. 2003).

Biochemical characterization

To identify the unknown isolates a number of biochemical tests were performed which included catalase test, indole test, methyl red test, Voges-Proskauer test, oxidase test and citrate test.

Indole test

A tube of peptone water was inoculated with a small amount of a pure culture. Incubation at 35°C (+/- 2°C) for 24 to 48 h. To test for the indole production, 5 drops of Kovac's reagent was added directly to the tube. A positive indole test was indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent. The reagent layer remained yellow or slightly cloudy if the culture was indole negative.

Methyl red test

MR-VP broth was prepared and 5ml of broth was added in test tubes and was sterilized by autoclaving at 121°C (15psi) for 45 min. The test tubes were inoculated with the bacterial culture and incubated at 37°C for 24-48 h. For the result, test tubes with the isolates were taken and 5 drops of methyl red indicator was added to the tubes. A red colour indicated positive methyl red test and yellow colour indicated a negative methyl red test.

Voges-Proskauer Test

MR-VP broth was prepared and 5ml of broth was added in test tubes and were sterilized by autoclaving at 121°C (15psi) for 45 min. The test tubes were inoculated with the bacterial culture and incubated at 37°C for 24-48 h. For the results the test tubes with the isolates were taken and 1-2 drops of Barritt Reagent A and 1-2 drops of Barritt Reagent B were added and kept in the shaker incubator and allowed for the reaction to complete for about 30 min. Pinkish red colour indicated positive test and no change in colour indicated a negative test (Usha *et al.* 2008).

Citrate utilization test

A single isolated colony was lightly streaked on the surface of the Simmons Citrate Agar slant. The tubes were incubated at 35°C (+/- 2°C) for 18 to 48 h. A Citrate positive growth was visible on the slant surface and the medium turned intense Prussian blue. Citrate negative culture had minimal trace or no growth visible and colour change had not occurred; the medium remained deep forest green colour of the uninoculated agar.

Catalase test

Catalase activity was observed by adding a few drops of 3 % H_2O_2 to the broth cultures, kept on the glass slides. Formation of oxygen bubbles confirms the positive result (Singh *et al.* 2012).

Oxidase test

The oxidase test was carried out by placing the colony of bacteria onto an oxidase disc which are sterile paper disc impregnated with N N-dimethyl phenylenediamine oxalate, ascorbic acid and a-naphthol (Usha *et al.* 2008). A sterile wooden cotton swab was used to pick a part of the colony of the unknown isolates and touched on the filter paper. If the colour of the disk changes to purple or dark blue within 10 seconds, it indicates a positive result. No colour change indicates a negative result.

Triple sugar iron agar (TSI) test

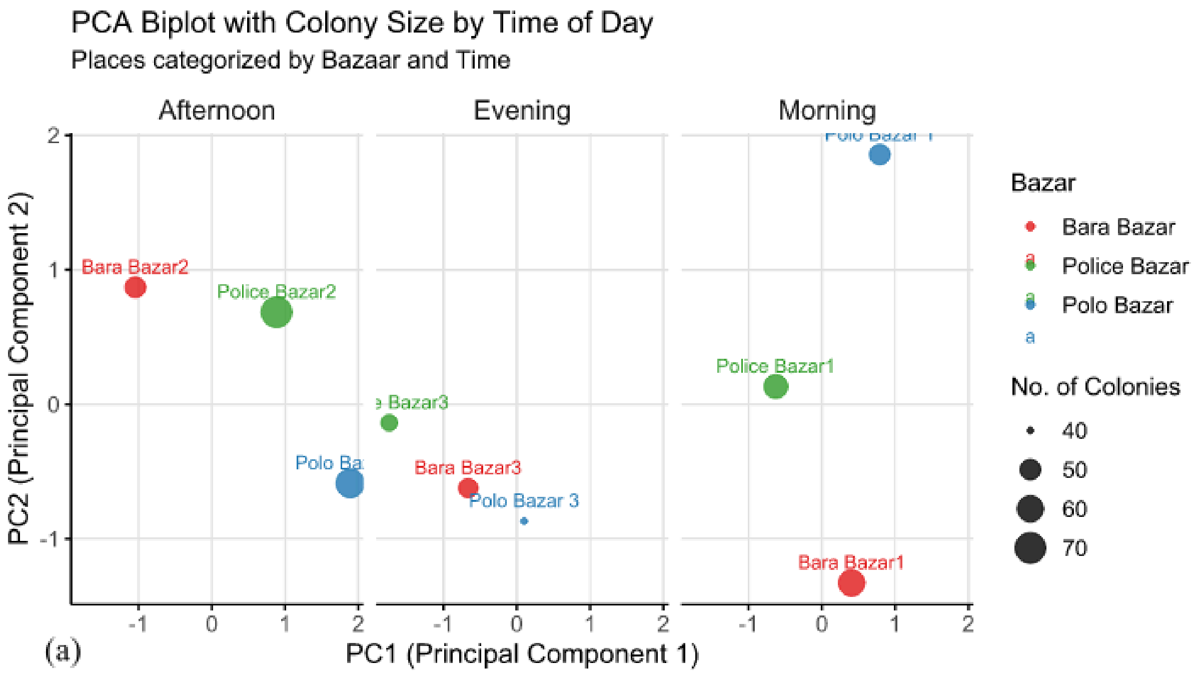
TSI agar was stabbed at the centre of the slant butt and streaked on the slope of the tube with a fresh culture of the test organism. The tubes were incubated at 37°C for 24 to 48 h. The glucose fermentation is shown by a yellow butt and red slant (Usha *et al.* 2008). Yellow slant and red butt indicate lactose/sucrose fermentation while red slant and red butt indicate no fermentation of any of the three sugar sources.

Results

Sampling areas show variation in the number of colonies collected in the morning, afternoon and evening (**Table 1**).

Table 1. Sampling sites and the bacterial colonies isolated at different times of the day.

Places	No of colonies		
	Morning	**Afternoon**	**Evening**
Polo Bazar 1	4	51	6
Polo Bazar 2	87	67	3
Polo Bazar 3	82	42	17
Police Bazar1	26	4	10
Police Bazar2	18	21	4
Police Bazar3	30	0	14
Bara Bazar1	160	6	5
Bara Bazar2	10	5	15
Bara Bazar3	54	29	29



(a)

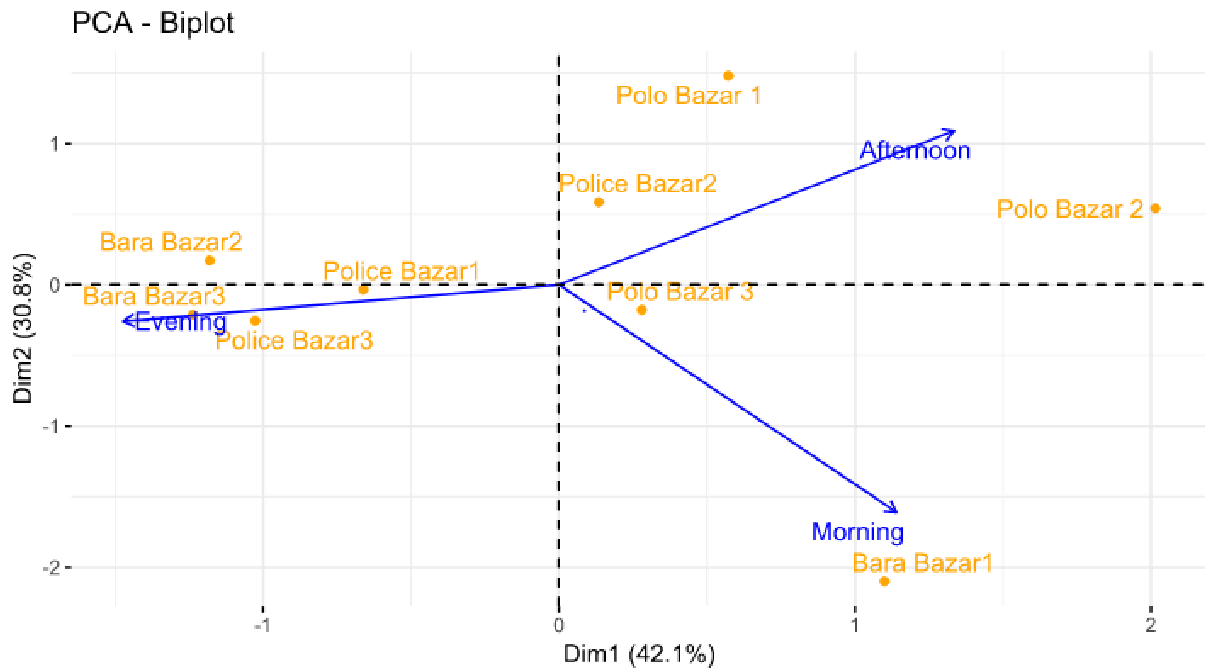
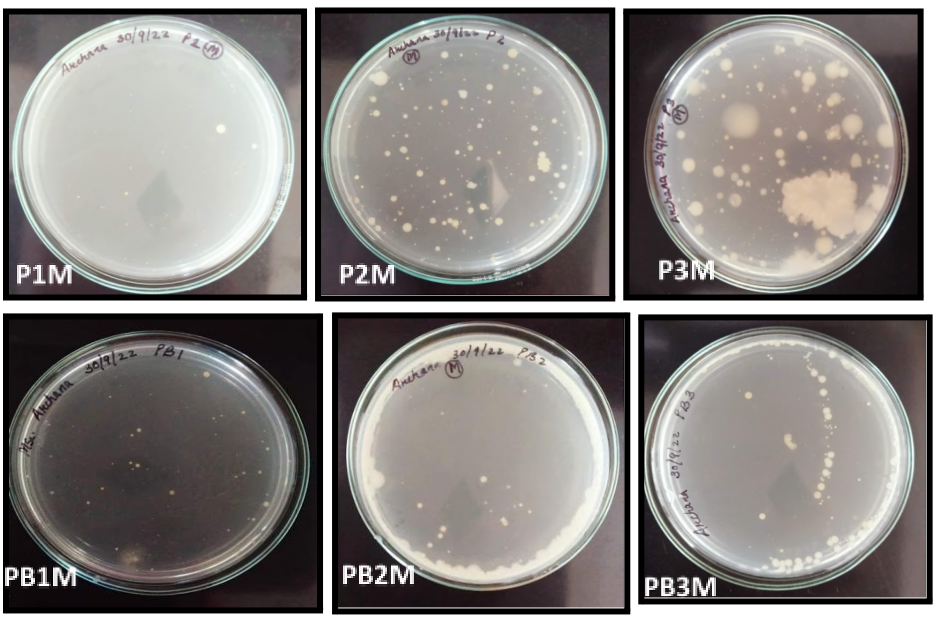


Fig. 1. Plot showing distribution of airborne microbes in colony distribution (a) and various locations (b).

The Normality Test (Shapiro-Wilk Test) and ANOVA (Analysis of Variance) showed that the data for morning, afternoon, and evening were normally distributed. There was a significant difference in the number of colonies across different times of the day. The Pearson Correlation coefficient indicated that there was no significant correlation between the sampling times of the day. All correlations were weak and not statistically significant ($p > 0.05$), suggesting no meaningful relationship between the number of colonies observed at different times of the day.



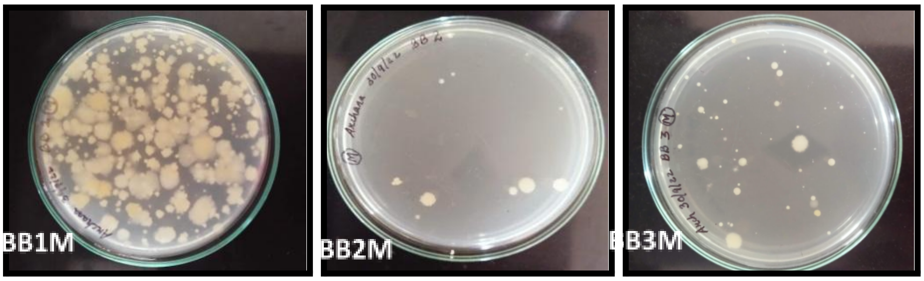


Fig. 2. Bacterial growth for morning sampled isolates.

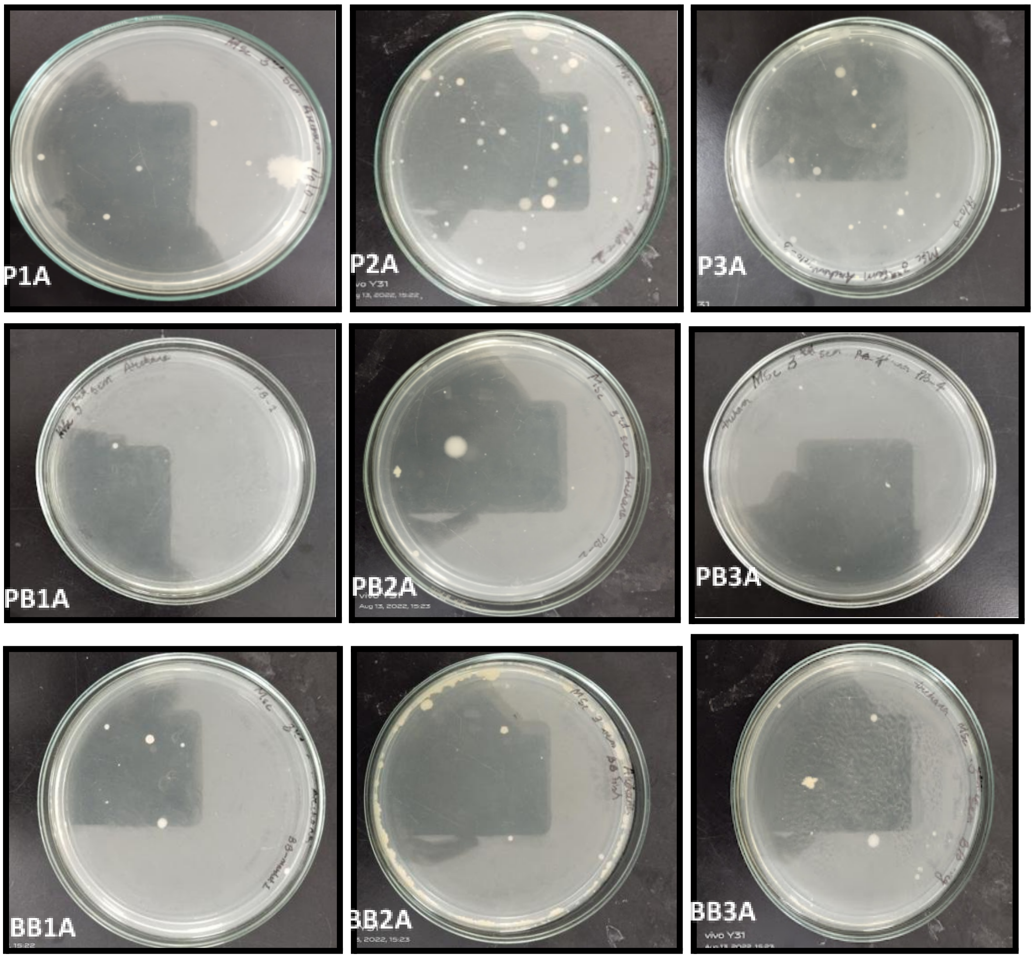


Fig.3. Bacterial growth for afternoon sampled isolates.

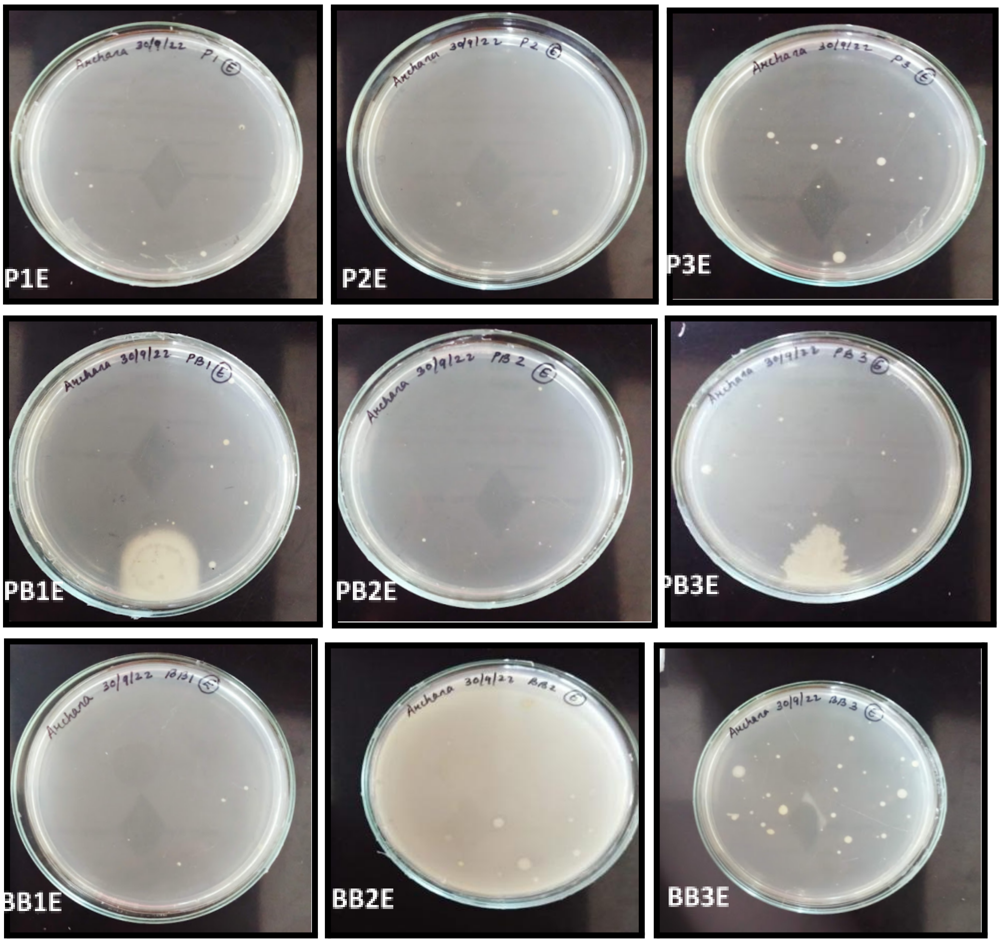
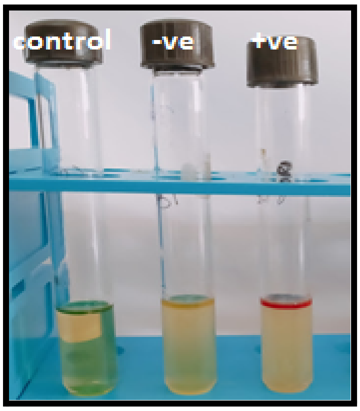
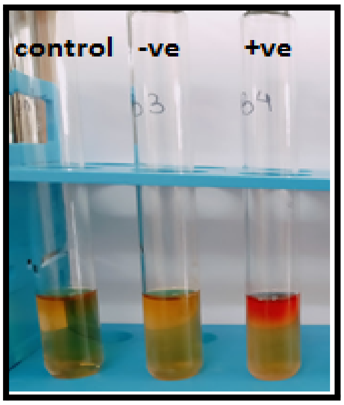


Fig. 4. Bacterial growth for evening sampled isolates.

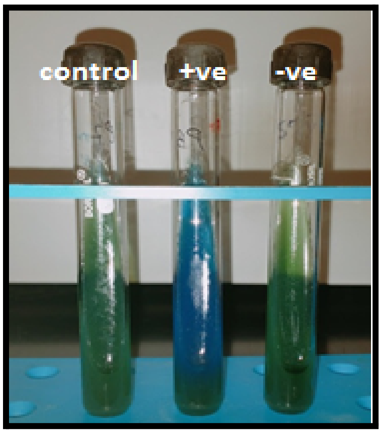


a. Indole test

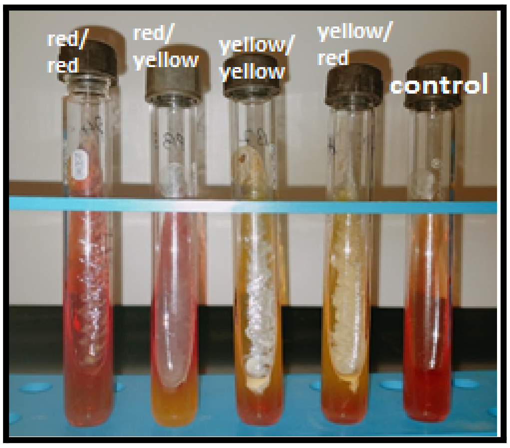


b. Methyl red test

c. Voges-proskaeur test

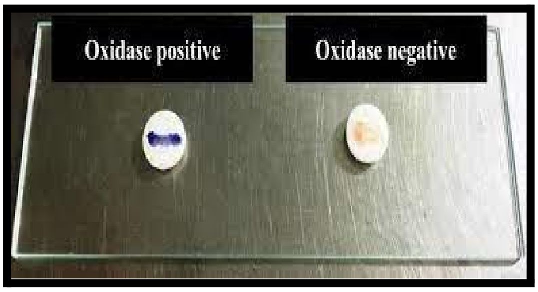


d. Citrate utilization test



e. Triple sugar iron test (TSI)

f. Catalase test



g. Oxidase test

Fig. 5. (a-g) Some biochemical tests (a) Indoletest (b) Methyl red test (c) Voges-Proskaeur test (d) Citrate utilization test (e)Triple sugar iron test (TSI) (f) Catalase test (g) Oxidase test.

Table 2a. Biochemical parameters characterized for the bacterial isolates from different market settings.

Isolates (M)	**Indole**	**Methyl red**	**Voges-Proskaeur test**	**Citrate utilization test**	**catalase**	**oxidase**	**Triple sugar iron test (TSI)**	**Gram nature**	**Presumptive identification**
P2_M	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
P4_M	-ve	-ve	-ve	-ve	+ve	-ve	R/R/-	Gram +ve, cocci	*Gemella*
P6_M	-ve	+ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
P7_M	-ve	+ve	+ve	-ve	+ve	+ve	Y/Y/-	Gram +ve, cocci	*Cellobiosococcus*
P9_M	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
P12_M	-ve	-ve	-ve	+ve	+ve	-ve	R/R/-	Gram +ve, rod	*Bacillus*
P13_M	-ve	+ve	-ve	-ve	+ve	+ve	Y/Y/-	Gram +ve, rod	*Bacillus*
P14 _M	-ve	-ve	-ve	-ve	+ve	+ve	Y/Y/-	Gram +ve, cocci	*Micrococcus*
PB1_M	-ve	-ve	+ve	-ve	+ve	+ve	Y/Y/-	Gram +ve, cocci	*Micrococcus*
PB4_M	-ve	+ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, cocci	*Saccharococcus*
PB7_M	-ve	-ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Staphylococcus*

PB8_M	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
PB9_M	-ve	+ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Saccharococcus*
PB10_M	-ve	-ve	-ve	+ve	+ve	-ve	R/R/-	Gram +ve, rod	*Bacillus*
PB11_M	-ve	+ve	-ve	-ve	+ve	+ve	Y/Y/-	Gram +ve, cocci	*Micrococcus*
PB12_M	-ve	+ve	+ve	+ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Cellobiosococcus*
BB3_M	-ve	+ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, cocci	*Saccharoccus*
BB11_M	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y/-	Gram +ve, cocci	*Trichococcus*
BB12_M	-ve	-ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, cocci	*Enterococcus*

Table 2b. Biochemical parameters characterized for the bacterial isolates from different market settings.

P1_A	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, rod	*Bacillus*
P2_A	-ve	+ve	+ve	-ve	+ve	-ve	Y/Y/-	Gram +ve, rod	*Bacillus*
P4_A	-ve	-ve	-ve	+ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Staphylococcus*
P22_A	-ve	-ve	-ve	-ve	+ve	-ve	R/R/-	Gram +ve, cocci	*Gemella*
PB1_A	-ve	+ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, rod	*Bacillus*
PB2_A	-ve	+ve	-ve	-ve	+ve	+ve	R/Y/-	Gram +ve, cocci	*Micrococcus*
PB3_A	-ve	+ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Trichococcus*
PB4_A	-ve	-ve	-ve	-ve	+ve	-ve	R/R/-	Gram +ve, cocci	*Gemella*
BB3_A	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
BB4_A	-ve	+ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Micrococuus*
BB5_A	-ve	+ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Trichococcus*
BB11_A	-ve	+ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Trichococcus*
BB12_A	-ve	-ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Staphylococcus*
BB13_A	-ve	-ve	+ve	-ve	+ve	+ve	R/Y/-	Gram +ve, rod	*Bacillus*

Table 2c. Biochemical parameters characterized for the bacterial isolates from different market settings.

Isolates (E)	Ind ole	Methyl red test	Voges-Pro skaeur test	Citrate utilization test	Catalase test	Oxidase	Triple sugar iron test	Gram nature	Presumptive identification
P1_E	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, rod	*Bacillus*
P2_E	-ve	+ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, cocci	*Saccharococcus*
P4_E	-ve	-ve	-ve	-ve	+ve	-ve	R/R/-	Gram +ve, cocci	*Gemella*
P5_E	-ve	-ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, rod	*Bacillus*
P7_E	-ve	+ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Staphylococcus*
PB1_E	-ve	-ve	-ve	+ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Staphylococcus*
PB3_E	+ve	+ve	-ve	-ve	+ve	+ve	R/Y/-	Gram +ve, rod	*Bacillus*
PB6_E	-ve	-ve	-ve	-ve	+ve	-ve	R/R/-	Gram +ve, cocci	*Gemella*
PB8_E	-ve	-ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Staphylococcus*
PB11_E	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*
BB3_E	-ve	-ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Staphylococcus*

BB4_E	-ve	+ve	-ve	-ve	+ve	-ve	Y/Y/-	Gram +ve, cocci	*Trichococcus*
BB7_E	-ve	+ve	-ve	-ve	+ve	-ve	Y/R/-	Gram +ve, cocci	*Trichococcus*
BB13_E	-ve	+ve	-ve	-ve	+ve	+ve	Y/R/-	Gram +ve, rod	*Bacillus*
BB14_E	-ve	-ve	-ve	-ve	+ve	+ve	R/R/-	Gram +ve, cocci	*Enterococcus*

Biochemical characterization of isolates collected in morning, afternoon and evening

The majority of the isolates showed negative results for methyl red, citrate, Voges-Proskauer test and indole test (except PB3_E). Additionally, all the isolates were tested positive for catalase activity. Most of the isolates exhibited a positive reaction for the oxidase test (**Table 2a-c**).

Discussion

This study highlights the variation in airborne bacterial diversity across three marketplaces in Shillong-Polo Bazar, Police Bazar, and Bara Bazar based on time of day and market conditions. Bara Bazar showed the highest bacterial count due to dense crowds and perishable goods, while Police Bazar had the lowest which could be attributed to its focus on non-perishable items like clothing and accessories and better hygiene conditions. The observation of higher morning bacterial count aligns with findings by Chmiel and Lenart-Boroń (2021), indicating a diurnal pattern where bacterial presence peaks in the morning and diminishes in the evening. Eight bacterial genera were identified, with *Bacillus* and *Staphylococcus* consistently present across all locations and times. All isolates were gram-positive, consistent with findings by Fengxiang (1990), indicating resilience in airborne environments. The dominance of Gram-positive cocci and rods aligns with previous atmospheric bacterial studies, where Gram-negative bacteria are a minority. *Staphylococcus,* commonly found on human skin and respiratory tracts, is abundant in the atmosphere due to shedding and coughing, as well as from organic waste. *Bacillus*, widely distributed in soil, is also prevalent in the air. Other significant bacteria include *Micrococcus*, *Enterococcus*, and *Gemella*, found in diverse environments such as soil, water, and the human body which align with the findings by Yongyi *et al.* (1993). These findings underscore the need for regular monitoring and improved hygiene practices in urban marketplaces to reduce potential health risks. The composition of airborne microbes in urban marketplaces like Polo Bazar, Police Bazar, and Bara Bazar in Shillong is influenced by various factors such as local sources of microbes from soil, human activity, and the presence of organic matter like garbage.

Furthermore, this study emphasizes the importance of understanding the microbial community in urban areas, as these environments host a large number of people who may inadvertently spread diseases as well as antibiotic and heavy metal resistance genes carried by these microbes. Monitoring microbial presence helps detect potential pathogenic strains and supports efforts to control their spread.

Conclusion

The present study revealed a wide microbial diversity present in the air in the bustling areas of Shillong. This study further emphasizes the microbial risks prevailing in the air around Shillong as well as necessitates adequate air quality monitoring and appropriate measures to improve the air quality such as establishment of green belt in and around the busy areas of Shillong. However, further studies are needed to assess and characterize the risk posed by the aerial microflora present in Shillong.

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